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# THE ACETYLATION OF S-ALKYLCYSTEINES BY THE RAT

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**Abstract**—Rats have been dosed with S-ethyl-, S-propyl-, S-butyl-, S-pentyl- and S--hexyl-cysteine, and the mercapturic acids excreted have been determined by paper and gas–liquid chromatography. *N*-(Ethyl thioacetyl)glycine is a metabolite of S-ethyl cysteine and has been determined by GLC; the higher cysteines did not form *N*-alkylthioacetylglycine metabolites. Rats dosed with S-butyl- and S-pentyl- cysteines excreted traces of the sulphoxide of the corresponding mercapturic acid. The amounts of mercapturic acid formed by rat liver slices from S-butyl, S-pentyl- and S-hexyl-L-cysteine have been determined. The synthesis and properties of *N*-(ethyl-, *N*-(propyl- and *N*-(butylthioacetyl)glycine, and of the sulphoxides of pentyl- and hexyl-mercapturic acid are described.

1-HALOGENO-ALKANES[1–5] are metabolised by the rat with the formation of *N*-acetyl-S-alkylcysteines (alkylmercapturic acids) and it is found that the percentage of the dose excreted in urine as mercapturic acid varies with the length of the alkyl chain, rising to a maximum at $C_4$ and $C_5$ and then decreasing with increasing chain length. The probable pathway of mercapturic acid synthesis involves the initial formation of an S-alkylglutathione followed by its conversion to the corresponding S-alkylcysteine which is then acetylated. It seemed possible that the extent of this acetylation might also vary with the length of the alkyl chain and so regulate the amount of mercapturic acid formed from the halogeno-alkanes, or alternatively that the percentage of the dose excreted as mercapturic acid might be determined by the extent to which other metabolic pathways are followed.

S-Methyl-L-cysteine[6,7] is extensively metabolised by the rat, half the sulphur content being excreted as sulphate and about 15 per cent as the metabolites methyl mercapturic acid and its sulphoxide, methylthioacetic acid, its sulphoxide and its *N*-glycine conjugate and the sulphoxide of 2-hydroxy-3-methylthiopropionic acid. Rats dosed with S-ethyl-L-cysteine[8] excrete 30 per cent of the dose as ethylmercapturic acid and 35 per cent of the dose as ethylmercapturic acid and 35 per cent as the corresponding sulphoxide while S-propyl-L-cysteine[9] is converted to propylmercapturic acid, the amount isolated from urine corresponding to 14 per cent of the dose. Some propylmercapturic acid sulphoxide is formed. A non-specific method for determining mercapturic acid suggested that rats excreted 61 per cent of a dose of S-butyl-L-cysteine[9] as butylmercapturic acid. In the present work the amount of mercapturic acid excreted by two strains of rats dosed with S-alkyl cysteines (from $C_2$ to $C_6$) has

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been measured using a gas chromatographic method which also permits the detection and determination of any alkylthioacetylglycine formed. The results are also reported of determinations (using a paper chromatographic method[5]) of both the amounts of mercapturic acid excreted in urine by similarly dosed rats and also of the amounts formed on incubation of alkylcysteines with rat liver slices.

## MATERIALS

All melting points are uncorrected. Elementary analyses were carried out by Weiler and Strauss, Oxford, and the Analytical Laboratory, School of Pharmacy, London W.C.1. The S-alkyl-L-cysteines and *N*-acetyl-S-alkyl-L-cysteines were prepared as described previously.[3,5,9,10] *N*-ethyl, *N*-propyl- and *N*-butyl-thioacetylglycines were prepared as described for *N*-methylthioacetylglycine[6] and their properties are listed in Table 1 with those of the sulphoxides of S-pentyl- and S-hexyl- mercapturic acids which were prepared as described for the sulphoxide of butylmercapturic acid.[11] *N*-Acetyl-S-(3-hydroxybutyl)-L-cysteine was prepared as previously described.[11]

