Journal of Medicinal and Pharmaceutical Chemistry

VOL. 4, No. 1 (1961

The Metabolism of Arylthioureas— II. The Metabolism of ^{14}C- and ^{35}S-Labelled 1-Phenyl-2-Thiourea and its Derivatives

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The oral LD_{50} of 1-phenyl-2-thiourea is 8·6 mg/kg for wild Norway rats and 3·1 mg/kg for domestic rats.[1] It is supposed to be less toxic to other species and we have observed during this investigation that the oral LD_{50} for rabbits is in the region of 40 mg/kg. This is in great contrast with 1,3-diphenyl-2-thiourea, which is non-toxic to rats at 2000 mg/kg.[1] We have already shown that diphenylthiourea is relatively non-toxic to rabbits and that this compound is not desulphurized *in vivo* to any great extent.[2] We shall show in this paper that phenylthiourea is desulphurized to a considerable extent in both the rat and rabbit. Furthermore, we shall show that none of its desulphurized metabolites which still contain an aromatic ring or its metabolic hydroxylation product, 1-(*p*-hydroxyphenyl)-2-thiourea, is highly toxic. *p*-Hydroxyphenylthiourea is not desulphurized *in vivo* to any great extent. It will appear from the work reported here either that phenylthiourea is toxic *per se* or that its toxicity is intimately related to its desulphuration. No previous work on the metabolism of phenylthiourea has been reported apart from that of Carroll and Noble[3] who found a small proportion of phenylthiourea (16 per cent estimated by Grote's reagent which is not specific for phenylthiourea) to be excreted unchanged by rats made resistant to it by pretreatment with sub-lethal doses; no metabolites were reported.

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Materials and Methods

Radioactive Phenylthiourea

(*a*) *Phenyl-[2-^{14}C]thiourea.* Potassium [^{14}C]thiocyanate (48·6 mg; 500 μc) (Radiochemical Centre, Amersham) was dissolved in dry acetone (1 ml) contained in a 25-ml Pyrex test-tube which was attached to the arm of a mechanical shaker. To this was added drop by drop with shaking benzoyl chloride (70·3 mg) in dry acetone (1 ml). The mixture was refluxed for 5 min using a current of warm air. Aniline (46·6 mg in 1 ml of dry acetone) was added drop by drop and followed by 10 ml of water. The precipitate of 1-benzoyl-3-phenyl-2-thiourea was centrifuged and the supernatant was decanted and discarded. Boiling 2·5N NaOH (2 ml) was added to the crystals and the mixture boiled for 5 min. After cooling, 0·6 ml of concentrated HCl was added and then the solution was made slightly alkaline with 0·4 ml of ammonia solution (s.g. 0·88). Phenylthiourea, m.p. 147–149°, separated on standing (yield, 46 mg containing 308 μc or 6·7 μc/mg). Phenylthiourea (200 mg) was added to the mother liquor and the solution boiled. On cooling, 172 mg of phenylthiourea (98 μc or 0·57 μc/mg), m.p. 151°, was recovered. The overall recovery of radioactivity was 81·2 per cent.

(*b*) *Ring-labelled [^{14}C]phenylthiourea.* Benzoyl chloride (1·51 g in 3 ml of dry acetone) was added drop by drop with stirring to ammonium thiocyanate (0·899 g) in dry acetone (5 ml). The mixture was refluxed for 5 min. [^{14}C]Aniline (1 g, *ca.* 12 μc) was then added slowly. The mixture and washings were poured into water and the precipitate was filtered. The benzoylphenylthiourea was then hydrolysed and the phenylthiourea recovered as above. It was recrystallized from water (m.p. 151°; yield 55 per cent of aniline used; specific activity, 6·85 μc/g).

(*c*) *[^{35}S]Phenylthiourea.* This compound was synthesized essentially in the same way as phenyl-[2-^{14}C]thiourea but using 243 mg of K^{35}SCN containing 6·85 mc of radioactivity. Two crops of phenylthiourea were recovered: 252 mg of m.p. 150–151° and containing 4·54 mc, and 157 mg of m.p. 150–151° containing 0·49 mc. The overall recovery of ^{35}S was 73·5 per cent.

The following compounds were prepared by standard methods: triphenylisomelamine,[4] m.p. 210°, *p*-hydroxyphenylthiourea,[5]

m.p. 219–220°; *p*-hydroxyphenylurea,[6] m.p. 167·5–168·5°; 2-aminobenzothiazole,[7] m.p. 125–126°; 2,4-di-imino-3,5-diphenyl-1,2,4-thiadiazolidine,[8] m.p. 182–183°; and *S*-methylphenylisothiourea,[9] m.p. 86–87° and picrate,[9] m.p. 171–172°. Phenylcyanamide was obtained from phenylthiourea by treatment with hot alkaline lead acetate solution (cf. Kurzer[10]). It was obtained as the hemihydrate, m.p. 42–44°, after recrystallization from wet ether. On keeping, the hemihydrate polymerizes to triphenylisomelamine,[11] and this occurs in 0·5 h at 100° and in about one week at room temperature. The anhydrous compound, which is a thick syrup, polymerizes in two days at room temperature. In slightly acid solution it is converted to phenylurea, but it dissolves in alkali to form a salt and can be precipitated unchanged by careful addition of acid. For feeding experiments it was prepared fresh from phenylthiourea and administered within 3 h.

Animals

Chinchilla rabbits and white rats were used. The rabbits were kept on a diet of 200 ml of water and 100 g of cubes (Diet No. 41, Associated Flour Millers) daily. All the compounds given to rabbits were administered by mouth as an aqueous suspension with the addition of a little bile salt. Phenylthiourea dissolved in 0·85 per cent NaCl was injected intraperitoneally into the rats.

Observations on Toxicity

During the experiments, the following observations were made on the toxicity of the various compounds used. With phenylthiourea, 300 and 500 mg/kg orally killed 3/3 rabbits at each level, 150 mg/kg killed 2/3, and 25–30 mg/kg killed none. With *p*-hydroxyphenylthiourea, 300 mg/kg killed 1/4, 150 mg/kg, 0/3, and 100 mg/kg, 0/3. Phenylurea was non-toxic when fed to three rabbits at 600 mg/kg. Phenylcyanamide had no effect on three rabbits when it was fed at 100 mg/kg; no other dose levels were used.

Analytical Methods

Glucuronic acid and ethereal sulphates in urine were determined as previously described.[12] Thione compounds in urine were determined with buffered Grote's reagent (see preceding paper[2]).

