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SUMMARY

The tissue distribution of the biquaternary compounds paraquat (1,1'-dimethyl-4,4'-dipyridylium) and diquat (1,1'-ethylene-2,2'-dipyridylium) have been investigated after intravenous injection into mice using the technique of whole-body autoradiography. In the initial stages both compounds are rapidly distributed throughout most tissues with the exception of the brain and spinal cord. There is evidence, however, for preferential localisation in liver and cartilage. Whereas diquat is rapidly eliminated from all tissues a small amount of the administered paraquat is selectively retained at 24 h in lung and skeletal muscle from where it is slowly excreted.

Rats were administered diets containing 50, 120 and 250 ppm paraquat ion and 250 ppm diquat ion for 8 weeks. Analysis of tissues at selected intervals indicated the absence of any accumulation of either compound. When those animals that had survived the experimental diet were returned to a normal diet for 7 days neither paraquat nor diquat could be detected in any tissue despite detection limits of 0.1 μg/g and 0.05 μg/g respectively.

The significance of these observations to some aspects of paraquat toxicity is considered.

INTRODUCTION

Diquat and paraquat are non-selective rapidly acting herbicides and desiccants. Although the cations are only moderately toxic when administered to rats, they differ in their effects on tissues [1,2]. When administered orally to rats diquat and paraquat are poorly absorbed from the gastrointestinal tract and most of the absorbed material is rapidly excreted in the urine [3]. It

Abbreviations: diquat, 1,1'-ethylene-2,2'-bipyridylium; paraquat, 1,1'-dimethyl-4,4'-bipyridylium; ppm, parts per million.

has also been established that neither compound is metabolised when administered parenterally. Evidence obtained from human cases involving the ingestion of solutions of paraquat indicate, however, that urinary excretion of small quantities may persist for as long as 31 days [4—7]. This is presumably the result of binding of paraquat to tissue, evidence for which has been presented by Murray and Gibson[8] and by Sharp, Ottolenghi and Posner[9]. These two studies apart, little is known about the tissue distribution of diquat and paraquat.

This paper describes the distribution of diquat and paraquat in mice using the technique of whole-body autoradiography; the kinetics of excretion in rats and the effect of continuous feeding on tissue concentrations. This latter aspect was investigated to examine if the compounds might accumulate following the ingestion of dessicated crops containing small residues.

MATERIALS AND METHODS

Chemicals

Diquat dibromide, paraquat dichloride, [^{14}C]diquat (1,1-[U-^{14}C]ethylene-2,2'-bipyridylium dibromide; specific radioactivity 0.93 mCi/mmole) and [^{14}C]paraquat (1,1'-dimethyl-4,4'-[U-^{14}C]bipyridylium diiodide, specific radioactivity 2.62 mCi/mmole) were obtained from Plant Protection Limited, Bracknell, Berkshire.

Animals

Male mice and male and female Wistar albino rats of the Alderley Park specific pathogen-free strain were used in these studies.

Excretion of radioactivity

The excretion of radioactivity in the urine of male rats (200 g body weight) was determined at intervals of 24 h after the oral administration of an aqueous solution of either [^{14}C]paraquat or [^{14}C]diquat (60 mg cation/kg). Radioactivity in the urine was measured according to Daniel and Gage[10].

Whole-body autoradiography

Mice (15—18 g body weight) were injected intravenously with 0.2 ml of a solution in isotonic saline of 5 μCi of either [^{14}C]paraquat (20 mg cation/kg) or [^{14}C]diquat (50 mg cation/kg). Two animals were killed by exposure to diethyl-ether 10 min, 1 h, 5 h, 24 h, and 72 h after paraquat and 10 min, 1 h, 24 h and 72 h after diquat. After death the animals were immersed in a 2% (w/v) aqueous solution of carboxymethylcellulose maintained at −20°. Serial sections (100 μ) containing representative samples of all tissues were obtained as described by Ullberg[11]. The sections were placed in contact with X-ray film (Agfa-Structurix 5A) in a light-tight box and stored at −20° for 60 days. Films were developed in DX-80 Kodak developer and fixed.