## METHODS

*Chromatography.* Details of the solvent mixtures used for paper chromatography are given in Table 2. Chromatograms on Whatman No. 3 paper were developed by the descending method with solvents I for 16 hr, and on Whatman 3MM paper with solvents II & II for 16 hr and with solvent IV for up to 48 hr. The relative ratios of movement of the reference compounds are given in Table 2. The detecting reagents used were the $K_2Cr_2O_7$–$AgNO_3$ reagent [12] for bivalent sulphur compounds and the chloroplatinate reagent[8,13] for bivalent sulphur compounds and sulphoxides. The KI–HCl reagent[14] was also used to detect sulphoxides. Amino acids were detected by ninhydrin (0·2% w/v in acetone to which pyridine 2% v/v had been added).

*Animals and dosing.* Two strains of male albino rats matched for age were used. Strain A was from a closed colony of specific pathogen-free animals; strain B was originally a Wistar strain and had been inbred for many years. These were maintained on a diet of Oxoid pasteurised mouse cubes with water *ad lib.* The compounds were administered by injection in the lumbar region. To prepare the material for injection weighed amounts of S-alkyl cysteine were homogenised with arachis oil in a tissue homogeniser and the suspension diluted to a known volume with arachis oil to give suspensions which settled only very slowly. Each animal received about 1·4 m-moles/kg body weight the maximum volume of suspension injected being 1·7 ml. The animals were rested for 1 week between doses of with the different alkylcysteines. Twenty-four-hour urine samples were collected separately from faeces, combined with the water washings from the urine–faeces separator and made up to 25 ml. The samples were filtered and some used immediately for the separation of metabolites, the remainder being kept at −25°. Female rats of strain B were dosed with the higher alkylcysteines at the same dose levels as for the males. The doses were administered by stomach tube as suspensions in water or by injection as described above.

*Paper chromatographic separation of thio-ether metabolites.*

To 5·0 ml of urine was added 1·0 ml of 20m M-alkylmercapturic acid marker (propylmercapturic acid was used for urine containing metabolites of ethyl, butyl,

TABLE 1. PROPERTIES OF *N*-ALKYLTHIOACETYLGLYCINES AND SULPHOXIDES OF MERCAPTURIC ACIDS

| Compound | Melting point | C% | | H% | | N% | | S% | | Equiv. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Found | Required | Found | Required | Found | Required | Found | Required | Found | Required |
| *N*-(Ethylthioacetyl)glycine | 92–92·5° | 41·0 | 40·6 | 6·3 | 6·3 | 8·3 | 7·9 | — | | 178 | 177 |
| *N*-(Propylthioacetyl)glycine | 82° | 44·2 | 43·9 | 6·9 | 6·9 | 7·8 | 7·3 | — | | 192 | 191 |
| *N*-(Butylthioacetyl)glycine | 77° | 47·1 | 46·8 | 7·4 | 7·4 | 7·0 | 6·8 | — | | 206 | 205 |
| Pentylmercapturic acid sulphoxide | 128–9° | — | | — | | — | | 12·8 | 12·8 | 255 | 249 |
| Hexylmercapturic acid sulphoxide | 106–108° | — | | — | | — | | 12·4 | 12·2 | — | |

(— indicates analysis not performed)