Phenylurea (and when present, phenylcarbamic acid) was determined, after alkaline hydrolysis, as aniline. A solution of phenylurea containing 0·3–1·4 mg in 1–5 ml of water or of rabbit urine was mixed with 20 ml of 40 per cent (w/v) NaOH and the mixture steam distilled until 100 ml of distillate had been collected. The aniline in the distillate was determined colorimetrically with *N*-(1-naphthyl)-ethylenediamine,[13] the red colour being allowed to develop for 24 h and read at 535 mμ in a Unicam spectrophotometer SP 600. The mean recovery of phenylurea added to water was 95 per cent and to urine, 93·3 per cent. Under these conditions, the amount of aniline released from phenylthiourea was 4 per cent (range 3–6 per cent) and from phenylcyanamide, 4 per cent (range 3–7 per cent).

p-Hydroxyphenylurea was determined as *p*-aminophenol after acid hydrolysis. Urine or water (5 ml) containing 3–8 mg of *p*-hydroxyphenylurea was heated under reflux with 5 N HCl (20 ml) for 3 h. The solution was cooled and diluted with water to 100 ml. Aliquots (1–2 ml) were treated with N Na_2CO_3 (5 ml) and made up to 10 ml with water. Phenol, (2 ml of 5 per cent w/v aqueous solution) and 0·1 per cent potassium ferricyanide (8 ml) were then added. After 30 min, the volume was made up to 25 ml with water and the blue colour measured at 610 mμ in a Unicam spectrophotometer. The mean recovery of *p*-hydroxyphenylurea was 96 ± 2 per cent from water and 94 ± 2 per cent from urine. Under the same conditions, *p*-hydroxyphenylthiourea was converted somewhat irregularly into *p*-aminophenol to the extent of 8–20 per cent. In the presence of *p*-hydroxyphenylthiourea, the estimation of *p*-hydroxyphenylurea is thus subject to error, the readings being slightly high.

Isotope dilution methods. Radioactive samples were counted as described in earlier papers from this laboratory.[14]

Phenylthiourea. The carrier (0·5 g) dissolved in 5 per cent NaOH (10 ml) was added to the urine (10 ml) and an excess of concentrated HCl then added. After keeping at 0° for 1 h, the crystals of phenylthiourea which separated were recrystallized from water to constant activity (m.p. 151–152°). The phenylthiourea was then dissolved in methanol (3–4 ml) and a few drops of methyl iodide added with warming. A few drops of 10 per cent $NaHCO_3$ followed by an excess of hot saturated aqueous

picric acid solution were added and *S*-methylphenylisothiourea picrate separated. It was crystallized (m.p. 174–175°) from aqueous alcohol (50 per cent v/v) to constant activity.

Phenylurea. The carrier (0·5 g) was added to the urine (10 ml) diluted with water (10 ml) and the mixture raised to the boil and then cooled. The phenylurea, m.p. 147–148°, was recrystallized from water to constant activity. It was then converted into 1-benzoyl-3-phenylurea (m.p. 191°) with benzoyl chloride and pyridine, and this was recrystallized to constant activity from hot ethanol.

p-*Hydroxyphenylthiourea.* The hydroxy compound (0·5 g) was dissolved in 10 per cent NaOH (10 ml) and added to 10 ml of urine. The solution was neutralized with concentrated HCl and the precipitated hydroxy compound collected and recrystallized from water to constant activity (m.p. 219–220°). It was then converted into *p*-hydroxyphenyl-*S*-methylthiourea picrate (m.p. 201–202°) as described for phenylthiourea, and the picrate was recrystallized from aqueous ethanol (50 per cent v/v) to constant activity.

p-*Hydroxyphenylurea.* To 10 ml of hot urine diluted with an equal volume of water, 0·5 g of the carrier was added. The solution was cooled and the hydroxyphenylurea recrystallized from hot water (m.p. 167–169°). It was further purified as the *O*-acetyl derivative (m.p. 201–203°) prepared with acetic anhydride, and recrystallized from ethanol.

2,4-Di-imino-3,5-diphenyl-1,2,4-thiadiazolidine. This compound (0·5 g) was dissolved in water with the addition of a few drops of 10 per cent HCl. The solution was neutralized with sodium bicarbonate and the precipitate filtered, washed with water, and recrystallized repeatedly from methanol (m.p. 178–180°). The product was not radioactive.

2-Aminobenzothiazole. The compound (0·5 g) was dissolved in water (10 ml) with the addition of a little dilute HCl and warming. This was added to urine (10 ml) and the mixture neutralized with $NaHCO_3$ solution. The precipitated 2-aminobenzothiazole was recrystallized repeatedly from water (m.p. 127–128°). The product was not radioactive.

S-*Methylphenylisothiourea.* The carrier (0·5 g) was dissolved in a little dilute HCl and added to 10 ml of urine. The base was

then precipitated with $NaHCO_3$ solution. It was recrystallized from aqueous ethanol as its picrate (m.p. 174–175°), and the radioactivity disappeared.

Phenylisothiocyanate. This compound (0·4 g) in a little acetone was added to urine (10 ml), and then extracted with 2 × 20 ml of ether. Aniline (0·4 ml) was added to the ether extract and then the ether was evaporated by warming. The residual thiocarbanilide was recrystallized from ethanol and its activity fell to zero (m.p. 253–254°).

Urea. Urea (0·5 g) was added to urine (5 ml). Concentrated HNO_3 was added to precipitate the nitrate (m.p. 152–153°). The latter was recrystallized from ethanol to constant activity. The urea nitrate was also converted into the xanthydrol derivative by dissolving it in water (5 ml) and adding a little solid excess $NaHCO_3$. Glacial acetic acid (10 ml) was followed by a solution of xanthydrol in methanol (10 per cent w/v). The dixanthylurea was recrystallized from aqueous dioxane, m.p. 276–277°.

Phenylcyanamide. This compound (0·5 g) dissolved in a little 10 per cent NaOH was added to urine (10 ml). Acetic acid was added to make the solution just acid, and the precipitate was filtered and washed with cold water. The precipitate was further purified by alternately dissolving it in 10 per cent NaOH, heating, and allowing the material to separate out on acidifying with 10 per cent acetic acid, and cooling. The phenylcyanamide was counted as the hemihydrate. Because of the low m.p. of this material, it was found convenient to count it as derivatives. Thus the material obtained on acidifying was dissolved in pyridine (1 ml) followed by *p*-toluenesulphonyl chloride (1·0 g) in acetone (5 ml), and the solution was heated on a water-bath for 20 min. It was cooled, 5 per cent HCl (25 ml) was added and then the solution was made basic with 10 per cent w/v NaOH solution. The material separating was the *p*-tolyl derivative (*N*-cyano-*p*-toluenesulphonanilide[15]), m.p. 85–87°. This was recrystallized from water to constant activity. A second sample of phenylcyanamide was benzoylated in aqueous suspension with benzoyl chloride to give *N*-benzoylphenylcyanamide,[16] m.p. 125–127°, which was recrystallized from aqueous alcohol to constant activity.

1-(p-*Hydroxyphenyl*)-3-*phenylurea.* The compound (0·5 g) was dissolved in a little sodium hydroxide, 10 per cent w/v, and

10 ml of radioactive urine was added. The urine was acidified with 10 per cent v/v HCl and the precipitate filtered and washed with cold water. It was recrystallized from aqueous alcohol until all radioactivity was lost, m.p. 219–220°.

Triphenylisomelamine. The carrier (0·5 g) was added to a mixture of urine (10 ml) and ethanol (10 ml) and the solution boiled until it dissolved. The material which separated on cooling was filtered off, washed with cold water and repeatedly recrystallized from aqueous alcohol (m.p. 210°). The product was not radioactive.

Aniline. (*a*) *Free.* Aniline hydrochloride (1 g) was added to one-fifth of the total urine from rabbits given ring-labelled phenylthiourea. The solution was brought to pH 10 and the aniline was extracted continuously with ether for 6 h. The ether was evaporated and the residue dissolved in 10 ml of water containing 0·67 ml of conc. HCl. Acetic anhydride (0·94 ml) was added and the solution neutralized with sodium acetate. Acetanilide, m.p. 113–114°, separated and was crystallized from water to constant activity. It was converted to *p*-bromoacetanilide, m.p. 166–167°, which was crystallized from water to constant activity.