Feeding experiments

Male and female rats weighing 210—240 g were fed diets containing paraquat dichloride monohydrate at concentrations equivalent to 50, 120 and 250 ppm paraquat ion and diquat dibromide monohydrate at 250 ppm diquat ion. The diets were analysed at regular intervals to ensure that the required bipyridyl content was attained.

The diets were fed to groups of 40 rats at the 250-ppm paraquat and diquat concentrations and to groups of 30 rats for the remaining paraquat levels. Food intake was measured daily and individual body weights recorded at weekly intervals throughout the experiment. At 2, 4 and 8 weeks ten rats from each group and five controls were killed, and brain, lungs, liver, kidneys, hind leg muscle, stomach, small and large intestines were analysed for paraquat and diquat. Livers, kidney and lungs were weighed at *post mortem.*

Colorimetric determination of paraquat and diquat in tissues

Blood (10 ml) was added to 5% (w/v) trichloroacetic acid solution (40 ml) and the contents mixed. The solution was centrifuged and the supernatant retained. The pellet was resuspended in 5% trichloroacetic acid (25 ml) and centrifuged. The two supernatant solutions were combined. A 10% (w/v) homogenate of tissues (10—20 g) was prepared in 10% (w/v) trichloroacetic acid. The homogenate was centrifuged, the precipitate washed with 10% trichloroacetic acid and the supernatant and washings combined.

The trichloroacetic acid extracts were allowed to percolate through a column (1—2 ml) of the ion-exchange resin, Zeo-Karb 225 (H^+-form) at a rate not exceeding 5 ml/min. The column was washed successively with distilled water (25 ml), 2 *N* HCl (50 ml), distilled water (25 ml), 2.5% ammonium chloride (25 ml) and distilled water (25 ml). Paraquat and diquat were then eluted with saturated ammonium chloride (25 ml) at a rate of 1 ml/min.

Paraquat was determined by adding a solution (2 ml) of 1% sodium dithionite in *N* NaOH to 10 ml of the ammonium chloride eluate and measuring the absorbance at 600 nm in 40 mm cells while diquat was similarly estimated at 379 nm after the addition of a solution (2 ml) of 0.2% sodium dithionite in 5% sodium tetraborate.

Determinations carried out on the organs of the 30 control animals showed no absorbance reading greater than 0.005 in 4 cm cells, and the mean absorbance for all organs was less than 0.002. To allow for adventitious contamination an absorbance of 0.01 was considered to be the lowest reading attributable to a bipyridyl in the test organs, thus setting limits of detection of 0.1 μg/g and 0.05 μg/g for paraquat and diquat respectively when 10 g tissue was taken for analysis.

The recovery of paraquat and diquat when injected into various tissues at the 1-ppm level was within the range 90—95%.