TABLE 2. RETENTION TIMES AND $R_f$ VALUES OF REFERENCE COMPOUNDS

| | Retention times of methylester (min) | | $R_f$ values in solvent | | | |
|---|---|---|---|---|---|---|
| | 140° | 160° | I | II | III | IV* |
| *N*-Acetyl-S-ethyl-L-cysteine | 14·2 | 6·0 | 0·86 | — | 0·54 | 1·0 |
| *N*-(Ethylthioacetyl)glycine | 12·3 | 5·4 | 0·88 | — | 0·45 | 0·87 |
| Ethylthioacetic acid | — | — | 0·97 | — | — | — |
| S-Ethyl-L-cysteine | — | — | 0·51 | — | — | — |
| Glycine | — | — | 0·23 | — | — | — |
| *N*-Acetyl-S-propyl-L-cysteine | 20·4 | 8·8 | 0·92 | — | — | — |
| *N*-(Propyl hioace yl)glycine | 18·0 | 8·0 | 0·91 | — | — | — |
| *N*-Acetyl-S-butyl-L-cysteine | 28·8 | 12·5 | 0·90 | 0·89 | 0·63 | 1·0 |
| *N*-(Butylthioacetyl)glycine | 26·4 | 11·7 | 0·88 | 0·86 | 0·55 | 0·94 |
| Sulphoxide of *N*-Acetyl-S-butyl-L-cysteine | — | — | — | 0·74 | 0·47 | 0·23 |
| *N*-Acetyl-S-pentyl-L-cysteine | 41·3 | 18·8 | 0·92 | 0·90 | 0·66 | 1·0 |
| Sulphoxide of *N*-acetyl-S-pentyl-L-cysteine | — | — | — | 0·79 | 0·51 | 0·27 |
| *N*-Acetyl-S-hexyl-L-cysteine | 59·6 | 26·8 | 0·92 | 0·92 | 0·71 | 1·0 |
| Sulphoxide of *N*-acetyl-S-hexyl-L-cysteine | — | — | — | 0·81 | 0·56 | 0·29 |

* Solvent allowed to run off edge of paper. For each set of compounds the rate of movements of the spots is related to that of the mercapturic acid, which is taken as 1·0.

G.L.C. chromatography was carried out on a 5% $QF_1$ column at the temperatures indicated. The solvents for paper chromatography were I, butan-1-ol–acetic acid–water (12:3:5, by vol.); II, butan 1-ol–water–acetic-acid–butylacetate (24:10:5:2, by vol.); III, butan-1-ol–ethanol aq. $NH_3$ (sp.gr· 0·88)–water (10:10:1:4, by vol.); IV, butan-2-one saturated with aq. 2N $NH_3$.

pentyl and hexyl-cysteines; and ethylmercapturic acid for urine containing metabolites of propylcysteine).

About 3 ml of this urine was applied as a streak 24 cm wide to Whatman No. 3 paper and the chromatogram was developed by the descending method with *n*-butanol–acetic acid–water (12:3:5 by vol.) (Solvent I). This system does not separate the individual metabolites. The solvent was allowed to run about 40 cm and then the outer 2 cm from each side of the sheet were cut off and dipped in the platinum reagent. The end 15 cm which contained all the thioether metabolites ($R_f$ 0·63–1·0) was cut off and extracted.

*Extraction of metabolites.* The paper (15 × 20 cm) was attached to wicks, and suspended in a chromatography tank and eluted with methanol–acetic acid–water (90:5:5 by vol.) as for descending chromatography. After the collection of 40 ml of eluate the paper and wicks gave a negative test with the platinum reagent. The eluate was evaporated at 50–55° and then dissolved in methanol (20 ml) and again evaporated to remove acetic acid. The residue dissolved in methanol (10 ml) was treated with a slight excess of diazomethane dissolved in ether. The product obtained on evaporation of the reaction mixture was dissolved in methanol (1 ml) and samples of this solution were used for the determination of mercapturic acid and *N*-(alkylthioacetyl)glycine as their methyl esters.

*Determination of metabolites.* (*a*) *By gas–liquid chromatography.* A Pye series 104 dual column flame ionisation-chromatograph (W. G. Pye & Co., Cambridge) was used. The column packing was 5% $QF_1$, on acid-washed Chromosorb W[15]. The instrument was fitted with a Disc Chart Integrater Model 201 B (Disc Instruments Ltd., Hemel Hempstead.). Duplicate samples of the methylated fractions from urine were applied to the column which was maintained at a temperature which gave a good separation of the metabolites and reference compound. The areas under the curves corresponding to the alkylmercapturic acid and *N*-alkylthioacetylglycines were measured and compared with the area of the reference mercapturic acid peak. From the ratio of the area of metabolites to the area of mercapturic acid the amount of metabolite excreted was calculated since it was shown that equimolecular amounts of the compounds examined gave the same peak areas.

