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METABOLISM AND EXCRETION OF DI(*p*-AMINOPHENYL) SULPHOXIDE IN DIFFERENT ANIMAL SPECIES

BY

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Rabbits, rats and guinea-pigs were treated with di(*p*-aminophenyl) sulphoxide and their urines examined by an analytical method which permits the simultaneous determination of this compound and of dapsone [di(*p*-aminophenyl) sulphone] which is a possible product of metabolic oxidation. The method gives for each drug the total of free compound plus acid-labile conjugates. All three species excreted unchanged drug together with dapsone. With rats and guinea-pigs about 33% of the excretion is dapsone, but with rabbits only 6 to 12%. The rate of combined excretion is much greater in rabbits than in the other two species. These results are discussed in relation to the significance of di(*p*-aminophenyl) sulphoxide as a drug in the treatment of leprosy.

Di(*p*-aminophenyl) sulphoxide has received favourable preliminary reports in clinical trials against leprosy (Buu-Hoï, Khuyen, and Xuong, 1955; Davey, Kissaun, and Moneta, 1957; Laviron, Lauret, Kerbastard, and Jardin, 1957), and there has been a suggestion that its action might be in some ways different from that of dapsone, di(*p*-aminophenyl) sulphone. Various workers have shown the sulphoxide to be almost without action *in vitro* against micro-organisms including pneumococci, haemolytic streptococci, and *Mycobacterium tuberculosis* H37Rv, in contrast to the considerable activity of dapsone in these species. However, the sulphoxide has definite activity *in vivo* against these same organisms in the mouse and the guinea-pig. These findings have led to the suggestion that the sulphoxide is activated by conversion to the sulphone in the animal body (Jensen and Schmith, 1943; Youmans, Feldman, and Doub, 1946; Wagner and Kimmig, 1946).

In order to evaluate the specific usefulness of the sulphoxide in leprosy it is desirable to know the extent of its conversion to dapsone in the human body. The metabolic fate of sulphoxides varies with different compounds and in different species. A number of instances are known where thioethers are oxidized to sulphoxides and are excreted in this form rather than as sulphones, for example, in the metabolism of phenothiazine (Whitten, Filmer, and Clare, 1947), chlorpromazine (Salzman and Brodie, 1956), and a pyrazolidinedione containing an alkylthio group (Burns, Yü, Ritterband, Perel, Gutman, and Brodie, 1957); the oxidation of di(*p*-aminophenyl) sulphide by a guinea-pig liver microsome preparation is said to go to the sulphoxide but no further (Gillette, Kamm, and Brodie, 1959). On the other hand, sulphides can be oxidized *in vivo* to the sulphone, for example, *p*-methylthioaniline (Rose and Spinks, 1948), and the catabolism of ethanethiol probably follows this route after methylation (Snow, 1957; Lowe, 1960). These oxidations probably involve formation of the sulphoxide as an intermediate step.

A method has been developed for the determination of di(*p*-aminophenyl) sulphoxide and dapsone in the presence of each other. It has been used to investigate metabolism in three animal species and is now available for investigation of metabolism in man.

Bushby and Woiwod (1956) showed that dapsone is largely excreted as the mono-*N*-glucuronoside. Our method therefore includes a preliminary acid treatment to hydrolyse such conjugates, and the results show the sum of free plus acid-labile compounds.

METHODS

Various methods of distinguishing between di(*p*-aminophenyl) sulphoxide and dapsone were considered. The sulphoxide gives a colour reaction with concentrated sulphuric acid (100° for 10 min.) whilst the sulphone does not. However, the intensity of the colour is far too low for convenience ($\varepsilon = 670$ at 520 mμ). A method has now been

developed for separating the sulphoxide from the sulphone by differential extraction from an organic solvent with acids of differing strength; this enables a diazotization method similar to that of Francis and Spinks (1950) to be applied to both components.

Procedure.—Urine samples (5 ml.) are acidified with N HCl (2.5 ml.); they are left for 1 hr. at 20 to 25° to allow for hydrolysis of acid-labile conjugates. After neutralization with N NaOH (2.5 ml.) the samples are diluted to contain 3 to 40 μg./ml. of either sulphoxide or sulphone. For estimation 1 ml. is mixed with 3 ml. 0.2 M phosphate buffer *p*H 7.0. Blood samples (3 ml.) are mixed with 0.8 M phosphate buffer *p*H 7.0 (1 ml.).