(*b*) *Total.* One-fifth of the urine (*ca.* 100 ml) was steam-distilled in 20-ml portions with 25 ml of 40 per cent NaOH until 600–700 ml of distillate collected. Aniline hydrochloride (1 g) was dissolved in the distillate which was made alkaline with solid $NaHCO_3$ and then continuously extracted with ether for 6 h. The extract was evaporated, water added and tribromoaniline prepared by slowly adding bromine. Tribromoaniline, m.p. 121–122°, was recrystallized from aqueous ethanol to constant activity. This was converted to the *N*-acetyl derivative which was recrystallized from aqueous ethanol.

Total p-*aminophenol.* One-fifth of the urine (*ca.* 100 ml) was heated under reflux for 8 h with 300 ml of conc. HCl. This process had been shown in preliminary experiments to convert *p*-hydroxyphenyl-thiourea and -urea and their conjugates into *p*-aminophenol. After refluxing for 2 h, the volume of hydrolysate was reduced to 200 ml by distillation, and this had the effect of removing any free aniline present. Heating under reflux was then continued. The hydrolysate was cooled and *p*-aminophenol hydrochloride (1 g base) was added. The solution was neutralized with solid K_2CO_3

and finally adjusted to pH 10. The *p*-aminophenol was extracted continuously with ether for 6 h and then the extract was evaporated. The residual *p*-aminophenol (m.p. 184–186°) was recrystallized to constant activity from water (charcoal) and then converted to the *O,N*-diacetyl derivative (m.p. 151–152°) which was recrystallized to constant activity from water.

Determination of sulphates and neutral sulphur. The general procedure was to add carrier sodium sulphate to the urine and to precipitate the sulphate with barium chloride. Preliminary experiments, using ^{14}C-phenylthiourea and urine from animals dosed with ^{14}C-phenylthiourea, showed that barium chloride did not precipitate phenylthiourea and its metabolites to more than 0·7 per cent.

Inorganic sulphate was determined by adding 0·5 ml of 10 per cent (w/v) HCl to 10 ml of urine, followed by 8·5 ml of 3·5 per cent (w/v) Na_2SO_4 solution and then 10 ml of 6 per cent (w/v) $BaCl_2$ solution. The precipitate was centrifuged at 2000 rev/min and the mother liquor poured off and filtered. The precipitate was washed twice with water and three times with acetone. It was plated out for counting as an acetone slurry and dried in a stream of cold air. For ethereal sulphate determination, the filtered mother liquor was treated with 3 ml of concentrated HCl and the solution placed on a boiling water bath for 1 h (reflux condenser). The solution was cooled and the procedure for inorganic sulphate followed. The mother liquor from the ethereal sulphate should contain only neutral sulphur compounds and was counted as such.

Determination of phenylurea + phenylcarbamic acid in rat urine. Phenylthiourea solution (aqueous, containing 1 mg/ml) was injected intraperitoneally into 6 rats at a dose level of 5 mg/kg. Three of these rats survived and their urine was collected daily for 3 days. The urine was analysed colorimetrically for aniline after steam-distillation in the presence of 40 per cent NaOH as already described for rabbit urine (for results see Table VIII).

Qualitative Examination of Urines

Phenylthiourea. Rabbits were given oral doses of 40 mg/kg of phenylthiourea and the urine was collected for 24 h. The urine volume was considerably above normal, and the pH was 5·5–7·0.

The urine gave a positive Grote's and naphthoresorcinol reaction, indicating the excretion of C=S compounds and of glucuronides. No distinct colour was obtained with ferric chloride, suggesting the absence of free phenols and of thiocyanate. The indophenol test for free *p*-aminophenol was negative. On diazotizing and coupling with *N*-(α-naphthyl)ethylenediamine, the urine slowly developed a pink colour characteristic of aniline, and this suggested the presence of a free diazotisable amino group or one set free by dilute acid.

Chromatography of the urine in solvent A (see Table I) revealed four spots (R_f 0·03, 0·28–0·34, 0·76, and 0·87) giving a blue colour with Grote's reagent. The first spot was weak and disappeared when the urine was first mildly hydrolysed with N HCl, and its disappearence was accompanied by an increase in the spot corresponding to *p*-hydroxyphenylthiourea (R_f 0·71). This spot may be due to the ethereal sulphate of *p*-hydroxyphenylthiourea. The second spot was the largest and also gave the naphthoresorcinol test, which suggested that it was the glucuronide of *p*-hydroxyphenylthiourea. The third spot corresponded to free *p*-hydroxyphenylthiourea and the fourth to free phenylthiourea. These four spots were confirmed with the sodium azide–iodine and silver nitrate sprays.

Using the same solvent and spraying with modified Ehrlich's reagent, five spots were revealed (R_f 0·06, 0·22–0·28, 0·49–0·55, 0·76 and 0·88). The first was weak and was possibly an ethereal sulphate. The second spot also gave a positive naphthoresorcinol reaction and was probably due to the glucuronide of *p*-hydroxyphenylurea. The third large spot corresponded to urea, the fourth to free *p*-hydroxyphenylurea and the fifth to phenylurea. On spraying another chromatogram with Brentamine Fast Blue 2B reagent, two phenolic spots, R_f 0·74 and 0·78, were found and these corresponded approximately to *p*-hydroxyphenylthiourea and *p*-hydroxyphenylurea, respectively. These results were confirmed with solvent system C.

When the urine was chromatographed in solvent A and the paper sprayed with 10 per cent HCl followed by diazotization and coupling with *N*-(α-naphthyl)ethylenediamine, a large pink spot was found at the origin which also gave a good naphthoresorcinol test. This suggested the presence of a labile glucuronide yielding

Table I. R_f values and colour reaction of phenylthiourea and its possible metabolites

	R_f values in solvent						Colour reactions					
	A	B	C	D	E	F	Grote's reagent	Iodine–sodium azide	Silver nitrate	Ehrlich's reagent	Brent-amine Fast Blue 2B	Diazo test
Phenylthiourea	0·87	--	0·89	—	0·38	—	blue	white spot on brown background	brown	—	—	—
Phenylurea	0·84	0·61	0·84	—	0·29	0·39	--	--	—	yellow	—	sometimes pink spot
p-Hydroxyphenylthiourea	0·71	--	0·87	—	0	—	blue	white spot on brown background	brown	—	pink	—
p-Hydroxyphenylurea	0·74	---	0·77	—	0	—	--	--	—	yellow	pink	—
Phenylcyanamide	0·80	0·72	—	--	--	0·75	—	—	--	yellow slowly	—	—
2-Aminobenzothiazole	—	0·10	—	--	—	—	—	--	brown on heating	yellow[a]	—	—
S-Methylphenylisothiourea	—	—	--	0·93	--	—	—	—	--[b]	—	—	—
2,4-Di-imino-3,5-diphenyl-1,2,4-thiadiazolidine	—	0·11	—	—	—	—	—	—	—	--[c]	--	--
Urea	0·47	—	0·28	—	0	—	—	—	—	yellow	--	—

[a] 2-aminobenzothiazole: with Ehrlich and heat—*salmon red* spot.
[b] *S*-methylphenylisothiourea: silver nitrate and heat—*brown* spot.
[c] 2,4-di-imino-3,5-diphenyl-1,2,4-thiadiazolidine: with Ehrlich and heat—*pink* coloured spot.

