THE COMPARATIVE TOXICITY OF PARAQUAT IN RATS, GUINEA PIGS AND MONKEYS

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ABSTRACT

THE ACUTE LETHALITY OF
PARAQUAT IN RATS, GUINEA
PIGS AND MONKEYS

By

Ronald Eugene Murray

Paraquat, a widely used contact herbicide, has been shown to induce fatal lung lesions in mammals regardless of the route of administration or species. The purpose of this study was to characterize the toxicity of paraquat in rats, guinea pigs and monkeys in order to show whether or not differences in lethality between species correlated with differences in the clinical syndrome, pharmacokinetics and pathology of paraquat intoxication.

The acute lethality of orally administered paraquat varied with each species studied and was 143, 22 and 50 mg/kg in rats, guinea pigs and monkeys respectively. The lethality in rats was not influenced by fasting or strain differences.

Clinical signs of intoxication were anorexia, adipsia, diarrhea, hyperpnea, tachycardia and dyspnea and were seen in all species. Rats and guinea pigs showed signs of hypoxia. The signs of paraquat intoxication varied with the dosage in monkeys. Monkeys which received greater than 63 mg/kg of

paraquat died acutely following convulsive seizures, those that received paraquat dosages of 50-53 mg/kg showed signs of dyspnea for several days prior to death while those monkeys that were given 30-40 mg/kg paraquat showed signs of dyspnea for a few days and developed pulmonary fibrosis.

The histopathology produced by paraquat intoxication was compared in each species. The liver, kidney and gastrointestinal tract showed occasional necrosis in local areas of all species studied. The primary lesion occurred in the lungs. Animals that died in less than 7 days showed hemorrhage, edema and congestion. Rats and monkeys developed trabecular collapse, alveolar collapse and fibrosis after 7-10 days.

The pharmacokinetics of paraquat were compared in rats, guinea pigs and monkeys. The absorption of paraquat was poor in the rat and guinea pig with peak serum levels of 5.0 μg/ml and 2.5 μg/ml respectively following LD50 dosages. Peak serum levels were reached at 48 min and 30 min after administration of paraquat in rats and guinea pigs respectively. The concentration of paraquat in heart, kidney, liver, lung and serum was measured for each species following LD50 dosages. The concentration of paraquat in the lung showed continual uptake of the compound. In the rat, lung concentration of paraquat increased steadily for 32 hr as compared to the guinea pig with two peaks at 1 and 16 hr.

The excretion of paraquat in urine and feces was measured in each species. In the rat, concentrations of paraquat in the urine and feces could be measured for as long as 14 and 19

days respectively. No detectable metabolites were found in rat urine during the first 12 hr after paraquat administration. Paraquat excretion in urine and feces of the guinea pig was considerably less than that in the rat. In the primate, constant low levels of paraquat were eliminated in urine for 2 weeks.

The paraquat intoxication syndrome was characterized in rats, guinea pigs and monkeys, and the clinical manifestations of the intoxication were related to hypoxia and dyspnea. The absorption, distribution and elimination of paraquat varied with each species, but the toxicity was principally related to the extent of paraquat absorption. Fasting and strain differences did not influence the toxicity of paraquat in rats. It was concluded that the primary pathology in the lung was related to uptake of the chemical by that organ.

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I. INTRODUCTION

Paraquat, 1,1'-dimethyl-4,4'-dipyridylium dichloride, is a broad spectrum, contact herbicide widely used in agriculture. 4,4'dipyridyl compounds were first synthesized in 1881 by A.W. Hofmann (Michaelis and Hill, 1933). The physical and chemical properties of dipyridyl compounds were studied by Michaelis and Hill in 1933, and paraquat was described as the oxidation-reduction indicator, methyl viologen. In 1955, the herbicidal activity of paraquat was discovered (Calderbank, 1968), and since that time the compound has been used extensively in Europe.

Pure paraquat, a colorless, shiny crystalline powder, divalent cation, consists of two quaternized pyridine rings. The anions associated with paraquat may be bromide, chloride, iodide, methylsulfate or sulfate. The associated anion has no effect on the herbicidal activity (Akhavein and Linscott, 1968).

Paraquat has redox properties and, upon uptake of a single electron during reduction, gives rise to a stable, intensely colored, water soluble free radical. All the nuclear carbon positions of the rings share the electron (Calderbank and Crowdy, 1962). Complete reduction of paraquat may occur by the addition of a second electron to the molecule (Akhavein

and Linscott, 1968). The reduction of paraquat is completely reversible and independent of pH and the nature of the associated anion (Homer et al., 1959).

Paraquat is water soluble (70 g/100 ml, 20 degrees C) and stable in aqueous acid or neutral solutions but insoluble in most organic solvents.

The mechanism of action of paraquat in plants has been extensively studied (Akhavein and Linscott, 1968; Calderbank, 1968; Homer et al., 1960; Conning et al., 1969). The compound is absorbed quickly by plant tissue, and all green growth is susceptible. The speed of action depends on light conditions. In bright sunlight, leaf kill is usually complete within a few days (Black and Meyers, 1966; Conning et al., 1969; and Calderbank, 1968). Paraquat is reduced by electrons from $NADPH_2$ (Black, 1966). The free radicals formed are readily reoxidized by molecular oxygen to the original diquaternary ion, generating hydrogen peroxide or intermediate radicals which may be responsible for the destruction of plant cells (Calderbank, 1968).

Recently, paraquat has been introduced into the United States for herbicidal use. Among the desirable features are: 1) rapid inactivation by soil bacteria (Calderbank, 1968); 2) minimal residues in edible crops (Calderbank and Slade, 1966); and 3) non-toxic residue levels (McKean, 1968).

Spectrophotometry and ion-exchange techniques have been combined for paraquat analysis of animal tissue (Daniel and Gage, 1966). In the presence of reducing agents, the generated

colored radical is stable and has absorption peaks at 396 and 600 mμ (Calderbank, 1968).

The acute toxicity of paraquat has been studied in different animal species, and the syndrome of toxicity appears the same regardless of route of administration (Clark et al., 1966). The acute toxicity of paraquat in laboratory animals was manifested as: anorexia, diarrhea, dyspnea, hyperpnea, tachycardia and convulsions. The most outstanding lesion from paraquat toxicity occurred in the lung. The lesion consisted of congestion, hemorrhage and edema which preceded cellular proliferation and fibroblastic overgrowth. Among the species studied were rats, mice, guinea pigs, cats, dogs, sheep, cattle, horses, chickens and fish (Thomas and Amor, 1968; Conning et al., 1969; Clark et al., 1966; Dunachie and Fletcher, 1970; Akhavein and Linscott, 1968; McIntosh, 1967; and Calderbank et al., 1968). Wide variability, however, in the lethal dose of paraquat among the different species has been found. For example, the acute oral LD50 of paraquat in rats was 440 mg/kg but only 30 mg/kg in guinea pigs (Tadjer, 1967 and Conning et al., 1969). Furthermore, the acute lethality for orally administered paraquat may vary among strains or colonies of rats since LD50's ranging from 57 to 440 mg/kg have been reported (Bailey and White, 1965 and Tadjer, 1967). The absorption of orally administered paraquat in the rat was slight since only 10% of the ingested paraquat was found absorbed and the rest was excreted in the feces (Daniel and Gage, 1966). Paraquat concentrations were measured

for 2 weeks in the urine of humans after acute intoxication (Pasi and Hine, 1971 and Matthew et al., 1968). Possibly continued absorption of paraquat temporarily retained by the gut or slow release from tissues was responsible for the slow excretion.

Chronic intoxication with paraquat in dogs led to progressive consolidation of the lungs. The subsequent development of fibrosis led to impairment of pulmonary function (Calderbank, 1968). Repeated doses of paraquat to laboratory animals (0.03 percent or more in the diet) produced pulmonary fibrosis in most species. Repeated instillation of 0.3 percent paraquat into the conjunctival sac produced a mild conjunctivitis with no damage to the cornea (Swan, 1969). Dermal application at the same concentration did not produce skin lesions in rabbits (Clark et al., 1966). However, 286 ppm paraquat added to the drinking water of rabbits produced inflammation of the tongue and esophagus and eventually resulted in death due to starvation. Inhalation of paraquat aerosols in concentrations above 1-2 μg/liter for several hours produced severe pulmonary congestion, alveolar edema and bronchial irritation (Gage, 1968). However, it was not possible to induce pulmonary fibrosis by exposing animals to respirable aerosols of paraquat Conning et al., 1969).

As rats developed pulmonary fibrosis, pulmonary compliance increased, respiratory minute volume decreased and pulmonary surfactant decreased (Cambar and Aviado, 1970). The lungs showed extensive intra-alveolar hemorrhage, diffuse fibrosis,

multinucleated epithelial cells, proliferation of the epithelium and periarteritis or perivascular lymphocyte infiltration (Kimbrough and Gaines, 1970). In rats that died within 5-6 days after a single oral dose, pulmonary edema, congestion and intra-alveolar hemorrhage developed. In animals that survived 10 or more days, fibrosis predominated in the lungs (Kimbrough and Gaines, 1970). No mammalian species, including man, has been found resistant to these paraquat-induced lesions, yet the mechanism responsible for the pulmonary lesions has remained unknown. In addition to lung lesions, chronic exposure in mice, rats and rabbits to paraquat resulted in slight liver and kidney necrosis (Clark et al., 1966). The typical pathology seen with oral ingestion of paraquat in the human included pharyngeal, esophageal and gastric submucosal erosions, small myocardial infarcts, edema and cellular proliferation in the lung, central zonal necrosis of the liver and tubular necrosis of the kidney (Hargreave et al., 1969; Campbell, 1968; Kerr et al., 1968; Toner et al., 1970; Bullivant, 1966 and McKean, 1968). Matthew et al. (1968) reported on the death of a child that had ingested paraquat after fasting 8 hours. Another child had ingested a comparable amount of paraquat and survived. The difference in lethality was attributed to the effect of fasting.

The purpose of this study was to characterize the toxicity of paraquat in rats, guinea pigs and monkeys. The working hypothesis of the present study was that differences between

species in acute lethality, clinical syndrome and pathology produced by paraquat were due to differences in pharmacokinetic parameters.