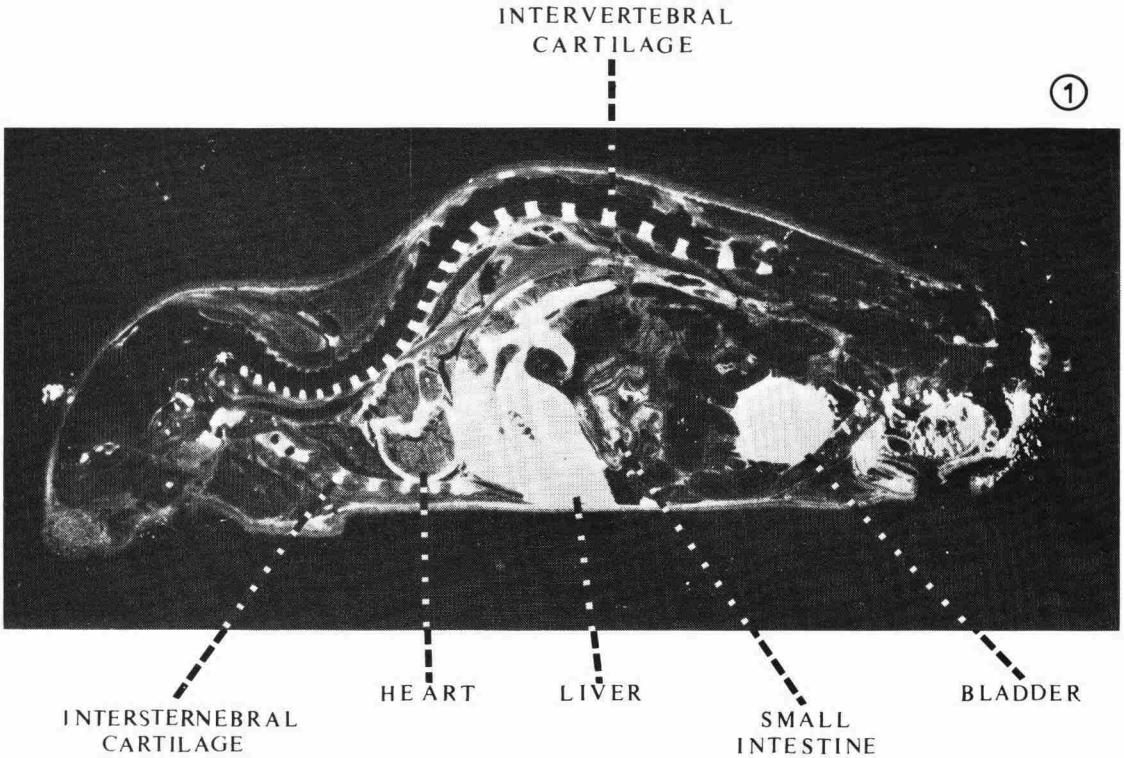

Fig. 1. Whole-body autoradiogram of a mouse frozen 10 min after i.v. injection of paraquat.

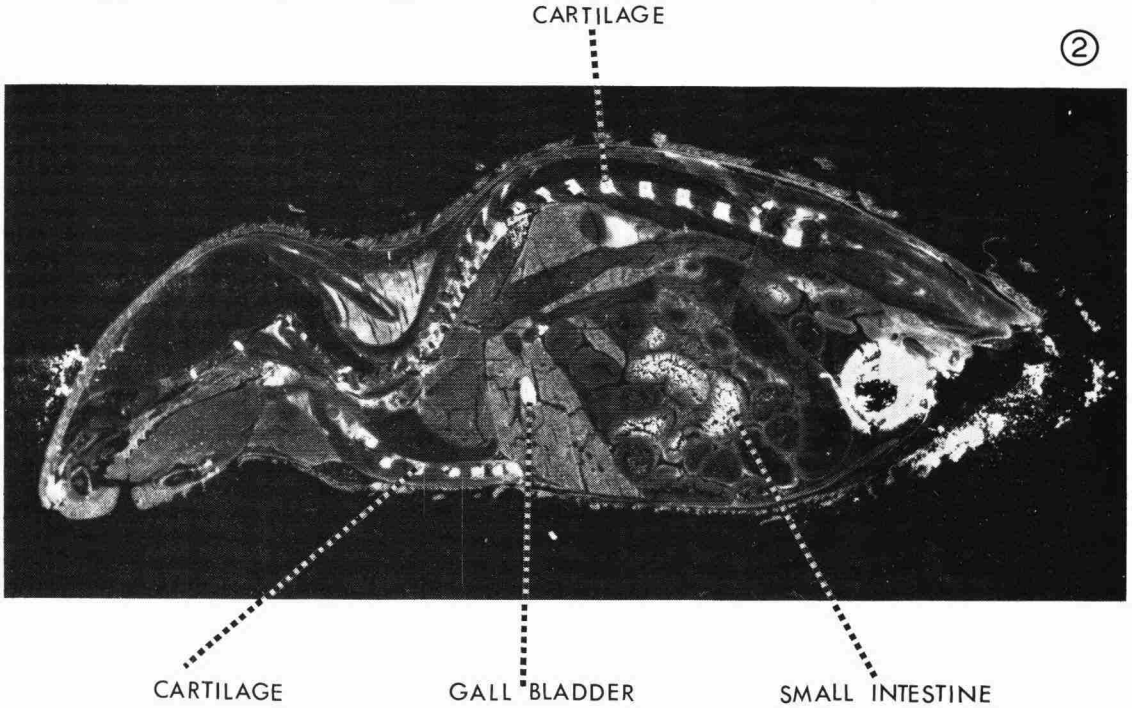

Fig. 2.Whole-body autoradiogram of a mouse frozen 10 min after i.v. injection of diquat.

