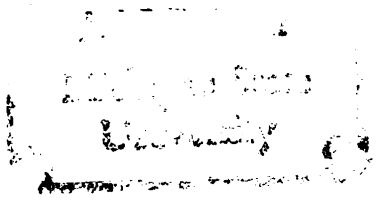
THE RENAL HANDLING AND NEPHROTOXICITY OF PARAQUAT

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

James Lee Ecker

1975

THESIS



ABSTRACT

THE RENAL HANDLING AND

NEPHROTOXICITY OF PARAQUAT

By

James Lee Ecker

Paraquat (1,1'-dimethyl 4,4'-bypyridylium) is a broad spectrum herbicide which is highly toxic to man and other animals. The objectives of this investigation were: (1) To determine the processes involved in the renal elimination of paraquat; (2) to evaluate the nephrotoxic potential of this compound; and (3) to utilize the information from these studies to gain a better understanding of the mechanisms responsible for paraquat toxicity.

Accumulation of various compounds by renal cortical slices *in vitro* is related to the capacity of the kidney to actively secrete these same compounds *in vivo*. Accumulation of paraquat by mouse renal cortical slices was determined to identify an active secretory component that might be involved in the renal elimination of paraquat. Paraquat, an organic base, was accumulated by slices. The amount accumulated was related to the concentration of paraquat in the medium and the duration of incubation. Paraquat accumulation was depressed by incubation of slices under nitrogen or by addition of metabolic inhibitors. Accumulation of a second organic base, N-methylnicotinamide (NMN), was depressed by a concentration of paraquat which failed to influence accumulation of the organic acid p-aminohippurate (PAH). The uptake component of NMN accumulation was inhibited by paraquat. The data suggest that paraquat is accumulated by an energy-

requiring process and that this accumulation occurs via the organic base transport system.

In addition, an apparently toxic effect of paraquat on cortical slice function was observed when the incubation temperature was raised from 25° to 37°C. At this temperature, 10^{-3}M paraquat depressed not only NMN accumulation but PAH accumulation and slice oxygen consumption as well. Thus, paraquat can be toxic to slice function and this effect appears to be temperature-dependent.

The nephrotoxic potential of paraquat was evaluated by determining renal function in mice acutely and chronically poisoned with paraquat. Renal function was estimated utilizing both *in vitro* and *in vivo* techniques. Proximal tubular function was monitored *in vitro* by measuring accumulation of PAH and NMN into renal cortical slices. Disappearance of phenolsulfonphthalein (PSP) and ^{14}C-paraquat from plasma was used to monitor tubular function in intact animals. Glomerular function was approximated using disappearance of iothalamate from plasma.

Slices prepared from mice chronically poisoned with paraquat (50 ppm of paraquat in the drinking water) were not different from control slices in their ability to accumulate PAH or NMN.

Renal function was also evaluated in mice surviving an LD50 (7 day) dose of the herbicide. Renal cortical accumulation of PAH and NMN *in vitro* was not markedly altered following acute paraquat poisoning. In contrast, dramatic effects were measured *in vivo*. The rate of disappearance of both ^{14}C-paraquat and PSP from plasma was

significantly reduced in poisoned mice. In contrast, the rate of disappearance of iothalamate was not affected by paraquat. However, the concentration of iothalamate was higher in the plasma of treated animals, suggesting that paraquat poisoning results in a smaller volume of distribution for iothalamate but fails to alter glomerular filtration.

The renal excretion of paraquat apparently involves an active secretory process. In addition, paraquat appears to interfere with renal function in the proximal tubule of the kidney. Since secretion of paraquat from the body involves accumulation of the herbicide within the proximal tubule cells, it is not surprising that paraquat interferes with the function of these same cells. Furthermore, it follows that, should toxic concentrations of paraquat be reached in the kidney, subsequent impairment of renal function would impede elimination of the herbicide, leading to more profound toxicity in organs other than the kidney.

THE RENAL HANDLING AND NEPHROTOXICITY OF PARAQUAT

by

James Lee Ecker

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pharmacology

1975

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the graduate committee members: Drs. Theodore M. Brody, James E. Gibson, Jerry B. Hook and David R. Rovner for their helpful assistance in the preparation of this thesis. I would especially like to acknowledge Dr. James E. Gibson for his guidance, constructive criticism and encouragement throughout the course of this investigation. In addition, the efforts of Dr. Jerry B. Hook throughout my college education have been greatly appreciated.

The technical assistance of Ms. Harriet Sherman, Ms. Carla Gauger, Mr. Robert Clark, Mr. Grant Moore and the typing skills of Ms. Joan Ecker are gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The herbicide, paraquat (1,1'-dimethyl 4,4'-bypyridylium dichloride) has caused more than 200 poisonings in man and numerous farm animals since its introduction (Rogers, *et al.*, 1973 and Kimbrough, 1974). The fatal dose in man has been estimated to be between 4 mg/kg (Editorial, 1972) and 50 mg/kg (Murray and Gibson, 1972) with the overall mortality in reported poisonings to be approximately 33-50% (Editorial, 1971).

The clinical symptoms following acute paraquat poisoning are similar in man and animals. Initially, vomiting is observed with ulceration of the mouth and pharynx observed later. Patients show signs of renal functional impairment, often including oliguria, albuminuria, increased blood urea nitrogen and altered serum electrolyte concentrations. After a latent interval, signs of respiratory failure ensue, including dyspnea and cyanosis. Death, usually attributal to respiratory failure resulting from progressive pulmonary fibrosis, often occurs from 1 to 4 weeks later. Additional symptoms sometimes noted in laboratory animals include anorexia, diarrhea and tachycardia. Pathological examinations have consistently demonstrated lung and kidney lesions, with occasional mention of alterations from normal in the liver, spleen, thymus and adrenal cortex. Histologically, the lungs appear hemorrhagic and edematous showing signs of increased fibroblastic activity and proliferation of the alveolar lining epithelium (Toner, *et al.*, 1970).

The distribution of ^{14}C-labeled paraquat in the mouse has been studied following i.p. and p.o. administration by Bus, *et al.* (1975a). The highest concentrations of paraquat (^{14}C) were obtained in the liver and kidney. These values were severalfold greater than those seen in other tissues or plasma. The concentrations of paraquat (^{14}C) in the

liver and kidneys declined quickly; whereas in the lung, the concentration of the herbicide apparently increased with time. Similar observations using whole body radioautography have been made by Litchfield, *et al.* (1973) following an intravenous injection of paraquat (^{14}C) in mice. Sharp, *et al.* (1972) reported that the concentration of paraquat in the kidney was greater than that observed in plasma for several days following the administration of the herbicide to rats.

In laboratory animals, paraquat is poorly absorbed following oral administration (Daniel and Gage, 1966 and Murray and Gibson, 1974). When paraquat was given subcutaneously to rats in a single dose, 80 to 98% of the dose was excreted in the urine within 24 hrs. No significant biliary excretion or metabolism of the compound was noted (Daniel and Gage, 1966). Significant urine concentrations of paraquat have been detected weeks following administration in both man and animals (Beebeejaum, *et al.*, 1971; Fisher, *et al.*, 1971 and Murray and Gibson, 1974.

Many procedures for the treatment of acute paraquat poisoning in man have been attempted, yet no specific antidote has been found and no therapeutic procedure has been shown to be exceptionally effective. Rational therapeutic procedures have not been developed partly because of the lack of information concerning the tolerable dose, precise mechanism of action and optimal methods for removal of the poison (Fisher, *et al.*, 1971). Following paraquat ingestion, attempts have been made to reduce absorption by the use of gastric lavage, cathartics and adsorbents. Staiff, *et al.* (1973) screened various materials to assess their adsorbent potential. The ion exchange resin, Amberlite CG-120 (100-200 or 200-400 mesh) was shown to be most effective, adsorbing paraquat *in vitro* and *in vivo*. On the other hand, Browne (1971)

recommended Fuller's Earth over Amberlite as an adsorbent. Smith, *et al.* (1975) recently suggested a treatment program to prevent the adsorption of paraquat into the plasma. Treatment consists of a stomach wash followed by the adsorbant bentonite and purgatives. Apparently this treatment effectively reduces paraquat lethality in rats even when treatment is delayed 10 hrs after paraquat administration. Forced diuresis has been shown to increase urinary excretion of paraquat in man and dog (Kerr, *et al.*, 1968; Fisher, *et al.*, 1971 and Ferguson, 1971). Most commonly mannitol has been used; however, the use of furosemide has also been reported (Beebeejaum, 1971). Apparently, peritoneal dialysis is ineffective in reducing the paraquat plasma concentration; however, hemodialysis may be effective (Grundies, *et al.*, 1971). Various therapeutic procedures have been recommended for paraquat poisonings including treatment with steroids, d-propranolol and superoxide dismutase (Editorial, 1973). Recently, Maling, *et al.* (1975) have shown that propranolol and other β-adrenergic blocking agents reduce paraquat mortality in rats.

Oxygen therapy is contraindicated in cases of paraquat poisonings unless significant cyanosis is present. Fisher, *et al.* (1973) have shown enhanced paraquat toxicity in rats during oxygen treatment. Bus, *et al.* (1974) recently presented evidence that paraquat toxicity may be a consequence of paraquat-induced lipid peroxidation. Paraquat has long been known to undergo oxidation and reduction under appropriate conditions. Paraquat (methyl viologen) is used as an indicator of oxidation-reduction reactions; the reduction of paraquat produces a deeply colored compound. Paraquat exists in the form of a reactive radical in the reduced state (Figure 1). This reactive state may account for the herbicidal properties of the compound. It has been suggested that paraquat

is reduced in plants by certain enzyme systems, then reacts with oxygen, leading to the formation of free radicals and/or hydrogen peroxide (Calderbank, 1968). Free radicals formed are proposed to react with lipids producing more free radicals and subsequently resulting in destructive biochemical changes described as lipid peroxidation (Barber and Bernheim, 1967). The effectiveness of bypyridylium compounds as herbicides has been shown to depend on light and oxygen (Mees, 1960). Dodge, *et al.* (1970) demonstrated lipid peroxidation in plant membranes resulting from the toxic effect of paraquat. Recently, pulse radiolysis studies have confirmed the existence of free radicals in plants following paraquat, which could account for the resulting lipid peroxidation (Farrington, *et al.*, 1973).

A similar mechanism of action might account for paraquat toxicity in mammalian systems. Free radicals have been demonstrated in broken-cell rat liver preparations with the addition of paraquat or diquat (Gage, 1968). More recently, Bus, *et al.* (1974) have shown that paraquat is reduced by mouse lung microsomes when incubated anaerobically with NADPH by the action of NADPH cytochrome c reductase. Reduced paraquat apparently reacts with oxygen to form superoxide anion which may non-enzymatically dismutate to singlet oxygen which initiates lipid peroxidation. As predicted, reduced paraquat increases the *in vitro* peroxidation of rat liver microsomal lipid (Bus, *et al.*, 1974). Thus, the toxic effect of paraquat in animals may occur by superoxide and singlet oxygen induced lipid peroxidation. Specifically, the chain of events following paraquat poisoning resulting in toxicity might be as follows. Paraquat is first reduced in various tissues, then reoxidized by oxygen generating free radicals. These highly reactive compounds induce

membrane destruction via lipid peroxidation, resulting in impaired organ function and later morphological changes.

Paraquat-induced lipid peroxidation has not been demonstrated *in vivo*. However, there is indirect *in vivo* evidence to suggest paraquat toxicity is mediated via lipid peroxidation. Vitamin E and selenium are known to protect against lipid peroxidation *in vivo*. Mice placed on vitamin E and selenium-deficient diets were more susceptible to paraquat toxicity, in that mice on these deficient diets had a lower paraquat LD50 value compared to mice on control diets (Bus, *et al.*, 1975b). Superoxide dismutase is an enzyme known to detoxify superoxide radicals and thus prevents superoxide and single oxygen induced lipid peroxidation. Autor (1974) has shown that superoxide dismutase administered i.v. reduced paraquat mortality in rats. Fisher, *et al.* (1973) have shown enhanced paraquat toxicity in rats during oxygen treatment and Rhodes (1974) has shown that hypoxia protects against paraquat poisoning. Thus, several lines of evidence suggest that paraquat toxicity may result from lipid peroxidation.

Renal Elimination of Paraquat

Elimination of paraquat from the body occurs via the kidneys with little or no biliary excretion of the compound (Daniel and Gage, 1966). Sharp, *et al.* (1972) observed that the elimination of paraquat from the plasma was biphasic. Murray and Gibson (1974) likewise reported that the disappearance of paraquat from plasma in several species was characterized by an initial rapid component followed by a period of prolonged excretion lasting several days. Ferguson (1971) studied the renal handling of paraquat in anesthetized dogs following an i.v. infusion of the drug. He suggested that paraquat was filtered at the glomerulus, then

35 to 65% of the filtered load was passively reabsorbed in the proximal portion of the nephron. Based on the observations that the renal excretion of paraquat was independent of the plasma concentration and that excretion was closely related to urine flow, he concluded that an active component of paraquat secretion was unlikely. In the oxidized form, paraquat is ionic in nature with a plus 2 charge. Due to the ionic nature of paraquat, it seems unlikely that paraquat is passively reabsorbed. Structurally, paraquat is similar to other bases known to be actively secreted into the urine (Peters, 1960). Thus, the renal excretion of paraquat may involve an active secretory component in addition to glomerular filtration. An active secretory component is consistent with the initial rapid disappearance of paraquat from plasma and the high renal concentration of paraquat compared to plasma and other tissues (Bus, *et al.*, 1975a).