II. METHODS

A. Materials

1. Paraquat dichloride (commercial formulation)

29.1% paraquat dichloride
53.0% water
8.8% sodium chloride
4.4% methanol
0.4% 1-methyl-4,4'bipyridylium cation
0.1% 4,4'-bipyridyl
1.5% sodium metaborate
2.2% sodium benzoate
0.5% unknown organic impurity

Chevron Chemical Co.
Richmond, California

2. Paraquat dichloride salt (pure)

Imperial Chemical Industries Limited
Macclesfield, Cheshire, England

3. bis-(N-Methyl-^{14}C)-4,4'-bipyridylium chloride
Specific activity 14.7 mCi/mM and 15.5 mCi/mM
Code CFA.344 Batch 3 and 4

Amersham/Searle
Arlington Heights, Illinois

4. 2,5-diphenyloxazole (PPO)
Lot number 2681

New England Nuclear
Boston, Massachusetts

5. 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene
(Dimethyl POPOP)
Lot number 4329

Packard Instrument Company, Inc.
Downers Grove, Illinois

6. Soluene ^{TM}100
   Sample Solubilizer
   Lot 33053

   Packard Instrument Co., Inc.
   Downers Grove, Illinois

7. Dow AG-50W-X8 cation exchange resin
   Lot number 50C-1130

   Sigma Chemical Company
   St. Louis, Missouri

B. Animals

Male Sprague-Dawley[1] or Long-Evans[2] rats (125-150 gm), male and female Hartley guinea pigs[3] (300-400 gm)or male and female Macaca fascicularis monkeys[4] (3-10 kg) were used in these studies. All animals were housed in facilities with constant temperature (70-75 degrees F) and relative humidity (40-60%). The dark-light cycle was 12 hours. Food and water were provided ad libitum except during fasting experiments in rats.

C. Acute Toxicity

Fifty Sprague-Dawley and fifty Long-Evans rats were divided into groups of ten, and each group received one of the following dosages of paraquat by oral gavage: 101, 126, 159, 200 and 252 mg/kg (expressed as the cation). Solutions

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1. Spartan Research Animals, Inc., Haslett, Mi. Rats were housed in stainless steel cages and fed Lab-Blox (Wayne Co., Chicago, Ill.).

2. Center for Laboratory Animal Resources, Michigan State University, East Lansing, Mi. Long-Evans rats were used for acute toxicity studies only and housed in stainless steel cages and fed Lab-Blox.

3. Center for Laboratory Animal Resources, Michigan State University, East Lansing, Mi. Guinea pigs were housed in groups of 3 or 4 on pine chips in plastic boxes for the LD50 studies. During the pharmacokinetic studies they were housed individually in stainless steel metabolism cages. The guinea pigs were fed Purina Guinea Pig Chow (Ralston, Purina Co., Richmond, Ind.) during all experiments.

4. Center for Laboratory Animal Resources, Michigan State University, East Lansing, Mi. The primates were restrained in primate restraint chairs (Forringer Co., Inc., Rockville, Md.) and fed Monkey Chow (Wayne Co., Chicago, Ill.). These animals were young adults 3-5 years of age. They were tested for parasites and tuberculosis, and given thorough physical examinations before inclusion in the studies.

of paraquat for administration were prepared from the commercial formulation and diluted with water to the final concentration. Particular care was taken during administration to avoid aspiration of the paraquat solution by the experimental animals. The solutions were administered in a volume of 1.0 ml/100 g body weight.

In another experiment 30 guinea pigs were divided into five groups of six. Each group received one of the following dosages of paraquat: 20.0, 21.2, 22.4, 23.7 or 25.0 mg/kg by oral gavage. Solutions for administration were prepared as described above.

Treated rats and guinea pigs were observed several times each day for 14 days and gross symptomatology and lethality noted. Seven day LD50 values and their 95% confidence limits were determined by the method of Litchfield and Wilcoxon (1949).

The acute toxicity of paraquat in monkeys was determined by the up-down method of Brownlee et al., (1953). Using this method each monkey received a single, specific dosage of paraquat. The first dosage was 126 mg/kg of paraquat cation which was the seven day LD50 in fasted rats. Since this dosage was lethal, each succeeding monkey received a one-third decrease in this dosage until a primate survived. When this occurred, the dosage difference between the survivor and last fatality was divided by three and that dosage added to the dose given the survivor. The newly calculated dosage was administered to the next monkey. Paraquat was given by oral

gavage via a No. 10 French polyethylene stomach tube. The monkeys were held until death ensued, or until they were sacrificed for tissue analysis.

D. Clinical Methods

For the purpose of determining heart, liver and kidney function, 5 ml blood samples were collected from monkeys 3 days before and up to 21 days after treatment with paraquat. The blood was allowed to clot and serum was obtained by centrifugation at 1200 g for 10 min for the measurement of serum glutamic oxalacetic transaminase (SGOT, method of Reitman and Frankel, 1957), blood urea nitrogen (BUN, method of Chancy and Marbach, 1962) and serum glutamic pyruvic transaminase (SGPT, method of Reitman and Frankel, 1957). The analyses were preformed using the Ames/BMI Blood Analyzer (Ames Company, Elkhart, Indiana).

E. Pathology

Two rats each were sacrificed by cervical dislocation at 2, 4, 6, 8, 10, 12 and 14 days after the oral administration of 143 mg/kg paraquat (LD50). Tissues were examined grossly, and selected samples from the lungs, liver, heart and kidney were fixed in 10% formalin. Following fixation tissues were sectioned into 10 micron sections, mounted on glass slides and stained with hematoxylin and eosin (Diagnostic Laboratories, Veterinary College, Michigan State University). Treated tissues were compared to normal tissues by light microscopy.

In similar experiments guinea pigs were given 22 mg/kg paraquat orally (LD50), and tissues were examined grossly and microscopically. Tissues were examined from treated guinea pigs at 3, 5, 7 and 14 days after paraquat administration. These

tissues were compared to tissues from non-treated guinea pigs.

Selected monkeys were immobilized by an intramuscular injection of 1 mg/kg phencyclidine hydrochloride (Parke-Davis Co., Detroit, Michigan), sacrificed by exsanguination via the jugular vein, and examined for gross pathology. Necropsy was performed on all primates whether they were sacrificed or succumbed to paraquat intoxication. Lung, heart, liver and kidney tissue samples were examined for histopathology. The histopathology slides were prepared as described above.

F. Pharmacokinetics

The disposition of paraquat was determined in rats and guinea pigs using ^{14}C-labeled paraquat [bis-(N-methyl-^{14}C)-4, 4'-bipyridylium chloride] . Rats and guinea pigs were divided into groups of three and administered ^{14}C-labeled paraquat (0.175 and 1.250 μCi/mg, specific activity respectively) at a dosage of 143 or 22 mg/kg respectively. Groups were sacrificed at 0.13, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 hours (no guinea pigs at 0.13, 128, 256 or 512 hours). Following paraquat administration animals were placed in stainless steel metabolism cages (Acme Metal Company, Chicago, Illinois) and urine and feces collected (for times greater than 32 hours samples were collected daily). Blood was collected by cardiac puncture under ether anesthesia for each animal before sacrifice by cervical dislocation. Heart, liver, lung and kidney samples (50-100 mg) were collected. In addition the skin, carcass and intestinal tract were collected (128, 256 and 512 hour groups only). The carcass and gastrointestinal

tract were homogenized in 1 volume of water using a Waring Blender. Serum was obtained by centrifugation of clotted blood samples. All tissues and excreta were assayed for radioactive paraquat using liquid scintillation techniques. Tissue samples were collected, minced with scissors and 50-100 mg transferred to tared glass liquid scintillation vials. Serum and urine samples (0.1 ml) were transferred directly to glass vials. Feces were weighed, homogenized in 2 volumes 1 N HCl in a glass grinder and samples (50-100 mg) were transferred to tared vials. After preparation of samples, one ml Soluene was added to each vial, and the solution was incubated at 45 degrees C for 24 hours to ensure solubilization. After cooling, 15 ml toluene counting solution (1 liter toluene, 5 g PPO and 200 mg POPOP) was added to each vial and the samples were counted in a Beckmann LS-100 liquid scintillation counter to obtain the count rate (counts per minute, CPM). A known quantity of ^{14}C-labeled toluene standard was then added to each vial and each vial was recounted. The disintegrations per minute per mg (DPM/mg) of sample were calculated using the following formulas.

$$\frac{\text{(second CPM-background) - (first CPM-background)}}{\text{DPM } ^{14}\text{C-toluene standard added}} = \text{counting efficiency}$$

$$\frac{\text{first CPM-background}}{\text{counting efficiency}} = \text{DPM for sample}$$

$$\text{DPM/sample size (mg)} = \text{DPM/mg sample}$$

The Michigan State University Control Data 6500 computer was utilized for these computations.

Serum and tissue levels of paraquat were determined in treated monkeys using a colorimetric assay (Daniel and Gage, 1966). Weighed samples were homogenized in 2 volumes of water with a Potter-Elvehjem homogenizer. Homogenates were diluted with water to 25 ml and 12.5 ml of 25% trichloroacetic acid was mixed with each sample and the samples centrifuged at 2000 g for 10 minutes. The supernatant was saved. The precipitate was resuspended in 6 ml 5% trichloroacetic acid and centrifuged again. Both supernatants were placed on an ion exchange column containing 1.5 ml Dow Ag-50W-X8 cation exchange resin (water slurry). The solution was allowed to flow through the column at a rate of 1-2 ml/minute. Each column was rinsed with 25 ml distilled water. The paraquat cation was eluted with 25 ml of 5 M ammonium chloride at a flow rate of 0.5 ml/minute. A 5 ml aliquot of the eluate was removed, and 1 ml of 0.2% freshly prepared sodium dithionite in N sodium hydroxide was added. The optical density was immediately measured at 600 nanometers in a double beam Coleman model 124 spectrophotometer (Perkin-Elmer Corp., Maywood, Illinois). The optical density measured was compared to a standard curve obtained as above using pure paraquat dichloride (Yuen et al., 1967).