RESULTS

Whole-body autoradiography

Autoradiograms prepared within 10 min of the injection of paraquat (Fig. 1) and diquat (Fig. 2) showed that the radioactivity was distributed throughout most tissues. Both compounds were concentrated in cartilaginous tissue and in the liver; although paraquat appeared to be distributed throughout the liver parenchyma, diquat was selectively located in the gall-bladder. The concentration of paraquat in cardiac muscle was appreciably greater than in skeletal muscle. Radioactivity was present in the bladder, in the intestine and in mucosal cells of many tissues including stomach, buccal and nasal cavities, but particularly those of the small intestine. Smaller amounts of radioactivity were present in the lung, thymus, hair-follicles, dentine, retina and sclera of the eye. Low levels of diquat were present in brain and spinal cord; no paraquat was detected in the central nervous system.

After 1 h, the general level of radioactivity of both paraquat (Fig. 3) and diquat (Fig. 4) had visibly declined, although the distribution among individual tissues was essentially similar to that at 10 min. The amount in urine and in the intestinal epithelium had, however, increased. The concentration of diquat in cartilage had declined more rapidly than that of paraquat.

In autoradiograms prepared 5 h after the administration of paraquat (Fig. 5), the amount in cardiac muscle appeared about equal to that in skeletal muscle and no radioactivity was present in cartilage.

After 24 h the excretion of diquat was virtually complete and radioactivity was only present in the small and large intestine and bladder.

One significant feature in the 24-h autoradiograms from paraquat-treated mice was the presence of radioactivity in the lung (Fig. 6). There was in addition evidence of the presence of radioactivity in both the brain and spinal cord.

In autoradiograms prepared 72 h after the administration of paraquat or diquat only the stomach and intestinal contents were labelled.

Excretion of radioactivity

In four experiments in which rats were given a single oral dose of [^{14}C]paraquat, an average of 9.6% of the radioactivity was excreted in the urine in the 4 days after dosing. An additional 0.34% was excreted during days 5—8; 0.05% in days 9—17 and 0.017% in days 18—28. In comparable experiments with [^{14}C]diquat 5.5% and 0.03% of the dose was excreted in the urine in days 1—7 and 8—14, respectively.

Feeding experiments

Apart from those killed at the intervals specified in Table I, all animals survived administration of the test diets. The food consumption by the rats on the 250-ppm paraquat diet was about 10% less than that of the controls and the weight gain over the 8-week period was slightly less, probably due to the reduced food intake. There was no change in organ:body weight ratio for liver or kidney. The value for lung was slightly increased, probably due to

