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The Metabolism of Arylthioureas— III. (a) The Toxicity of Hydrogen Sulphide in Relation to that of Phenylthiourea. (b) The Protection of Rats against the Toxic Effects of Phenylthiourea, with 1-Methyl-1-Phenylthiourea

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In the preceding paper,[1] it was concluded that the toxicity of phenylthiourea could be due to the release of hydrogen sulphide in the tissues. It therefore became necessary to determine the toxicity of aqueous solutions of hydrogen sulphide. No previous determinations of the toxicity of hydrogen sulphide solutions appear to have been made. Gaseous hydrogen sulphide is a well known toxic agent and Ljunggren and Norberg[2] have shown that a concentration of 1·52 mg/l. in air is fatal to rats in 10–20 min. We shall show that the toxicity of aqueous hydrogen sulphide is such that it could explain the toxicity of phenylthiourea in the rat.

A number of compounds have been screened for ability to antagonize the toxic action of phenylthiourea in rats with a view to obtaining an insight into the mechanism of toxic action. 1-Methyl-1-phenyl-2-thiourea, but not its isomer, 1-methyl-3-phenyl-2-thiourea, was found to have such an action when injected simultaneously with phenylthiourea. Potassium iodide and propylthiouracil given to rats for three days before injection of phenylthiourea were also protective.

Methods and Materials

Animals. White albino rats were used for most of the experiments described in this paper.

Materials. 1-Methyl-1-phenylthiourea, m.p. 104–106°, and 1-methyl-3-phenylthiourea, m.p. 110–111°, were prepared according to Gebhardt.[3,4] Other compounds were purchased or prepared as described earlier.[1]

Preparation of H_2S solutions. Pure hydrogen sulphide was prepared by the action of 5N HCl on antimony trisulphide. The gas was passed through water contained in three Drechsel bottles in series. The first two bottles were used to wash the gas, and the solution of H_2S which formed in the third was used for injection into rats. The H_2S content of this solution was determined iodometrically with N/20 iodine. The solution was kept in a tightly stoppered flask and was used as soon as possible after preparation. The H_2S content of the solution was about 1·0–1·25 mg/ml. On keeping overnight, the solution oxidized and elementary sulphur separated as a fine precipitate.

Determination of toxicity of H_2S. Rats were injected intraperitoneally or intravenously with the above solution. For intravenous injection, the femoral vein was exposed whilst the rat was under light ether anaesthesia. As the rat was recovering from the anaesthesia, the H_2S solution was injected into the vein. If rats were poisoned by the H_2S they died within 20 sec after intravenous injection and within 30–60 sec after intraperitoneal injection.

Determination of the toxicity of phenylthiourea. Rats were injected intraperitoneally with aqueous solutions of phenylthiourea containing 1 or 2 mg/ml. The animals were observed for 24 h after the injection. Usually, if animals were poisoned by phenylthiourea they died within 24 h. Administration of other compounds is described in Table III.

Determination of urinary ^{35}S-compounds. Inorganic and ethereal sulphate and neutral sulphur in the urine of rats dosed with ^{35}S-phenylthiourea were determined as described in the preceding paper.[1]

Results and Discussions

Toxicity of H_2S. The results of injecting aqueous solutions of H_2S into rats are shown in Tables I and II. The LD_{50} of aqueous H_2S for female rats is about 2·3–2·8 mg/kg by intraperitoneal injection and about 0·27–0·55 mg/kg by intravenous injection

Table I. The toxicity of aqueous hydrogen sulphide in female rats. Each rat weighed 210–250 g

Dose of H_2S		No. of rats			Mortality, %
mg	vol. of solution, ml	used	killed		
(a) By intraperitoneal injection					
0·21	0·2	3	0		0
0·35	0·3	3	0		0
0·53	0·5	4	2		50
0·64	0·6	4	2		50
1·06	1·0	2	2	(died in 15–30 sec)	100
(b) By intravenous injection					
0·064	0·1	3	1		33
0·127	0·1	3	2		66
0·25	0·2	2	2	(died immediately)	100

Table II. Toxicity of aqueous hydrogen sulphide in male rats. Nine rats were injected intraperitoneally with a fresh aqueous solution of H_2S containing 0·98 mg/ml

Wt. of rat, g	Dose of H_2S		Result
	mg	mg/kg	
360	0·98	2·7	killed
320	0·98	3·0	survived
400	1·47	3·7	survived
340	1·47	4·3	survived
320	1·47	4·6	killed
300	1·47	4·9	killed
310	1·96	6·3	killed
350	2·94	8·3	killed
320	2·94	9·2	killed

(Table I). According to Table II, H_2S appears to be less toxic by intraperitoneal injection to male rats than to female rats and the LD_{50} is roughly about 2·7–4·9 mg/kg. Now the LD_{50} of phenylthiourea is about 5 mg/kg and if the compound were completely converted *in vivo* into H_2S, then it would release 1·12 mg of H_2S/kg. However, about 60 per cent of the sulphur of a sublethal dose of phenylthiourea is excreted as urinary sulphate (see Table V) and if it is assumed that this sulphate is formed *via* H_2S, then one LD_{50} of phenylthiourea would release about 0·7 mg of H_2S/kg. This figure is greater than the approximate LD_{50} of H_2S by intravenous injection into female rats given above. These findings would thus give some support to the view that phenylthiourea is toxic to rats through release of H_2S in the tissues.