Thin layer chromatography was used in an attempt to analyze rat urine for paraquat metabolites. Two different thin-layer adsorbants were used; cellulose[5] and silica

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5. Cellulose with fluorescent indicator, Eastman chromagram sheet #6065, Rochester, New York.

gel[6]. Standards of 10 µl ^{14}C-paraquat (0.775 µCi/mg, specific activity) were used. Urine samples were collected at 1 and 12 hours from three 150 gram rats given 92 mg/kg ^{14}C-paraquat (0.364 µCi/mg specific activity). Urine samples (10 µl) were spotted on cellulose or silica gel thin-layer plates containing fluorescent indicator and developed in two solvent systems: 1) butanol, acetic acid and water (6:2:2) or 2) (8:1:1). After visual examination under long and short wave ultraviolet light, each chromatogram was cut into 10 equal squares, placed in scintillation vials, and 0.5 ml distilled water was added to elute the radioactive material from the adsorbant. Ten ml of modified Brays solution (100g napthalene, 6g PPO dissolved in 1 liter of dioxane) was added to each sample. These samples were counted using the liquid scintillation techniques previously described.

The extent of paraquat serum protein binding in vitro was determined by dialysis. Normal rat serum, 4.9 ml, was added to 0.1 ml paraquat solution (0.775 µCi/mg, specific activity), and placed in cellulose dialysis tubes one-half inch in diameter by 12 inches long, tied at both ends. The tubes were placed in 125 ml Erlenmeyer flasks with 50 ml phosphate buffer, pH 7.4, (132 ml of 0.067 M KH_2PO_4 and 868 ml of 0.067 M Na_2HPO_4) and placed in a water bath at 37 degrees C with mechanical shaking. One-half ml samples were removed from the buffer at time intervals of 0.5, 1, 2, 4 and 8 hours. In addition at 8

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6. Silica gel - MN silica gel (N-HR/UV254) Brinkmann Instruments, Inc., Westbury, New York.

hours the tubes were opened and 0.5 ml serum removed. All samples were placed in liquid scintillation vials and 10ml of modified Brays solution was added. Equilibration was achieved at 8 hours. Serum protein binding was calculated from the 8 hour values. The percent free paraquat in serum was found from the ratio of the paraquat concentration outside (equal to free paraquat inside) to the total paraquat concentration in serum. Percent protein bound was obtained by difference. In addition, rat serum from paraquat treated rats was dialyzed to determine the extent of in vivo serum protein binding. Six rats were given 92 mg/kg ^{14}C-paraquat orally (0.364 µCi/mg). Serum samples were taken one hour later from 2 groups of three rats each and pooled. Five ml of rat serum were dialyzed against 50 ml phosphate buffer (pH 7.4). Samples of 0.5 ml were taken from the buffer at 0.5, 1, 2, 4, and 8 hr and from the serum at 8 hours. Serum protein binding was calculated in the same manner as above.

G. Effect of fasting on paraquat lethality in rats

Fifty rats were divided into 5 groups of 10. The rats were fasted from 4 a.m. until 8 a.m. (4 hour fast). These rats were given paraquat orally at doses of 80, 101, 126, 159 and 200 mg/kg, and were maintained for 7 days. In another experiment animals were fasted for 8 hours. Their food was removed at 12 P.M. the evening prior to paraquat administration. These rats were divided into five groups of 10 and given paraquat at doses of 63, 80, 101, 126 and 159 mg/kg. LD50's

and their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (1949).

III. RESULTS

A. Acute toxicity of paraquat

The 7 day LD50's for orally administered paraquat in rats, guinea pigs and monkeys were 126, 22 and 50 mg/kg respectively (Table 1). Among rats, however, strain differences were not obvious. Sprague-Dawley and Long-Evans rats had LD50's of 126 (102-156, 95% confidence limits) and 116 (98-137) mg/kg (Table 2). The overlapping 95% confidence limits indicated there was no significant difference in paraquat lethality between strains.

The individual responses of 10 non-fasted primates to an acute dose of paraquat are presented individually in Table 3. Monkeys given more than 50 mg/kg survived at least seven days. The exception was a monkey which vomited 1 hour after paraquat administration and died three days later.

Table 1

Acute Oral Lethality of Paraquat in

Rats, Guinea Pigs and Monkeys

Species	Number of Animals Treated	LD50 mg/kg	(95% confidence limits)[a]
Rat	50	126[b]	(102-156)
Guinea Pig	50	22[b]	(15-33)
Monkey	10	50[cd]	

[a]Method of Litchfield and Wilcoxon (1949).

[b]Seven day test period.

[c]See Table 3 for individual data.

[d]Method of Brownlee, et al. (1953).

Table 2

Acute Oral Lethality of Paraquat

in Two Strains of Rats[a]

Rat Strains	LD50 mg/kg (95% confidence limits)[b]	
Long-Evans[c]	116	(98-137)
Sprague-Dawley[c]	126	(102-156)

[a] Fasted 8 hours.

[b] Method of Litchfield and Wilcoxon (1949).

[c] Seven day test period.

Table 3

Acute Oral Lethality of Paraquat in

Non-Fasted *Macaca fascicularis*

Monkey Number	Sex	Wt. kg	Dosage mg/kg	Survival time (days)
1	M	3.2	126	1
2	M	4.7	76	2
3	M	3.7	63	1
4	M	8.5	53	8[a]
5	M	3.7	50	14[a]
6	F	3.9	50	10
7	F	4.4	45	7
8	M	10.4	40	3[b]
9	F	4.6	40	21[a]
10	F	3.6	35	21[a]

[a]Sacrificed.

[b]Vomited 1 hour after paraquat administration.

B. Clinical manifestations of paraquat intoxication

The clinical manifestations of orally administered paraquat were observed for all species. A few (less than 5%) rats showed convulsions and/or dyspnea within 72 hours after oral administration of paraquat and died. Three to four days after oral administration, the rats began to show signs of hypoxia, decreased activity, labored breathing and later hyperpnea. They stopped eating and drinking. Most of the rats died within 3-7 days after paraquat administration, and a few rats died 7-10 days after administration. During the 3-10 day period after paraquat administration, most of the rats lost 20-30% of their body weight.

Guinea pigs showed signs of weakness, cyanosis, hyperpnea and tachycardia after paraquat administration. Death losses occured from 2-8 days. If any sign of dyspnea developed the guinea pigs died within 24-48 hours. No guinea pigs survived once they began to show signs of intoxication.

The primates showed various clinical signs depending upon the dosage of administered paraquat (Table 3). Primates which received high doses died within 1-3 days. The major clinical sign was convulsions which preceded death by a few hours. Signs of hypoxia were noted and included tachycardia and hyperpnea. In addition, anorexia and adipsia were seen. Monkeys that lived 3-7 days showed diarrhea, some with mucous or blood. After a few days the stool and the appetite returned to normal. Primates that lived longer than 7 days were given doses of paraquat 50 mg/kg or less. These monkeys also went through

a period of anorexia, dehydration and depression. Within the first week they recovered from the symptoms and began to increase in activity, appetite and hydration. These monkeys showed no signs of dyspnea or cyanosis.

In general, measurement of BUN, SGOT and SGPT in paraquat poisoned monkeys revealed no definite dysfunction of liver, heart or kidneys (Tables 4, 5, 6). The blood urea nitrogen levels rose slightly 48-72 hours after paraquat intoxication but returned to normal levels within a few days in the animals which survived paraquat exposure.

C. Pathology

The gross pathological manifestations of paraquat poisoning in the rat were limited to lesions in the kidney, gastrointestinal tract and lung. The kidney showed congestion. The gastrointestinal tract was inflammed and showed erosions of the mucosal lining. Lung lesions varied with the duration of intoxication. Rats that died within 3-5 days showed swollen, hyperemic lungs. Rats surviving 5-7 days developed progressive pulmonary lesions, with atelectasis, emphysema, congestion and fibrosis.

Tissues from rats sacrificed at various time intervals between 1 and 14 days after oral administration of paraquat showed lesions in the lung, kidney and liver. The lesions of the liver and kidney showed small areas of centrolobular necrosis and tubular necrosis, respectively. Lung lesions

Table 4

Blood Urea Nitrogen Levels in Macaca fascicularis after Oral Administration of Paraquat

Monkey[a] Number	Blood Urea Nitrogen Days After Paraquat										
	-2	-1	0	1	2	3	4	5	6	7	14
	mg/100 ml serum										
1	20	15	5	30	--[b]	--	--	--	--	--	--
2	19	21	23	45	--	--	--	--	--	--	--
3	20	15	25	30	--	--	--	--	--	--	--
4	25	25	27	25	17	21	15	19	31	31	--
5	20	20	15	20	15	5	20	35	15	25	25
6	20	0	10	20	20	30	35	20	20	35	--
7	40	35	30	30	40	40	50	50	60[d]	--	--
8	20	15	18	30	25	--	--	--	--	--	--
9	21	20	14	18	17	18	12	18	13	14	11
10	25	35	55	60[d]	40	30	45	30	25	20	20
	[c]23±2		20±3		22±4						

[a]Monkey numbers same as Table 3.

[b]Animal succumbed.

[c]Mean ± S.E. for all monkeys during pre-exposure period. Range during pre-exposure period was 0-55 mg/100 ml serum.

[d]Value exceeded normal range.

Table 5

Serum Glutamic Oxalacetic Transaminase Activity in *Macaca fascicularis* After Oral Administration of Paraquat

Monkey Number[a]	Enzyme Activity Days After Paraquat										
	-2	-1	0	1	2	3	4	5	6	7	14
	Karmen Units[b]										
1	56	75	3	30	--[c]	--	--	--	--	--	--
2	24	49	47	28	--	--	--	--	--	--	--
3	50	75	5	400[d]	--	--	--	--	--	--	--
4	35	21	45	37	25	15	19	37	101	57	--
5	10	110	90	90	20	5	10	0	180	0	10
6	35	85	50	0	0	0	50	35	40	35	--
7	80	150	200	175	200	150	125	125	90	--	--
8	100	50	20	15	20	--	--	--	--	--	--
9	40	15	13	9	11	15	15	5	4	15	20
10	175	15	75	60	35	35	55	30	40	25	20
[e]60±15	65±14	52±19									

[a]Monkey number same as Table 3.

[b]One unit is equal to the enzyme activity necessary to produce a decrease in the optical density (oxidation of reduced nicotinamide-adenine dinucleotide, NADH, to NAD) at 340 mμ of 0.001 optical density/min/ml (Karmen, 1955).