(b) *By paper chromatography.* This was carried out directly on samples of urine using the method[5] previously described. The corresponding alkyl mercapturic acid was used to construct a calibration curve for each set of determinations.

*Acetylation by rat-liver slices.* Duplicate digests were prepared[10] each containing about 2 mg of substrate accurately weighed and 2 g rat liver slices. The digests were incubated at 37° for 4 hr; each digest was then adjusted to pH 5·0 by the addition of acetic acid and homogenised using a Potter–Elvehjem type homogeniser for 2 min. The homogenate was heated at 70–75° for 2 min to precipitate the protein and the mixture was filtered. The precipitate was washed thoroughly with water. The volume of the filtrate and the washings was made up to 175 ml and aliquots were continuously extracted with ether for 6 hr after the addition of 1 ml conc. HCl per 25 ml solution. The ethereal extract was evaporated and the residue dissolved in dichloromethane. The whole of the extract was applied as a streak to a strip of Whatman 3 MM paper, 5 cm wide. The chromatograms were developed in solvent III and the mercapturic acid was located by dipping in the chloroplatinate reagent. The width of the mercapturic acid streaks was measured. A calibration curve was prepared by similarly applying known

amounts (100–200 $\mu$g) of the appropriate mercapturic acids to paper strips which were developed with the sample strips. When the mercapturic acids were added to digests in which the liver slices had been inactivated by boiling, the average percentage recovery of the acids was 92%, 100% and 106% for butyl-, pentyl- and hexylmercapturic acids respectively. When the S-alkyl-L-cysteines were incubated with inactivated liver slices the formation of mercapturic acid was not detected.

## RESULTS

### *Identification of metabolites*

N-(*Ethylthioacetyl*)*glycine*. The 24-hr urine of rats dosed with S-ethyl-L-cysteine was collected and the sulphur-containing metabolites separated from a 12 ml sample by paper chromatography in solvent I as previously described. The separated material was dissolved in water (4 ml) and applied to a column (50 cm × 3 cm diam.) of CG-400 formate form (Type I) resin equilibrated with 0·1M-sodium formate buffer, pH 4·1. The column was washed with 2l. of this buffer and then the adsorbed material eluted with 0·5M-buffer, 25 ml fractions being collected. Application of the nitroprusside reaction[15] to 0·5 ml samples of each fraction gave faint positive reactions in fraction 11 and 18. Fractions 10, 11 and 12 were combined [metabolite (i)] as were fractions 17, 18 and 19 [metabolite (ii)]. The two solutions were de-salted on zeo-Karb 225 ($H^+$ form), the eluates evaporated and the residues dissolved in water (1 ml) to give solutions of metabolites (i) and (ii). A sample of each solution was heated with an equal volume of conc. HCl in a sealed tube at 100° for 3 hr and 50 $\mu$l samples were chromatographed with 10 $\mu$l samples of the original solution on Whatman No 3. paper in solvent I. Metabolite (i) had $R_f$ 0·86 identical with that of ethylmercapturic acid and on acid hydrolysis gave ethylcysteine ($R_f$ 0·53). The $R_f$ value of metabolite (ii) was 0·84 corresponding to that of *N*-(ethylthioacetyl)glycine and the metabolite on treatment with acid gave products having $R_f$ values 0·91 and 0·24 corresponding to those of ethylthioacetic acid and glycine respectively. Both gas–liquid chromatography and paper chromatography in solvent III confirmed that metabolites (i) and (ii) were identical with ethylmercapturic acid and *N*-(ethylthioacetyl)glycine respectively. The mass spectrum of the methyl ester of metabolite (i) gave peaks at m/e 205, 146 ,117, 114, 88, 75 and 43 which were seen in the mass spectrum of the methyl ester of ethylmercapturic acid; that of the methylester of metabolite (ii) showed the same peaks, m/e 191, 131, 103, 88 and 75, as those of the methylester of *N*-(ethylthioacetyl)glycine.