Protection of rats against the toxicity of phenylthiourea. A number of compounds were tested to see if they antagonized the toxic action of phenylthiourea and the results are given in Table III. In most cases, the rats were injected with the compound to be tested just before injection with $2LD_{50}$, that is 10 mg/kg, of phenylthiourea. This amount of phenylthiourea alone was always fatal to rats as can be seen from the numerous control tests quoted in this table. It can be seen that the antihistamines promethazine and diphenhydramine, the adrenergic blocking agent phenoxybenzamine, the parasympatholytic agent atropine sulphate, the tranquillizer chlorpromazine, the anti-inflammatory agent hydrocortisone, and the microsomal enzyme blocking agent SKF 525A afford no protection against phenylthiourea.

If phenylthiourea is toxic through release of H_2S, then it is possible that toxicity could be the result of the blocking of enzymes containing metals such as copper, iron and zinc. Table III suggests the possibility that copper may be involved, as rats allowed to drink 0·2 per cent copper acetate solution for 7 days before injection with phenylthiourea were partially protected. Ferrous sulphate and zinc acetate appeared to offer little protection.

The antithyroid agents, propylthiouracil and potassium iodide, under certain conditions seemed to afford protection. If these compounds were injected just before phenylthiourea they afforded no protection, but if they were present in the drinking water for several days or if propylthiouracil was injected daily for three

Table III. The influence of various compounds on the toxicity of phenylthiourea (PTU) in the rat. All compounds administered by injection were given by the intraperitoneal route

Compound	Dose/rat, mg	Dose of PTU injected in LD_{50}'s	No. of rats used	No. of rats died	Mortality, %
(a) Analogous compounds, injected just before PTU					
None	—	2	8	8	100
Thiourea	20	2	4	4	100
Phenylurea	10	2	5	5	100
1,3-Diphenylthiourea	20	2	5	5	100
1-Methyl-3-phenylthiourea	10	2	5	5	100
1-Methyl-1-phenylthiourea	10	2	10	0	0
S-Methyl-*N*-phenylisothiourea	10	2	5	5	100
p-Hydroxyphenylthiourea	10	2	5	5	100
(b) Various drugs, injected just before PTU					
None	—	2	5	5	100
Promethazine	5	1	5	3	60
Diphenhydramine	10	1	5	2	40
Atropine sulphate	0·46	2	5	5	100
Phenoxybenzamine	10	2	5	5	100
Chlorpromazine	10	1	5	3	60
Hydrocortisone	20	1	5	3	60
None	—	1·5	9	7	79
None	—	1·5	8	6	75
SKF 525A injected 5 min before PTU	1	1·5	9	5	75
SKF 525A 5 min before PTU	20	1·5	8	6	75
SKF 525A 1 h before PTU	20	1·5	8	6	75
(c) Antithyroid agents					
(i) Propylthiouracil					
none	—	2	5	5	100
injected just before PTU	15	2	5	5	100
injected 10 mg a day for 3 days before PTU	30	5	5	5	100
allowed to drink water containing 0·1% (w/v) propylthiouracil for 5 days before PTU	*ca.* 30	2	5	1	20
(ii) Potassium iodide					
none	—	1·5	8	6	75
injected just before PTU	100	1·5	8	5	63
1% KI in drinking water for 3 days before PTU	420	1·5	6	0	0

Table III—*continued*

Compound	Dose/rat, mg	Dose of PTU injected in LD_{50}'s	No. of rats used	No. of rats died	Mortality, %
(d) Metallic salts					
None	—	2	6	6	100
Ferrous sulphate, 0·2% in drinking water for 5 days	166	2	6	5	83
Copper sulphate 0·2% in drinking water for 5 days before PTU	50	2	6	5	83
intraperitoneally 30 min before PTU	10	2	4	4	100
None	—	2	6	6	100
Copper acetate, 0·2% in drinking water for 7 days before PTU	160	2	5	2	40
Zinc acetate, 0·2% in drinking water for 7 days before PTU	280	2	5	5	100

days before the toxic agent was given, they afforded almost complete protection. The reason for these observations is not clear.