Descending chromatography was used with Whatman No. 4 paper

Solvent systems: A, butanol–acetic acid–water (4 : 1 : 5, by volume); B, benzene–acetic acid–water (1 : 1 : 2, by volume).; C, butanol–pyridine–benzene–water (5 : 1 : 3 : 3, by volume using the top layer); D, propanol–ammonia solution, s.g. 0·88 (7 : 3, by volume); E, isopropyl ether saturated with water; F, benzene saturated with formic acid (98%).

Spraying Agents: Thione compounds were detected with Grote's reagent, silver nitrate solution and iodine–sodium azide reagent and glucuronides by the naphthoresorcinol method.[2] Monoarylureas were detected with a modified Ehrlich's reagent. This consisted of *p*-dimethylaminobenzaldehyde (1 g) dissolved in ethanol (30 ml) and mixed with conc. HCl (30 ml) and *n*-butanol (200 ml). Monoarylureas showed up as yellow spots, but monoarylthioureas did not react. Phenolic metabolites were detected with a spray of 2 per cent aqueous Brentamine Fast Blue 2B followed by 5 per cent (w/v) aqueous sodium carbonate. Diazotizable amino groups were detected by spraying first with nitrous acid (made with 20 ml cold 10 per cent HCl and 5 ml of 0·1 per cent aqueous sodium nitrate). After 10 min, the paper was sprayed with 0·5 per cent ammonium sulphamate solution and 5 min later with 0·1 per cent aqueous *N*-(α-naphthyl)ethylenediamine hydrochloride. Where in this test preliminary hydrolysis with NaOH was necessary the paper was sprayed with 5 per cent NaOH, warmed, and then sprayed with 10 per cent HCl to acidify it.

aniline on hydrolysis. Using butanol–ethanol–aqueous 3N NH_3/3N ammonium carbonate buffer (40 : 11 : 19)[17] as solvent, this spot gave an R_f of 0–0·1, and in ethanol–water (1 : 1) the R_f was 0·75. The same results were obtained if the paper was sprayed with 5 per cent NaOH instead of 10 per cent HCl. As will be seen later, this spot may be due to the ester glucuronide of phenylcarbamic acid, since it was found in phenylcyanamide and in phenylcarbamic acid urine, but not in phenylurea urine.

Chromatography of the urine in solvent F and spraying with Ehrlich's reagent was used to detect phenylcyanamide (R 0·75). The phenylcyanamide spot was eluted with ethanol and rechromatographed in the same solvent and again eluted. The eluate showed the characteristic ultraviolet absorption spectrum of phenylcyanamide (strong band at 231 mμ and a weak one at 265 mμ in ethanol replaced by a strong band at 260 mμ in 0·01N KOH in 90 per cent ethanol).

S-Methylphenylisothiourea, 2-aminobenzothiazole and 2,4-diimino-3,5-diphenyl-1,2,4-thiadiazolidine were not found in phenylthiourea urine or in ether extracts of the urine brought to pH 9 by chromatography (solvent B and D) or by isolation.

Metabolites of [^{35}S]*phenylthiourea.* Paper chromatograms were prepared with the 24-h urine specimen of rats and rabbits which had received sublethal doses of [^{35}S]phenylthiourea. The urines (0·1 ml) were analysed on Whatman No. 4 paper by the descending method using two solvent systems, namely: (*a*) ethanol–ammonia solution (s.g. 0·88)–water, 80 : 4 : 6 by volume; and (*b*) butanol–pyridine–2N ammonia solution, 2 : 1 : 2 by volume. The chromatograms were run for 5–6 h. The papers were dried and radioautographs were prepared from them. Rat and rabbit urines gave similar results.

In solvent system (*a*), most of the radioactivity was found in one spot of R_f 0·09–0·1. This spot was identical in R_f value with a spot produced by $Na_2{}^{35}SO_4$ and was therefore identified as inorganic sulphate. Two other small spots of R_f 0·9 and 0·76 corresponded to phenylthiourea and *p*-hydroxyphenylthiourea, respectively. A very weak spot of R_f 0·54 was also found which corresponded with the thiocyanate ion.

In solvent system (*b*), a strong spot was found of R_f 0·07 corresponding to inorganic sulphate, two weak spots corresponding

to phenylthiourea (R_f 0·82) and *p*-hydroxyphenylthiourea (R_f 0·71) and a very weak spot corresponding to thiocyanate (R_f 0·52).

p-*Hydroxyphenylthiourea.* This compound was fed at a level of 150 mg/kg. The urine (pH 6·5–9·0) was normal in appearance and gave strongly positive Grote's and naphthoresorcinol reactions, but negative indophenol tests for *p*-aminophenol and ferric chloride tests for thiocyanate. Benedict's and Fehling's reagents gave black precipitates on boiling with urine. Chromatography of the urine in solvent A gave two large spots with Grote's reagent (R_f 0·26–0·33 and 0·78), two naphthoresorcinol spots (R_f 0·15–0·2 and 0·28–0·33) and three spots with Ehrlich's reagent (R_f 0·22–0·28, 0·49, and 0·75). These spots correspond to *p*-hydroxyphenylthiourea and *p*-hydroxyphenylurea and their glucuronides, and the one at R_f 0·49 to urea. Similar results were obtained with solvent C.

Phenylurea. The dose used was 100 mg/kg. The urine was normal in appearance and gave negative Benedict's, Fehling's, ferric chloride, and indophenol tests. The naphthoresorcinol test was positive. A weak pink colour was obtained in diazotization and coupling with *N*-(α-naphthyl)ethylenediamine. Paper chromatography in solvent A revealed a large spot of R_f 0·22–0·32, which gave a naphthoresorcinol test and was probably conjugated *p*-hydroxyphenylurea. *p*-Hydroxyphenylurea (R_f 0·77) and a small amount of phenylurea (R_f 0·89) were also detected with the appropriate spraying agents. No spot corresponding to 'phenylcarbamic acid glucuronide' was found.

Phenylcyanamide. The freshly prepared compound was fed at a dose level of 100 mg/kg. The 24-h urine specimen was larger in volume than normal. The naphthoresorcinol test was positive and urine readily reduced Benedict's and Fehling's solution. Ferric chloride gave a deep brown colour, but the indophenol reaction was negative. Treatment of the urine with either dilute acid or alkali followed by diazotization and coupling with *N*-(α-naphthyl)ethylenediamine gave a typical colour reaction for aniline, but this reaction was not given by the untreated urine.

Paper chromatography of the urine in solvent A showed the presence of small amounts of phenylurea and *p*-hydroxyphenylurea, but no phenylcyanamide or *p*-aminophenylglucuronide.

However, large amounts of an acid and alkali labile glucuronide giving positive tests for aniline, similar in R_f value to that found in phenylthiourea urine, were detected. In fact, the main metabolite of phenylcyanamide appeared to be the 'phenylcarbamic acid glucuronide'.

