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PARAQUAT ACCUMULATION: TISSUE AND SPECIES SPECIFICITY

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Abstract Rat tissues have been examined *in vitro* for their ability to accumulate paraquat or diquat to concentrations in excess of those present in the incubation medium. With a concentration of 10^{-6} M, lung slices were able to accumulate paraquat to concentrations nearly ten times that of the medium, and brain slices to concentrations double that of the medium, over a period of two hours. Neither slices of lung nor brain accumulated diquat significantly from a concentration in the medium of 10^{-6} M. The accumulation of paraquat by brain slices, like that of lung slices, has been shown to be energy-dependent. Other organs examined showed little, if any, ability to accumulate either paraquat or diquat. Lung slices from dog, monkey and rabbit have also been shown to possess the ability to accumulate paraquat *in vitro*. After oral dosing of paraquat to rats, the lung concentration increased with time to six times that of the plasma after 30 hr. Other organs, with the exception of the kidney, did not concentrate paraquat to the same extent. Kidney concentrations after oral dosing of both paraquat and diquat were high throughout the period of time studied. It is, therefore, suggested that the apparent selectivity exhibited by paraquat for the lung is associated with the accumulation process.

Paraquat (1,1′-dimethyl-4,4′-bipyridilium) and diquat (1,1′-ethylene-2,2′-bipyridilium) are closely related non-selective herbicides with a similar mode of action against plants [1]. Despite very similar chemical, herbicidal and biochemical effects [2], they have different toxic effects in mammals; paraquat seriously damages the lung [3, 4], whereas diquat does not [5].

After oral administration to rats, the compounds are poorly absorbed from the gastrointestinal tract [6, 7]. After subcutaneous injection into rats, 90% is excreted unchanged into the urine in the first 24 hr and there is no evidence of metabolism [6].

The discovery of energy-dependent accumulation of paraquat by slices of rat lung [8] suggests a possible reason for the propensity exhibited by paraquat for damaging the lung. In this study, other organs have been examined for their ability to accumulate paraquat and diquat. Lung slices from other species have also been compared with those from rat in their ability to accumulate paraquat. The relevance of accumulation to tissue damage is discussed in the light of these measurements.

MATERIALS AND METHODS

Special chemicals

Methyl-^{14}C paraquat (sp. act. 30 mCi/mmole) and ethylene-^{14}C diquat (sp. act. 29 mCi/mmole) were purchased from the Radiochemical Centre, Amersham.

Animals

The animals used in this study were all males and were as follows: rats, Alderley Park (Wistar-derived) specific pathogen free; dogs, (inbred beagles); rabbits (New Zealand Whites); monkeys cynomologus (*Macaca fascicularis*).

Methods

Preparation of tissue slices. Animals were killed with halothane and selected organs rapidly removed and placed in Krebs–Ringer phosphate buffer at r.t. Tissue slices of lung, liver, skeletal muscle, kidney cortex, heart, brain cortex and spleen were prepared by hand or by using a modified Stadie–Riggs tissue slicer [9]. Adrenals were quartered and small intestine was cut into rings. Slices of skin were cut using a keratotome. The wet weights of tissue slices ranged from 20 to 60 mg.

Incubation. Slices were weighed and incubated in a modified Krebs–Ringer phosphate medium (3 ml) containing NaCl (130 mM), KCl (5·2 mM), $CaCl_2$ (1·9 mM), $MgSO_4$ (1·29 mM), Na_2HPO_4 (10 mM) and glucose (11 mM). The pH of the buffer was adjusted to 7·4 with HCl. In addition the incubation medium contained 0·1 μCi of either ^{14}C-paraquat or ^{14}C-diquat, together with the required concentration of unlabelled bipyridyl. Incubation was carried out in air, or in the case of brain tissue, in oxygen, with shaking, at 37°.

Bipyridyl measurement in slices. Slices were removed from the incubation medium and washed by transferring them to fresh medium without bipyridyl. They were then carefully blotted, dissolved in 1 ml Soluene (Packard Instrument Co Ltd) and radioactivity measured after addition of 10 ml Dimilume scintillator (Packard Instrument Co Ltd) using a liquid scintillation spectrometer. Samples of the medium (0·1 ml) were diluted to 1·0 ml with water and radioactivity measured after addition of 10 ml Instagel scintillator (Packard Instrument Co Ltd). Counting efficiency was determined by the addition of an internal standard and all counts were converted to disintegrations per minute.

Bipyridyl measurement in tissues after oral administration to rats. Rats were starved for 24 hr before being

dosed with 680 μmoles/kg body-weight of ^{14}C-bipyridyl orally as described previously [7]. They were killed with halothane at various times up to 30 hr after dosing, the organs removed and bipyridge determined either by oxidation of the tissue to $^{14}CO_2$ using an Oxymat (Intertechnique Ltd) or after solubilisation of the tissue in Soluene as described for tissue slices.