[c]Animal succumbed.

[d]Value exceeded normal range.

[e]Mean ± S.E. for all monkeys during pre-exposure. Range during pre-exposure period was 3-200 Karmen units.

Table 6

Serum Glutamic Pyruvic Transaminase Activity in *Macaca fascicularis* After Oral Administration of Paraquat

Monkey[a] Number	Enzyme Activity Days After Paraquat										
	-2	-1	0	1	2	3	4	5	6	7	14
	Karmen Units[b]										
1	5	10	190	25	--[c]	--	--	--	--	--	--
2	28	40	20	37	--	--	--	--	--	--	--
3	5	10	150	400	--	--	--	--	--	--	--
4	19	11	309	107	107	5	103	109	301	31	--
5	500	0	10	30	10	0	10	10	100	0	5
6	50	75	50	0	0	10	50	10	30	20	--
7	125	100	175	125	100	70	90	70	40	11	--
8	70	40	35	30	40	--	--	--	--	--	--
9	55	12	15	17	5	15	3	5	20	10	19
10	75	10	10	15	10	15	10	10	10	5	20
	[d]43±12			31±10			55±22				

[a]Monkey numbers same as Table 3.

[b]One unit is equal to the enzyme activity necessary to produce a decrease in the optical density (oxidation of reduced nicotinamide-adenine dinucleotide, NADH, to NAD) at 340 mμ of 0.001 optical density/min/ml (Karmen, 1955).

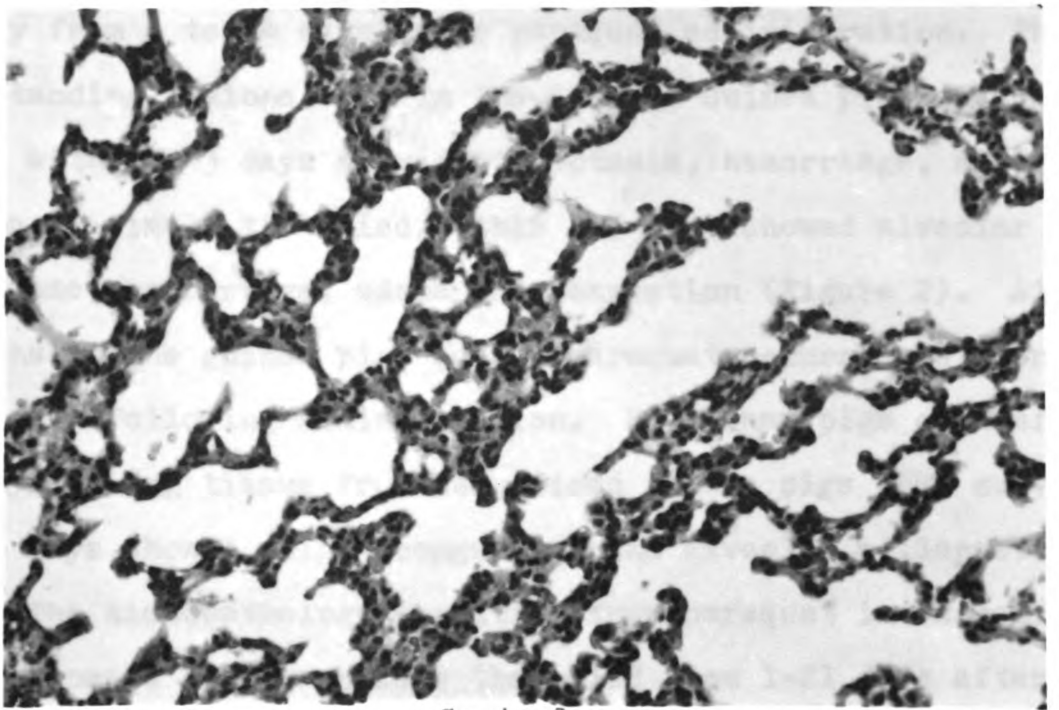
[c]Animal succumbed.

[d]Mean ± S.E. for all monkeys during pre-exposure. Range during pre-exposure period was 0-500 Karmen units.

varied depending upon the survival time after oral administration. Rats that died 3-5 days after paraquat administration showed hemorrhage and edema of the lungs. Rats that died 5-7 days after paraquat administration showed additional lesions of ruptured alveolar spaces, atelectasis, congestion and fibrosis. Fibrosis, alveolar collapse and congestion were the predominant lesions of rats that survived 7-10 days (Figure 1).

No guinea pigs died within two days after paraquat administration. Gross pathological examination of paraquat poisoned guinea pigs showed swollen, hyperemic lungs if they died within 2-5 days. If they lived longer than 5 days, emphysema, edema and congestion of the lung tissue was found. Fibrosis was not apparent in guinea pigs surviving longer than 1 week.

The gross pathological lesions in primates were limited primarily to the lung, liver, and kidney. Occasional slight necrosis of the liver and kidney tissue was found. Animals that died within 1-3 days showed emphysema, congestion and hemorrhage of the lung. One monkey had slightly hyperemic lung tissue when sacrificed 21 days after paraquat. The rat and monkey are the only two of the three species that developed fibrotic changes in the lung due to paraquat exposure. The other pathological lesions in tissues were not sufficient to cause death in the animals that succumbed to paraquat intoxication.



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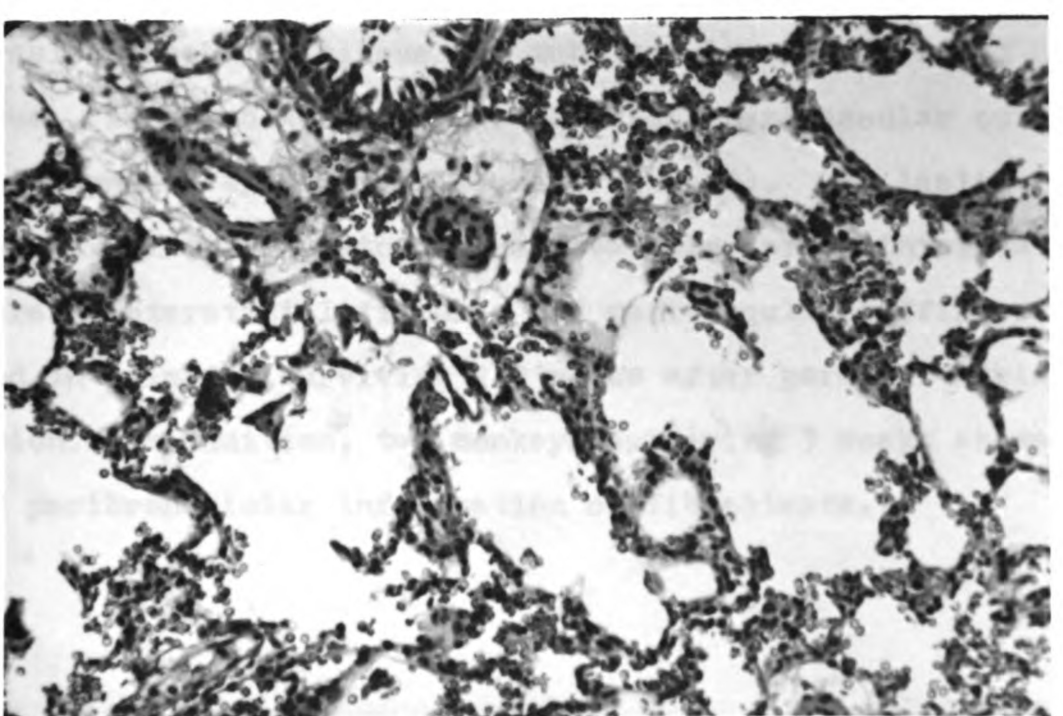
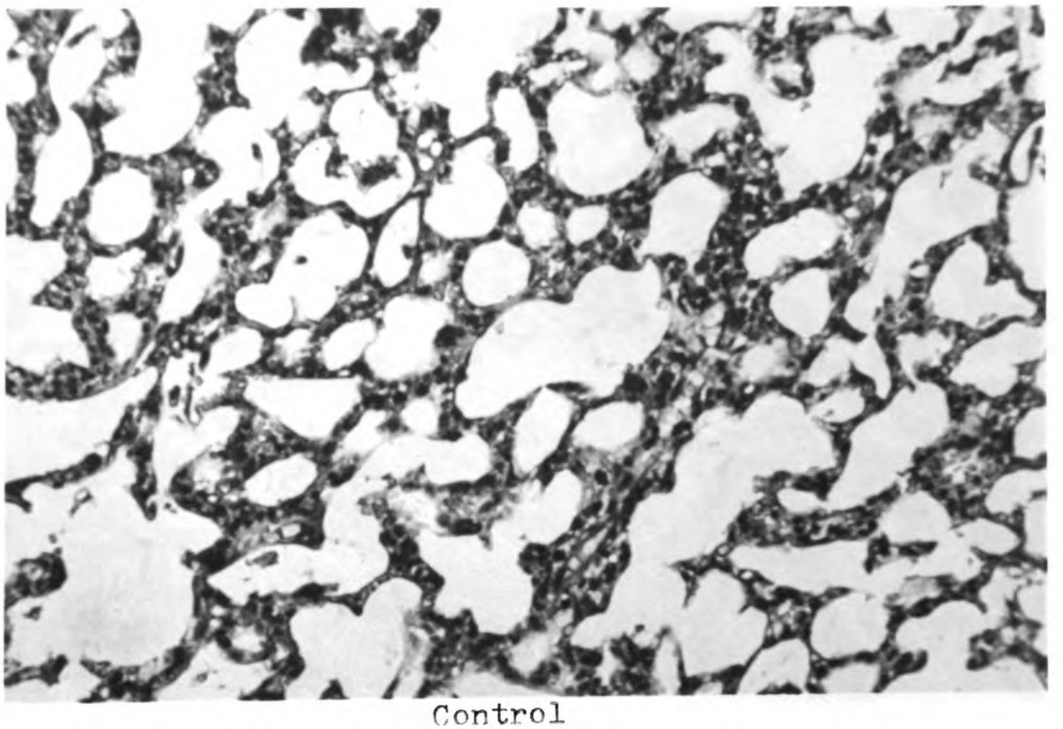


Treated

Figure 1. Lung tissue from control and paraquat treated rats. The treated rat was given paraquat orally at a dose of 143 mg/kg and sacrificed 7 days later. The treated lung tissue shows changes of alveolar membrane structure, atelectasis, congestion and fibrosis. Hematoxylin and eosin. X125.