Nephrotoxic Effects of Paraquat

The ingestion of paraquat by man has typically resulted in signs of renal functional impairment and histological changes in the proximal tubule of the kidney (Bullivant, 1966 and Beebeejaum, *et al.*, 1971). Clinical symptoms of kidney damage in man following paraquat poisoning include elevated plasma levels of blood urea nitrogen and creatinine, proteinuria, oliguria and altered serum electrolytes. Proximal renal tubular necrosis was also reported in mice chronically poisoned with paraquat (Fowler and Brooks, 1971). In mice, paraquat administration induced proximal tubular necrosis, increased amount of smooth endoplasmic reticulum and produced a number of large lamellate cytosomes, presumably filled with a lipid material. The histological picture was similar in several respects to that seen following the administration of other

nephrotoxic compounds like carbon tetrachloride, phenacetin and cephaloredine. Morfamquat, another bypyridylium herbicide, apparently also induces similar changes (Balogh and Merk, 1973).

Since the primary route for paraquat elimination is via the kidneys, perhaps the nephrotoxic properties of paraquat impair renal function resulting in the retention of the poison. This might partially explain the biphasic nature of paraquat elimination. Sharp, *et al.* (1972) showed a relationship between paraquat toxicity and the extended exposure to low plasma and tissue concentrations of the chemical. These observations suggest that effective treatment of paraquat poisoning may require removal of the toxin from the body. The toxic properties of the compound may interfere with its own excretion enhancing toxicity in the lungs and other organs.

Renal Secretion of Organic Compounds

The elimination of a considerable number of organic compounds occurs via the urine by active energy-requiring processes located in the proximal tubule of the kidney, as well as by the passive process of glomerular filtration. These transport processes have been studied extensively and two specific transport mechanisms have been described. The tubular excretion of compounds like PAH and PSP occurs via a transport mechanism specific for several organic acids. Several organic bases including various amines and quaternary ammonium compounds, like hexamethonium and NMN, are excreted by a second mechanism which apparently acts independent from organic acid excretion (Peters, 1960).

In Vitro Slice Technique

The secretory mechanism for organic compounds can be studied utilizing both *in vitro* and *in vivo* techniques. One commonly used *in vitro*

technique is the cortical slice method developed by Cross and Taggart (1950). In this technique, thin renal cortical slices are prepared free-hand and incubated in a physiological medium containing dilute concentrations of the drug or drugs to be studied. Accumulation of a compound against a concentration gradient is used as an indication of active transport. Results are expressed as the slice/medium (S/M) concentration ratio. Results obtained *in vitro* utilizing this technique correlate well with results obtained *in vivo*. Foulkes and Miller (1959) have shown that the maximum capacity of rabbit kidney cortex to accumulate PAH is similar to the concentrating capacity of rabbit renal cortical slices. Park, *et al.* (1971) studied various competitive inhibitors of PAH transport utilizing a kinetic model for studying organic acid transport *in vitro*. Compounds which inhibit PAH transport *in vitro* are often effective inhibitors of PAH secretion *in vivo*. Other inhibitors of PAH transport, for example, dinitrophenol which uncouples oxidate phosphorylation, inhibit PAH transport both *in vitro* and *in vivo*. The slice technique employing mouse renal cortical slices has been used to characterize the renal handling of compounds similar in structure to paraquat. Holm (1970) observed that mouse renal cortical slices accumulate the organic base, hexamethonium. The uptake of hexamethonium by cortical slices could be blocked by sodium azide and dinitrophenol (McIssac, 1965) and by incubating slices under nitrogen (Holm, 1970), suggesting an active energy-requiring component in the uptake process. Tetraethylammonium and decamethonium antagonized the renal accumulation of hexamethonium *in vitro* presumably by competitive inhibition of the organic base transport process (McIssac, 1965). Due to the structural similarity between paraquat and hexamethonium, it seemed likely that paraquat might

be accumulated by mouse renal cortical slices by the same active energy-requiring process.

The *in vitro* slice technique can also be used to assess the nephrotoxic potential of various compounds. Watrous and Plaa (1972) injected mice and rats with halogenated hydrocarbons, then measured the ability of renal cortical slices to actively accumulate organic ions. Hirsch (1973) has performed these and other renal functional tests following the administration of various nephrotoxic agents. Possibly paraquat is nephrotoxic in the mouse and this toxicity can be demonstrated utilizing the *in vitro* slice technique.

In Vivo Measurements of Renal Function

Many techniques are available for determining various aspects of renal function *in vivo*. Most of these techniques require extensive experimental procedures which are typically best performed on large experimental animals. For example, the classical clearance technique for determining renal blood flow and/or glomerular filtration requires cannulation of arteries and veins, catheterization of the ureters or bladder and the maintenance of relatively constant plasma concentrations by infusing drugs. To assess the nephrotoxic potential of paraquat in the mouse, it is important to determine renal function both *in vitro* and *in vivo*. Thus, it was necessary to employ *in vivo* techniques which could be applied to mice in order to determine the effect of paraquat poisoning on renal function.

Sakai, *et al.* (1969) have used a simplified clearance technique in children. The rate of glomerular filtration was estimated by determining the disappearance of iothalamate (^{125}I) from plasma following a single i.v. injection of the compound. There was good correlation

between this technique and the classical clearance technique. Utilizing a similar approach, Silkalns, *et al.* (1973) have accurately estimated renal plasma flow by determing the disappearance of PAH from plasma. It was of interest to determine if paraquat poisoning might alter renal function in the mouse as indicated by a change in the plasma disappearance of a compound known to be excreted via the kidney.

Purpose

The basic thesis underscoring this research is that a rational therapeutic program for paraquat poisoning is contingent on a better understanding of the renal handling and nephrotoxicity of paraquat. The renal handling of paraquat may be similar to the excretion of other bases which are known to be handled by the organic base secretory system of the kidney. The nephrotoxic effects of paraquat may be related to the relatively high concentrations of paraquat found in the kidney, which subsequently might impair renal function, leading to paraquat retention and more profound toxicity in other organs in the body.

METHODS

In Vitro Determination of Renal Function

1. Description of the Slice Technique

Female Swiss-Webster mice[1] weighing 25-35g were stunned by a blow on the head and the kidneys quickly removed and placed in ice-cold saline. Thin renal cortical slices were prepared free-hand and placed in beakers containing 2.0 ml of Ringer solution containing 10 mM Na acetate and the drug(s) to be studied. Beakers were incubated in a Dubnoff-metabolic shaker at 25° or 37°C under a gas phase of 100% oxygen except for the few experiments when a gas phase of 100% nitrogen was employed. The duration of incubation and concentration of drug(s) in the medium varied with the experiment being conducted. Following incubation, slices were removed from the medium, blotted on gauze and weighed. Tissue and medium were treated as outlined by Cross and Taggart (1950). Concentrations of PAH[2], NMN[3] and paraquat[4] were determined using ^{14}C-labeled compounds except for the few experiments where PAH concentrations were determined by the spectrophotometric method of Smith *et al.* (1945) following acid hydrolysis of samples to ensure recovery of the PAH. Radioactivity was determined by placing a 0.5 ml aliquot into a vial containing 10 ml PCS^{R}-solubilizer and the disintegrations per

---

[1]Spartan Research Animals, Inc., Haslett, Michigan.

[2]New England Nuclear, Boston, Massachusetts.

[3]New England Nuclear, Boston, Massachusetts.

[4]Amersham/Searle Corporation, Arlington Heights, Illinois.

minute (dpm) in each sample were determined using a Packard model 3380 liquid scintillation spectrometer. Data concerning accumulation of drugs were expressed as the slice to medium (S/M) ratio, calculated by dividing the concentration of drug per g of tissue by the concentration of drug per ml of incubation medium, or, in the case of radio-labeled compounds, by dividing dpm per g of tissue by dpm per ml of medium.

2. Effect of Paraquat on NMN and PAH Accumulation

Accumulation of PAH and NMN by slices incubated for 90 min was determined in medium containing 7.4×10^{-5}M PAH and 6.0×10^{-4}M NMN. The effect of various concentrations of paraquat on PAH and NMN accumulation was determined at 25° and 37°C.

3. Paraquat Accumulation

Accumulation of paraquat by renal cortical slices was determined after 30, 90 and 180 minutes of incubation at 25°C. Paraquat accumulation was also determined at 37°C and compared to accumulation of paraquat by slices incubated under nitrogen.

4. Effect of Inhibitors on Paraquat Accumulation

Slices were incubated for 180 minutes at 25°C in the presence of 10^{-5}M paraquat. The inhibitors cyanine dye #863, 2,4-dinitrophenol and iodoacetic acid were present at final medium concentrations of 5 µg/ml, 10^{-4}M and 10^{-3}M respectively.

5. Uptake of NMN

Uptake of NMN into slices was determined following pre-incubation of slices for 30 min. ^{14}C-Labeled NMN was added to the medium to produce final concentrations of 0.75, 1.5, 3.0 and 6.0×10^{-4}M.

Slices were assayed for ^{14}C-NMN following 30 min incubation in the presence of the drug. Results were expressed as µg NMN per g tissue per min.

6. Determination of Slice O_2 Consumption

Oxygen consumption of slices was measured using a Yellow Springs model 53 oxygen monitor employing a Clark-type polarographic electrode. Stirring rate of the sample was slowed in order to minimize tissue damage. The instrument was calibrated before each determination with 4 ml of bathing medium saturated with oxygen at either 25 or 37°C. Slices were weighed and added in a volume of 2 ml to 4 ml of saturated medium. Oxygen consumption was then measured over a 10 min period. Data were expressed as µl of O_2 consumed per mg tissue.

7. Slice Function as an Indicator of Nephrotoxicity

Renal function was determined *in vitro* following acute and chronic paraquat poisoning. The ability of renal tissue to accumulate PAH and NMN was measured in renal cortical slices prepared from various treated and control animals. In chronic toxicity studies, pregnant mice were obtained from timed pregnancies started in this laboratory. Paraquat was added to the drinking water supply of pregnant mice at 50 ppm beginning on day 8 of gestation. Animals were placed in individual cages on day 19 and allowed to litter. Paraquat in the drinking water was continued. At weaning, the litter was separated from the mother and maintained on paraquat water until the experimental age (7 weeks). In acute toxicity studies, adult female mice were treated i.p. with an LD50 (7 day) dose of paraquat (30 mg/kg)

and renal function determined *in vitro* at 1, 3, 5 and 7 days following the paraquat poisoning.

In Vivo Determination of Renal Function

1. Disappearance of Paraquat from Plasma

Mice were anesthetized with ether and bilaterally nephrectomized or sham-operated using the dorsal approach (Becker and Gibson, 1967). Paraquat (50 mg/kg) was injected via the tail vein 16 hr following surgery. The concentration of paraquat in the plasma was determined at 5, 10, 15, 20 and 30 minutes using individual mice for each time point. Blood was collected by cardiac puncture under ether anesthesia. Plasma was separated from blood by centrifugation. The concentration of paraquat in the plasma was determined colorimetrically following the addition of 0.5 ml of 2% Na dithionite in 1N NaOH to 2.5 ml of a 1:25 water dilution of plasma. Optical density was measured at 395 nm. The addition of plasma did not alter the standard curve for paraquat.

In other experiments, the effect of paraquat poisoning on the ability of the kidney to excrete a second paraquat dose was determined. At 2 and 24 hr following paraquat poisoning (30 mg/kg i.p.; 7 day LD50), mice were challenged with 50 mg/kg paraquat i.v. and the plasma disappearance of paraquat determined as described above. Control mice were treated with an equal volume of water (5 ml/kg i.p.) 24 hr prior to the i.v. injection of paraquat.

2. Disappearance of PSP from Plasma

The disappearance of phenolsulfonphthalein (PSP) from plasma, 50 mg/kg i.v., was determined in a similar manner 24 hr following paraquat poisoning (LD50 dose) or a water injection.

3. Disappearance of Inulin from Plasma

In control and paraquat-poisoned mice, the disappearance of inulin from plasma was also determined. Twenty-four hr following an LD50 dose of paraquat or a water injection, ^{3}H-(methoxy)-inulin[5] (503.6 mCi/g specific activity) was injected i.v. (25 μCi/kg) and the plasma concentration of inulin was determined at various times. Inulin concentrations were determined by dissolving 100 μl of plasma in 1 ml of Soluene 100^{R}. Toluene-counting solution (15 ml) was added and radioactivity determined employing a Packard model 3380 liquid scintillation spectrometer.