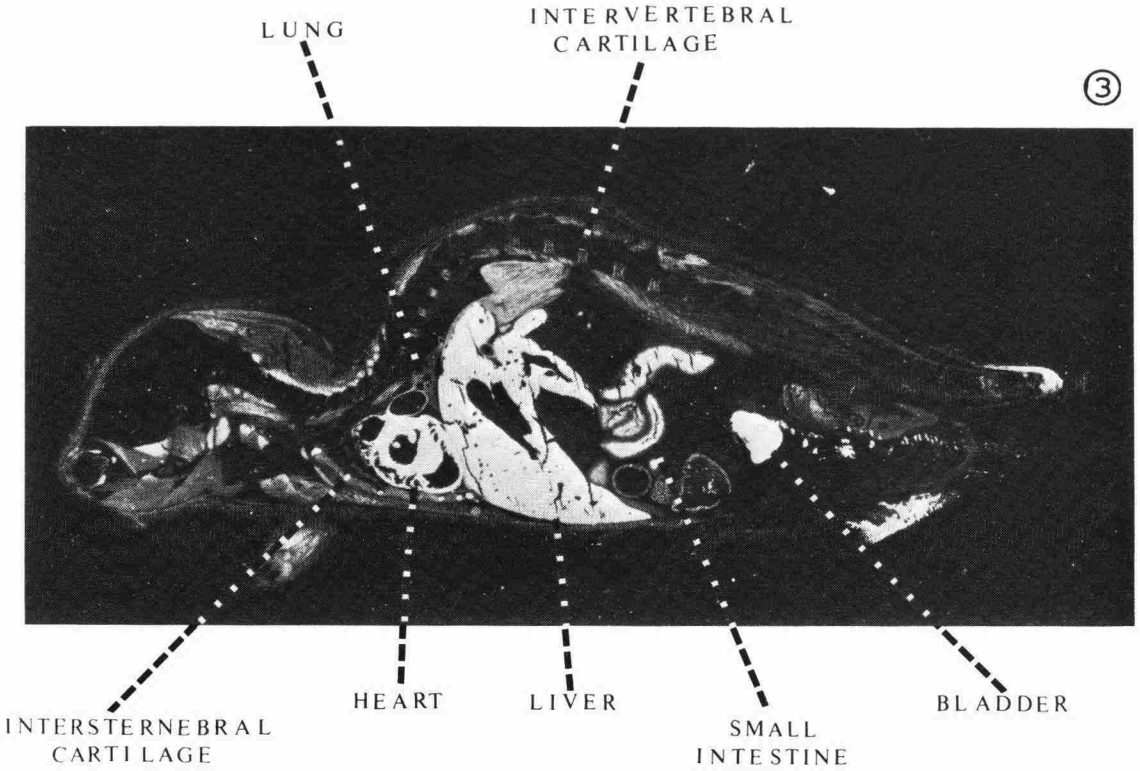

Fig. 3. Whole-body autoradiogram of a mouse frozen 60 min after i.v. injection of paraquat.

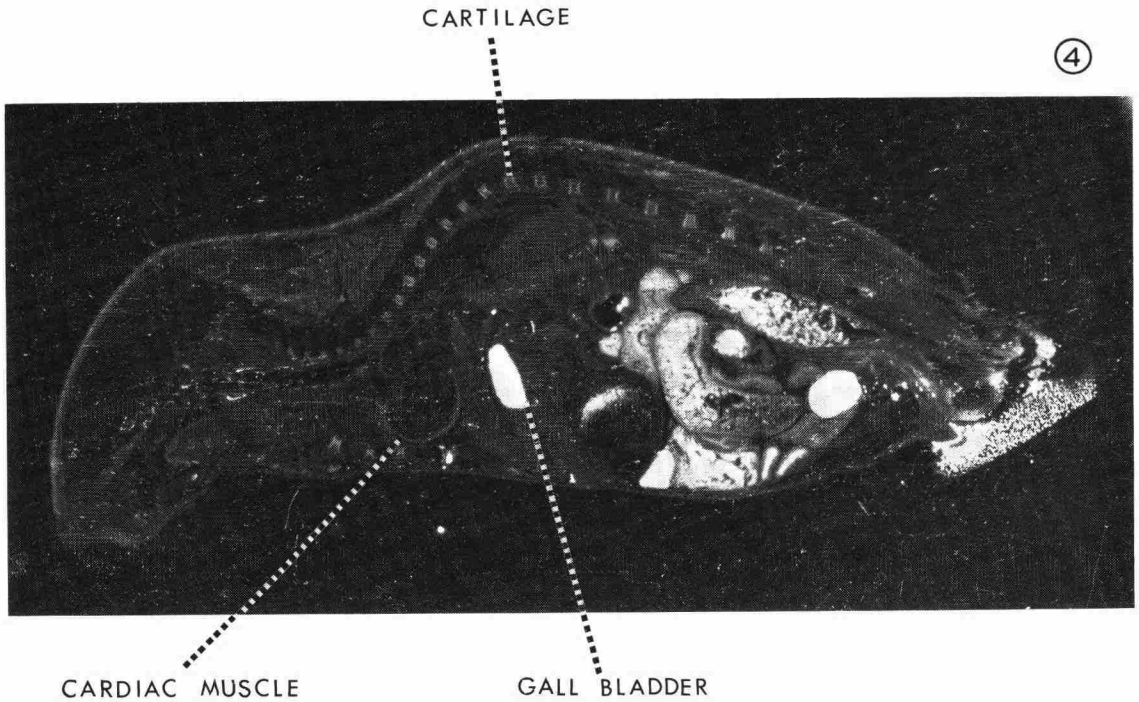

Fig. 4. Whole-body autoradiogram of a mouse frozen 60 min after i.v. injection of diquat.