Guinea pig tissues were studied from animals sacrificed daily from 1 to 14 days after paraquat administration. The outstanding lesions were in the lungs. Guinea pigs that died within 2-3 days showed atelectasis, hemorrhage, and edema. Animals that died within 3-7 days showed alveolar collapse, hemorrhage, edema and congestion (Figure 2). All deaths in the guinea pigs due to paraquat occurred between 2-8 days following administration. No guinea pigs died after 8 days. Lung tissue from sacrificed guinea pigs that survived 8-14 days showed slight congestion and alveolar collapse.

The histopathology resulting from paraquat intoxication was compared in ten monkeys that died from 1-21 days after paraquat ingestion. The paraquat was administered orally at varying dosages (Table 3). Primates showed mild centrolobular necrosis of hepatic tissue and tubular necrosis of renal tissue. Lungs showed edema, congestion, perivascular cuffing, hemorrhage and alveolar collapse (Figure 3). The lesions showed a relationship to the survival time after intoxication. Moderate interstitial fibrosis and perivascular cuffing was noted in primates surviving 2-3 weeks after paraquat administration. In addition, two monkeys surviving 3 weeks showed mild peribronchiolar infiltration of fibroblasts.



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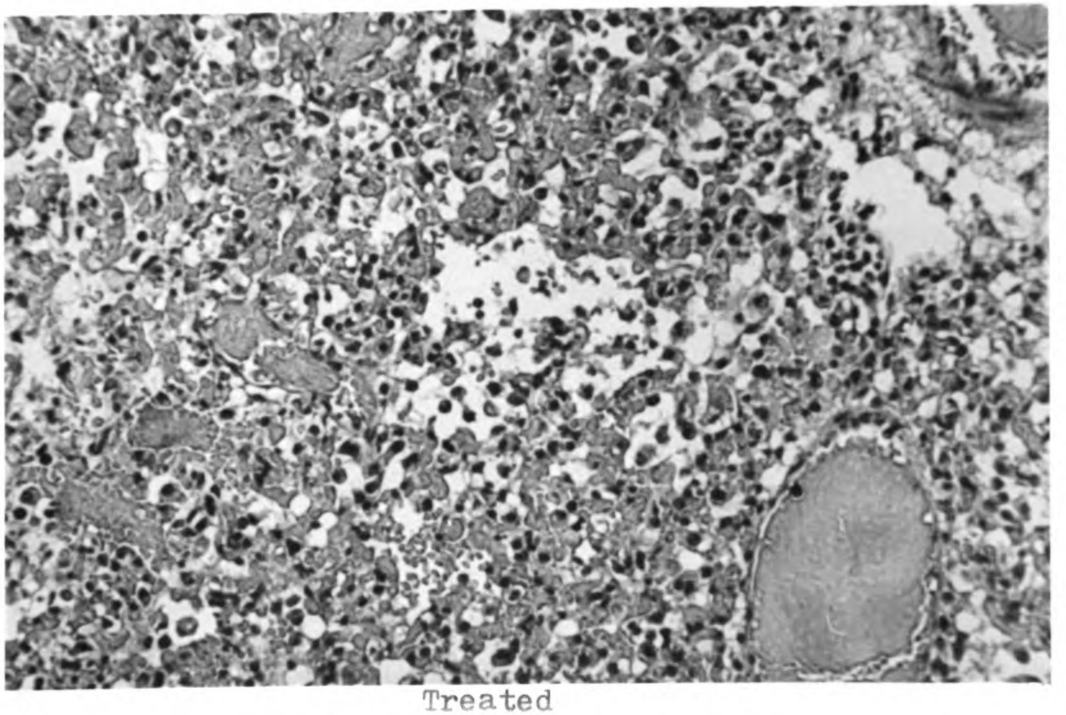
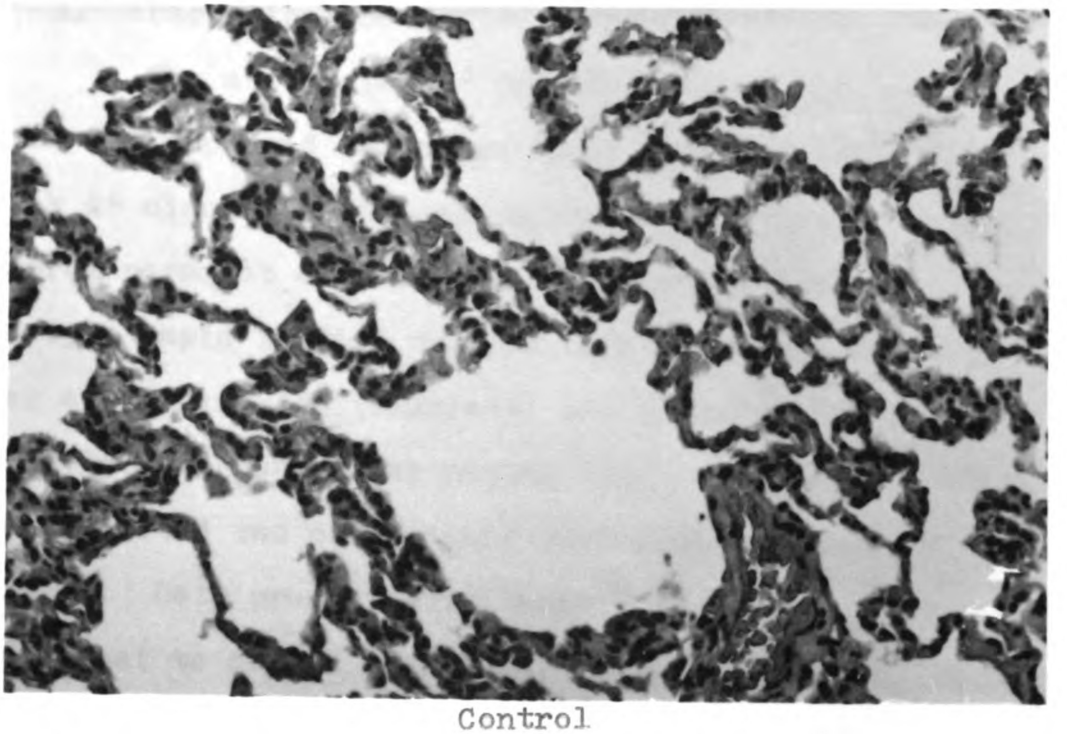


Treated

Figure 2. Lung tissue from control and paraquat treated guinea pigs. The treated guinea pig was given paraquat by oral gavage at a dose of 22 mg/kg and sacrificed 4 days later. The outstanding lesions from the treated guinea pigs were edema, congestion, and hemorrhage. Hematoxylin and eosin. X125.



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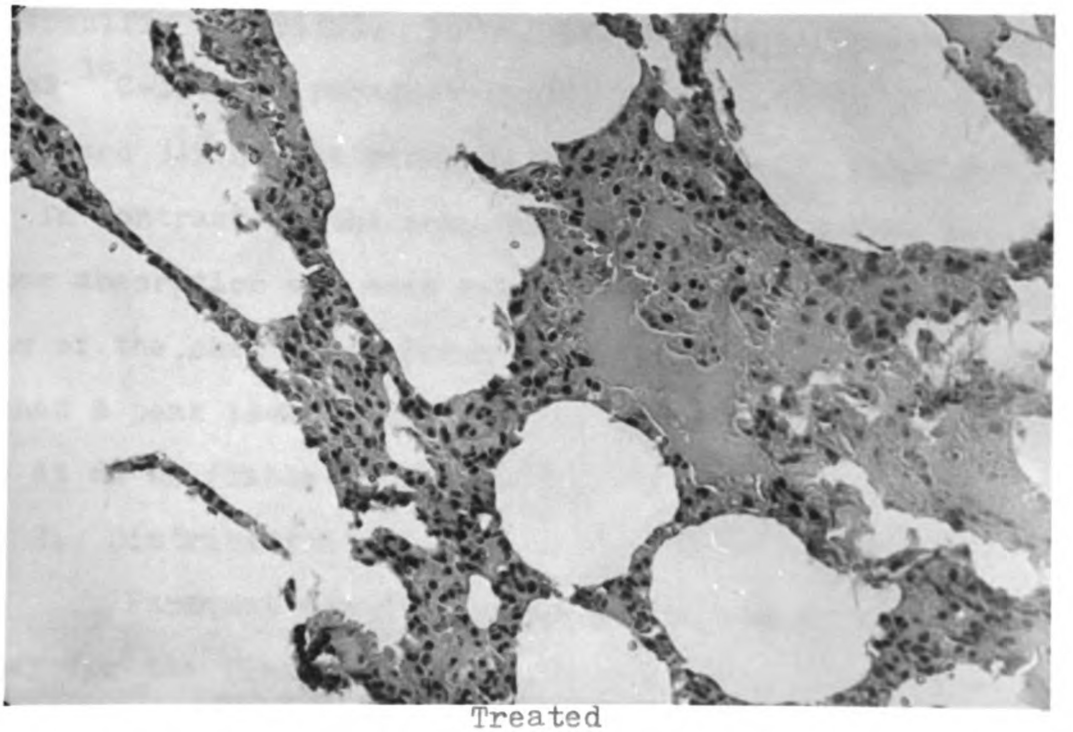

Treated

Figure 3. Lung tissue from control and paraquat treated Macaca fascicularis. Paraquat was administered by oral gavage at a dose of 53 mg/kg 7 days prior to death. The lungs from the treated animal showed congestion, fibrosis, alveolar and trabecular collapse. Hematoxylin and eosin. X125.

D. Pharmacokinetics

1. Absorption

The absorption of paraquat after oral administration in the rat appeared to follow first order kinetics and reached a peak 48 minutes after administration (Figure 4). The decline in serum paraquat of rats followed complex kinetics characterized by a rapid initial decline between 48 min and 4 hours after administration (Figure 4) and a prolonged slow decline from 4 to 256 hours after administration (Table 7). These data suggested two compartment distribution of absorbed paraquat. Data presented in Table 8 indicated in vivo binding of paraquat to serum, presumably to serum protein. Rat serum binding of paraquat was measured by dialysis and found to be 79% for rats given 92 µg/kg ^{14}C-labeled paraquat (0.364 µCi/mg, specific activity). Normal rat serum was dialysed with 0.1 ml ^{14}C-labeled paraquat (0.775 µCi/mg, specific activity) and showed 31% of the paraquat bound to serum proteins (Table 8).