4. Disappearance of Iothalamate from Plasma

The disappearance of ^{125}I iothalamate[6] from plasma was determined in mice 24 hr following an LD50 dose of paraquat or a water injection. Iothalamate (229 μCi/ml specific activity) was administered i.v. in a dose of 0.03 mg/kg and radioactivity in the plasma was determined 5, 10, 20, 30 and 60 minutes later. A similar experiment was conducted utilizing longer time intervals (30, 60, 90 and 180 min) and a higher dose of iothalmate (1.5 mg/kg). Radioactivity in the plasma was determined by counting 0.1 ml of a plasma in a well-type crystal scintillation counter.

Statistical Analyses

Statistical analyses were performed using analysis of variance followed by Student-Newman-Keuls test for the difference between means

---

[5]New England Nuclear, Boston, Massachusetts.

[6]Glofil-125^{R}, Abbott Laboratories, North Chicago, Illinois.

(Sokal and Rohlf, 1969). Regression lines were determined by the method of least squares and slopes were compared using Student's "t" test (Steel and Torrie, 1960). The 0.05 level of probability was used as the criterion of significance.

RESULTS

In Vitro Analysis of the Renal Handling of Paraquat

1. Paraquat Accumulation by Mouse Renal Cortical Slices

Slices prepared from mouse renal cortical tissue and incubated under oxygen at 25°C, accumulated ^{14}C-labeled paraquat, achieving S/M ratios significantly greater than 1 (Fig. 2). Incubation of slices under nitrogen at 37°C produced paraquat S/M ratios not significantly different than 1 (Fig. 3). The magnitude of aerobic accumulation was related to the duration of incubation and the concentration of paraquat in the medium, the most dilute concentration of the herbicide yielding the greatest S/M ratios (Fig. 2). In addition, the magnitude of accumulation under oxygen was related to the incubation temperature with higher S/M ratios observed at 37° (Fig. 3) than 25°C (Fig. 2) at comparable medium concentrations. Various metabolic inhibitors and cyanine dye #863 significantly reduced the accumulation of paraquat (Table 1).

2. Effect of Paraquat on NMN and PAH Accumulation

Accumulation of ^{14}C-NMN by cortical slices incubated at 25°C was depressed by paraquat in a dose-related fashion (Fig. 4). In contrast, accumulation of PAH was not inhibited by 10^{-4} or 10^{-3}M paraquat. The inhibitory effect of paraquat was temperature-dependent (Table 2). At 25°C, both 10^{-4} and 10^{-3}M concentrations of paraquat inhibited NMN accumulation without affecting PAH accumulation. However, at 37°C, 10^{-3}M paraquat inhibited accumulation of both PAH and NMN.

3. Effect of Paraquat on NMN Uptake

The effect of paraquat on the uptake component of ^{14}C-NMN accumulation by cortical slices was estimated. The initial rate of NMN uptake was determined at 25° and 37°C. The uptake of NMN was directly related to the concentration of NMN present in the medium (Fig. 5 and 6). NMN uptake under oxygen was greater at 37°C than at 25°C, whereas uptake under nitrogen was similar at the two incubation temperatures. At both 25° and 37°C, uptake of NMN under oxygen was depressed by 10^{-3}M paraquat. Incubation of slices under nitrogen further depressed NMN uptake. The uptake of NMN under nitrogen at 37°C was not influenced by the presence of paraquat (Fig. 5), suggesting that the depressant effect of paraquat on NMN uptake was on the oxygen-dependent component of the uptake process.

4. Effect of Paraquat on Slice Oxygen Consumption

Slice oxygen consumption at 37°C was not altered by preincubation of slices at 25°C in the presence of paraquat (Table 3). However, slice oxygen consumption determined at the same temperature was depressed by 10^{-3}M paraquat when slices were preincubated at 37°C for 60-90 minutes.

Nephrotoxicity of Paraquat in Mice

1. Slice Function as an Indicator of Nephrotoxicity

The effect of paraquat administration on *in vitro* estimates of renal function was determined in two experimental protocols. In the first group, paraquat was administered chronically, beginning prenatally and extending until 7 weeks of age. In this treatment regimen, 50 ppm of paraquat in the drinking water had no significant

effect on accumulation of PAH and NMN by renal cortical slices. An interesting sidelight of this protocol was the observation that PAH accumulation was greater in renal cortical slices from male than female mice (Table 1). In the second protocol, the accumulation of PAH and NMN was determined in renal cortical slices at 1, 3, 5 and 7 days following the 7-day LD50 dose of paraquat (Table 2). Mice surviving the acute paraquat poisoning weighed significantly less than control mice at 1 and 3 days but this difference was not evident at 5 and 7 days following the poisoning (Table 3). One day following the LD50 dose of paraquat, PAH accumulation was significantly less than that in controls (Table 2). This difference was not maintained however, for at 3, 5 and 7 days there was no significant difference in accumulation of PAH or NMN by tissue from intoxicated animals.

2. Effect of Nephrectomy on the Disappearance of Paraquat from Plasma

The role of the kidney in elimination of paraquat from the plasma of intact mice was determined by comparing disappearance of the herbicide from the plasma in nephrectomized animals to the disappearance observed from plasma of sham-operated animals. Disappearance of paraquat from the plasma of sham-operated mice was rapid with an approximate half time of 7.0 minutes. The disappearance appeared to follow first-order kinetics over the time interval tested (Fig. 7). Following bilateral nephrectomy, however, mice were unable to significantly reduce the plasma concentration of the herbicide (Fig. 7).

3. Effect of Paraquat Poisoning on the Disappearance of Paraquat from Plasma

The effect of an LD50 dose of paraquat on renal function was estimated by measuring the disappearance of the herbicide from the plasma 2 and 24 hrs following the toxic dose. Two hours following a toxic dose of paraquat, the disappearance of a radiolabeled dose of the herbicide appeared to be somewhat depressed though this effect was not statistically significant. However, by 24 hrs the ability of the poisoned animals to eliminate the herbicide was significantly depressed (Fig. 8). Whereas, in the control animals the concentration of herbicide was reduced to less than 2 mg/100 ml of plasma within 30 min, in the intoxicated animals the concentration at 30 minutes was not significantly different than that observed at 5 minutes, i.e., the slope of the disappearance curve was not significantly different than zero.

4. Effect of Paraquat Poisoning on the Disappearance of PSP from Plasma

Pretreatment with the LD50 dose of paraquat also significantly retarded the elimination of PSP from the plasma (Fig. 9). Thirty minutes after an injection of PSP, the plasma concentration in the treated animals was approximately 4 times greater than that in controls, but the estimated volume of distribution was no different (Fig. 9).

5. Effect of Paraquat Poisoning on the Disappearance of Inulin from Plasma

The slope of the plasma disappearance curve for ^{3}H-methoxyinulin was not altered by paraquat treatment (Fig. 10). However, at each

time measured following inulin administration, the plasma concentration of inulin was significantly higher in the paraquat-treated mice. The disappearance of inulin over this 30-minute interval was very rapid ($t_{\frac{1}{2}}$ = 8.5 min).

6. Effect of Paraquat Poisoning on the Disappearance of Iothalamate from Plasma

The disappearance of iothalamate from plasma was determined between 5 and 60 min following administration, and in a second group of animals, between 30 and 120 min following administration. The disappearance of iothalamate appeared to be biphasic in that there was a rapid decrease in plasma concentration followed by a more prolonged period of disappearance from the plasma (Fig. 11). This pattern was changed somewhat by pretreatment with an LD50 dose of paraquat in that the initial rapid fall in plasma concentration was not observed. Consequently, higher concentrations of iothalamate were observed at each time measured in the plasma of paraquat-poisoned animals. Between 30 and 120 min, the slope of the disappearance was not influenced by paraquat administration.

DISCUSSION

Organic bases like NMN and tetraethylammonium are actively accumulated by renal cortical tissue *in vivo* and then excreted into the urine. This active accumulation of drug by renal cortical tissue is a process necessary for the phenomenon of tubular secretion (Peters, 1960). The *in vitro* accumulation of drugs by renal cortical slices correlates well with the *in vivo* process and was chosen as a model for characterizing paraquat transport in this study (Ross, *et al.*, 1959).

The structural similarity between paraquat and other organic bases like hexamethonium and decamethonium suggested that the herbicide may be transported by the organic base transport system (McIssac, 1969 and Holm, 1970). Indeed, paraquat was accumulated by mouse renal cortical slices (Fig. 2 and 3). This process appears to require cellular energy in that accumulation was depressed by incubation of slices under nitrogen or in the presence of the metabolic inhibitors iodoacetic acid and dinitrophenol (Table 1).

Cyanine dye #863 at low doses blocks the uptake of organic bases by slices, apparently by competing for the organic base transport system (Farah, *et al.*, 1959). Depression of paraquat accumulation by cyanine #863 could reflect competition between paraquat and the dye for uptake by the base transport system. In order to demonstrate the specificity of paraquat for this transport system, accumulation of PAH and NMN were determined in the presence of various concentrations of paraquat. As predicted from the structure of paraquat, NMN S/M ratios were selectively depressed whereas organic acid transport (PAH) was not affected (Fig. 4).

An S/M ratio does not reflect only active uptake of a drug by a transport system, but rather reflects net intracellular accumulation and the respective rates of influx and efflux of the compound studied (Ross, *et al.*, 1968). Perhaps depression of the NMN S/M ratio by paraquat resulted from more than a simple competition between the two compounds for a common transport system. To better characterize the interaction between paraquat and a second base for active transport, the effect of paraquat on the uptake component of NMN accumulation was determined. Paraquat depressed NMN uptake under oxygen at both incubation temperatures (Fig. 5 and 6). At 37°C, uptake under oxygen was greater than that observed at 25°C, although paraquat inhibition of NMN uptake was similar at both temperatures. Uptake under nitrogen was less than that observed under oxygen and was not influenced by incubation temperature or by paraquat. Thus, paraquat inhibition of NMN accumulation (S/M ratio) is apparently specific for the oxygen-requiring component of NMN uptake.

To evaluate the possibility that a portion of paraquat inhibition of NMN accumulation was secondary to depression of slice metabolism, the effect of paraquat on total slice oxygen consumption was determined. Incubation of slices at 25°C in the presence of paraquat did not alter slice oxygen consumption, supporting the contention that paraquat inhibits organic base transport by competition for a common transport mechanism (Table 3). However, at 37°C, 10^{-3}M paraquat depressed slice oxygen consumption. Subsequent to this observation, the effect of paraquat on NMN and PAH accumulation was determined at 25° and 37°C (Table 2). In contrast to 25°C, at 37°C PAH accumulation and slice oxygen consumption was depressed by the herbicide. This suggests that at 37°C paraquat may competitively inhibit

NMN transport and, in addition, produce a general depression in slice function. Bus, *et al.* (1975) have demonstrated that paraquat can undergo reduction when incubated with lung microsomes in the presence of NADPH and that the reduced paraquat formed reacts with oxygen to form superoxide and singlet oxygen which initiates lipid peroxidation. Perhaps the general depression of slice function under certain conditions is a consequence of paraquat-induced lipid peroxidation. Alternatively, paraquat might interfere with other processes essential for slice function.

The *in vitro* evidence for active transport presented here, in addition to the rapid disappearance of the poison from plasma observed *in vivo* (Fig. 7), suggests that paraquat is actively secreted into the urine via the organic base secretory system. The plasma disappearance of paraquat from plasma appears to be biphasic, with an initial component, followed by a phase of prolonged elimination (Sharp, *et al.*, 1972). The present results suggest active transport may be involved in the initial rapid removal of drug from plasma. However, the data fail to explain the prolonged period of paraquat excretion. Perhaps other tissues initially accumulate paraquat then slowly release the compound. Or perhaps paraquat is tightly bound to a fraction of plasma. Furthermore, the nephrotoxic properties of paraquat may result in a diminished capacity of the kidney to excrete the poison.

Sharp, *et al.* (1972) reported a correlation between paraquat concentrations in the lung and kidney and the toxicity of the drug. A high concentration of drug in these organs may result from a compromised capacity of the kidney to excrete the poison. Apparently paraquat excretion and the nephrotoxic properties of the compound are

related in that paraquat poisoning altered the excretion of a second paraquat dose administered 24 but not 2 hrs later (Fig. 8). Thus, it appears that the nephrotoxic properties of the herbicide are not expressed initially when the kidney concentrations are greatest, but rather are observed later when most of the poison has been removed from the body.