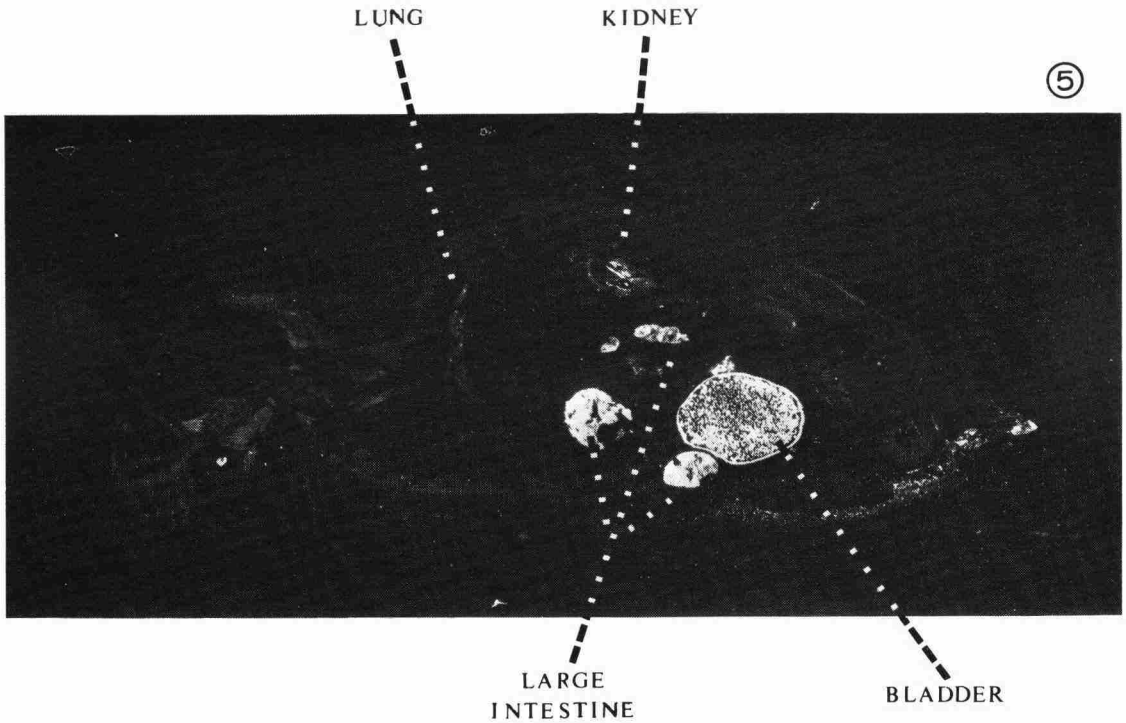

Fig. 5. Whole-body autoradiogram of a mouse frozen 5 h after i.v. injection of paraquat.