In contrast to the rat, paraquat in the guinea pig showed better absorption and more rapid attainment of serum concentrations of the chemical. Serum concentrations of paraquat reached a peak level of 2.47 µg/ml serum in 0.5 hr and decreased to 0 at 64 hr (Table 9).

2. Distribution

Paraquat concentrations in the rat were higher in the kidney for the first 16 hours than any other tissue analysed. In general, kidney concentrations of paraquat were 5-10 times higher than serum concentration (Table 7).

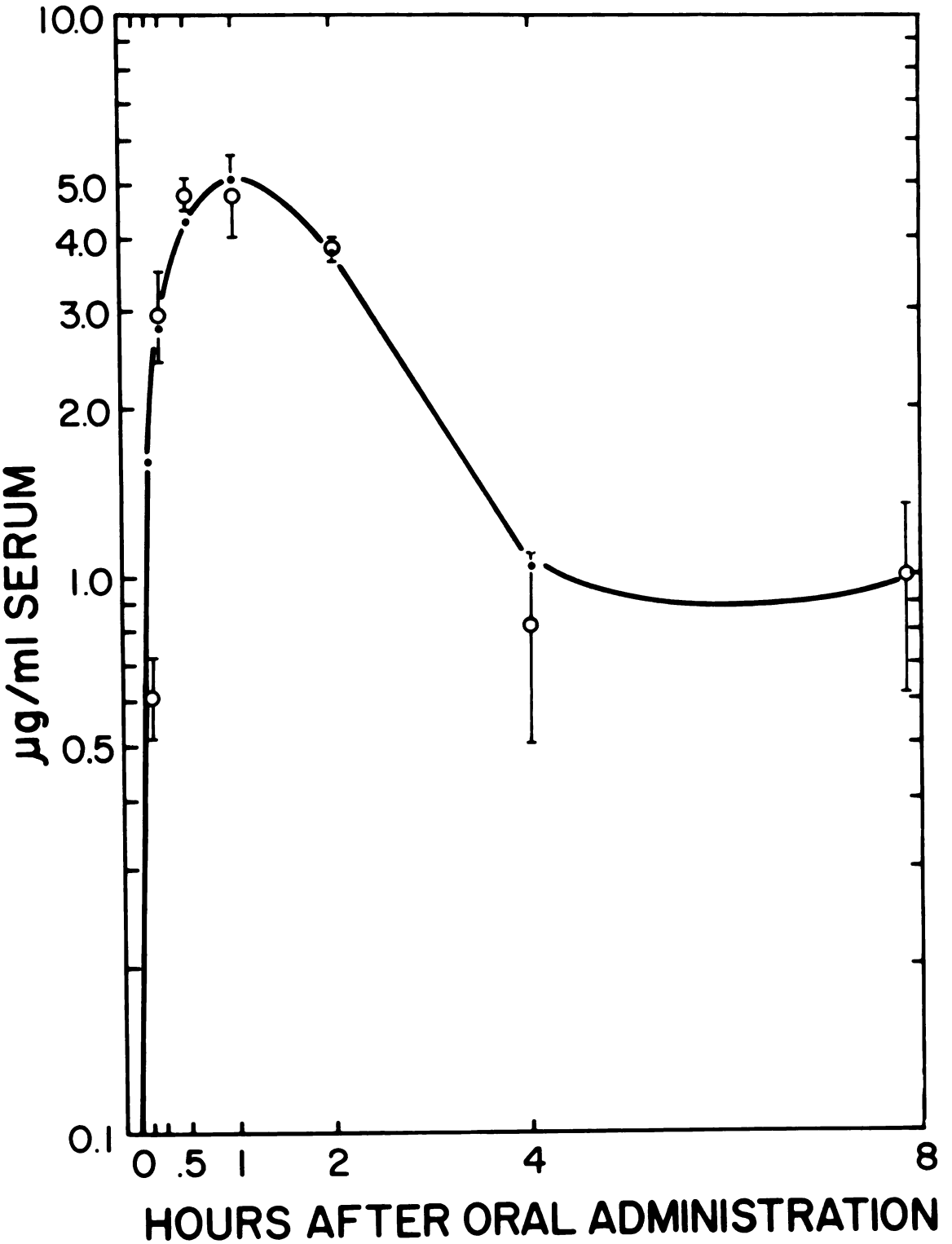

Figure 4. Paraquat concentration in rat serum 0 to 8 hours after administration. Twenty-four rats were divided into groups of 3 and given 143 mg/kg ^{14}C-labeled paraquat orally. The mean serum levels ± S.E. were determined, and are shown by the open circles. The closed circles represent calculated values obtained by linear regression analysis using an iterative process (Wiegand and Sanders, 1964).

Table 7

Tissue Distribution of Paraquat in Rats

Time After Administration[a] (hours)	μg paraquat/gm tissue[b]				
	Serum	Lung	Kidney	Liver	Heart
.25	2.9±0.6[c]	2.2[b]±0.3	7.7± 1.7	1.5±0.5	1.3±0.4
.50	4.8±0.2	3.4 ±0.1	13.1± 1.5	3.2±0.3	2.2±0.4
1	4.7±0.8	3.3 ±0.2	27.5±16.0	2.0±1.3	1.8±0.4
2	3.9±0.3	4.2 ±0.3	13.7± 1.0	5.0±0.3	0.5±0.4
4	0.8±0.3	3.7 ±1.5	4.5± 1.3	4.4±1.2	0.9±0.4
8	1.0±0.2	4.3 ±0.3	6.6± 1.1	1.9±0.4	1.5±0.2
16	0.9±0.1	5.0 ±1.1	7.0± 1.5	2.1±0.3	2.7±0.5
32	1.1±0.1	13.6 ±3.9	9.4± 2.6	5.7±1.6	2.8±0.4
64	0.1±0.0	1.7 ±1.0	1.0± 0.6	7.7±4.7	0.2±0.1
128	0.1±0.1	0.6 ±0.3	1.5± 0.8	0.1±0.1	0.1±0.1
256	0	0.1 ±0.1	0.3± 0.1	0.1±0.1	0
512	0	0	0.1± 0.1	0	0

[a] 143 mg/kg p.o. (LD50).

[b] 3-5 rats/group.

[c] Mean ± S.E.

Table 8

Serum Binding of Paraquat

In Rats

Sample	Total[a] Paraquat Dialyzed	Mean DPM/0.5 ml dialysate — Dialysis Time (hours)					% Free Paraquat[b]
		.5	1	2	4	8	
Standard[c]	1937[d]	1165	1522	1710	1795	1948	100
Serum In vivo[e]	850	134	150	150	178	175	20.6
Serum In vitro[f]	2067[d]	901	1067	1206	1354	1420	68.8

[a] Free and protein bound, DPM/0.5 ml.

[b] 8 hr.

[c] 0.1 ml ^{14}C-paraquat (0.775 µCi/mg, specific activity), $2.2X10^5$ DPM.

[d] Mean of six determinations.

[e] Serum pooled from 2 groups of 3 rats each given ^{14}C-paraquat 92 mg/kg (0.364 µCi/mg, specific activity), 1 hr before.

[f] 0.1 ml ^{14}C-paraquat (0.775 µCi/mg, specific activity), $2.2X10^5$ DPM mixed with normal rat serum.

Table 9

Tissue Distribution of Paraquat in Guinea Pigs

Time After Administration[b] (Hours)	µg paraquat/gm tissue[a]				
	Serum	Lung	Kidney	Liver	Heart
0.5	2.47±0.36[c]	1.05±0.59	7.82±1.42	2.12±0.40	0.80±0.20
1	0.45±0.05	0.41±0.21	2.89±0.92	0.05±0.05	0.05±0.03
2	0.25±0.06	0.71±0.14	2.04±0.74	0.07±0.07	0.13±0.03
4	0.05±0.02	0.85±0.15	2.47±0.45	0.14±0.01	0.07±0.01
8	0.03±0.02	0.52±0.10	1.03±0.52	0.14±0.05	0.15±0.03
16	0.03±0.03	1.29±0.86	1.99±1.07	0.08±0.07	0.31±0.26
32	0.01±0.01	0.61±0.40	0.54±0.15	0.09±0.06	0.01±0.01
64	0	0.48±0.34	0.30±0.13	0.09±0.07	0.01±0.01

[a]Three guinea pigs per group.

[b]22 mg/kg p.o. (LD50).

[c]Mean ± S.E.

Paraquat concentrations in the liver and heart of rats reached peak levels of 8 and 3 µg/gm respectively. Paraquat uptake by lungs was unique since levels in this organ increased steadily for 32 hours after paraquat administration. At 32 hours the concentration of paraquat was higher than in any other organ studied (Figure 5).

Paraquat concentration was measured in the skin, carcass, gastrointestinal tract and eliminations of rats at time intervals of 64, 128, 256 and 512 hr (Table 10). The total recovered paraquat included the paraquat in the lungs, liver, kidneys and heart. The total paraquat recovered was 41, 29, 19 and 27 percent of the administered dosage for the time intervals of 64, 128, 256 and 512 hr respectively. Identical percent recoveries were obtained for each group when the results were calculated on the basis of recovered radioactivity. There was no detectible concentration of paraquat in the skin, carcass or gut at 512 hr.

In guinea pigs, like in rats, lung concentration of paraquat increased to 1.05 µg/g in 0.5 hr and maintained a steady concentration for 64 hr. The kidney, liver and heart tissue concentrations of paraquat increased sharply to 7.82, 2.12 and 0.80 µg paraquat/g tissue respectively and decreased over the period of 64 hr (Table 9).

