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# THE METABOLISM OF SOME SULPHONAMIDES IN REGARD TO THE DURATION OF THEIR ACTION IN EXPERIMENTAL ANIMALS*

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**Abstract**—SMpyrazine, a new long-acting sulphonamide, is similar, in some pharmacological and metabolical aspects, to three other sulphonamides of long or short durations in dogs and rabbits. Absorption, concentration in blood and in some tissues, renal excretion, acetylation, protein binding and other data are considered here in order to contribute to the explanation of the different periods of duration in various animals.

RECENTLY some new long-acting sulphonamides have been introduced.[1] A product with analogous properties has also been synthetized in our laboratories[2] : the 3-methoxy derivative of sulphopyrazine (2-sulphanilamido-3-methoxy-pyrazine† (Fig. 1)).

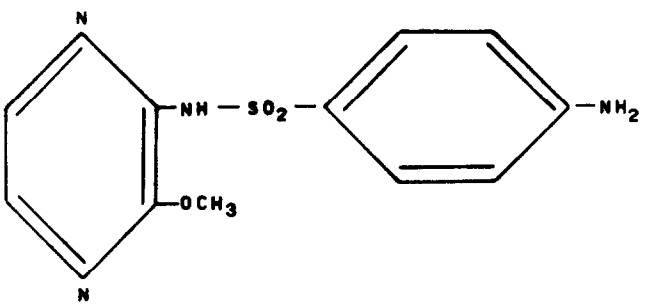


FIG. 1. 2-Sulphanilamido-3-methoxy-pyrazine (Mol. wt. 280.298).

The acute and chronic toxicological studies made on various animals, the therapeutic activity *in vitro* and *in vivo* and certain amounts of preliminary pharmacological data, experimental and clinical, referred to by us in a prior publication,[3] have demonstrated a more favourable behaviour compared to other well known long-acting sulphonamides.

In this report we feel it useful to refer to data gathered subsequently pertaining to the pharmacology and metabolism of our sulphonamide compared to other sulphonamides whether long-acting (sulphamethoxypyridazine) or short-acting (sulphadimidine and sulphisoxazole).

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† Registered name: Kelfizina-Farmitalia.

This is necessary, not only for a general examination of our product, but also to attempt thoroughly to analyse the mechanism permitting this prolonged action.

## METHODS

Animals were kept on a balanced diet, supplied with food and water *ad libitum*, and housed in our laboratories. The average weight of dogs was 20 kg, of rabbits 2 kg, of rats (Wistar strain) 200 g and of mice (Swiss strain) 20 g. For the absorption tests the animals were fasted 18 hr before and re-fed 12 hr after the beginning of the test.

Blood and fresh bloodless tissue samples were obtained with the usual care; the animals were sacrificed with injections of pentothal.

The sulphonamides were determined by the Bratton and Marshall method;[4] hydrolysis was carried out for the determination of the eventual conjugated fraction.