RESULTS

Uptake of paraquat by slices of rat tissue. Slices of rat lung accumulated paraquat linearly [8] for two hours achieving concentrations ten times higher than present in the incubation medium (Table 1). Apart from lung slices, slices of brain cortex were the only other tissue slices studied which were able to accumulate paraquat to a concentration in excess of that in the medium (Table 1). The uptake observed with brain slices was non-linear and was reduced when cyanide and iodoacetate were included in the incubation medium (Fig. 1). Brain slices are known to swell on incubation *in vitro* [10] and this swelling is exacerbated in the presence of inhibitors of energy production [11]. The water content of brain slices was, therefore, measured and increases in wet weight were assumed to be due to uptake of medium. The paraquat present in this volume of medium was then calculated and subtracted from the total paraquat found in the slice. Little, if any, difference was observed when corrections were applied to the uptake of paraquat in the absence of inhibitors but a considerable proportion (>30%) of the paraquat in slices in the presence of the inhibitors could be ascribed to uptake of medium through swelling (Fig. 1). Thus, after correction, it can be seen that accumulation of paraquat by brain slices was virtually stopped by the presence of inhibitors of energy production.

Uptake of diquat by slices of rat tissues. Diquat was not accumulated *in vitro* to any great extent by any of the tissues studied (Table 2). Kidney slices, however, appeared to concentrate diquat to levels higher than that present in the medium, but this process occurred within the first hr (Table 2) unlike accumulation of paraquat by lung or brain slices.

Tissue concentrations of paraquat and diquat after oral dosing to rats. After oral administration of 680 μmoles/kg body-weight to rats, the concentration of paraquat in the plasma remained relatively constant between two and thirty hours at 6·14 nmoles/ml (Table 3). The concentration of paraquat in the kidney was high during this time at approximately 50–100 nmoles/g wet tissue weight. The concentration in the lung increased progressively until, by 30 hr, it was six times the plasma concentration (Table 3). In contrast, the brain concentration was below that of the plasma at all times measured. After oral administration of 680 μmoles of diquat/kg body-weight to rats, the plasma concentration was lower than that obtained after paraquat (Table 4). The lung concentration followed closely the plasma concentration and there was no evidence of any accumulation. The kidney concentration of diquat was high at all time points measured and the liver and adrenal concentrations were also somewhat higher than that of the plasma (Table 4).

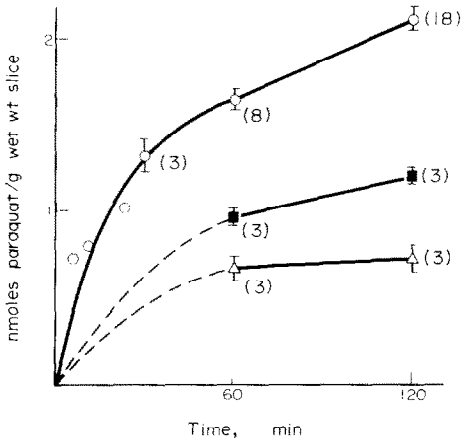

Fig. 1. Accumulation of paraquat by slices of rat brain. Slices of rat brain cortex were incubated in Krebs–Ringer phosphate–glucose medium as described in the Methods section, with 10^{-6}M paraquat (○) or with 10^{-6}M paraquat and KCN (10^{-3}M) plus iodoacetate (10^{-3}M) (■). The paraquat present in slices was also corrected for swelling in the presence of KCN plus iodoacetate (△). The points represent the means ± S.E.M. with the number of slices used in parentheses.

Table 1. Accumulation of paraquat by slices of rat tissues.

	nmoles of paraquat/g wet wt tissue	
	1 hr	2 hr
Lung	4·78 ± 0·41 (7)	9·91 ± 0·42 (7)
Brain cortex	1·62 ± 0·05 (5)	2·06 ± 0·05 (13)
Adrenal	0·62 ± 0·04 (8)	0·99 ± 0·11 (12)
Kidney cortex	0·91 ± 0·04 (8)	1·18 ± 0·08 (15)
Muscle (skeletal)	0·93 ± 0·03 (8)	1·15 ± 0·07 (8)
Liver	1·36 ± 0·08 (11)	1·30 ± 0·07 (12)
Skin	0·73 ± 0·05 (5)	0·98 ± 0·15 (5)
Heart	0·95 ± 0·05 (12)	1·03 ± 0·06 (8)
Small intestine	0·77 ± 0·07 (8)	0·98 ± 0·12 (8)
Spleen	0·44 ± 0·02 (5)	0·46 ± 0·04 (5)

Slices were prepared as described in the Methods Section and incubated in the presence of 10^{-6} M paraquat. The results are expressed as the mean ± S.E.M. with the number of slices used in parentheses.