The tissue distribution of paraquat in the monkey (serum, lung, liver, heart and kidney) was measured at death by a colorimetric method (Table 11). In addition, the same method was used to measure daily serum levels in the monkeys. A peak serum level of paraquat was measured on the 24 hr sample

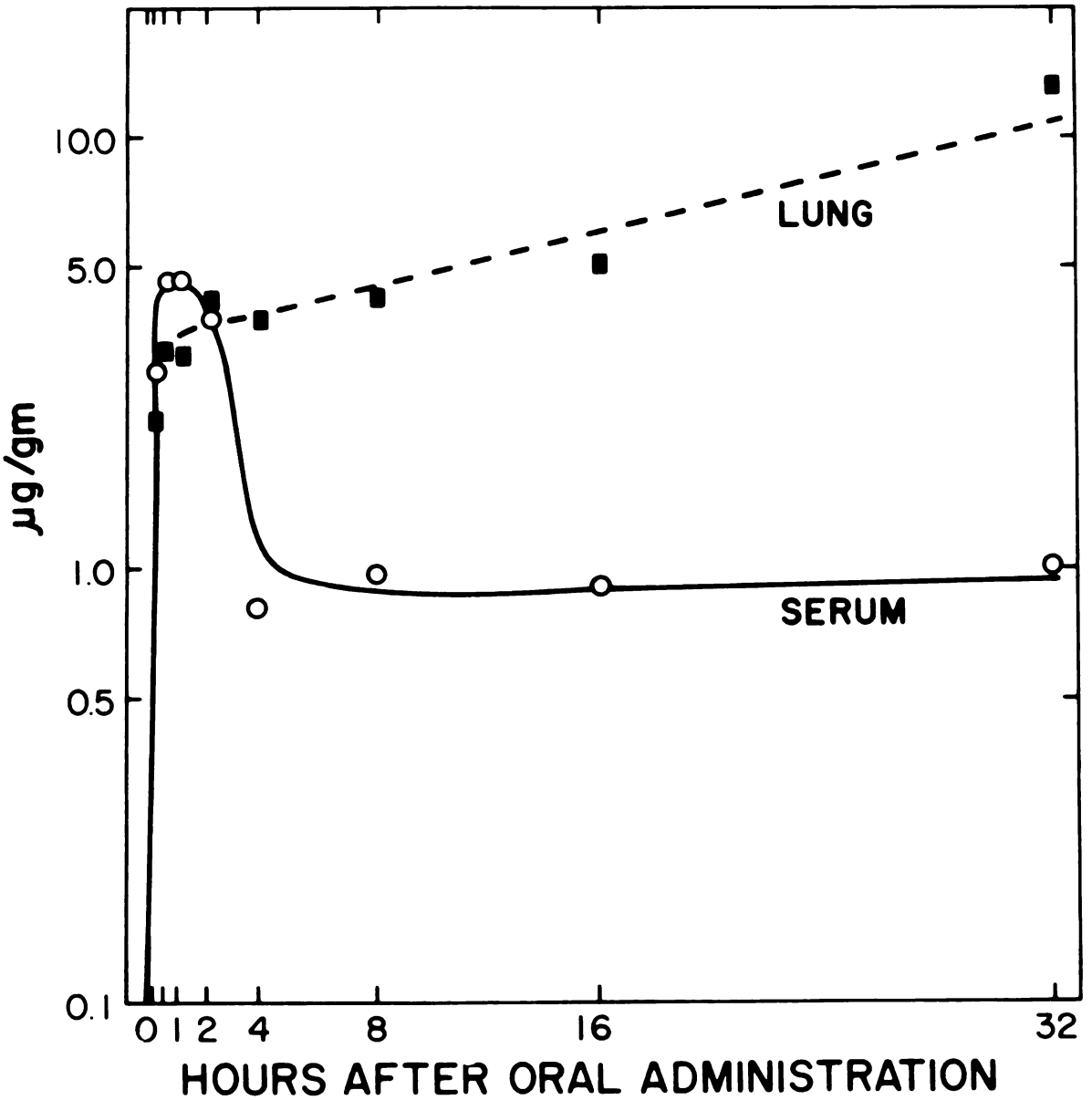

Figure 5. Concentration of paraquat in rat lung and serum 0 to 32 hours after administration. Twenty-seven rats were divided into groups of 3 and given 143 mg/kg ^{14}C-labeled paraquat orally. The mean serum levels $\pm$ S.E. were determined. After 4 hr the serum concentration of paraquat leveled off through 32 hr. The concentration in the lung increased sharply for 1 hr then increased steadily through 32 hr.

Table 10

Paraquat Recovery in Rats

64 to 512 Hours

After Administration

Time after Administration (Hours)	Paraquat (% Administered Dose)[a]				
	Carcass	Gastro-intestinal	Skin	Total Elimination	Total Recovery[b]
64[c]	1.48	17.55	.21	21.49	41.00
128	.55	.91	.22	27.29	29.01
256	.13	.43	.07	18.63	19.01
512	0	0	0	27.09	27.09

[a] 143 mg/kg (LD50) ^{14}C-paraquat orally.

[b] All tissues and eliminations.

[c] 3-5 rats at each time interval.

Table 11

Terminal Tissue Distribution of

Paraquat in Macaça fascicularis

Monkey Number	Dosage mg/kg	Survival Time (days)	µg paraquat/gm tissue				
			Serum	Lung	Heart	Liver	Kidney
1	126	1	0	1	12	2	2
2	76	2	0	1	0	2	1
3	63	1	0	8	2	4	10
4	53	8[a]	4	1	8	1	1
5	50	14[a]	0	0	0	0	0
6	50	10	0	0	0	0	0
7	45	7	0	1	0	0	1
8	40	3[b]	0	0	0	0	0
9	40	21[a]	0	0	0	0	0
10	35	21[a]	0	0	0	0	0

[a]Sacrificed.

[b]Vomited 1 hour after paraquat administration.

(Table 12), after which the level decreased daily. In one monkey serum levels of paraquat were measurable on the third and seventh day after administration. In most primates, the serum concentrations of paraquat fell below 1 μg/ml after 2-3 days (Table 11). Serum levels of paraquat were measured in only one monkey that was given less than 50 mg/kg (Table 11).

3. Excretion

Paraquat excretion in urine and feces of rats was measured for 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 hr (Table 13). Paraquat elimination in the urine of rats ranged from 0 to 18% of the administered dose. Paraquat excreted in the feces of rats increased with time (Table 13) and the total paraquat eliminated increased through 32 hr. Small amounts of paraquat were eliminated through 512 hr.

Paraquat excretion in feces and urine was measured in the guinea pig for 1, 2, 4, 16, 32 and 64 hr (Table 14). During 64 hr, 1.3% of the administered dose was recovered from the urine and 1.9% from the feces. Paraquat concentration increased gradually to a level of 3.2% in 64 hr. The rat, for the same time interval, eliminated 21.5% of the administered paraquat. There appeared to be a considerably higher percentage of the administered dose retained in the body of the guinea pig than in the rat.

Fecal and urine elimination of paraquat was measured in selected monkeys. Two monkeys surviving 3 weeks eliminated 3% and 10% of the administered dose in urine and feces respectively. Paraquat was measured in the urine at a constant daily rate for

Table 12

Serum Levels of Paraquat

In Macaca fascicularis

Monkey[a] Number	Dosage mg/kg	Survival time (days)	Paraquat concentration µg/ml serum Days after administration 1	2	3	4	5	6
1	126	1	2	-[b]	-	-	-	-
2	76	2	2	-	-	-	-	-
3	63	1	1	-	-	-	-	-
4	53	8	1	1	5	1	1	1
5	50	14	0	1	0	0	0	0
6	50	10	0	0	0	0	0	0
7	45	7	0	0	0	0	0	0
8	40	3	0	0	-	-	-	-
9	40	21	0	0	0	0	0	0
10	35	21	2	0	0	0	0	0

[a]Monkey numbers same as Table 3.

[b]Animal succumbed.

Table 13

Paraquat Excretion in Urine

And Feces of Rats

Time After Administration (hours)	Lethality[b]	% of Administered Dose[a] Excreted		
		Urine	Feces	Total
1	0/3	0.2 ± 0.0[c]	0.0	0.2
2	0/3	2.0 ± 0.6	1.2 ± 1.2	3.2
4	0/3	1.5 ± 0.4	0.3 ± 0.0	1.8
8	0/3	4.5 ± 0.7	1.8 ± 1.2	6.3
16	0/3	8.6 ± 4.2	9.3 ± 2.4	17.9
32	0/3	11.0 ± 1.2	12.0 ± 3.9	23.0
64	0/5	14.2 ± 1.0	7.3 ± 2.8	21.5
128	1/5	18.4 ± 5.9	8.9 ± 1.2	27.3
256	7/10	7.3 ± 2.2	11.3 ± 2.6	18.6
512	20/23	7.4 ± 0.5	19.7 ± 2.9	27.1

[a] 143 mg/kg p.o. (7 day LD50).

[b] Number dead/number treated.

[c] Mean ± S.E.

Table 14

Paraquat Excretion in Urine and Feces

In Guinea Pigs

Time After Administration (hours)	Percent of Administered Dose[a] Excreted		
	Urine	Feces	Total
1	0.0 ± 0.0[b]	0.0 ± 0.0	0
2	0.1 ± 0.1	0.0 ± 0.0	0.1
4	0.3 ± 0.3	0.0 ± 0.0	0.3
8	0.0 ± 0.0	0.8 ± 0.6	0.8
16	1.1 ± 0.4	0.7 ± 0.7	1.8
32	1.3 ± 0.5	1.3 ± 0.6	2.6
64	1.3 ± 0.7	1.9 ± 0.9	3.2

[a] 22 mg/kg p.o.

[b] Mean ± S.E. for three animals.

2 weeks. Fecal elimination of paraquat continued for 1 week.

4. Metabolism

Urine of rats was examined for possible metabolites of paraquat. Rats were given 92 mg/kg (LD5) p.o. ^{14}C-labeled paraquat, and urine collected for 12 hours. Visible examination of urine thin-layer chromatograms with short-wave length ultraviolet light revealed a blue-brown spot at the point of origin and a blue tailing spot (Rf 0.50) on cellulose and only a blue spot at the point of origin on silica gel. Long wave length ultraviolet and white light examination of rat urine did not reveal paraquat. These results were identical to those obtained with paraquat standards. No metabolites were observed. Radioactive measurements of the sectioned adsorbants confirmed the movement of isotope on the cellulose (Rf 0.50) but no movement on silica gel.