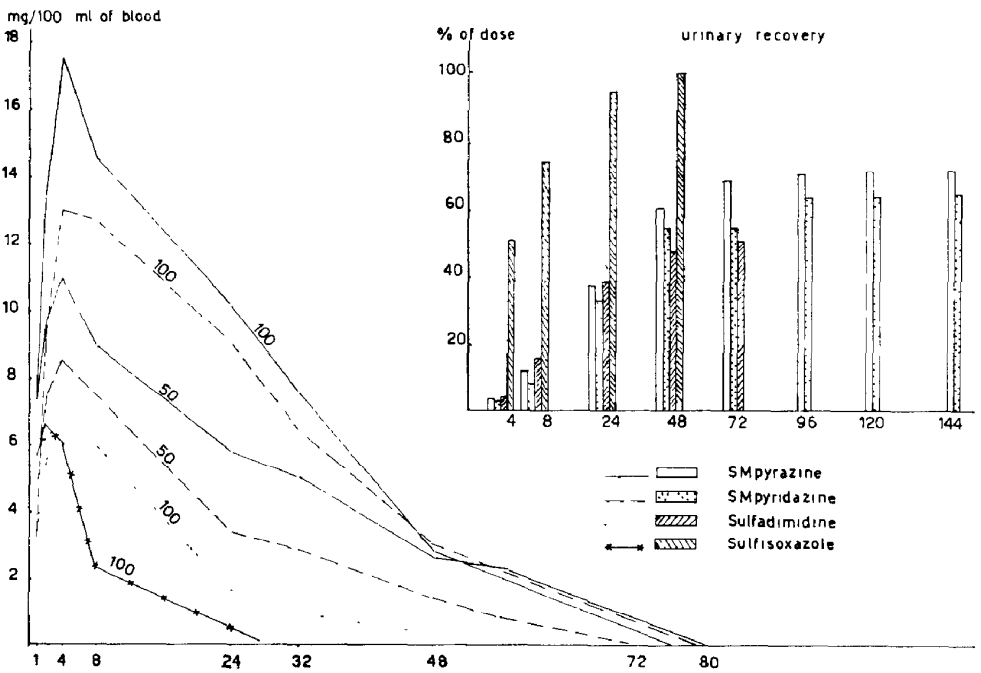


FIG. 2. Concentrations of sulphonamides in blood and cumulative urinary excretion in dogs following single oral doses of 50 or 100 mg/kg (abscissa: time in hours).

Protein binding was determined by dialysis[5] 16 hr after incubation at 37 °C; in the diffusibility test we controlled the passage of sulphonamide through the semipermeable membrane between ¼ and ½ hr of incubation, also by dialysis.

## EXPERIMENTAL DATA

*Absorption and excretion.*

SMpyrazine induces *in dogs* (Fig. 2), after single oral doses, blood concentrations with a peak at the fourth hour and a duration exceeding 72 hr; the maximum

concentration is of 17·6 mg % blood after doses of 100 mg/kg and 10·9 mg % after doses of 50 mg/kg.

The blood levels are higher, and, for doses of 50 mg, longer than those obtained employing equal doses of SMpyridazine.

The difference is particularly evident in the administration of sulphadimidine (about double) and sulphisoxazole (about triple).[6] As with other sulphonamides SMpyrazine is not acetylated in dogs.

Urinary excretion is complete within 3–4 days and reaches about 70 per cent of 100 mg doses (only these doses have been plotted in Fig. 2), against 60 per cent elimination of SMpyridazine, 50 per cent of sulphadimidine and, as noted 100 per cent of sulphisoxazole.

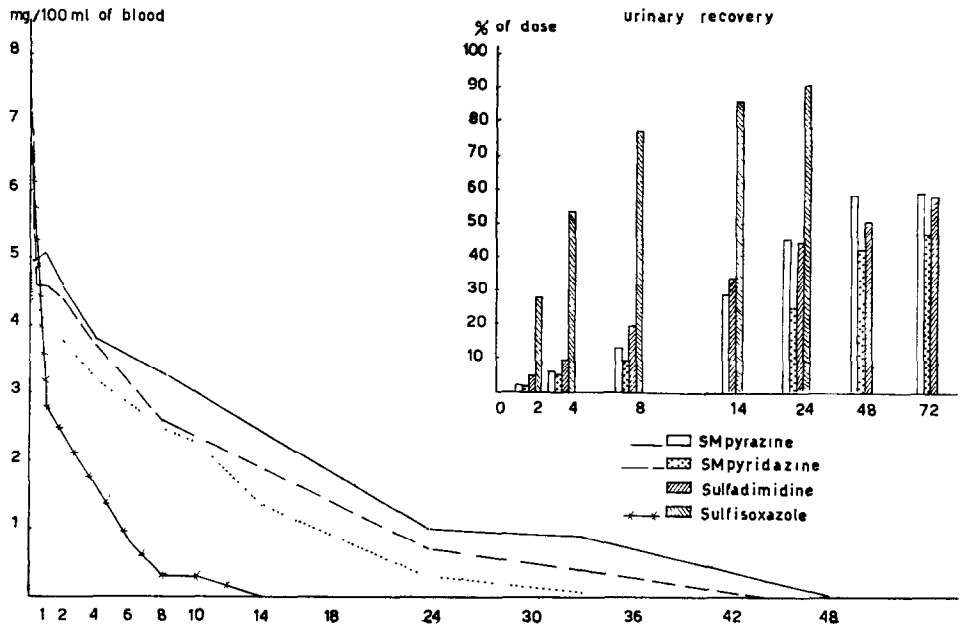


FIG. 3. Concentrations of sulphonamides in blood and cumulative urinary excretion in dogs following single i.v. doses of 20 mg/kg (abscissa: time in hours).

Sulphisoxazole is naturally excreted much more rapidly than all others.