Phenylcarbamic acid. This acid is very labile and therefore a buffered solution of the sodium salt was injected. Five per cent (w/v) NaOH (30 ml) was cooled in 4° in an ice-bath and phenylisocyanate (5·4 ml) slowly added with gentle shaking. After 20 min, the small amount of diphenylurea which had been formed was filtered off. This solution contained sodium phenylcarbamate (shown by the preparation of barium phenylcarbamate on adding excess $BaCl_2$ solution and analysing the precipitate), and was stable between pH 6 and 8. The solution (10 ml) which contained approximately 2 g of sodium phenylcarbamate was mixed with 5 ml of 10 per cent (w/v) sodium bicarbonate solution and extracted with ether to remove any free aniline. This solution was injected intraperitoneally into a rabbit (2·5 kg) and a 24-h urine specimen collected. The urine was slightly darker than normal and gave an intense naphthoresorcinol test and readily reduced Fehling's and Benedict's reagents on warming. It gave a brown colour with ferric chloride. On diazotization and coupling with *N*-(α-naphthyl)ethylenediamine, a strong red colour was obtained. Chromatography of the urine in butanol–ethanol–ammonium carbonate buffer and in ethanol–water, as described for phenylthiourea, revealed the presence of the same labile glucuronide as found in phenylthiourea and phenylcyanamide urine. This material appeared to be the major metabolite of phenylcarbamic acid.

Results and Discussion

The distribution of radioactivity in the urine of rats and rabbits receiving the ^{14}C- and ^{35}S-labelled forms of phenylthiourea is shown in Tables II–V. In the rabbit, using both [2-^{14}C] and ring-labelled phenylthiourea, 95 per cent of the ^{14}C given orally is eliminated in the urine and faeces, about 85 per cent of the dose being in the urine and about 10 per cent in the faeces. Table III shows that the compound behaves similarly in rats receiving it by

Table II. The recovery of ^{14}C in the urine and faeces of rabbits receiving ^{14}C-phenylthiourea orally

	[2-^{14}C]-Phenylthiourea			Ring-labelled phenylthiourea			Mean values
Rabbit no.	38	48	68	46	42	56	
Weight, kg	3·0	3·0	4·4	4·5	4·2	4·0	
Dose of phenylthiourea, mg	30	60	88	112	105	100	
Dose of ^{14}C, μc	17·1	17·1	25·1	0·77	0·69	0·69	
% of dose in:							
urine in 2 days	79·0[a]	87·4	91·6[a]	84·2	89·2	82·1	85·7
faeces in 7 days	16·4	6·1	3·9	12·6	6·7	12·4	9·7
total ^{14}C excreted	95·4	93·5	95·5	97·4	95·9	94·5	95·4

[a] In 4 days.

Table III. The recovery of ^{14}C in the urine and faeces of rats receiving ^{14}C-phenylthiourea intraperitoneally. The [2-^{14}C]phenylthiourea of activity 6·7 μc/mg was injected intraperitoneally dissolved in 5 ml of 0·85 per cent NaCl solution

	Mean value	Range[a]
Wt. of rats, g	190	145–325
Dose of phenylthiourea, mg/kg	2·12	1·53–2·78
Dose of ^{14}C, μc	2·70	
% of dose in:		
urine in 4–10 days	80·1[b]	69·0–88·6
faeces in 4–10 days	9·8	3·7–20·9
total ^{14}C excreted	89·9	89·6–94·9

[a] 9 rats were used.
[b] In 2 days the mean value for urinary excretion was 77 per cent of the dose.

Table IV. The fate of the sulphur of [^{35}S]phenylthiourea in rabbits. Dose of phenylthiourea, 20 mg/kg orally; dose of ^{35}S, 125·5 μc/rabbit. The results are the mean of three animals; ranges are given in parentheses

Time after dosing, days	% of dose of ^{35}S excreted						
	In urine	In faeces	Total[a]	In urine as			
				Inorganic SO_4	Ethereal SO_4	Neutral sulphur	Total sulphates + neutral sulphur
1	63 (54–68)	—	—	40 (32–45)	8 (6–10)	14 (13–15)	62
2	73 (72–74)	—	—	49 (46–52)	10 (8–11)	14 (13–15)	73
3	77 (75–78)	—	—	—	—	—	—
4	77·5 (75–79)	—	—	—	—	—	—
7	79 (77–81)	6 (4–8)	85	51 (48–55)	11 (10–12)	16 (15–18)	78
11	81 (77–83)	—	—	—	—	—	—
14	81·2 (77–83)[2]	8 (6–8)	89	—	—	—	—

[a] One of these rabbits was killed on the eighth day and its tissues were found to contain 5·2 per cent of the dose of ^{35}S. In this particular rabbit 90 per cent of the ^{35}S was accounted for in 8 days (urine 77 per cent, faeces 8 per cent, carcass 5 per cent).

injection. When ^{35}S is used as the label, a little less can be accounted for in the urine and faeces. Table IV shows that rabbits excrete about 80 per cent of the ^{35}S in the urine and about 8 per cent in the faeces and there is about 5 per cent of the dose left in the body 8 days after dosing. In rats, as shown in Table V, some 67 per cent of the ^{35}S is eliminated in the urine and about 14 per cent in the faeces in 5 days after dosing. In Fig. 1, the elimination of ^{14}C and ^{35}S in the urine of rabbits is shown graphically and it is clear that the ^{35}S is excreted more slowly than the ^{14}C. This suggests that the sulphur atom of phenylthiourea is being removed *in vivo* from the rest of the molecule. In fact over 60 per cent of the ^{35}S is excreted by the rabbit as sulphate (51 per cent as inorganic sulphate and 11 per cent as ethereal sulphate, see Table IV) and about 56 per cent (47 per cent as inorganic sulphate and 9 per cent as ethereal sulphate; see Table V) by the rat. An analysis of the tissues of rats receiving the ^{14}C- and ^{35}S-labelled compound also suggests rapid removal of the sulphur atom (see Figs. 2 and 3).

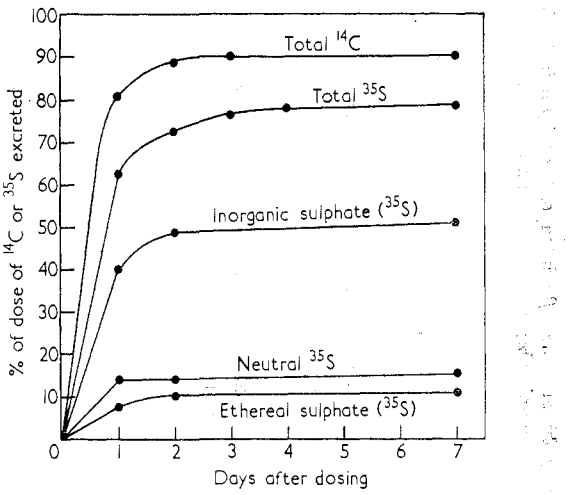


Fig. 1. The excretion of ^{14}C or ^{35}S by rabbits which had received either phenyl[2-^{14}C]thiourea or ^{35}S-phenylthiourea orally at a dose level of 20 mg/kg. The nature of the ^{35}S excreted is shown in the lower three curves.

In Tables IV and V, it will be noted that all the radioactivity of the urine is accounted for by the sulphates and neutral sulphur compounds excreted. The neutral sulphur compounds of the urine should be mainly unchanged phenylthiourea and its S-containing metabolite, *p*-hydroxyphenylthiourea (see Tables VI and VII). In rabbits, the neutral sulphur compounds amount to 16 per cent of the dose (Table IV). By isotope dilution for ^{14}C (see Table VI), phenylthiourea plus its hydroxy derivative amounts to 22 per cent, whereas an estimation of C : S compounds by Grote's reagent (see Table IX) gives a figure of 15 per cent of the dose. There is therefore reasonable agreement between these three methods of assessing the output of thione compounds after administration of phenylthiourea, and in the rabbit this output is about 15–20 per cent of the dose. For rats, the urinary neutral sulphur is 7–9 per cent of the dose (Table V), and phenylthiourea plus *p*-hydroxyphenylthiourea by isotope dilution for ^{14}C (Table VII) is about 9 per cent.