The renal functional impairment produced by paraquat was not limited to organic base excretion since elimination of the organic acid, PSP, was likewise affected (Fig. 9). Perhaps the nephrotoxic effects of paraquat are due to a direct effect on renal cortical tissue, impairing function in general. When renal function was examined *in vivo*, only a small change in PAH transport was noted (Table 5). Similarly, renal function measured *in vitro* was not affected following chronic paraquat poisoning (Table 4). Apparently, the effects of paraquat on renal function are best detected *in vivo*. The *in vitro* slice technique estimates only one step in drug excretory processes which occur *in vivo* and thus may lack the capacity or sensitivity to quantitate changes in function that occur *in vivo*. For instance, the slice technique measures steady-state accumulation; possibly the effect of paraquat is overcome in the steady-state and can only be measured in the dynamic state that occurs in the intact animal. Alternatively, paraquat poisoning may affect parameters other than the transport process. For example, paraquat poisoning may decrease renal blood flow and indirectly depress renal excretion of drugs.

The disappearance of inulin may be used to approximate glomerular filtration rate. Therefore, the effect of paraquat on the disappearance of inulin from plasma was determined in order to estimate changes in renal hemodynamics subsequent to herbicide intoxication. The initial

rate of disappearance of inulin from the plasma was not affected by paraquat pretreatment (Fig. 10). However, at each time point measured, the concentration of inulin in the plasma was higher in the paraquat-poisoned animals, suggesting a smaller volume of distribution for inulin following paraquat. A diminished volume of distribution is consistent with the marked loss in body weight noted 24 hrs following paraquat administration (Table 6). The disappearance of inulin over the 30-min interval measured was too rapid to reflect glomerular filtration in that the plasma half life for inulin was not different than that of PSP. Thus, the possibility existed that disappearance of inulin from plasma was not an adequate estimate of glomerular filtration rate in the mouse. The disappearance of iothalamate, like inulin, may also be used to approximate glomerular filtration (Sigman, *et al.*, 1965). Iothalamate elimination was biphasic with an initial rapid disappearance from plasma followed by a period of slower elimination (Fig. 11). Following paraquat, plasma concentration of iothalamate was higher at each time tested, possibly reflecting a smaller volume of distribution of the drug. Paraquat poisoning failed to alter the slope of the disappearance curve for iothalamate between 30 and 120 min, suggesting that depression of blood flow to the kidney cannot account for the diminished secretory capacity in paraquat-poisoned animals.

The curves for PSP and paraquat disappearance from plasma following paraquat administration lack the change in the apparent volume of distribution that was evident with inulin and iothalamate. This discrepancy probably lies in the difference of binding of these agents to plasma proteins. Paraquat, like PSP, may be tightly bound to plasma proteins in contrast to iothalamate and inulin, which are not. Thus, a paraquat-induced decrease in plasma volume would lead to an apparent

decrease in the volume of distribution for iothalamate and inulin. However, assuming that the amount of plasma protein would not be changed by paraquat, the apparent volumes of distribution of these highly protein-bound components would not be altered.

Since paraquat is accumulated by renal cortical tissue, this organ is likely to be more sensitive to the toxic effects of paraquat compared to other organs which do not accumulate the drug. In addition, since the kidney, like the lung, has a high oxygen tension relative to other organs, it seems reasonable that the kidney may be predisposed to paraquat-induced lipid peroxidation. If the nephrotoxic effects of paraquat could be prevented, then the elimination of paraquat via the kidney might be facilitated, decreasing the potential toxicity to the kidney and other organs.

SUMMARY

Paraquat, a bypyridylium herbicide, has caused numerous poisoning in man and farm animals since its introduction. Elimination of paraquat from the body occurs via the kidneys with little or no biliary excretion of the compound (Daniel and Gage, 1966). Paraquat is accumulated by mouse renal cortical slices by an active energy-requiring process. Accumulation of paraquat apparently occurs by the organic base secretory system of the kidney. Thus, the renal excretion of paraquat likely involves an active secretory component in addition to glomerular filtration.

Under certain conditions, paraquat has an apparently toxic effect on cortical slice function. This toxic effect appears to be concentration and temperature-dependent.

The nephrotoxic potential of paraquat was evaluated by determining renal function both *in vitro* and *in vivo* in mice acutely and chronically poisoned with paraquat.

Slices prepared from mice chronically poisoned with paraquat (50 ppm of paraquat in the drinking water) were not different from control slices in their ability to accumulate PAH or NMN. Similarly, renal cortical slices prepared from mice acutely poisoned with paraquat (LD50 - 7 day) were similar to control slices in their ability to accumulate PAH or NMN. In contrast, paraquat poisoning produced a marked effect on renal function when renal function was assessed *in vivo*. Disappearance of PSP and paraquat from plasma was significantly reduced in paraquat-poisoned mice (LD50 - 7 day), suggesting a depression of tubular function. In contrast, the rate of disappearance of iothalamate was not affected by paraquat poisoning,

indicating that the depression of tubular function was not secondary to a change in glomerular filtration.

The renal excretion of paraquat apparently involves an active secretory process. In addition, paraquat appears to interfere with renal function in the proximal tubule of the kidney. Since secretion of paraquat from the body involves accumulation of the herbicide within the proximal tubule cells, it is not surprising that paraquat interferes with the function of these same cells. Furthermore, it follows that should toxic concentrations of paraquat be reached in the kidney, subsequent impairment of renal function would impede elimination of the herbicide, leading to more profound toxicity in organs other than the kidney.

TABLE 1

Effect of inhibitors of organic base transport on paraquat accumulation (S/M)[a] by mouse renal cortical slices

Inhibitor	Paraquat S/M[b]
Control	2.39
Cyanine Dye #863	1.33[c]
2,4-Dinitrophenol	1.09[c]
Iodoacetic Acid	0.92[c]

[a]Slices were prepared and incubated at 25°C in the presence of 10^{-5}M paraquat for 180 minutes. Cyanine dye #863, 2,4-dinitrophenol and iodoacetic acid were present at final medium concentrations of 5 µg/ml, 10^{-4}M and 10^{-3}M respectively. Values represent the mean of 4 experiments.

[b]Coefficient of variability was 9%.

[c]Significantly different from control ($p < 0.05$).

TABLE 2

Effect of paraquat on PAH and NMN accumulation (S/M) by mouse renal cortical slices[a]

	PAH S/M Ratio				NMN S/M Ratio			
Paraquat Conc.	0	10^{-4}M	10^{-3}M	(C.V.)[b]	0	10^{-4}M	10^{-3}M	(C.V.)[b]
25°C	7.92	8.84	8.41	(23%)	18.02	13.15	7.47	(8%)
37°C	7.82	6.93	3.49	(19%)	8.00	5.78	3.35	(12%)

[a]Slices were prepared and incubated at 25° or 37°C with paraquat for 90 minutes. Values represent the mean of 4 experiments. Any values underscored by the same line were not significantly different.

[b]Coefficient of variability.

TABLE 3

Effect of paraquat on oxygen consumption by mouse renal cortical slices[a]

Preincubation Temperature	O_2 Determination Temperature	Paraquat Concentration 0	10^{-4}M	10^{-3}M	(C.V.)[b]
25°C	37°C	.077	.078	.086	(15%)
37°C	37°C	.068	.062	.048	(13%)

[a]Slices were prepared and incubated with paraquat for 60 to 90 minutes, then oxygen consumption (µl/mg tissue/min) was determined for a 6 to 10 minute period. Values represent the mean of 4 experiments. Any values underscored by the same line were not significantly different.

[b]Coefficient of variability.

TABLE 4

Accumulation of NMN and PAH by renal cortical slices[a] prepared from mice chronically poisoned with paraquat

Incubation Time	90				180			
S/M Ratio	PAH		NMN		PAH		NMN	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Control	11.5 ±0.6	5.7 ±0.6	20.2 ±0.9	18.7 ±1.1	28.2 ±5.7	12.0 ±0.5	29.4 ±3.3	28.4 ±2.2
Treated	12.5 ±1.4	6.8 ±1.0	19.4 ±0.5	16.8 ±2.2	23.7 ±0.7	12.4 ±1.8	37.5 ±1.8	27.7 ±1.9

[a]Pregnant mice were given 50 ppm paraquat in drinking water beginning on day 8 of gestation. At weaning the litter was separated from the mother and maintained on paraquat water until the experimental age at 7 weeks. Values represent the mean ± S.E. for 4 determinations.

TABLE 5

Accumulation of PAH and NMN by renal cortical slices prepared from mice acutely poisoned with paraquat[a]

S/M Ratio	PAH				NMN			
Days Following LD50 Dose	1	3	5	7	1	3	5	7
Control	9.7 ±0.8	9.5 ±1.4	7.3 ±1.1	7.1 ±1.4	14.4 ±0.9	16.9 ±2.6	13.4 ±2.1	12.6 ±0.6
Treated	6.3[b] ±1.1	7.4 ±1.2	6.4 ±1.3	10.3 ±1.5	14.2 ±2.7	10.6 ±1.9	10.2 ±1.9	11.9 ±1.3

[a]Adult female mice were treated i.p. with an LD50 dose of paraquat (30 mg/kg). Values represent the mean ± S.E. for 4 experiments.

[b]Significantly different from control ($p < .05$).

TABLE 6

Body and kidney weight of mice[a] acutely poisoned with paraquat

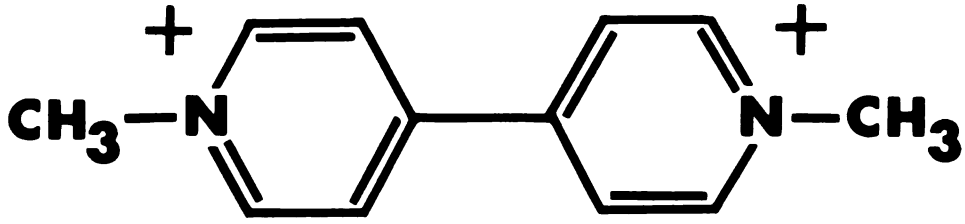
	Body Weight (g)				Kidney Weight (g)			
Days Following LD50 Dose	1	3	5	7	1	3	5	7
Control	32 ±1	31 ±1	31 ±1	30 ±1	0.39 ±0.01	0.39 ±0.01	0.40 ±0.01	0.39 ±0.02
Treated	27[b] ±1	26[b] ±1	30 ±1	32 ±1	0.38 ±0.01	0.37 ±0.02	0.39 ±0.02	0.41 ±0.01

[a]Adult female mice were treated i.p. with an LD50 dose of paraquat (30 mg/kg). Values represent the mean ± S.E. for 12 animals.

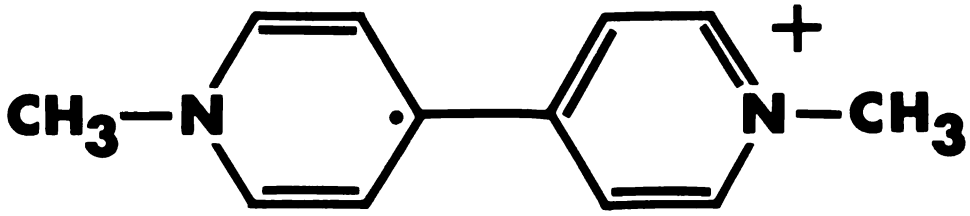
[b]Significantly different from control ($p < .05$).

Figure 1: Structure of paraquat in its oxidized and reduced states.

PARAQUAT



(oxidized)



(reduced)

Figure 1

Figure 2: Effect of medium concentration and duration of incubation on ^{14}C-labeled paraquat accumulation (S/M ratio) by mouse renal cortical slices incubated under oxygen at 25°C. Bars represent the mean ± S.E.M. of three experiments.