For the first extraction, 4 ml. of buffered urine or blood, prepared as above, is shaken with isobutyl methyl ketone (20 ml.). Both sulphoxide and sulphone pass quantitatively into the organic phase.

For the second extraction, a 15 ml. aliquot of the separated ketone layer is extracted with 0.1 N HCl (4 ml.); 88.9% of the sulphoxide and 2.3% of the sulphone pass from the ketone solution into the acid layer. Of the acid layer 2 ml. is mixed with 0.1% $NaNO_2$ solution (1 ml.), and 5 min. later 1% sulphatoethyl-*m*-toluidine solution (0.5 ml.) is added. After 20 min., ethanol (3 ml.) is added and the optical density is read at 520 mμ. A standard curve, which should be rectilinear, is prepared from readings on solutions containing 2 to 20 μg. di(*p*-aminophenyl) sulphoxide in 2 ml. 0.1 N HCl diazotized and coupled as above. The apparent sulphoxide content of the aliquot of 0.1 N HCl from the second extraction is read from the standard curve. Let this value be x μg.

For the third extraction, a 10 ml. aliquot of the ketone layer remaining after the extraction with 0.1 N HCl is further extracted with 2N HCl (4 ml.). The small residue of sulphoxide is quantitatively extracted into the stronger acid, together with 93.8% of the remaining sulphone. Of the acid layer, 3 ml. is taken and mixed with 0.1% $NaNO_2$ solution (0.5 ml.). After 5 min. 5% ammonium sulphamate solution (1 ml.) is added, followed 5 min. later by 0.2% *N*-1-naphthylethylenediamine solution (0.5 ml.). After a further 30 min. the solution is diluted to 6 ml. and the optical density read at 535 mμ. A standard curve (rectilinear) is constructed from readings on solutions containing 2 to 20 μg. of dapsone in 2N HCl (2 ml.) diazotized and coupled as above. The apparent dapsone content of the aliquot of 2N HCl from the third extraction is read from the standard curve. Let this value be y μg.

The amounts of sulphoxide and sulphone in the buffered solution taken for the first extraction are given by the equations:

di(*p*-aminophenyl) sulphoxide content (μg.) ... ... $=3.01x-0.075y$

dapsone content (μg.) ... ... $=2.92y-0.36x$

(The derivation of these equations is shown below.) From these figures the concentrations present in the original blood or urine may be calculated, taking into account the dilutions involved in making the buffered solution for extraction.

General Precautions.—The isobutyl methyl ketone is presaturated with phosphate buffer, and the 0.1 N and 2N HCl solutions with isobutyl methyl ketone, in order to avoid volume changes during the extraction. All extractions are carried out by shaking the solutions for 1 min. in stoppered tubes and centrifuging the mixtures to separate the layers cleanly. Nitrite and *N*-1-naphthylethylenediamine solutions are freshly prepared each day. Stock solutions of the pure drugs contained 1 mg./ml. in dilute acid; the sulphone may be kept several weeks, but the sulphoxide only for three days. Diluted solutions (10 μg./ml.) are prepared as required. Colours formed after coupling fade in strong daylight, and the tubes are kept in the dark except during the actual measurement. Solutions are thoroughly mixed after each addition of reagent. This is especially important after ethanol additions, where faulty mixing can easily occur.

Analysis of the Method.—Coupling of diazotized dapsone with sulphatoethyl-*m*-toluidine does not occur satisfactorily in 2N HCl, and for the third extraction a coupling component which requires this strength of acid was substituted. Di(*p*-aminophenyl) sulphoxide and dapsone at equal concentrations diazotize and couple with sulphatoethyl-*m*-toluidine to produce colours of almost equal intensity at 520 mμ. The same is true of the colours produced with *N*-1-naphthylethylenediamine measured at 535 mμ. Thus no appreciable error arises when measurements are made on mixtures containing both amino compounds.

The formula for calculating the results was derived from a series of experiments in which standard solutions of di(*p*-aminophenyl) sulphoxide and dapsone were extracted under conditions identical to those used in the procedure described above. Extraction into 0.1 N and 2N HCl was determined directly using standard solutions of the sulphoxide and sulphone in isobutyl methyl ketone (saturated with buffer). Extraction into isobutyl methyl ketone was determined indirectly: standard solutions of sulphoxide and sulphone in phosphate buffer *p*H 7.0 were extracted into isobutyl methyl ketone and an aliquot of the ketone layer was extracted with HCl (0.1 N for the sulphoxide, 2N for the sulphone). The efficiency of the first extraction step was calculated from knowledge of the efficiency of the overall extraction and that of the extractions with acid. Each extraction was carried out several times. Mean results are given in Table I.