Accumulation of paraquat by slices of lung from different species. Slices of lung from dog, monkey and rabbit all showed the ability to accumulate paraquat in the same way as slices of lung from rat and man [7, 8]. Kinetic analysis [12] of the accumulation process for each of these species indicates that it obeys saturation kinetics and an apparent V_{max} and K_m for each has, therefore, been calculated (Table 5). The apparent K_m values are all in the range of $2–7 \times 10^{-5}$ M, whilst the V_{max} values vary from 10 to 300 nmoles/g/h.

DISCUSSION

The metabolic activity of lung and brain slices is impaired if they are chilled during preparation [13, 14]. Accordingly, in this work, lung and brain slices were prepared without cooling the tissue and the same conditions were used for the preparation of slices from the other tissues studied. The uptake of either bipyridyl by liver or kidney cortex was, however, unaffected by whether the slices were cut from chilled tissue or tissue kept at room temperature.

Diquat was only accumulated significantly by kidney slices (Table 2). After oral dosing, the concentrations of diquat found in kidney, adrenal and liver relative to that of the plasma, suggest that all of these organs have some ability to either accumulate or retain diquat (Table 4).

Paraquat was markedly accumulated by lung slices and significantly accumulated by brain slices (Table 1), and energy is required for this uptake [8] (Fig. 1). After oral dosing, the lung concentration after

Table 2. Accumulation of diquat by slices of rat tissues.

	nmoles of diquat/g wet weight tissue	
	1 hr	2 hr
Lung	0·51 ± 0·02 (4)	0·57 ± 0·02 (4)
Brain cortex	1·19 ± 0·07 (5)	1·38 ± 0·05 (9)
Adrenal	0·58 ± 0·02 (5)	0·66 ± 0·08 (9)
Kidney cortex	2·00 ± 0·05 (5)	2·01 ± 0·13 (9)
Muscle (skeletal)	0·95 ± 0·08 (6)	0·89 ± 0·07 (8)
Liver	1·35 ± 0·19 (6)	1·62 ± 0·06 (8)
Skin	0·37 ± 0·02 (5)	0·53 ± 0·08 (5)
Heart	0·97 ± 0·04 (6)	1·12 ± 0·09 (8)
Small intestine	0·74 ± 0·04 (5)	0·97 ± 0·04 (5)
Spleen	0·22 ± 0·01 (5)	0·29 ± 0·01 (5)

Slices were prepared as described in the Methods section and incubated in the presence of 10^{-6} M diquat. The results are expressed as the mean ± S.E.M. with the number of slices used in parentheses.

Table 3. Paraquat concentrations in rat tissues after oral administration of 680 μmoles/kg body weight.

	nmoles paraquat/g wet weight tissue Hr after dosing			
	2	4	18	30
Brain	6·8 ± 3·2 (4)	0·81 ± 0·08 (4)	1·5 ± 0·1 (4)	3·1 ± 0·3 (4)
Lung	16·5 ± 2·2 (8)	17·0 ± 1·6 (8)	29·6 ± 2·7 (8)	86·6 ± 17·2 (7)
Liver	20·8 ± 6·8 (7)	8·9 ± 1·7 (8)	11·6 ± 1·7 (8)	20·4 ± 3·1 (7)
Kidney	75·0 ± 15·1 (8)	54·9 ± 18·1 (8)	57·7 ± 3·1 (8)	108 ± 22 (7)
Adrenal	12·8 ± 2·5 (8)	30·1 ± 15·4 (8)	16·1 ± 1·5 (8)	26·2 ± 6·5 (7)
Muscle	4·8 ± 0·4 (8)	5·2 ± 2·1 (8)	5·2 ± 1·1 (8)	11·0 ± 2·7 (7)
Plasma	14·0 ± 3·7 (8)	6·6 ± 1·0 (8)	8·0 ± 0·6 (8)	13·8 ± 2·9 (7)

Values are expressed as mean ± SEM with the number of animals in parentheses.