After i.v. administration of 20 mg/kg (sodium salt) the behaviour of SMpyrazine is the same as that of SMpyridazine, while both presented a more protracted blood level than sulphadimidine and sulphisoxazole (Fig. 3).

Urinary excretion is completed within 2 days and reaches 60 per cent of the dose, for SMpyrazine and sulphadimidine, about 50 per cent for SMpyridazine, while

sulphisoxazole is excreted in a larger quantity (90 per cent) and, after oral doses, with greater rapidity.

The correlation between the percentage of excretion after oral or i.v. administration makes it possible to evaluate indirectly and approximately that the absorption of SMpyrazine as well as that of sulphisoxazole is almost complete, while that of sulphadimidine and SMpyridazine reaches about 80 per cent of the dose.

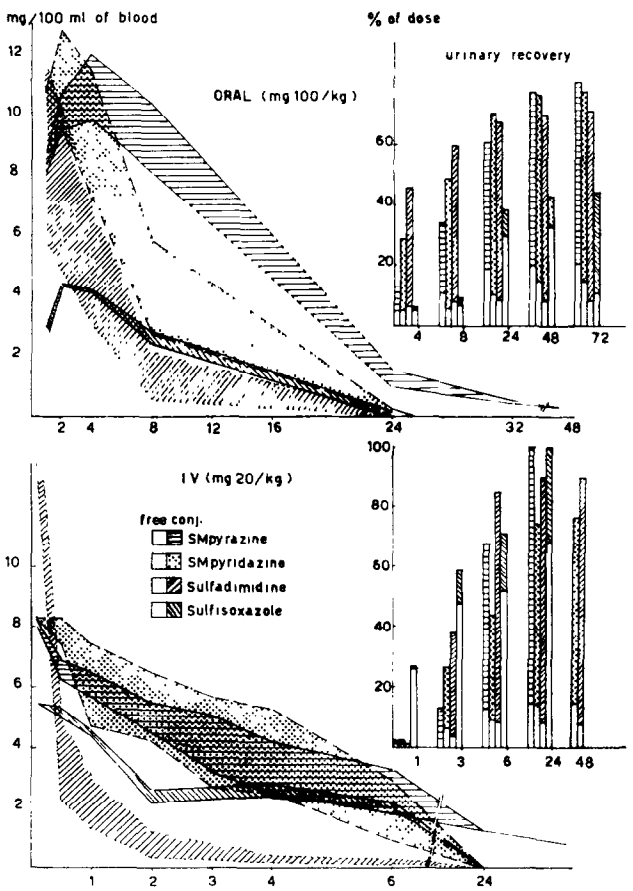


FIG. 4. Concentrations of sulphonamides in blood and cumulative urinary excretion in rabbit following single oral or i.v. doses (abscissa: time in hours).

The concentrations of SMpyrazine in the blood, following single oral doses of 100 mg/kg as well as single i.v. doses of 20 mg/kg are, *in the rabbit*, also generally higher than those of other sulphonamides (Fig. 4). Evidently this drug remains in the blood for the greatest length of time (over 32 hr).

The urinary excretion as a total sulphonamide is slower but more complete (83 per cent of the dose); only sulphisoxazole is excreted more slowly, but absorption is very limited (46 per cent of the dose).

Acetylated SMpyrazine in the blood in only 20–30 per cent of the total sulphonamide, against 35–40 per cent of SMpyridazine and 50–60 per cent of sulphadimidine; in the urine, these differences are less evident.

Sulphisoxazole, naturally, is hardly acetylated at all (6 per cent in blood, 30 per cent in urine).

Blood concentrations in the mouse are 127 mg % at the second hour following a single oral dose of 1 g/kg, and 71·3 mg % at the fourth hour. The acetylation in this animal, as in the rat, is very low, and reaches 6–10 per cent of the total sulphonamide in the blood.

The distribution of SMpyrazine between plasma and erythrocytes in dogs is almost equal with a ratio (0·96) slightly higher than that of other sulphonamides considered; distribution is practically uncorrelated to blood concentration. In the rabbit the ratio is a little less (0·80) for the free SMpyrazine and higher (2·2) for the acetylated form.

For blood levels of 5–15 mg % in dogs, about 24 per cent sulphonamide is protein bound; we found a similar binding for the other sulphonamides considered.

The binding is higher in the rabbit (39 per cent) especially for the acetylated form (75 per cent); higher values were obtained with SMpyridazine and sulphadimidine and, above all, with sulphisoxazole.