N-*acetyl-S-butyl-*, N-*acetyl-S-pentyl- and* N-*acetyl-S-hexyl-L-cysteines*.

The combined 24 hr urine of six rats which had each received S-butyl-L-cysteine (40 mg) was acidified (pH1) by the addition of HCl and continuously extracted with ether for 16 hr. The residue after evaporation of the ether was extracted with chloroform and the residue from this extract was extracted with water. Evaporation of the aqueous extract gave a syrup which crystallised. The crystals were separated and recrystallised from ether-petroleum ether (b.p. 40–60°) to give crystals of butylmercapturic acid (11 per cent of the dose) m.p. 67° alone and in admixture with an authentic specimen. The crystals showed the same chromatographic behaviour in solvents II, III & IV as authentic butylmercapturic acid and the infra-red spectrum in Nujol mull was identical with that of the synthetic material. The same identity was found when the infra-red spectra of the methyl esters of the biosynthetic and synthetic

materials in $CS_2$ solution were compared. By the same procedure pentyl mercapturic acid was isolated from the urine of six rats each dosed with S-pentyl-L-cysteine. The crystals (0·8 per cent of the dose) had m.p. 97–99° and did not depress the m.p. of synthetic pentylmercapturic acid. KBr discs of the biosynthetic and synthetic materials gave identical infra-red spectra. The chromatographic behaviour of the biosynthetic and synthetic materials was identical in solvents II, III & IV.

Hexylmercapturic acid was detected in the urine and ethereal extracts of acidified urine of rats dosed with S-hexyl-L-cysteine by paper chromatography in solvents, II, III & IV and by gas–liquid chromatography of the methyl ester (see Table 2).

*Sulphoxides of butyl, pentyl and hexyl mercapturic acids.* Traces of the sulphoxides of butyl and pentyl mercapturic acids were detected by paper chromatography in solvents III & IV in the urine of rats dosed with S-butyl- and S-pentyl-L-cysteine respectively, but hexylmercapturic acid sulphoxide was not detected in the urine of rats dosed with S-hexyl-L-cysteine.

*Hydroxyalkylmercapturic acids.* The urine of rats dosed with butyl-, pentyl- and hexyl-cysteine contained small amounts of metabolites which are probably hydroxyalkyl-mercapturic acids. That present in the urine of rats dosed with butylcysteine was identified by gas–liquid chromatography of the methylester as *N*-acetyl-S-(3-hydroxy-butyl)-L-cysteine, retention time 9·3 min at 196° on the 5 per cent $QF_1$ column. The corresponding metabolites formed from S-pentyl- and S-hexyl-L-cysteine have not been identified. An additional sulphur-containing metabolite of S-pentyl-L-cysteine having $R_f$ 0·16 in solvent III and two further metabolites of S-hexyl-L-cysteine, having $R_f$ values of 0·07 and 0·14 in solvent III were detected but their structure has not been established.

*Quantitative results.*

(a) In vivo *experiments.* As may be seen from Table 3 no significant difference in the excretion pattern was found between the male rats of strains A and B. The mercapturic acids formed were excreted in the 24 hr after the dose except in the case of pentyl- and hexyl-mercapturic acids, traces of which were detected in the urine on the second day. Female rats of strain B dosed with S-butyl-L-cysteine by stomach tube excreted the same amount of butylmercapturic acid as the males which had received the dose by injection. Female strain B rats receiving oral doses of S-pentyl- or S-hexyl-L-cysteine excreted much more mercapturic acid than did the male rats receiving similar doses by injection and the excretion was virtually complete in 24 hr. Female rats of strain B excreted an injected dose of pentylcysteine slowly only 10 per cent being recovered on the first, 17 per cent on the second and 9 per cent on the third day; with an injected dose of hexylcysteine less than 0·5 per cent was excreted on either the first or second day.

S-Ethyl-L-cysteine was excreted as *N*-(ethylthioacetyl)glycine but with the higher alkylcysteines examined the formation of *N*-alkylthioacetylglycines was not detected.