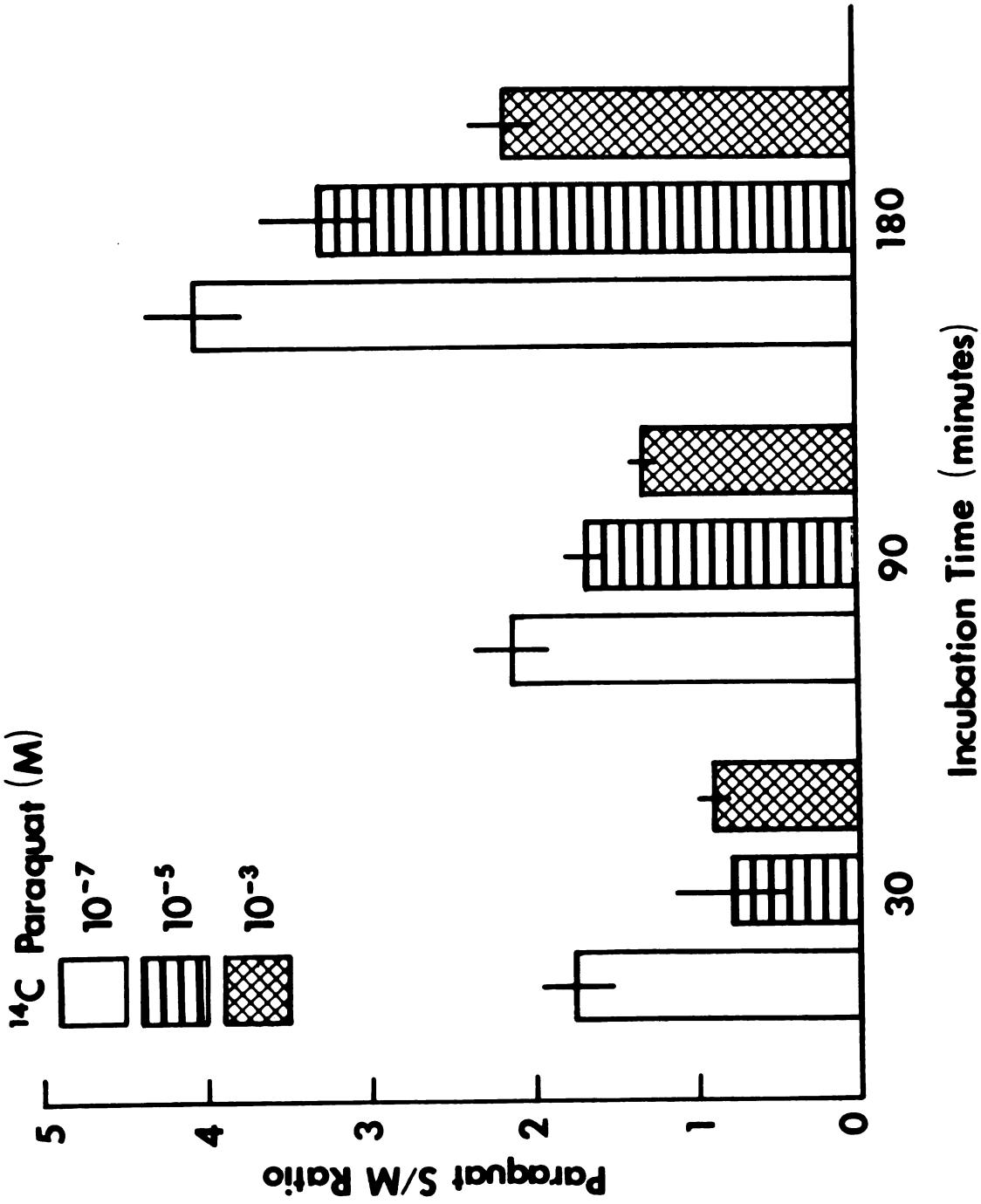

Paraquat S/M Ratio
0
1
2
3
4
5
30
90
180
Incubation Time (minutes)
^{14}C Paraquat (M)
10^{-7}
10^{-5}
10^{-3}

Figure 2

Figure 3: Accumulation (S/M ratio) of ^{14}C-labeled paraquat at an initial medium concentration of 10^{-5} M by mouse renal cortical slices incubated at 37°C under oxygen and nitrogen. Bars represent the mean ± S.E.M. of three experiments.

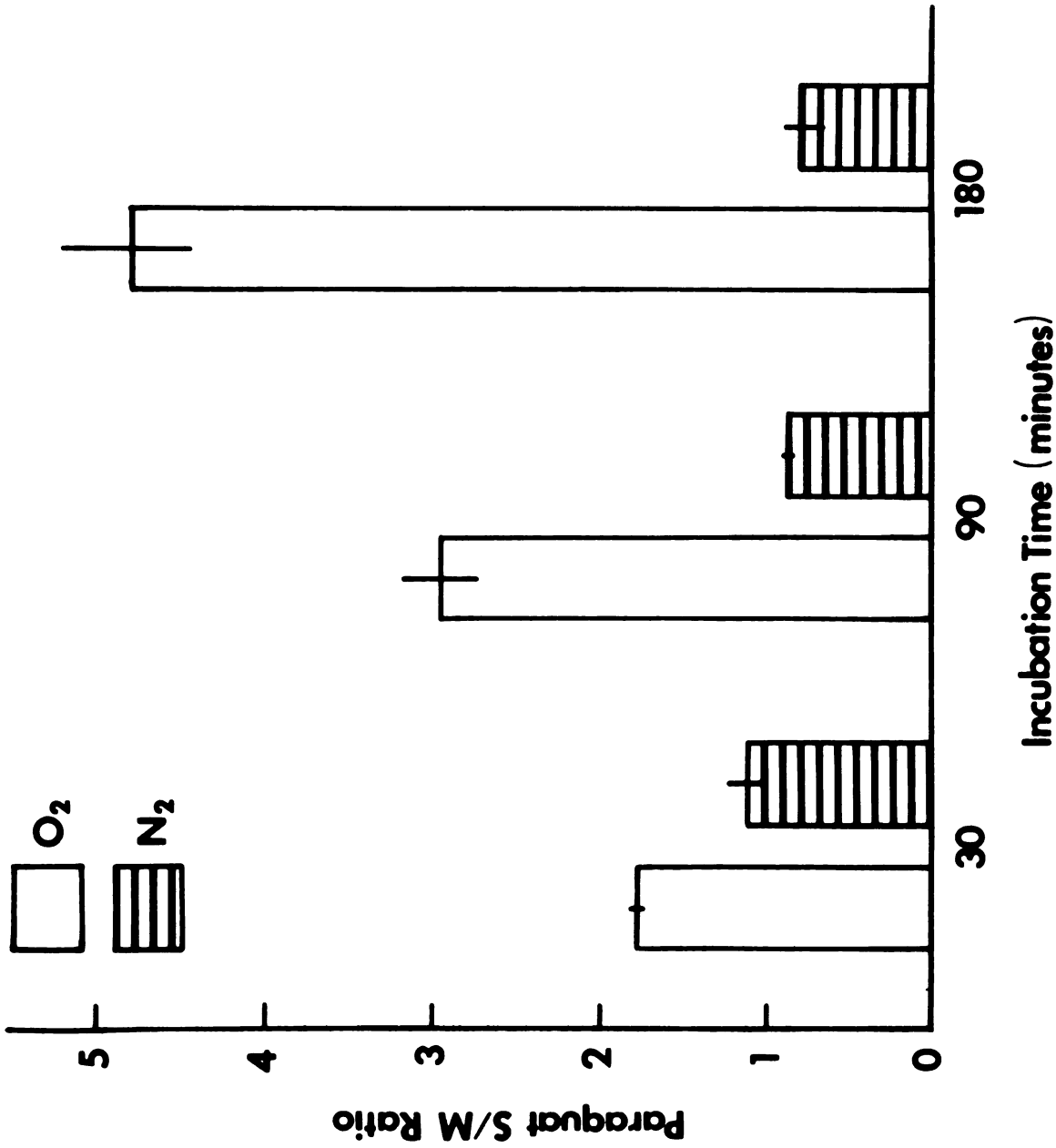

O2
N2
Paraquat S/M Ratio
0
1
2
3
4
5
30
90
180
Incubation Time (minutes)

Figure 3

Figure 4: Effect of paraquat on accumulation (S/M ratio) of PAH and NMN by mouse renal cortical slices incubated under oxygen at 25°C for 90 minutes. Points represent the mean ± S.E.M. of three (NMN) or seven (PAH) experiments. The S.E.M. was within the diameter of the circle for the points representing PAH S/M ratios.

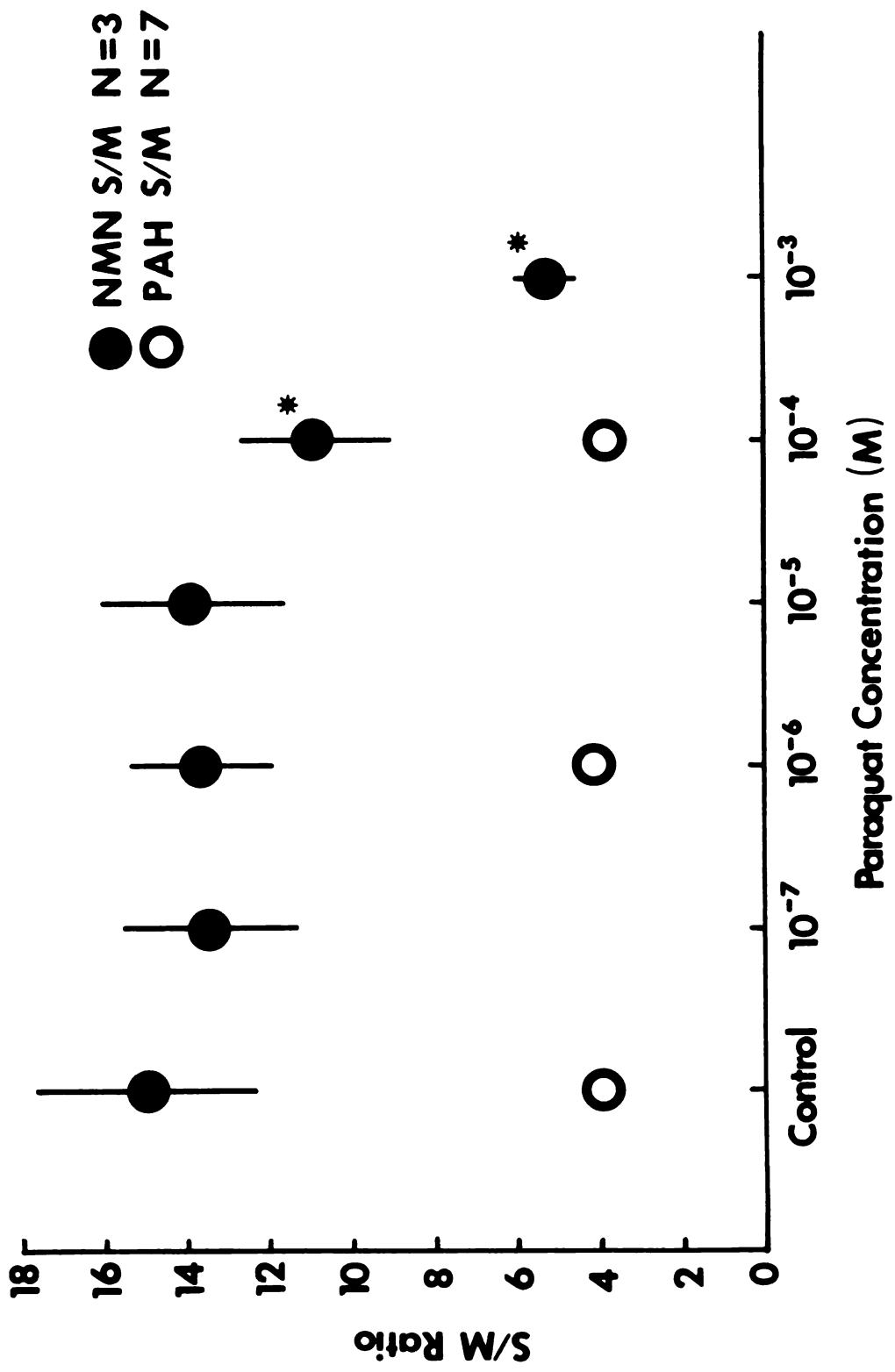

NMN S/M N=3
PAH S/M N=7
S/M Ratio
0
2
4
6
8
10
12
14
16
18
Control
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
Paraquat Concentration (M)
*
*

Figure 4

Figure 5: Effect of 10^{-3}M paraquat on the rate of ^{14}C-labeled NMN uptake by mouse renal cortical slices incubated under oxygen and nitrogen at 37°C. The clear area of each bar represents the uptake of NMN observed under a nitrogen atmosphere. The total area represents the uptake of NMN observed under oxygen. The difference between the oxygen and nitrogen uptake, represented by the shaded area, reflects the oxygen-requiring component of NMN uptake. Bars represent the mean ± S.E.M. of three experiments.