*Concentrations in various tissues*

Concentrations in the bile, in the cerebrospinal fluid and in various tissues such as liver, kidney, brain and the skeletal muscle of dogs and rabbits, as shown in Fig. 5, were obtained under the same conditions as in the absorptions studies.

Groups of animals were sacrificed 1, 4, 24 hr after administration. The drug is present in all tissues examined in both species at concentrations lower than that in the blood: for example, about 30–50 per cent in the kidney and liver.

The high concentration in the bile (especially dogs) and in the c.s.f. can be noted, but in rabbits the drug is not conjugated in the c.s.f.

Sulphadimidine (not represented graphically) by equal doses in the rabbit, induces much lower and less lasting concentrations, and the drug is almost all acetylated. Sulphadimidine is accumulated only in the liver, as a free drug.

The distribution of these sulphonamides between blood and c.s.f. has as an average ratio in dogs of 0·60 for SMpyrazine, 0·28 for SMpyridazine, 0·34 for sulphadimidine, and zero for sulphisoxazole; in rabbits of 0·33, 0·25, 0·50, and zero, respectively.

*Renal clearance*

The experiments were made in female dogs following continuous intravenous perfusion of drug in a saline solution (0·1 ml/kg per min).

Blood levels were kept between 6–12 mg %. Creatinine was subcutaneously administered in dose of 100 mg/kg, so as to obtain blood concentrations of about 5 mg %; diodrast was injected i.v. in an initial dose of 17 mg/kg followed by an i.v. perfusion of 0·7 mg/kg per min, so as to obtain a blood concentration of about 7 mg %.[7]

Table 1 compares the blood concentration against the total clearance, the clearance of filtrable sulphonamide (non-protein bound), the relative clearance (referring to creatinine clearance, which, in our experimental conditions, is about 70 ml/min) and the excretion index (relative clearance of filtrable sulphonamide).

TABLE 1. RENAL CLEARANCE ON A BLOOD LEVEL BASIS, IN DOGS

| Drug | No. of dogs | Blood concn. mg/100 ml | Free sulfonam. % of total | Clearance | | | |
|---|---|---|---|---|---|---|---|
| | | | | Total | Free sulfonam. | Relative creatine = 1 | Excretion index |
| SMpyrazine | 2 | 6·2 | 74·1 | 4·13 (3·5–4·7)* | 6·90 | 0·06 | 0·10 |
| | 3 | 10·6 | 74·3 | 8·82 (7·4–8·7) | 14·15 | 0·13 | 0 20 |
| Sulfadimidine | 2 | 7·2 | 64·8 | 10·75 (8·9–12·7) | 17·00 | 0·15 | 0·24 |
| | 4 | 11·9 | 70·5 | 11·20 (6·6–14·0) | 19·80 | 0·16 | 0·28 |
| SMpyridazine | 1 | 10·3 | 71·3 | 9·34 | 14·00 | 0·13 | 0·20 |
| Sulfisoxazole | 1 | 8·6 | 64·9 | 70·20 | 112·00 | 1·00 | 1·60 |

* Ranges in brackets.

SMpyrazine, as can be noted, has the lowest clearance of all, even though it is not very different from that of SMpyridazine and sulphadimidine, while that of sulphisoxazole is very high (more than ten times).

The excretion index, also, is equal or inferior to that of other sulphonamides; it is, however, greatly inferior to the unit, demonstrating a tubular reabsorption of 80–90 per cent.

It is interesting to note that our sulphonamide, has an increase in clearance proportional to the increased blood concentration; this behaviour is practically non-existant in sulphadimidine, while SMpyridazine has an opposite behaviour, at least in man.[8]

Tubular excretion and glomerular filtration are not influenced by SMpyrazine; in fact the average amount of creatinine and diodrast clearance before and during perfusion of SMpyrazine is almost constant (80–100 ml/min and 258–252 ml/min respectively).

It is also worthy of note that the protein-binding (expressed as a percentage of diffusible sulphonamide in the table) is not directly related to renal clearance in that, even though there is not a great difference among the sulphonamides themselves, an increase, if anything, is evident in the clearance which accompanies a reduction of the diffusible sulphonamide.