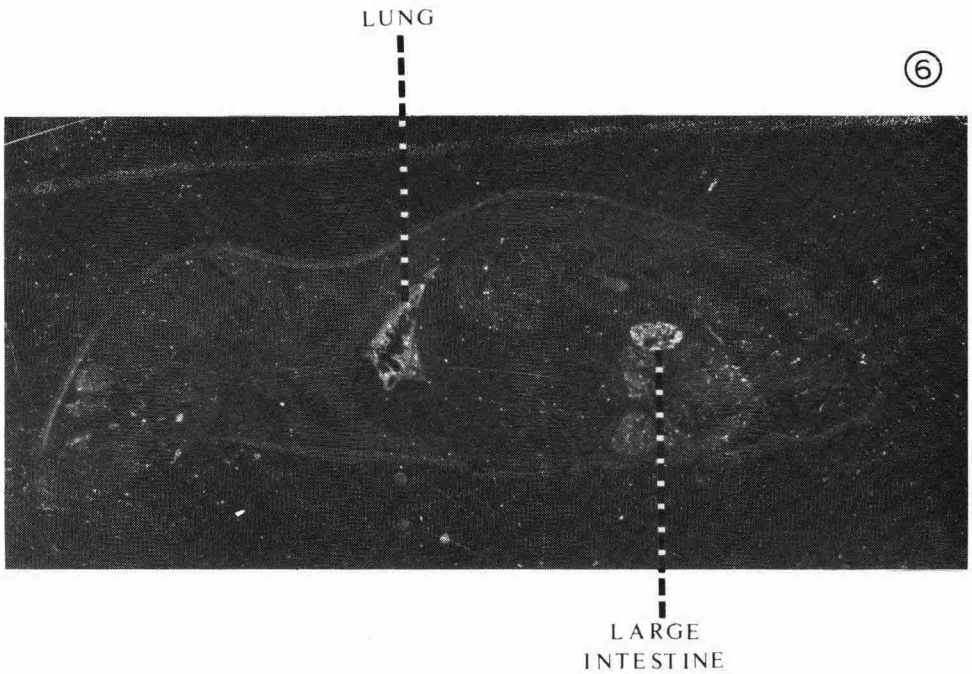

Fig. 6. Whole-body autoradiogram of a mouse frozen 24 h after i.v. injection of paraquat.

TABLE I
THE CONCENTRATION OF PARAQUAT OR DIQUAT IN TISSUE OF MALE RATS FED DIETS CONTAINING PARAQUAT OR DIQUAT FOR 2, 4, AND 8 WEEKS

Results, in ppm, are the means of determination on 2 batches of 5 tissues.

	Concentration in diet ppm											
	Paraquat									Diquat		
	50			120			250			250		
Week	2	4	8	2	4	8	2	4	8	2	4	8
Kidney	<0.10	<0.10	<0.10	0.36	0.10	0.23	0.96	0.36	1.80	0.18	0.25	1.17
Brain	<0.10	<0.10	<0.10	<0.10	<0.10	0.20	<0.10	<0.10	ND	0.08	<0.05	<0.05
Liver	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.28	0.21	1.15	0.07	0.22	0.08
Lung	<0.10	<0.10	<0.10	0.24	0.17	0.35	2.44	1.07	1.60	<0.05	0.53	0.16
Stomach	0.96	0.32	0.20	0.84	0.30	0.23	0.90	0.42	2.40	0.79	0.15	0.29
Small intestine	0.25	0.10	0.31	0.79	0.27	0.27	1.81	2.12	3.40	0.66	0.55	0.30
Large intestine	0.24	0.17	0.12	1.32	0.72	0.62	3.32	3.06	7.48	0.77	0.67	1.18
Muscle	0.14	<0.10	<0.10	0.10	<0.10	<0.10	ND	ND	1.46	ND	ND	<0.05
Blood	ND[a]	ND	ND	ND	ND	ND	<0.10	<0.10	<0.10	ND	ND	ND

[a]ND, not determined.

the fact that almost all of the lungs were congested from animals in this group. There were no significant changes in food consumption, weight gain or organ:body weight ratio in the rats on the lower paraquat diets or those on diquat diet compared with controls.

No paraquat was detected in kidney, brain, liver or lung after feeding paraquat at a dietary concentration of 50 ppm for a period of 8 weeks (Table I). A small (< 1 ppm) and variable amount was present in stomach, small and large intestine. At a dietary concentration of 120 ppm, paraquat was present in low concentrations in kidney, lung and the gastro-intestinal tract, and there was some evidence of paraquat in brain after 8 weeks of feeding. The presence of paraquat in tissue occurred within 2 weeks of feeding at 250 ppm and the content of paraquat was somewhat variable over the 8-week period and showed no clear pattern of accumulation. The tissue concentration of diquat administered at 250 ppm was lower than that of paraquat particularly in lung, where the average paraquat content over the 8-week period was 1.7 ppm, while that of diquat was 0.2 ppm. No sex differences were observed with either compound. Within 1 week of return to a normal diet no paraquat or diquat was detectable in any tissue.