Protection by 1-methyl-1-phenylthiourea. In Table III, it can be seen that 1-methyl-1-phenylthiourea, injected just before $2LD_{50}$'s of phenylthiourea, affords complete protection against the poison. Its isomers, 1-methyl-3-phenylthiourea and *S*-methyl-*N*-phenyl-isothiourea, however, did not protect, nor did thiourea, phenylurea, 1,3-diphenylthiourea and *p*-hydroxyphenylthiourea. The mode of action of this methyl derivative was therefore investigated. 1-Methyl-1-phenylthiourea, 1-methyl-3-phenylthiourea and *S*-methyl-*N*-phenylisothiourea were each injected intraperitoneally into groups of 5 rats at a level of 50 mg/kg. All the rats survived in each case. These compounds were therefore non-toxic to rats at this level of dosing. The first two compounds were also administered orally to rabbits at a level of 150 mg/kg. Two rabbits were used with each of the compounds and none of the rabbits showed any toxic effects.

The protective action of 1-methyl-1-phenylthiourea could be due to: (*a*) the prevention of the toxic action of H_2S already released *in vivo* from phenylthiourea, or (*b*) the prevention of the

release of H_2S from phenylthiourea by competing for the enzyme system metabolizing phenylthiourea.

That the first of these alternatives is unlikely to be true is shown in Table IV. 1-Methyl-1-phenylthiourea did not protect rats against a lethal dose of aqueous H_2S.

Table IV. *The effect of 1-methyl-1-phenylthiourea (MPTU) on the toxicity of aqueous* H_2S. Male rats were injected intraperitoneally with 10 mg of 1-methyl-1-phenylthiourea in water (10 mg/ml) before an injection of a lethal dose of H_2S in water

MPTU injected, mg	H_2S injected, mg	No. of rats		Mortality, %
		used	dead	
0	3·4	4	4	100
10 (10 min before H_2S)	3·4	3	3	100
10 (30 min before H_2S)	3·4	3	3	100

That the second alternative is a possible explanation is suggested by the figures in Table V. In experiment 3 quoted in this Table (taken from Scheline *et al.*[1]), the rats were given ^{35}S-phenylthiourea alone, and of the ^{35}S excreted in the urine during 2 days after the injection, 89 per cent was in the form of sulphates and 11 per cent as neutral sulphur compounds. When 1-methyl-1-phenylthiourea was injected before the same dose of ^{35}S-phenylthiourea (exp. 1), the sulphate output in 3 days was now 52 per cent and the neutral sulphur 46 per cent of the ^{35}S excreted. Similar figures (exp. 2) were obtained when $2LD_{50}$'s of phenylthiourea were injected with the methyl derivative, i.e. 50 per cent as sulphate and 49 per cent as neutral sulphur. These figures suggest that the methyl derivative retards the conversion of the sulphur of phenylthiourea to sulphate, which we assume is formed *via* H_2S. This would mean that more phenylthiourea is excreted unchanged and/or more of it is hydroxylated to *p*-hydroxyphenylthiourea[1] and therefore an increase in neutral sulphur excretion would be expected. In fact the neutral S excretion increased 4–5 times when the methyl derivative was administered with phenylthiourea (see Table V).

Table V. The effect of 1-methyl-1-phenylthiourea (MPTU) on the excretion of various sulphur compounds by rats given ^{35}S-phenylthiourea (PTU)

Exp. 1. Three rats injected intraperitoneally with 10 mg of MPTU in water, followed 1–2 min later by an intraperitoneal injection of 2 mg of ^{35}S-PTU/kg in water.

Exp. 2. Same as exp. 1 except that 10 mg of ^{35}S-PTU/kg, i.e. 2LD$_{50}$'s, were injected.

Exp. 3. ^{35}S-PTU/kg (2 mg) injected alone; data taken from Scheline *et al.*[1], Table V. ^{35}S-analyses were carried out as described by Scheline *et al.*[1]

The figures given below are the average of 3 rats; ranges are given in parentheses.