The results of isotope dilution experiments are shown in Tables VI and VII. In rabbits, it can be seen that most of the radioactivity of the urine can be accounted for, in order of importance,

Table V. The fate of the sulphur of [^{35}S]phenylthiourea in rats. Dose of phenylthiourea, 2 mg/kg intraperitoneally in isotonic saline; dose of ^{35}S, about 7 μc/rat. The results given are the mean of three animals; ranges are in parentheses

Time after dosing, days	% of dose excreted in						
				Urine as			
	Urine	Faeces	Total	Inorganic SO_4	Ethereal SO_4	Neutral sulphur	Total sulphates + neutral sulphur
First series							
1	49 (46–52)	—	—	36 (34–41)	7 (6–8)	6 (5–8)	49
2	62 (58–64)	—	—	47 (44–49)	9 (7–10)	7 (6–9)	63
3	64 (61–67)	—	—	—	—	—	—
5	67 (62–69)	—	—	—	—	—	—
6	67·3 (63–69)	14 (12–18)	81[a]	—	—	—	—
Second series							
4	63 (58–66)	—	—	—	—	—	—
5	66 (62–68)	14 (12–18)	80[b]	50 (48–52)	8 (6–9)	9 (5–12)	67

[a] The carcass contained 3 per cent (1·5–3·5) of the dose of ^{35}S, making the total 84 per cent.
[b] The carcass contained 3 per cent (2–5) of the dose of ^{35}S, making the total 83 per cent.

Table VI. The quantitative aspects of the metabolism of ^{14}C-phenylthiourea in rabbits determined by isotope dilution. Details of dosage are given in Table II

Compound sought	% of the dose excreted in two days after dosing						
	with [2-^{14}C]phenylthiourea			with ring-labelled phenylthiourea			Mean values
Rabbit no.	38	48	68	46	42	56	
Phenylthiourea	6·4	2·3	10·5	—	—	—	6·4
Phenylcyanamide	0·4	1·5	1·1	—	—	—	1·0
Phenylurea	3·3	5·0	2·8	—	—	—	3·7
Urea	2·5	4·6	2·8	—	—	—	3·3
p-Hydroxyphenylthiourea (free)	8·6	10·1	4·1	—	—	—	[7·6][b]
p-Hydroxyphenylthiourea (total)	15·0	23·3	8·8	—	—	—	15·7
p-Hydroxyphenylurea (free)	1·5	1·4	1·3	—	—	—	[1·4][b]
p-Hydroxyphenylurea + *p*-hydroxyphenylthiourea	—	—	—	38·5[a]	22·9[a]	28·6[a]	[30·0][b]
p-Hydroxyphenylurea (total) by difference	—	—	—	—	—	—	14·3
Aniline (free)	—	—	—	4·6	—	3·6	4·1
Phenylcarbamic acid + phenylurea + free aniline[c]	—	—	—	49·7	31·2	33·2	[38·0][b]
Phenylcarbamic acid, by difference	—	—	—	—	—	—	30·2
Sum of appropriate metabolites				87·2	54·1	61·8	78·7[d]
^{14}C in urine in 2 days	76·4	87·4	90·2	84·2	89·2	82·1	84·9

[a] Determined as *p*-aminophenol.
[b] These figures are not added to sum of metabolites.
[c] Determined as total aniline.
[d] Sum of mean values of metabolites.

Isotope dilution was also carried out for 2-aminobenzothiazole, phenylisothiocyanate, *S*-methylphenylisothiourea, 2,4-di-imino-3,5-diphenyl-1,2,4-thiadiazolidine, 4-hydroxy-*N*,*N*^{1}-diphenylurea and triphenylisomelamine, but these compounds were not found.

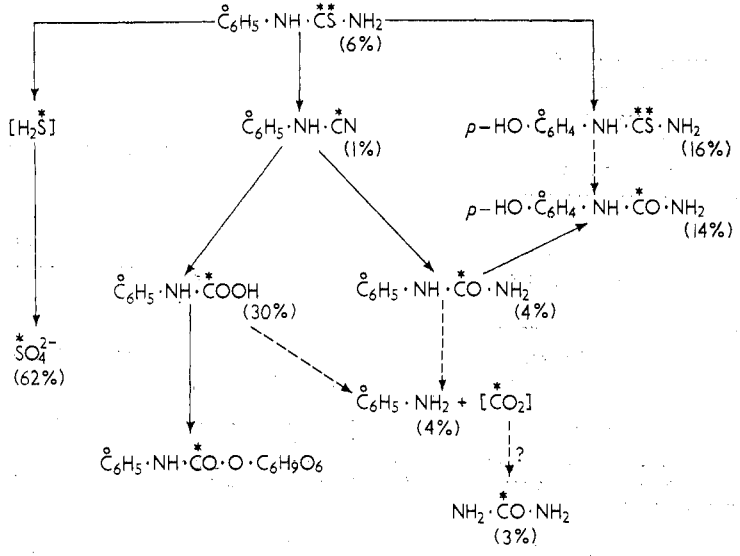
Table VII. Quantitative aspects of the metabolism of [2-^{14}C]phenylthiourea injected into rats determined by isotope dilution. Doses as in Table III

% of ^{14}C excreted in 2 days as	Mean	Range in 3 animals
Phenylthiourea	6·0	4·6– 7·8
p-Hydroxyphenylthiourea, free	2·7	2·4– 3·2
Phenylurea	3·1	1·4– 4·6
p-Hydroxyphenylurea, free	1·6	0·9– 2·1
Urea	3·0	2·5– 3·5
Phenylcyanamide	2·3	2·0– 2·5
Substances yielding aniline[a] with alkali	32·3	30·7–34·6
Phenylcarbamic acid by difference, (total aniline − phenylurea)	29·2	

[a] See Table VIII.

as phenylcarbamic acid (30 per cent), *p*-hydroxyphenylthiourea (16 per cent), *p*-hydroxyphenylurea (14 per cent), phenylthiourea (6 per cent), phenylurea, aniline, urea (about 3–4 per cent each) and phenylcyanamide (1 per cent). In rats, a complete coverage of all these compounds was not made but Tables VII and VIII suggest that as far as some of the metabolites are concerned rats and rabbits are similar.

Our results suggest that the routes of metabolism of phenylthiourea in the rabbit are as follows:



The metabolism of phenylthiourea. The percentages are the amounts excreted in the urine. Minor routes are shown as broken arrows.

$\overset{\circ}{C}$, ring labelled; $\overset{*}{C}$, 2-C-labelled; $\overset{*}{S}$, ^{35}S-labelled.

The main reaction is the loss of sulphur. In the rabbit at least 60 per cent of the dose of phenylthiourea is desulphurized. In Table VI, the difference between the thione compounds of the urine and the sum of the metabolites is 57 per cent, which is in close agreement with the figure of 62 per cent for total sulphates given in Table IV. If this sulphur were lost as H_2S, then phenylthiourea should give rise to phenylcyanamide and its metabolites.