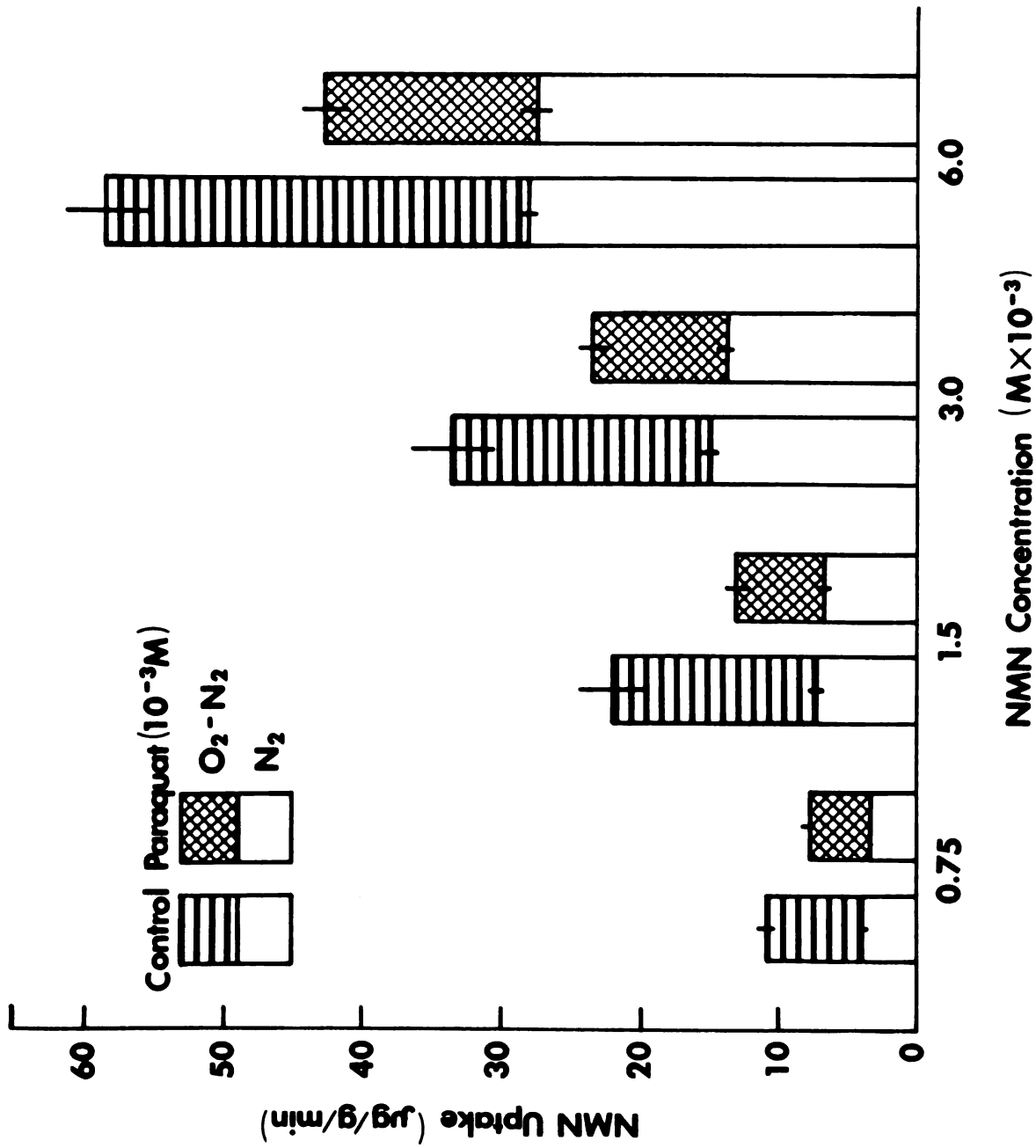

NMN Uptake (μg/g/min)
0
10
20
30
40
50
60
0.75
1.5
3.0
6.0
NMN Concentration (M×10⁻³)
Control
Paraquat (10⁻³M)
O₂ - N₂
N₂

Figure 5

Figure 6: Effect of 10^{-3}M paraquat on the rate of ^{14}C-labeled NMN uptake by mouse renal cortical slices incubated under oxygen (shaded area) and nitrogen (clear area) at 25°C. Bars represent the mean ± S.E.M. of four experiments.

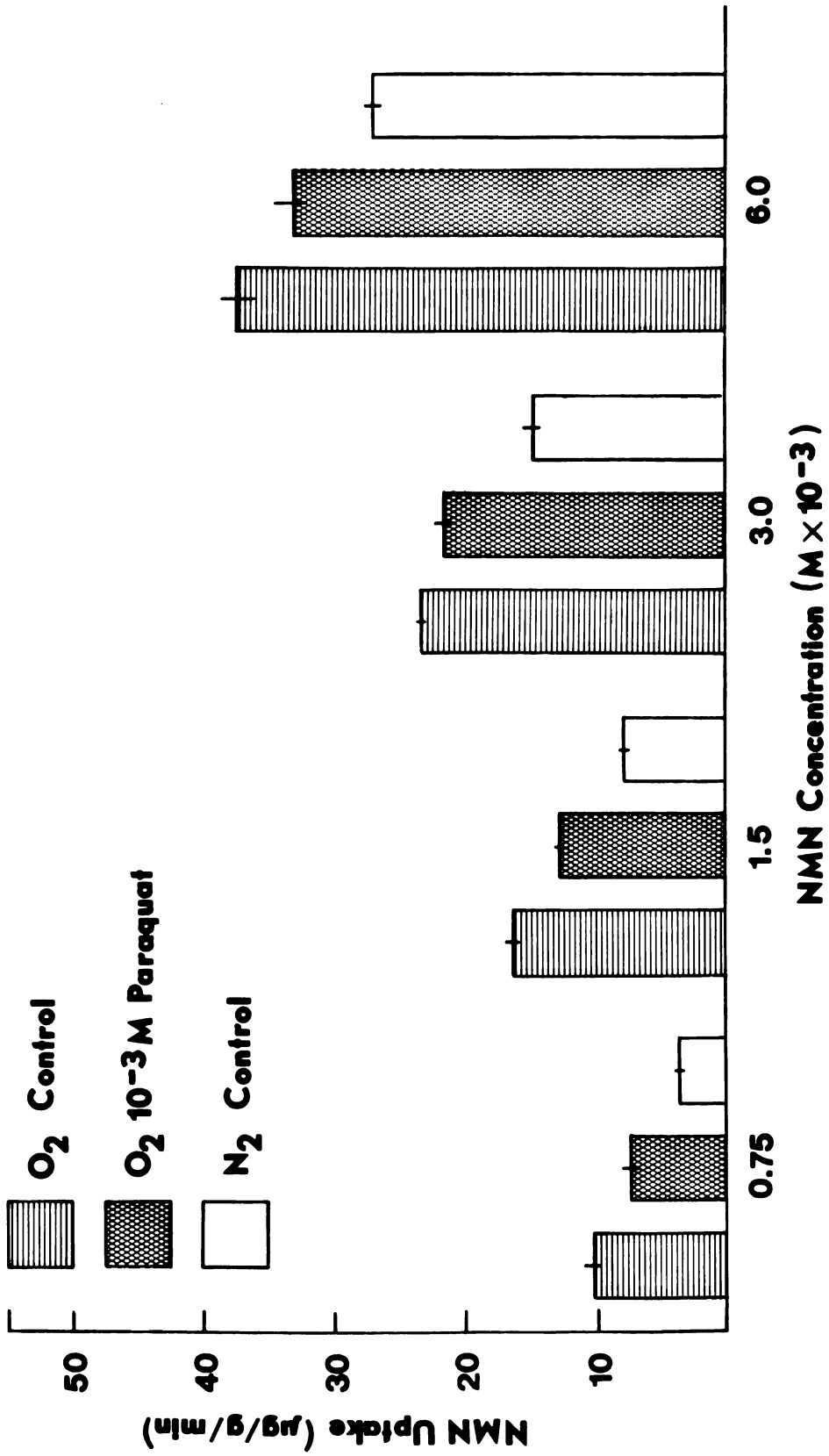

NMN Uptake (μg/g/min)
50
40
30
20
10
O_2 Control
O_2 10^{-3}M Paraquat
N_2 Control
0.75
1.5
3.0
6.0
NMN Concentration (M × 10^{-3})

Figure 6

Figure 7: Effect of bilateral nephrectomy or sham-operation on the disappearance of paraquat from plasma 16 hr later. Each point represents the plasma concentration of an individual animal following the i.v. injection of 50 mg/kg paraquat. Only the slope for the sham-operated group was significant ($p < .05$).

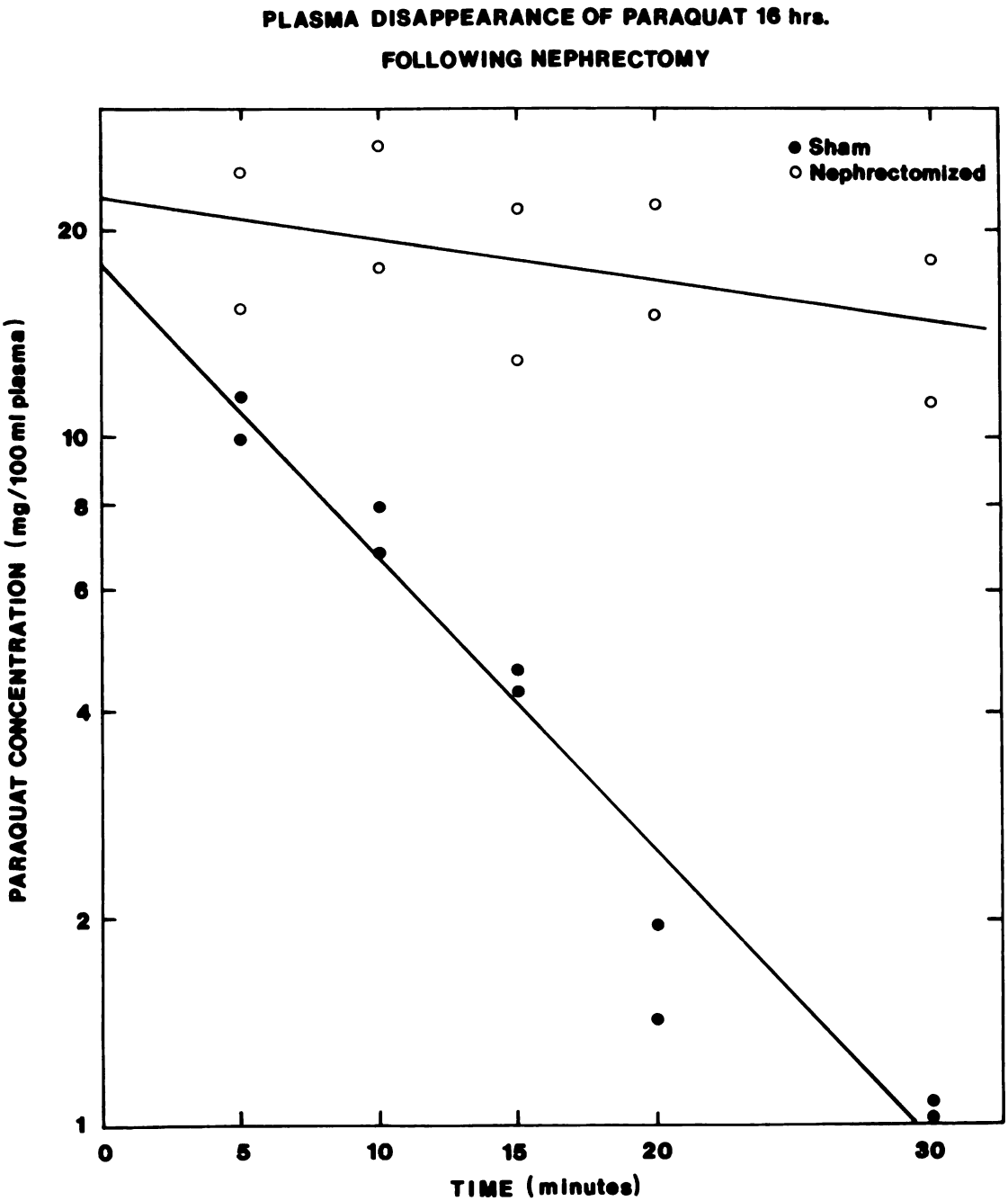

PLASMA DISAPPEARANCE OF PARAQUAT 16 hrs.
FOLLOWING NEPHRECTOMY
● Sham
○ Nephrectomized
PARAQUAT CONCENTRATION (mg/100 ml plasma)
20
10
8
6
4
2
1
0
5
10
15
20
30
TIME (minutes)

Figure 7

Figure 8: Effect of 30 mg/kg i.p. paraquat on the subsequent plasma disappearance of a second paraquat dose determined 2 and 24 hr later. The plasma concentration of paraquat was determined at various times following the second injection of paraquat, 50 mg/kg i.v. Each point represents the mean ± S.E.M. of 3 or 4 animals. Control values are shown on both figures. Only the slopes of the 2 hr and control groups were significant. Slopes of the 2 hr and control groups were not significantly different ($p < .05$).