Using these figures, equations may be derived as follows:

Let the prepared sample of blood or urine (4 ml.) contain A μg. of sulphoxide and B μg. of sulphone. This sample is quantitatively extracted into isobutyl methyl ketone (20 ml.). A 15 ml. aliquot thus contains 0.75 (A+B): it is extracted with 0.1 N HCl (4 ml.), and the measurement (x) is made on a 2 ml.

aliquot of the acid. From the determined extraction efficiencies

$$x = 0.5 \times 0.75\ (0.889A + 0.023B)$$
$$\therefore\ x = 0.334A + 0.0086B \quad \ldots\ldots(1)$$

After this extraction a 10 ml. aliquot of the ketone layer contains 0.5 [$(-0.889)\ A + (1 - 0.023)\ B$]; it is extracted with 2N HCl (4 ml.), and the measurement (y) is made on a 3 ml. aliquot of the acid. From the determined extraction efficiencies

$$y = 0.75 \times 0.5\ (0.111A + 0.938 \times 0.977B)$$
$$\therefore\ y = 0.0415A + 0.344B \quad \ldots\ldots(2)$$

Solving the simultaneous equations (1) and (2) gives the expressions for A and B quoted in the section on procedure.

The complete method was tested by determination of known mixtures of the two components in water and by determinations on blood and urine to which known amounts had been added. The results (Table II) show that some sets of figures have a small bias, but without any consistent trend. The standard deviation was quite similar for each set and could be taken as ±5% for the method as a whole. This is satisfactory for practical requirements.

Animal Experiments.—The animals were fed on normal laboratory diets and food was withdrawn 18 hr. before the administration of the drug. The compound was dosed in aqueous suspension by means of a stomach tube. The urine from the animals in metabolism cages was collected in a container chilled in ice, acidified, and extracted as soon as practicable after excretion. For rats and guinea-pigs the numbers of animals were sufficient to ensure a representative excretion of urine over the periods involved. With rabbits excretion was promoted by giving 15 ml. water at the time of treatment, but even so it was inconveniently erratic. This was partly overcome by repeating the dosing and collection from each individual rabbit after a rest period of three days. The urines from the two collections were combined as far as possible, as shown in Table IV. Where there was an excretion in only one of the pair of experiments over a given period figures are shown in brackets. These figures are based on the assump-

TABLE I

EXTRACTION OF DI(*p*-AMINOPHENYL) SULPHOXIDE AND DAPSONE

	1st Extraction. From *p*H 7·0 Buffer into Isobutyl Methyl Ketone		2nd Extraction. From Isobutyl Methyl Ketone into 0·1 N HCl		3rd Extraction. From Isobutyl Methyl Ketone into 2N HCl	
	Sulphoxide	Sulphone	Sulphoxide	Sulphone	Sulphoxide	Sulphone
Phosphate buffer *p*H 7·0 (ml.)	4	4	—	—	—	—
Isobutyl methyl ketone (ml.)	20	20	15	15	10	10
Hydrochloric acid	—	—	4 ml. 0·1 N	4 ml. 0·1 N	4 ml. 2 N	4 ml. 2 N
Wt. of compound taken (μg.)	10–20	10–20	10–20	125–750	10–20	10–20
No. of determinations ..	9	9	9	12	9	9
Mean % extraction	101·6	99·9	88·9	2·31	101·8	93·8
Standard error on mean ..	0·6	0·8	0·6	0·01	1·6	0·5

TABLE II

RESULTS OF ESTIMATIONS ON SYNTHETIC MIXTURES OF DI(*p*-AMINOPHENYL) SULPHOXIDE AND DAPSONE

	In Water		In Urine		In Blood	
Number of determinations ..	12		10		6	
Component	Sulphoxide	Sulphone	Sulphoxide	Sulphone	Sulphoxide	Sulphone
Range of amounts of components in mixtures (μg.) ..	5·2–48	7·4–37·2	1·6–21·6	15·4–46·2	10·5–101·5	20·8–131
Mean % error on result ..	−2·4	2·7	3·4	−3·3	−5·5	−0·6
Standard deviation of % error	5·0	4·4	3·9	6·2	2·1	4·5

TABLE III

URINARY EXCRETION FROM GUINEA-PIGS AND RATS DOSED WITH DI(*p*-AMINOPHENYL) SULPHOXIDE

Guinea-pigs (wt. 250 g.) each received 2·5 mg. di(*p*-aminophenyl) sulphoxide orally. 'Rats (wt. 300 g.) each received 3 mg. of the drug orally. Figures for both species refer to 2 separate groups of 6 animals. Cumulative % figures for dapsone are based on the sulphoxide equivalent.