Phenylcyanamide was detected in the urine by paper chromatography and by isotope dilution, but it is not as toxic as phenylthiourea, since rabbits given doses of 100 mg/kg orally are unaffected by it.

The main urinary metabolite of phenylthiourea appears to be a phenylurethane and on our evidence we believe it to be the ester glucuronide of phenylcarbamic acid. This metabolite is formed in major amounts when phenylcyanamide or sodium phenylcarbamate is administered, but it is not formed from phenylurea. The

Table VIII. The excretion of substances yielding aniline with alkali in the urine of rats receiving phenylthiourea. Rats injected intraperitoneally with aqueous phenylthiourea (1 mg/ml) at a dose level of 5 mg/kg

Rat no.	Wt, g	Dose of phenylthiourea, mg	Aniline[a] in urine, % of dose		
			On day 1	On days 2 & 3	Total
1	170	0·85	27·3	4·8	32·1
2	200	1·0	29·1	5·5	34·6
3	190	0·95	24·2	6·5	30·7
					Mean 32·5

[a] The urine was steam-distilled with 40 per cent NaOH and the aniline in the distillate determined colorimetrically. The precursors of the aniline are probably phenylcarbamic acid and phenylurea (see text).

amount of phenylcarbamic acid formed and excreted in the urine is in the region of 30 per cent of the dose as determined by isotope dilution (Table VI) and colorimetric estimation after conversion to aniline (Table IV) (see also Table VIII for rats). Phenylurea is largely converted (65 per cent) into *p*-hydroxyphenylurea and its conjugates (Table IX). Phenylcyanamide when administered is largely converted (50 per cent) into phenylcarbamic acid glucuronide; it is also the probable precursor of the phenylurea and *p*-hydroxyphenylurea both of which are found in phenylthiourea urine, for about 18 per cent of administered phenylcyanamide is excreted as *p*-hydroxyphenylurea and its conjugates (Table IX).

Phenylthiourea also gives rise to aniline (4 per cent) and urea (3 per cent) (Table VI). These could be expected to arise by the

decomposition of phenylcarbamic acid which would yield equimolar amounts of aniline and CO_2. It is assumed that this CO_2 would be converted into urea.

Apart from desulphuration, phenylthiourea undergoes another reaction *in vivo*, namely hydroxylation to *p*-hydroxyphenylthiourea which accounts for about 16 per cent of the dose (Table VI). *p*-Hydroxyphenylthiourea is at least five times less toxic to rats and rabbits than phenylthiourea, and is metabolized mainly

Table IX. Quantitative aspects of the metabolism of phenylthiourea and its metabolites in rabbits

Compound fed	Dose, mg/kg	% of dose excreted in 2 days as				Total
		C=S Compounds	Free aniline	Phenylurea and/or phenylcarbamic[a] acid	*p*-Hydroxy-phenylurea[b]	
Phenylthiourea	40	15 (12–19)	—	34 (32–35)[c]	15 (12–16)	64
Phenylurea[d]	100	—	3 (2·6–3·4)	6 (5– 7)[e]	65 (60–70)	74
Phenylcyanamide	100	—	—	48 (45–49)[f]	18 (13–21)[g]	66
p-Hydroxyphenyl-thiourea	100	70 (62–76)	—	—	small[h] amounts	70

[a] Determined as aniline after alkaline hydrolysis. [b] Determined as *p*-aminophenol. [c] Mainly phenylcarbamic acid (phenylurea is about 4 per cent, see Table IV). [d] About 0·4 per cent (0·3–0·6)[8] of phenylurea is excreted in the faeces. [e] Mainly phenylurea. [f] Mainly phenylcarbamic acid as shown by chromatography. [g] Could arise from *p*-hydroxyphenylcyanamide. [h] Detected by paper chromatography.

by conjugation with glucuronic acid and sulphuric acid (with three rabbits it was found that 100 mg/kg oral doses of the hydroxy compound were excreted to the extent of 27 per cent (22–23) conjugated with glucuronic acid, and 20 per cent (15–26) with sulphuric acid; see also Table IX) although appreciable amounts (20–30 per cent) were excreted unchanged. Only very small amounts of *p*-hydroxyphenylthiourea were desulphurized to *p*-hydroxyphenylurea.

Isotope dilutions were also carried out (see Table VI) for 2-aminobenzothiazole, which could be formed by cyclization of one molecule of phenylthiourea;[7] for 2,4-di-imino-3,5-diphenyl-1,2,4-thiadiazolidine, which could be formed by condensation of

two molecules of phenylthiourea;[8] for triphenylisomelamine, a polymerization product of phenylcyanamide;[11] for *S*-methylphenylisothiourea, which could be formed by an S-methylation of phenylthiourea; and for phenylisothiocyanate, which could be formed by loss of ammonia from phenylthiourea. None of these compounds were found as metabolites.

It appears from this work that none of the organic metabolites of phenylthiourea is toxic. It is therefore concluded that its toxicity is due either to the compound itself or to the H_2S released in the reaction to phenylcyanamide. The toxicity of H_2S is discussed in the succeeding paper, for H_2S could well be the toxic agent.[18]

Tissue distribution of ^{14}C *and* ^{35}S. The elimination of ^{14}C and ^{35}S in the urine of rabbits has been discussed (see Fig. 1). The

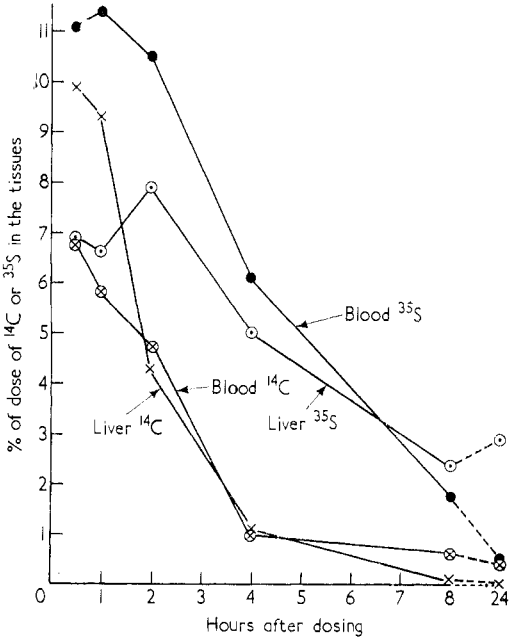


Fig. 2. ^{14}C or ^{35}S in the liver and blood of rats at various times after the injection of 1·5 mg/kg of phenyl-[2-^{14}C]thiourea or 2 mg/kg of ^{35}S-phenylthiourea. Six female rats (weight, 150–280 g) were used and one was killed and tissues analysed after 0·5, 1, 2, 4, 8 and 24 h after injection. The ^{14}C falls to half-value in about 2 h and the ^{35}S in about 4 h.

distribution of these labels in the tissues of rats at various intervals after the intraperitoneal injection of ^{14}C- or ^{35}S-labelled phenylthiourea has also been examined. The results for some of the tissues examined are shown in Figs. 2 and 3. Two groups of six female rats were used. Each rat in the first group was injected with 1·5 mg/kg of $Ph \cdot NH \cdot {}^{14}CS \cdot NH_2$ in isotonic saline, and each