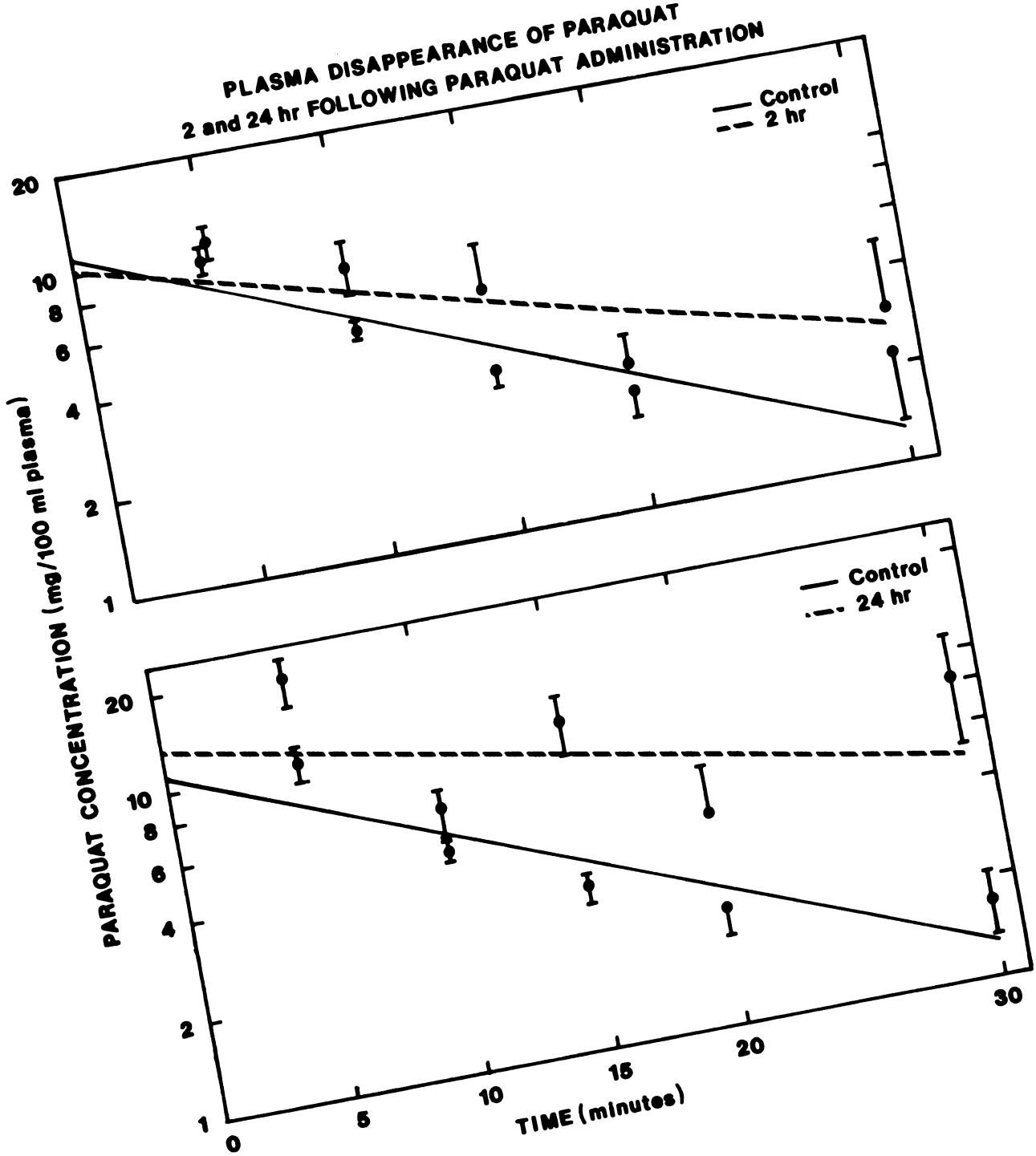

PLASMA DISAPPEARANCE OF PARAQUAT
2 and 24 hr FOLLOWING PARAQUAT ADMINISTRATION
Control
2 hr
Control
24 hr
PARAQUAT CONCENTRATION (mg/100 ml plasma)
TIME (minutes)
20
10
8
6
4
2
1
0
5
10
15
20
30

Figure 8

Figure 9: Effect of 30 mg/kg paraquat i.p. on the disappearance of phenosulfonphthalein (PSP) from plasma 24 hr later. The plasma concentration of PSP was determined at various times following the i.v. injection of 50 mg/kg PSP. Each point represents the mean ± S.E.M. of 3 animals. Slopes of the plasma disappearance curves were significantly different ($p < .05$).

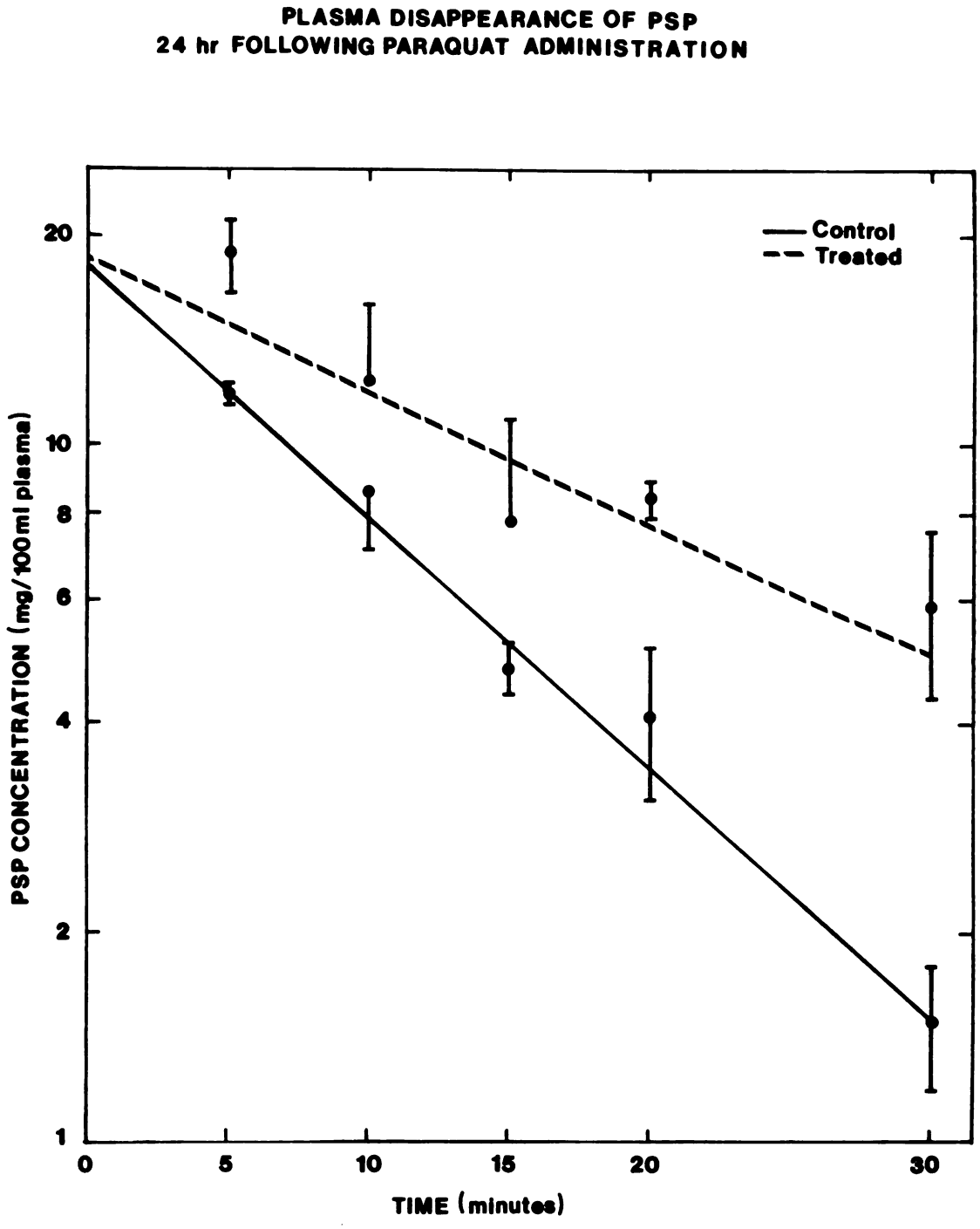

PLASMA DISAPPEARANCE OF PSP
24 hr FOLLOWING PARAQUAT ADMINISTRATION
Control
Treated
PSP CONCENTRATION (mg/100ml plasma)
20
10
8
6
4
2
1
0
5
10
15
20
30
TIME (minutes)

Figure 9

Figure 10: Effect of 30 mg/kg paraquat i.p. on the disappearance of ^{3}H-inulin from plasma 24 hr later. Each point represents the mean ± S.E.M. of 3 animals. Slopes of the plasma disappearance curves were not different.

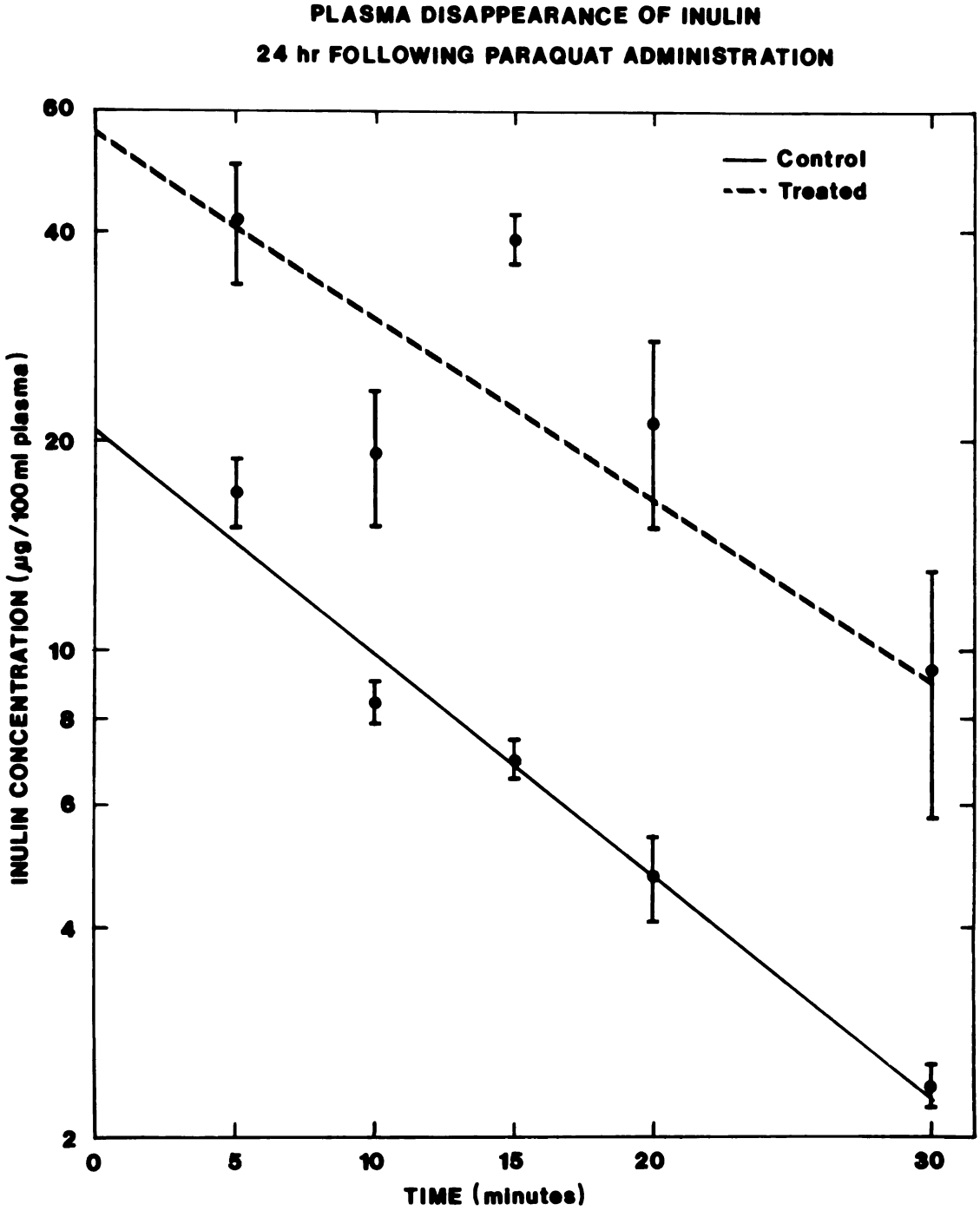

PLASMA DISAPPEARANCE OF INULIN
24 hr FOLLOWING PARAQUAT ADMINISTRATION
Control
Treated
INULIN CONCENTRATION (µg/100ml plasma)
60
40
20
10
8
6
4
2
0
5
10
15
20
30
TIME (minutes)

Figure 10

Figure 11: Effect of 30 mg/kg paraquat i.p. on the disappearance of ^{125}I iothalamate from plasma 24 hr later. Two separate experiments are presented. Closed circles and squares represent the disappearance of iothalamate from plasma following a 0.03 mg/kg dose i.v. The right hand ordinate corresponds to the open circles and squares and represents the disappearance of iothalamate from plasma following a 1.5 mg/kg dose i.v. Each point represents the mean ± S.E.M. of 3 animals. Slopes of the plasma disappearance curves between 30 and 120 min (open circles and squares) were not different.