Table 4. Diquat concentrations in rat tissues after oral administration of 680 μmoles/kg body weight

	nmoles diquat/g wet weight tissue Hr after dosing			
	2	4	17	30
Brain	1·1 ± 0·3 (4)	0·86 ± 0·08 (3)	1·2 ± 0·4 (4)	1·2 ± 0·5 (4)
Lung	3·8 ± 0·7 (4)	5·6 ± 0·8 (3)	5·9 ± 1·6 (4)	6·0 ± 2·5 (4)
Liver	6·1 ± 1·6 (4)	7·9 ± 1·7 (3)	13·9 ± 4·0 (4)	9·6 ± 3·1 (4)
Kidney	23·8 ± 4·6 (4)	59·0 ± 21·1 (3)	48·4 ± 10·8 (4)	54·0 ± 33·6 (4)
Adrenal	7·5 ± 2·0 (4)	14·3 ± 8·6 (3)	12·8 ± 7·0 (4)	16·0 ± 7·5 (4)
Muscle	1·2 ± 0·2 (4)	2·9 ± 1·8 (3)	2·2 ± 0·4 (3)	4·6 ± 1·3 (4)
Plasma	5·0 ± 1·2 (4)	5·3 ± 2·1 (3)	6·3 ± 2·3 (4)	6·6 ± 3·5 (4)

Values are expressed as mean ± S.E.M. with the number of animals in parentheses.

Table 5. Kinetic constants for the accumulation of paraquat by lung slices from different species.

	K_m(M)	V_{max} (nmoles paraquat/g tissue/hr)
Dog	6×10^{-5}	10
Monkey	7×10^{-5}	50
Rabbit	2×10^{-5}	200
Man*	4×10^{-5}	300
Rat**	7×10^{-5}	300

Lung slices were prepared as described in the Methods section, and incubated with a range of concentrations of paraquat (c). Rates of accumulation (v) were measured over 2 hr and the above constants derived from plots of 1/v against 1/c.

* Data from *BMJ* **4**, 369–371.

** Data from *Nature* **252**, 5481 314–315.

30 hr clearly reflected the ability of this organ to accumulate paraquat (Table 3). The brain, however, did not show any evidence of accumulating paraquat *in vivo*, the concentration present in brain being below that of the plasma at each time point measured (Table 3). This almost certainly reflects the poor penetration of paraquat through the blood–brain barrier. The kidney, adrenal and liver, as with diquat, appear to have some ability either to accumulate or retain paraquat since at most times the concentration present in these organs exceeded that of the plasma (Table 3).

Following equimolar oral doses of paraquat and diquat, the concentration of diquat in the plasma was approximately half that of paraquat. Kidney, adrenal and liver do not discriminate between paraquat and diquat, and concentrate or retain the compounds to the same extent in relation to the concentration present in the plasma. These organs have also been reported to be damaged after both paraquat and diquat poisoning in man and experimental animals [15, 4, 16, 5]. The lung, however, accumulates paraquat very much more effectively than any other organ examined. This selectivity must be a primary factor in the development of lung damage and explains why this organ is the most severely affected.

Lung slices from the species so far examined have all shown the ability to accumulate paraquat (Table 5). Lung slices from dog or monkey are relatively poor at accumulating paraquat when compared with those from man or rat. Thus, from the point of view of paraquat accumulation by the lung, the rat is a good experimental model for man.

Although the lungs of all species studied showed the ability to accumulate paraquat, rabbits have been reported to be resistant to lung damage after paraquat [17] whilst dogs, monkeys, rats and man are susceptible to lung damage [4, 18]. Other factors apart from the intrinsic ability of the lung to take up paraquat must therefore play a part in determining whether damage will occur in a given species. Two such factors are:

(1) the attainment and maintenance of significant concentrations of paraquat in the lung *in vivo*, and

(2) the sensitivity of those cells accumulating paraquat to damage by a given concentration of paraquat.

The rabbit lung does not appear to retain paraquat as effectively as that of the rat [19] and may also be more resistant to damage. However, the difference between the rabbit and other species might be more apparent than real since, in those studies carried out with rabbits, the paraquat was administered either intraperitoneally or intravenously, and this will have led to very high initial plasma concentrations followed by a rapid fall [20], conditions which will tend to maximise damage to organs other than the lung and minimise accumulation. It is perhaps relevant that most of the mortality in these studies occurred in the first 24 hr after dosing, with no apparent histological damage that could account for death [17].

From studies of the uptake of paraquat by slices of rat lung [8], *in vitro*, a rate of 30–40 nmoles paraquat/g wet weight/hr is predicted from a concentration of 10 μM in the incubation medium. A concentration close to this was maintained in the plasma after oral dosing (Table 3) yet the apparent rate of accumulation was 4–6 nmoles/g/hr. This difference in rate of uptake might reflect differences in the behaviour of tissue slices *in vitro* from lung *in vivo* or it might be due to the presence of circulating endogenous inhibitors which slow down paraquat uptake *in vivo*. Addition of plasma to rat lung slices *in vitro* has been shown to slow down paraquat accumulation (Lock and Rose, unpublished work) indicating that such inhibitors may well be present in plasma.

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