Species	Time after Dose hr.	Sulphoxide Excretion				Dapsone Excretion			
		mg.		Cumulative % of Dose		mg.		Cumulative % of Dose	
Guinea-pig	0–3	0·38	0·59	2·5	4·0	0·19	0·21	1·0	1·5
	3–6	1·07	0·58	9·5	8·0	0·68	0·27	5·5	3·0
	6–12	0·40	0·46	12·5	11·0	0·26	0·30	7·0	5·0
	12–24	0·35	0·26	14·5	12·5	0·18	0·10	8·0	5·5
Rat	0–3	1·72	1·61	9·5	9·0	0·61	0·68	3·0	3·5
	3–6	2·12	1·36	21·5	16·5	1·11	0·74	9·0	7·5
	6–12	1·39	1·06	29·0	22·5	0·88	0·64	13·5	10·5
	12–24	0·59	0·51	32·5	25·0	0·37	0·34	15·5	12·5

TABLE IV

URINARY EXCRETION FROM RABBITS DOSED WITH DI(*p*-AMINOPHENYL) SULPHOXIDE

Each animal received 10 mg. per kg. of di(*p*-aminophenyl) sulphoxide orally on two separate occasions. Cumulative % figures for dapsone are based on the sulphoxide equivalent. Values in parentheses refer to a single experiment only.

Collection Period (hr. after Dose)	Expt. No.	Sulphoxide Excretion		Dapsone Excretion	
		mg.	Cumulative % of Dose Excreted	mg.	Cumulative % of Dose Excreted
Rabbit 1. *Dose:* 30 *mg. in each experiment*					
0–2	1+2	20·9	35	0·0	0
2–5	2	3·68	(48)	0·33	(1·0)
2–12	1	2·62	47	0·32	2·0
5–12	2	1·03		0·21	
12–24	1+2	1·15	49	0·28	3·1
Rabbit 2. *Dose:* 20 *mg. in each experiment*					
0–2	3+4	16·1	40	1·69	4·2
2–6	3+4	2·99	48	0·50	5·5
6–12	3	0·49	(50)	0·15	(6·2)
6–24	4	1·77	51·5	0·46	7·3
12–24	3	0·26		0·14	

tion that the rate of excretion was identical in the two experiments with the same animal. Results from two rabbits are presented in Table IV as typical.

RESULTS

The analytical method has been used to investigate the oxidation of di(*p*-aminophenyl) sulphoxide to dapsone in guinea-pigs, rats, and rabbits. The dose of drug (10 mg./kg.) was comparable, on the basis of the surface area of the body, with therapeutic doses used in man. At this dose the blood levels were very low, and only urinary excretion has been studied in detail. Results are given in Tables III and IV.

The total excretion of sulphoxide and sulphone is much less with the guinea-pig than with the rat, but the pattern of excretion is similar in these species. In both, the excretion at 3 hr. is about one third, and at 6 hr. two thirds, of the total 24 hr. excretion. There is considerable oxidation of the compound in the body and, of the detectable excretion products, about one third is dapsone and two thirds sulphoxide. The excretion in rabbits is quite different. Elimination is much more rapid; about 75% of the 24 hr. excretion occurs in the first 2 hr. Also the conversion to dapsone is much less. The fraction of diazotizable material excreted in the sulphone form was 6, 12, 10, and 6% in four different experiments.

DISCUSSION

There are clearly differences in the metabolism of di(*p*-aminophenyl) sulphoxide in different species, though some conversion to dapsone may perhaps be expected in all cases. The smaller extent of oxidation in the rabbit is probably the result of the more rapid excretion of the sulphoxide.

Since this work was carried out a paper has appeared (Jardin, 1958) reporting the chromatographic investigation of urine from patients receiving di(*p*-aminophenyl) sulphoxide. The results show qualitatively that the unchanged drug is probably the principal excretion product; dapsone is found in somewhat smaller amounts along with some relatively minor metabolites. Jardin expresses his opinion that the amount of dapsone excreted is not sufficient to account for the antileprotic activity of the sulphoxide. This point, however, can only be convincingly proved when quantitative information on the blood levels and urinary excretion of the two compounds is obtained. The method described in this paper should enable this to be done.

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