E. Effect of fasting

The effect of fasting on the lethality of paraquat in Sprague-Dawley rats was evaluated by fasting rats 0, 4 and 8 hr. LD50's and their 95% confidence limits were calculated for each group. There were no significant differences between groups (Table 15).

Table 15

Effect of Fasting on Lethality of

Paraquat in Sprague-Dawley Rats

Fast Period (hours)	LD50[a] (95% confidence limits) (mg/kg p.o.)	
0	143	(123-166)
4	130	(106-159)
8	126	(102-156)

[a]Seven day test period.

IV. DISCUSSION

The species variability in the acute toxicity of paraquat previously noted by others (Conning, et al., 1969) was confirmed in the present studies. LD50 values were 143, 50 and 22 mg/kg paraquat cation for rats, monkeys, and guinea pigs. In the literature, these values for orally administered paraquat in rats vary from 57 to 440 mg/kg (Bailey and White, 1965; Tadjer, 1967). The wide range may have been due to strain differences, although the present study showed that there was no significant difference between two strains. Possibly the wide range in reported LD50 values was due to the time interval used to compute the acute LD50. The peak death rate occurred in rats 3-4 days after oral administration, but rats continued to die for 2-3 weeks. Very few rats died on the first or second day after paraquat.

The LD50 for orally administered paraquat in guinea pigs in this study was in close agreement to that reported by Conning, et al. (1969). These values were 22 and 30 mg/kg respectively. The present results therefore indicate greater sensitivity of the guinea pig to paraquat intoxication as compared to the rat.

The LD50 for paraquat in Macaca fascicularis was 50 mg/kg. This value was in good agreement with the human data of

Oreopoulos and McEvoy (1969) who reported the death of a child from paraquat intoxication at a dosage reported to be 40-50 mg/kg. Therefore the lethal dose of paraquat in monkeys may closely approximate that for man, although death has been reported in man at levels estimated to be 3-4 mg/kg (Hargreave et al., 1969 and Almog and Tal, 1967).

The clinical manifestations of paraquat intoxication have not been adequately reported in most species, although clinical manifestations have been characterized in detail for man (Campbell, 1968; Matthew et al., 1968; McKean, 1968; and Oreopoulos and McEvoy, 1969). The clinical syndrome observed in rats, guinea pigs and monkeys closely approximated the syndrome reported in man. Rats and guinea pigs showed signs of lethargy, anorexia, adipsia, hyperpnea and tachycardia. Clinical signs in the primate were related to the dosage and time after administration of paraquat. At high doses i.e., 126 mg/kg, the syndrome was characterized by convulsions and death. At doses near the LD50 i.e., 50 mg/kg, there were signs of respiratory distress. The animals showed signs of dyspnea, tachycardia, hyperpnea and cyanosis. If the animals survived the critical period of 3-10 days after administration, they proceeded to recover from the stress.

The clinical chemistry changes in man caused by paraquat intoxication were elevated bilirubin, alkaline phosphatase, BUN, SGOT and SGPT (Matthew et al., 1968 and Oreopoulos and McEvoy, 1969). Jaundice and increased serum transaminase

concentrations were interpreted as evidence of liver damage. Elevated BUN levels indicated renal necrosis. Obvious changes in BUN, SGOT and SGPT however were not confirmed in this study in monkeys fatally poisoned with paraquat.

The pathology induced by paraquat intoxication varied in rats, guinea pigs and monkeys. The pathology seen in rats was similar to that reported by Kimbrough and Gaines (1970). Rats which died 3-7 days after paraquat administration showed pulmonary edema, congestion and intra-alveolar hemorrhage. Rats surviving 7-10 days showed additional lesions of pulmonary fibrosis. Guinea pigs showed pathological changes primarily consisting of edema, congestion and hemorrhage. Fibroblastic infiltration was not evident on histopathological examination of lung tissue from guinea pigs surviving 7-14 days. Other tissues examined did not show significant pathological changes. The pathological lesions in primates were primarily in the gastrointestinal tract, lung, kidney and liver. Occasional diffuse centrolobular necrosis of the liver and tubular necrosis of the kidney were seen in primates. The histopathological changes seen in monkey lungs were edema, hemorrhage, congestion, ruptured alveoli, trabecular collapse and peribronchiolar fibrosis.

All of the lesions reported in this study have been reported for paraquat intoxication in man (Campbell, 1968; Matthew et al., 1968; and McKean, 1968). Toner et al., (1970) however reported additional lesions in man not seen in this

study. These lesions were hyaline membrane formation and combined intra-alveolar and interstitial fibrosis. Large ulcers of the pharyngeal, esophageal and gastric mucosa have been reported in man (Matthew et al., 1968). These lesions were not seen in primates even at relatively high doses of paraquat (126 mg/kg). Presumably these differences were due to the method of paraquat administration. Since monkeys received paraquat by gastric gavage, no direct contact between the pharynx or esophagus and paraquat was made.

The absorption of paraquat in rats was poor as demonstrated by low peak serum levels and high residual paraquat content in the gastrointestinal tract. This coincided with the results of Conning et al. (1969) and Daniel and Gage (1966). The absorption of paraquat in the guinea pig was also poor (2.5 µg and 5 µg paraquat/ml serum for guinea pigs and rats respectively). The guinea pig had one-half the peak serum level of the rat but was given one-seventh the dosage, which indicated slightly better absorption in the guinea pig than in the rat. Also, the time to peak serum level of paraquat in guinea pigs (0.5 hr) was more rapid than the rat (1 hr). Since the guinea pig has a larger caecum than the rat, paraquat could be retained for a longer period of time in the gut and then be more extensively absorbed. In addition, Schanker et al., (1958) have reported that cations of strong bases are very slowly absorbed from rat small intestine.

The distribution of paraquat in rat tissue was measured at time intervals up to 512 hr. The results indicated that the concentration of paraquat in serum and kidney reached a peak 1-2 hr after administration and then proceeded to decrease. Paraquat concentration in the heart and liver increased to 3-5 μg/gm tissue and maintained this level for 32 hr and 64 hr respectively. Paraquat concentration in the lung however was of primary interest. The lung had the highest of any tissue concentration, 14 μg/gm at 32 hr. This value was in contrast to heart, liver, kidney and serum concentrations of 3, 6, 9 and 1 μg/gm respectively. The concentration of paraquat increased in the lung for 32 hr then decreased through the remainder of the test period. Lung tissue concentrations of 0.85 μg/gm of paraquat in humans have been reported by Matthew et al. (1968), which also showed tissue specificity when compared to the concentration of paraquat in other tissues.

The distribution of paraquat in guinea pigs was similar to the distribution seen in rats. The lung paraquat concentration, however, was different in the guinea pig than in the rat. The guinea pig lung paraquat concentration increased sharply in the first 0.5 hr and decreased slowly for 64 hr. In contrast, the rat lung paraquat concentration increased sharply for 1 hr but continued to increase for 32 hr. The concentration of paraquat in each tissue in the guinea pig was about one-half the rat tissue concentration.

The excretion of paraquat in the rat was measured at time intervals up to 512 hr. Urinary paraquat concentrations were measurable for as long as 14 days and fecal elimination

persisted for 19 days. Paraquat excretion in urine and feces was measured in guinea pigs for 64 hr, and the results indicated considerably less excretion in urine and feces of guinea pigs than rats during the same time intervals. The storage of paraquat in the caecum of the guinea pig could account for the delay in paraquat elimination as compared to the rat. Furthermore, tissue binding could account for low urinary elimination of paraquat in guinea pigs.

The excretion of paraquat by primates surviving 2-3 weeks showed measurable paraquat in the urine for as long as 14 days. This was similar to the results reported by Pasi and Hine (1971) in a human case where paraquat concentrations were measurable for 12-14 days after non-fatal intoxication. Oreopoulos and McEvoy (1969) suggested that in man there is storage of paraquat in the tissues with a gradual liberation into the blood stream and excretion by the kidneys. This may account for measurable concentrations in the urine after the serum level has dropped below a measurable amount.

In the present study no metabolites of paraquat were detected in urine of rats using thin layer chromatography. Daniel and Gage (1966) however claimed that gastrointestinal bacteria were capable of metabolizing 30% of administered paraquat. These metabolites were apparently not absorbed through the gastrointestinal mucosa but instead excreted in feces.

Daniel and Gage (1966) reported 40-50% degradation of paraquat in fecal homogenates by microbiological action during

24 hours in vitro. Similar honogenates which had been subjected to previous heat treatment produced only minor losses of paraquat for the same period. In that study in vivo results indicated 9 to 30% loss of radioactivity from feces in 24 hr. Therefore significant decreases in the radioactivity measured in rat feces could be expected. The loss of radioactivity from feces in the present study could explain the low percent recoveries of paraquat.

The effect of fasting on paraquat intoxication was studied. Matthew et al. (1968) reported the results of two children poisoned with paraquat. One child died that had not eaten in 8 hr. Fasting was postulated to have affected the lethality of paraquat by influencing absorption of paraquat. However, rats fasted 0, 4 and 8 hr showed no significant difference in LD50's between the groups.

The mechanism of action for paraquat induced pulmonary fibrosis is not known, but it has been suggested to involve a loss of pulmonary surfactant (Conning et al., 1969). Manketlow (1967) suggested that the specific action of paraquat on lung tissue was to block the production of lung surfactant. However, changes in surfactant would result in earlier primary damage, such as found with pulmonary irritants and therefore would not totally explain the paraquat lesion (Conning, et al., 1969). In addition, Kimbrough and Gaines (1970) reported no loss of pulmonary surfactant in rats that developed pulmonary fibrosis.

Hexamethonium has caused fibrinous edema in humans (Goodman and Gilman, 1970). Since the pathology produced by hexamethonium resembles that of paraquat, there may be some structural requirements for the chemical induction of pulmonary fibrosis.

In summary, the paraquat intoxication syndrome was characterized in rats, guinea pigs and monkeys, and the clinical manifestations of the intoxication were related to hypoxia and dyspnea. The absorption, distribution and elimination of paraquat varied with each species, but the toxicity was principally related to the extent of paraquat absorption. Fasting and strain differences did not influence the toxicity of paraquat in rats. It was concluded that the primary pathology induced by paraquat in the lung was related to uptake of the chemical by that organ.

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