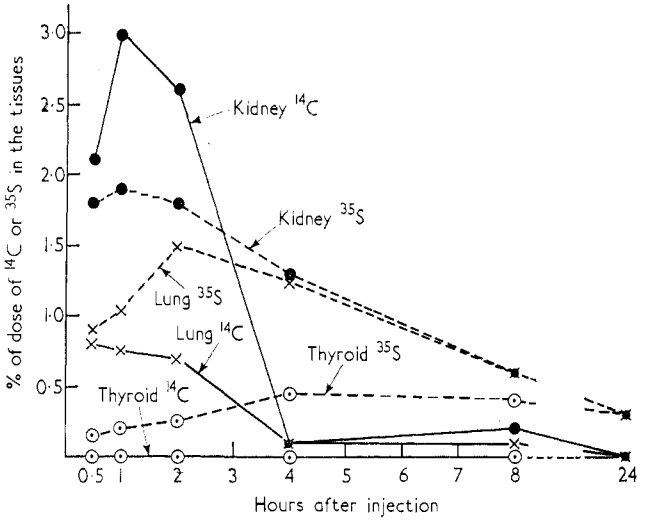


Fig. 3. ^{14}C or ^{35}S in the kidney, lung and thyroid of rats at various times after the injection of 1·5 mg/kg of phenyl-[2-^{14}C]thiourea or 2 mg/kg of ^{35}S-phenylthiourea. The rats were the same as in Fig. 2.

● kidney × lung ○ thyroid

rat in the second group with 2 mg/kg of $Ph \cdot NH \cdot C^{35}S \cdot NH_2$. The rats in each group were killed at intervals and the radioactivity of the various tissues determined. Fig. 2 shows the results for blood and liver and it can be seen that the ^{14}C concentration falls off more rapidly than the ^{35}S. The ^{14}C concentration falls to half its maximum value in roughly 2–3 h whereas the ^{35}S concentration falls to half in roughly 4–5 h. This suggests fairly rapid removal of sulphur from the molecule. Fig. 3 shows the results for kidney, lung and thyroid. In the kidney the ^{14}C concentration rapidly falls to a small value in 4 h, whereas the ^{35}S concentration falls

slowly and is still present after 24 h. In the lung, the ^{14}C concentration again falls rapidly, but the ^{35}S concentration increases during the first 2 h and then slowly diminishes. This observation is significant because the most spectacular pathological change in rats poisoned with phenylthiourea is marked oedema of the lung and pleural effusion.[19] In the thyroid, the ^{14}C concentration was very low, but significant amounts of ^{35}S were found (see Fig. 3).

A more detailed examination was made of the thyroid. Two groups of five rats were each injected as before with 10 mg/kg of ^{14}C- or ^{35}S-phenylthiourea, and an animal in each group was killed at 0·5, 1, 2, 4 and 8 h after dosing and the radioactivity of the thyroid was determined. (It should be noted that a lethal dose of phenylthiourea takes about 12 h to kill a rat.) The following results were obtained:

Time after dosing, h	0·5	1	2	4	8
Ratio, $^{35}S/^{14}C$ in thyroid	2·3	1·5	3·6	2·8	3·5

These figures show that nearly three times as much ^{35}S as ^{14}C enters the thyroid. This suggests that much of the sulphur enters the thyroid after separation from the phenylthiourea molecule. Phenylthiourea appears to have considerable antithyroid activity, for Richter and Clisby[19] found that rats given this compound in drinking water showed marked hypertrophy of the thyroid glands.

Note on the metabolism of phenylthiourea in hens. Phenylthiourea is not highly toxic to hens[20, 21] and the oral LD_{50} is about 1000 mg/kg. Some experiments were therefore carried out on hens and are briefly reported. A hen (1·47 kg), given 1 g of [2-^{14}C]-phenylthiourea orally, excreted 48 per cent of the ^{14}C in 1 day, 82 per cent in 3 days and 85 per cent in 6 days. Another hen (1·2 kg) given 1 g of [^{35}S]phenylthiourea excreted 15 per cent of the ^{35}S in one day, and 41 per cent (two fifths of this was neutral S and the rest sulphates) in 3 days. These results suggest the desulphuration of phenylthiourea in the hen. Three other hens, starved for 24 h, were each given 1 g/kg of phenylthiourea. One of these died overnight and post-mortem examination showed much fluid in its lungs. The urine of the surviving two hens was analysed daily for *p*-hydroxyphenylurea and for compounds yielding aniline on alkaline hydrolysis (i.e. phenylurea + phenylcarbamic acid). In three days only 4 and 8 per cent of the dose

was found as these compounds. The urine of these hens showed only weak tests for C=S compounds, glucuronides, and, after acid hydrolysis, diazotizable amines. Chromatography of the urine showed the presence of small amounts of phenylurea and *p*-hydroxyphenylurea and larger amounts of phenylthiourea and *p*-hydroxyphenylthiourea. These results suggest that desulphuration of phenylthiourea does occur in the hen but that the main metabolite found in rats and rabbits, i.e. phenylcarbamic acid, is not excreted by hens. It is hoped to investigate this problem further at a future date.

Summary. The ^{35}S, 2-^{14}C and ring-^{14}C forms of the highly toxic phenylthiourea have been synthesized and administered to rats and rabbits. With the ^{14}C-label it was found that 86 per cent of the ^{14}C was eliminated in the urine by rabbits in 2 days (dose 10–25 mg/kg orally), and 10 per cent in the faeces. Similar results were obtained with rats (dose about 2 mg/kg by injection). With the ^{35}S-label, excretion of radioactivity was slower, indicating the separation of the sulphur from the rest of the molecule. The urinary metabolites of phenylthiourea in rabbits two days after dosing were shown to be total sulphate (62 per cent of the dose of ^{35}S-phenylthiourea), phenylcarbamic acid glucuronide (30 per cent of the ^{14}C-phenylthiourea), *p*-hydroxyphenylthiourea (16 per cent), *p*-hydroxyphenylurea (14 per cent), phenylthiourea (6 per cent), phenylurea (4 per cent), phenylcyanamide (1 per cent), aniline (4 per cent) and urea (3 per cent). Similar results were obtained in rats. Studies were also made of the metabolism of *p*-hydroxyphenylthiourea, phenylurea, phenylcyanamide, and phenylcarbamic acid, and from the results it was concluded that the main route of metabolism of phenylthiourea was

$$\mathrm{Ph{\cdot}NH{\cdot}CS{\cdot}NH_2} \xrightarrow{-H_2S} \mathrm{Ph{\cdot}NH{\cdot}CN} \longrightarrow \mathrm{Ph{\cdot}NH{\cdot}COOH.}$$

Hydroxylation to *p*-hydroxyphenylthiourea was not a major route of metabolism but could account for about a fifth of the dose. None of the metabolites of phenylthiourea were highly toxic and it was concluded that the toxicity of phenylthiourea could be due to rapid release of H_2S in the tissues. This point is discussed in the succeeding paper.[18] Phenylthiourea is not highly toxic to hens and preliminary experiments suggest that its metabolism in hens is different from that in rats or rabbits.

Acknowledgements. This investigation was supported by a research fellowship number EF-8328 (to R.R.S.) from the National Institute of Allergy and Infectious Diseases, United States Public Health Service. R.L.S. is grateful to the Smith, Kline and French Laboratories for a scholarship. The financial support of Ciba Laboratories Limited is also acknowledged.

(*Received 16 January, 1961*)

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