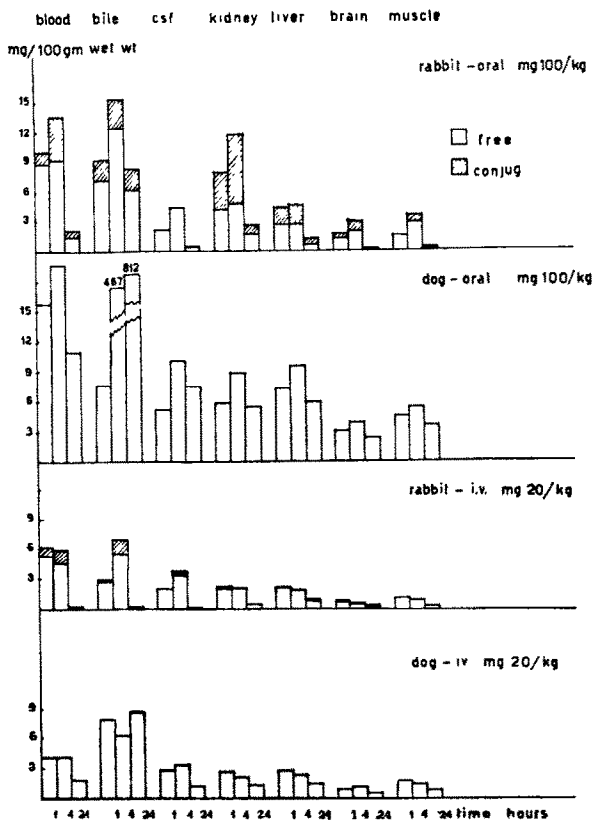


FIG. 5. Concentrations of SMpyrazine in tissues of dog and rabbit following single oral or i.v. dose.

*Urinary metabolites*

In dogs, after either i.v. or oral administration, about 60–70 per cent of the dose is recovered in the urine.

Of this quantity it is possible, through chromatographic analysis, to distinguish several small fractions, one of SMpyrazine unmodified, one of sulphonamide, one of a glycuronic derivative.

This last has a chromatographic $R_f$ 0·31 in 75% ethanol–ammonia (95–5): it is separable by glycuronidases and subsequently shows a positive glycuronic acid reaction.[7]

One last part (about 80 per cent) consists of a metabolite not yet identified ($R_f$ 0·55 in the aforementioned solvent).

This metabolite has the $—NH_2$ group free or not closely bound and not oxidated: its extraction with organic solvents is difficult, it is highly soluble in water and does not appear to be closely bound to either glycuronic, sulphuric or phosphoric acids.

## DISCUSSION

Among the four sulphonamides considered, SMpyrazine demonstrates more lasting blood concentrations, especially after oral administration, and particularly in the rabbit and with smaller doses.

This is strictly correlated with the subtotal absorption, with the low renal clearance which, moreover, decreases in agreement with the fall of the blood concentration and to the inferior acetylation.

The $N^4$-acetyl-sulphonamides have, in fact, a much higher renal clearance: the clearance of acetyl-SMpyrazine can be indirectly evaluated in the rabbit as ten times that of the non-acetylated form.

This ratio is high but the absolute figure (3 ml/min) is lower than that presented by all other sulphonamides.

An indirect and, at this moment, unexplained correlation was noted between duration and clearance on the one hand and protein binding on the other; in other words, as the binding rises, the clearance increases and the duration decreases.

Perhaps this behaviour may be an expression of a higher reactivity of the molecules with biological substrates and hence of higher production of more eliminable metabolites.

The solubility *in vitro* of SMpyrazine and of its $N^4$-acetylated form in citrate–phosphate buffer, as reported in our previous work, is high and corresponds, for example, to 625 and 700 mg % respectively for a pH of 7·4

The corresponding values for SMpyridazine and sulphadimidine are much lower, and only sulphisoxazole is more soluble.

On the other hand the *in vitro* diffusibility of SMpyrazine in the plasma in the first 15 min of the dialysis is about 20 per cent of the total concentration, against 10 per cent for SMpyridazine and sulphisoxazole and 3 per cent for sulphadimidine.

SMpyrazine is also highly diffusible *in vivo*, as a result of the distribution of the drug between plasma and red cells, of the CSF concentration, and of the low ratio between the toxicity after oral and i.v. administration in the mouse (DL 50 g 2·2/kg and 1·6, respectively).

Both the solubility and diffusibility of the drug, and of its metabolites at physiological pH, are important factors in the mechanism of renal excretion.

In regard to the differences of duration between the two species considered, we think that this depends, above all, on the ability of the animals to absorb and excrete the drug, and to transform the drug into specific metabolites, each of which has a particular pharmacological behaviour.

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