DISCUSSION

Whole-body autoradiograms obtained from mice injected with either diquat or paraquat show that both compounds are rapidly distributed throughout most tissues with the exception of brain and spinal cord. Although the compounds are concentrated in the liver within a relatively short period diquat appears to be localised in the gall-bladder whereas paraquat is distributed throughout the liver parenchyma. The reason for this difference is not known. The localisation of both diquat and paraquat in cartilage is presumably the result of binding to polyanionic mucopolysaccharides and, possibly, acidic phospholipids[1,2]. The affinity of both compounds for cartilage is however relatively weak for autoradiograms obtained 1 h after dosing show a substantial reduction in the level of radioactivity. When the autoradiograms obtained within 10 min of dosing (Figs. 1 and 2) are compared with those after 1 h (Figs. 3 and 4) and 5 h (Fig. 5) it is apparent that both compounds are rapidly eliminated from all tissues. This is in agreement with the observations of Murray and Gibson[8], who quoted a value of 0.9 h^{-1} for the elimination rate constant (K_e) for paraquat, and of those of Sharp, Ottolenghi and Posner[9] who quoted a value of 20—30 min for the half-life of paraquat in the plasma, lung, kidney, liver and muscle of rats. The presence of radioactivity in the mucosa and lumen of the small-intestine indicate that both compounds are secreted into the intestine. This would account for the substantial faecal excretion of radioactivity that occurs in rats given either compound by subcutaneous injection in the absence of any significant biliary excretion[3].

A comparison of the autoradiograms obtained 1 h after dosing indicates that the concentration of paraquat in cardiac muscle is substantially greater

than that observed with diquat. This preferential uptake of paraquat by cardiac muscle may be responsible for the toxic myocarditis noted in several cases of human paraquat poisoning[13,14].

The affinity of muscle for paraquat and, to a lesser extent diquat, has been previously described by Sharp, Ottolenghi and Posner[9]. The 24-h autoradiograms indicate the presence of low levels of paraquat and diquat in muscle and in the gastro-intestinal tract. These two sources clearly provide the major pools of residual radioactivity. However, Ferguson[15] has shown that paraquat is re-absorbed from the proximal tubule in dogs and this might provide an additional reservoir. The extent of the residual pool after day 4 is estimated to be approx. 0.5% of the dose for paraquat and 0.03% for diquat.

The most conspicuous difference between paraquat and diquat concerns the persistence of paraquat in the lungs. This is particularly apparent in the autoradiograms obtained 24 h after dosing. This selective retention of paraquat is supported by chemical analysis of the lungs of rats fed diets containing either diquat or paraquat where, at a dietary concentration of 250 ppm, the concentration of paraquat is consistently greater than that of diquat. Whereas all lungs from animals fed 250 ppm paraquat were oedematous and histological examination revealed changes that varied from mild perivascular oedema to complete consolidation, no such changes were noted in animals fed either 50 or 120 ppm. Damage would therefore appear to be associated with a paraquat concentration in the lung greater than 1–2 μg/g. There are insufficient data to explain the observation that the concentration of paraquat in the lungs of rats maintained on diets containing 250 ppm of the herbicide was 5—10 times that found after feeding at a dietary concentration of 120 ppm. It is considered unlikely that the haemorrhagic and oedematous condition of the lungs of animals from this group would in itself account for the high concentration of paraquat present. It is apparent that additional studies into the nature of the binding of paraquat in the lung are necessary.

The data obtained from the feeding experiments indicate that the amount of bipyridyl in the lungs attains a relatively constant value within a short period. Neither compound was detected in the lungs of animals that had been maintained on the experimental diet and then transferred to a normal diet for 7 days. The data also illustrate that no accumulation is likely to occur following the ingestion of foods containing low residues of either compound.

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