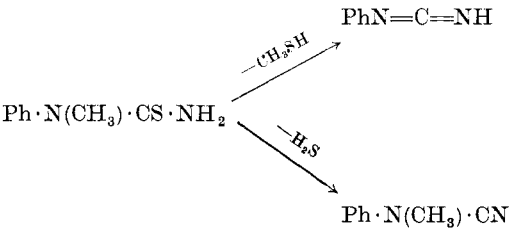
Exp. no. ...	1	2	3
Dose of ^{35}S-PTU, mg/kg	2	10	2
Dose of MPTU, mg/rat	10	10	0
Period of urine collection, days	3	3	2
^{35}S excreted in urine as:			
Inorganic SO_4 — % of dose	38 (37–39)	33 (37–34)	47 (44–49)
Inorganic SO_4 — % of total urine ^{35}S	45	41	75
Ethereal SO_4 — % of dose	6·4 (5·7–7·0)	7·1 (7·0–7·3)	9 (7–10)
Ethereal SO_4 — % of total urine ^{35}S	7	9	14
Total SO_4 — % of total urine ^{35}S	52	50	89
Neutral S — % of dose	39 (37–41)	38 (37–39)	7 (6–9)
Neutral S — % of total urine ^{35}S	46	49	11
Sum of sulphates and neutral S — % of dose	83	78	63
Total ^{35}S in urine, % of dose	84 (81–85)	76 (72–78)	62
^{35}S in urine in 6 days, % of dose	86 (85–87)	77 (74–79)	67 (63–69)
^{35}S in faeces in 6 days, % of dose	9 (7–10)	11 (8–14)	14 (12–18)
Total ^{35}S accounted for, % of dose	95 (92–97)	88 (83–93)	81 (75–87)

Table VI. The excretion of thione compounds by rabbits given the methylphenylthioureas orally

Compound fed	Dose, mg/kg	CS compounds excreted, % of dose[a]
1-Methyl-1-phenylthiourea	150	1·4[b] (0·8 – 1·9)
1-Methyl-3-phenylthiourea	150	33·4[b] (30·5–36·5)

[a] Excreted in 24 h after dosing; no thione compounds were found in the second day's urine.
[b] These figures are the mean of three animals, the ranges are given in parentheses.

The question now arises of why only one of the methyl derivatives of phenylthiourea exhibits this protection. Some evidence on this point was obtained from a study of the desulphuration of the two methyl derivatives in the rabbit. When the methyl derivative, $C_6H_5N(CH_3)CS \cdot NH_2$, is fed to rabbits at a dose level of 150 mg/kg, only about 1 per cent of the dose is excreted in the urine as thione compounds in 24 h (see Table VI), whereas 33 per cent of the methyl derivative, $C_6H_5NH \cdot CS \cdot NHCH_3$, is excreted as thione compounds determined by Grote's reagent.[5] This would suggest that the first compound is readily desulphurized *in vivo*, but that the second compound is only slowly and incompletely desulphurized. 1-Methyl-1-phenylthiourea could decompose in two ways, thus:



If it lost H_2S rapidly it could be expected to be toxic, but if it lost CH_3SH, it might be less toxic than phenylthiourea, because methanethiol is much less toxic than H_2S.[2] On the evidence available, it is suggested that 1-methyl-1-phenylthiourea competes strongly with phenylthiourea for a 'desulphurizing enzyme system' and thus prevents the rapid formation of H_2S from phenylthiourea. It is possible that the relatively low toxicity of 1-methyl-1-phenylthiourea itself is due to its being metabolized to CH_3SH and not to H_2S. The lack of any protective action by 1-methyl-3-phenylthiourea may be because it has relatively less affinity for the 'desulphurizing enzyme system'. Further evidence on these points can only be obtained by a detailed study of the metabolism of the *N*- and *S*-methyl phenylthioureas.

Summary. The toxicity of aqueous solutions of hydrogen sulphide containing 1·0–1·25 mg of H_2S/ml in rats has been investigated. In female white albino rats the LD_{50} is about 0·27–0·55 mg/kg intravenously and about 2·3–2·8 mg/kg intraperitoneally. Aqueous H_2S is less toxic to male rats by intraperitoneal injection than to females. The intravenous

toxicity of H_2S is such that it could account for the toxicity of phenylthiourea if it is assumed that phenylthiourea releases H_2S in the tissues.

Rats could be protected against the lethal effect of $2LD_{50}$'s of phenylthiourea by a previous injection of 1-methyl-1-phenylthiourea. The isomers of this compound, 1-methyl-3-phenylthiourea and *S*-methyl-1-phenylisothiourea, had no protective action. A number of other arylthioureas and various drugs including promethazine, diphenhydramine, phenoxybenzamine, chlorpromazine, hydrocortisone and SKF 525A had no protective action. The antithyroid agents, propylthiouracil and potassium iodide, afforded slight protection. Iron and zinc salts had little effect, but copper salts showed a slight effect.

1-Methyl-1-phenylthiourea, which is not highly toxic, appeared to retard the metabolic loss of sulphur from ^{35}S-phenylthiourea in rats, possibly preventing rapid formation of H_2S in the tissues. 1-Methyl-1-phenylthiourea itself is completely desulphurized in rats, but 1-methyl-3-phenylthiourea is only partly desulphurized. The findings quoted in this paper support the view that phenylthiourea is toxic through release of H_2S in the tissues. The protective action of 1-methyl-1-phenylthiourea may be due to competition for a desulphurizing enzyme.

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