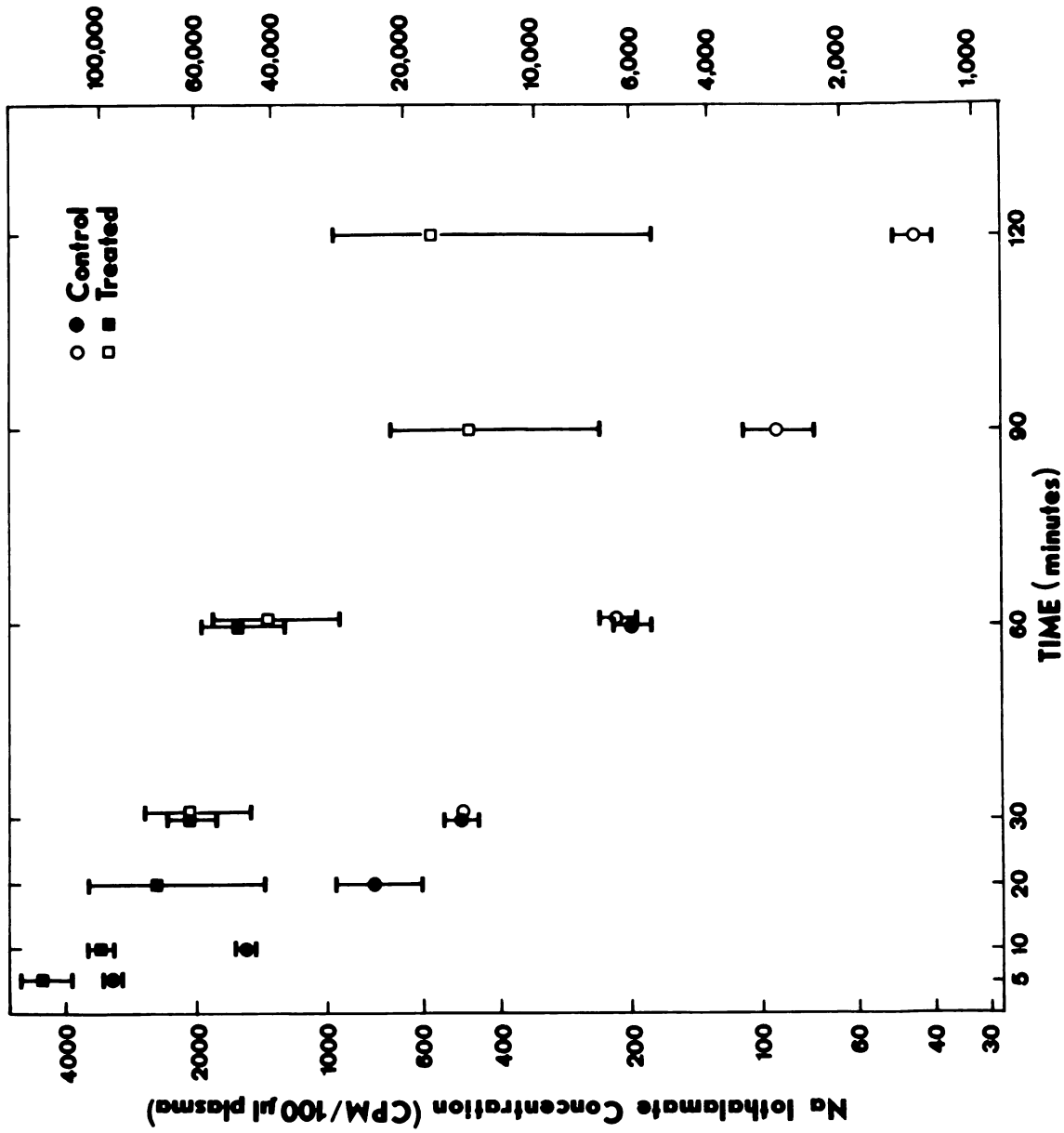

100,000
60,000
40,000
20,000
10,000
6,000
4,000
2,000
1,000
Control
Treated
5
10
20
30
60
90
120
TIME (minutes)
4000
2000
1000
600
400
200
100
60
40
30
Na Iothalamate Concentration (CPM/100µl plasma)

Figure 11

BIBLIOGRAPHY

BIBLIOGRAPHY

Autor, A.P.: Reduction of paraquat toxicity by superoxide dismutase. Life. Sci. 14, 1309-1319, 1974.

Balogh, K. and Merk, F.B.: Ultrastructure of renal collecting tubules following ingestion of a bipyridinium herbicide (Morfamquat). Experientia 29, 1101-1103, 1973.

Barber, A.A. and Bernheim, F.: Lipid peroxidation: Its measurement, occurrence and significance in animal tissues. *Advances in Gerontological Research.* Academic Press, New York, 1967.

Becker, B.A. and Gibson, J.E.: A simple method for the production of anuria in mice. Proc. Soc. Exp. Biol. Med. 124, 296-298, 1967.

Beebeejaum, A.R., Beevers, G. and Rogers, W.N.: Paraquat poisoning - prolonged excretion. Clin. Toxicol. 4, 397-407, 1971.

Browne, T.D.: Treatment of paraquat ingestion. Brit. Med. J. 3, 580, 1971.

Bullivant, C.M.: Accidental poisoning by paraquat. Brit. Med. J. 1, 1272-1273, 1966.

Bus, J.S., Aust, S.D. and Gibson, J.E.: Superoxide- and singlet oxygen-catalyzed lipid peroxidation as a possible mechanism for paraquat (methyl viologen) toxicity. Biochem. Biophys. Res. Commun. 58, 749-755, 1974.

Bus, J.S., Preache, M.M., Cagen, S.Z., Posner, H.S., Eliason, B.C., Sharp, C.W. and Gibson, J.E.: Fetal toxicity and distribution of paraquat in mice and rats. In press, Toxicol. Appl. Pharmacol., 1975a.

Bus, J.S., Cagen, S.Z., Aust, S.D. and Gibson, J.E.: Lipid peroxidation: A possible mechanism for paraquat toxicity. In press, Abst., Toxicol. Appl. Pharmacol., 1975b.

Calderbank, A.: The bipyridylium herbicides. Advan. Pest. Contr. Res. 8, 127-235, 1968.

Copland, G.M., Kolin, A. and Shulman, H.S.: Fatal pulmonary intra-alveolar fibrosis after paraquat ingestion. N. Engl. J. Med. 291, 290-292, 1974.

Cross, R.J. and Taggart, J.V.: Renal tubular transport: Accumulation of p-aminohippurate by rabbit kidney slices. Amer. J. Physiol. 161, 181-190, 1950.

Daniel, J.W. and Gage, J.C.: Absorption and excretion of diquat and paraquat in rats. Brit. F. industr. Med. 23, 133-136, 1966.

Dodge, A.D., Harris, N. and Baldwin, B.C.: (Par. Mech.) Biochem. J. 118, 43P-44P, 1970.

Editorial: Paraquat poisoning. Lancet 2, 1018-1019, 1971.

Editorial: Touching up the paraquat picture. Fd. Cosmet. Toxicol. 10, 700-705, 1972.

Editorial: Paraquat attacked. Nature 245, 64, 1973.

Farah, A., Frazer, M. and Porter, E.: Studies on the uptake of n-methylnicotinamide by renal slices of the dog. J. Pharmacol. Exp. Ther. 126, 202-211, 1959.

Farrington, J.A., Ebert, M., Land, E.J. and Fletcher, K.: Bipyridylium quaternary salts and related compounds. V Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implications for the mode of action of bipyridyl herbicides. Biochim. biophys. Acta. 314, 372-381, 1973.

Ferguson, D.M.: Renal handling of paraquat. Brit. J. Pharmac. 42, 636P-637P, 1971.

Fisher, H.K., Humphries, M. and Bails, R.: Paraquat poisoning: Recovery from renal and pulmonary damage. Ann. Int. Med. 75, 731-736, 1971.

Fisher, H.K., Clements, J.A. and Wright, R.R.: Enhancement of oxygen toxicity by the herbicide paraquat. Amer. Rev. Resp. Dis. 107, 246-252, 1973.

Foulkes, E.C. and Miller, B.F.: Transport of p-aminohippurate from cell to lumen in kidney tubule. Am. J. Physiol. 196, 83-85, 1959.

Fowler, B.A. and Brooks, R.E.: Effects of the herbicide paraquat on the ultrastructure of mouse kidney. Am. J. Path. 63, 505-512, 1971.

Gage, J.C.: The action of paraquat and diquat on the respiration of liver cell fractions. Biochem. J. 109, 757-761, 1968.

Grundies, H., Kolmar, D. and Bennhold, I.: Paraquat intoxication. Case report with special reference to hemodialysis. Dtsch. Med. Wachenschr. 96, 588-589, 1971.

Hirsch, G.H.: Effect of chronic lead treatment on renal function. Toxicol. Appl. Pharmacol. 25, 84-93, 1973.

Holm, J.: The uptake of decamethonium and hexamethonium by slices of mouse kidney. Acta. pharmacol. toxicol. 28, 192-202, 1970.

Kerr, F., Patel, A.R., Scott, P.D.R. and Tompsett, S.L.: Paraquat poisoning treated by forced diuresis. Brit. Med. J. 3, 290-291, 1968.

Kimbrough, R.D.: Toxic effects of the herbicide paraquat. Chest 65, 655-675, 1974.

Litchfield, M.W., Daniel, J.W. and Longshaw, S.: The tissue distribution of the bipyridylium herbicides diquat and paraquat in rats and mice. Toxicology 1, 155-165, 1973.

McIssac, R.J.: The uptake of hexamethonium-C^{14} by kidney slices. J. Pharmacol. 150, 92-98, 1965.

McIssac, R.J.: The binding of organic bases to kidney cortex slices. J. Pharmacol. Exp. Ther. 168, 6-12, 1969.

Maling, H.M., Saul, W., Brown, E.A.B. and Gillette, J.R.: Propranolol treatment of experimental paraquat poisoning in rats. Fed. Proc. 34, 226, 1975.

Mees, G.C.: Experiments on the herbicidal action of 1-1'-ethylene-2'-2'-dipyridylium dibromide. Ann. Appl. Biol. 48, 601-612, 1960.

Murray, R.E. and Gibson, J.E.: A comparative study of paraquat intoxication in rats, guinea pigs and monkeys. Exp. Mol. Pathol. 17, 317-325, 1972.

Murray, R.E. and Gibson, J.E.: Paraquat disposition in rats, guinea pigs and monkeys. Toxicol. Appl. Pharmacol. 27, 283-291, 1974.

Park, Y.S., Yoo, H.S. and Hong, S.K.: Kinetic studies on transport of organic acids in rabbit kidney slices. Am. J. Physiol. 220, 95-99, 1971.

Peters, L.: Renal tubular excretion of organic bases. Pharmacol. Rev. 12, 1-35, 1960.

Rhodes, M.L.: Hypoxic protection of paraquat poisoning: A model for respiratory distress syndrome. Chest 66, 3-4, 1974.

Rogers, P.A.M., Spillane, T.A., Fenlon, M. and Henaghan, T.: Suspected paraquat poisoning in pigs and dogs. Vet. Rec. 93, 44-45, 1973.

Ross, C.R., Pessah, N.I. and Farah, A.: Studies of uptake and runout of p-aminohippurate and n-methylnicotinamide in dog renal slices. J. Pharmacol. Exp. Therap. 160, 381-386, 1968.

Sakai, T., Leumann, E.P. and Holliday, M.A.: Single injection clearance in children. Pediatrics 44, 905-911, 1969.

Sharp, C.W., Ottolenghi, A. and Posner, H.S.: Correlation of paraquat toxicity with tissue concentrations and weight loss of the rat. Toxicol. Appl. Pharmacol. 22, 241-251, 1972.

Sigman, E.M., Elwood, C.M., Reagan, M.E., Morris, A.M. and Catanzaro, A.: The renal clearance of I^{131}-labelled sodium iothalamate in man. Invest. Urol. 2, 432-438, 1965.

Silkalns, G.I., Jeck, D., Earon, J., Edelmann, C.M., Chervu, L.R., Blaufox, M.D. and Spitzer, A.: Simultaneous measurement of glomerular filtration rate and renal plasma flow using plasma disappearance curves. J. Pediatrics 83, 749-757, 1973.

Smith, H.W., Finkelstein, N., Aliminosa, L., Crawford, B. and Graber, M.: The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest. 24, 388-404, 1945.

Smith, L.L., Wright, A. and Rose, M.S.: An effective treatment for paraquat poisoning in rats. In press, 1975.

Sokal, R.R. and Rohlf, F.J.: *Biometry*. Freeman and Co., San Francisco, 1969.

Staiff, D.C., Irle, G.K. and Felsenstein, W.C.: Screening of various adsorbents for protection against paraquat poisoning. Bull. Environ. Contam. Toxicol. 10, 193-199, 1973.

Steel, R.G.D. and Torrie, J.H.: *Principles and Procedures of Statistics*. McGraw-Hill Book Co., New York, 1960.

Toner, P.G., Vetters, J.M., Spilg, W.G.S. and Harland, W.A.: Fine structure of the lung lesion in a case of paraquat poisoning. J. Path. 102, 182-185, 1970.

Watrous, W.M. and Plaa, G.L.: Effect of halogenated hydrocarbons on organic ion accumulation by renal cortical slices of rats and mice. Toxicol. Appl. Pharmacol. 22, 528-543, 1972.