(b) In vitro *experiments.* When the higher alkylcysteines were incubated with rat liver slices the percentages of the substrate converted to the corresponding mercapturic acid were as follows (ranges are given in parenthesis and the number of experiments is indicated by the subscript): S-butylcysteine, 22 $(9–42)_{16}$; S-pentylcysteine, 25 $(14–36)_3$; S-hexyl-L-cysteine, 11 $(4–18)_5$. The mercapturic acids were identified by chromatography in sovlents II, III and IV. Small amounts of other metabolites were

TABLE 3. EXCRETION OF ALKYLMERCAPTURIC ACID AND *N*-ALKYLTHIOACETYLGLYCINE BY RATS DOSED WITH S-ALKYL-L-CYSTEINES

| | | Method a | | | | Method b |
|---|---|---|---|---|---|---|
| | | Strain A (Males) | | Strain B (Males) | | Strain B (Females) |
| Dose 1·4 m-mole/kg | Route | *N*(alkylthioacetyl)glycine | Alkylmercapturic acid | *N*-alkylthioacetylglycine | Alkylmercapturic acid | Alkylmercapturic acid |
| S-Ethyl-L-cysteine | s.c.i. | 5·5 (4–8)$_4$ | 19 (12–23)$_4$ | 7 (5–10)$_4$ | 16 (8–28)$_4$ | — |
| S-Propyl-L-cysteine | ,, | N.D. | 80 (65–97)$_4$ | N.D.$_4$ | 60 (49–67)$_4$ | — |
| S-Butyl-L-crysteine | ,, | N.D.$_4$ | 52 (43–61)$_4$ | N.D.$_4$ | 60 (49–67)$_4$ | — |
| ,, ,, | s.t | — | — | — | — | 63 (47–80)$_4$ |
| S-Pentyl-L-cysteine | s.c.i. | N.D.$_4$ | 6 (2–12)$_4$ | N.D.$_3$ | 5 (4–7)$_3$ | 37* (25–48)$_3$ |
| ,, ,, | s.t | — | — | — | — | 54† (43–63)$_9$ |
| S-Hexyl-L-cysteine | s.c.i | N.D.$_3$ | 0·3 (0·2–0·4)$_3$ | N.D.$_4$ | 0·3 (0–0·6)$_4$ | N.D.$_2$ |
| ,, ,, | s.t | — | — | — | — | 6 (3–11)$_5$ |

* Excreted over three days (11 per cent 1st day; 17 per cent 2nd day; 9 per cent 3rd day).
† not more than 1 per cent on second day.
Amounts are expressed as percentages of the dose, ranges are given in parentheses and numbers of experiments are indicated by subscript numbers. Unless otherwise stated the amounts given were excreted in 24 hr after the dose. N.D. indicates not detected;—indicates not examined; s.c.i. indicates subcutaneous injection; s.t. indicates dose administered by stomach tube.

formed; those from S-pentyl- and S-hexyl-L-cysteine have not been identified but it has been shown[11] previously that 2- and 3-hydroxybutylmercapturic acids are formed from S-butyl-L-cysteine on incubation with rat liver slices.

## DISCUSSION

The quantitative results obtained by gas–liquid chromatogarphy of the methyl esters of the sulphur-containing metabolites suggest that the two strains of rats used behaved in the same way towards the S-alkylcysteines administered. Only from the lowest member of the series examined was an *N*-alkylthioacetylglycine formed. *N*-(Ethylthioacetyl)glycine was clearly identified by its retention time and by the mass spectrum obtained after the separation of the metabolite. Paper chromatographic results supported the identification. Thus, in this respect, the metabolism of S-ethyl-L-cysteine resembles that of S-methyl-L-cysteine[6,7] which gives rise to *N*-(methylthioacetyl)-glycine.

The extent of the acetylation of the S-alkylcysteines varied with the length of the alkyl chain. The present work suggests that with S-ethyl-L-cysteine about 18 per cent of the dose is converted to ethylmercapturic acid and as it has been shown[8] that 35 per cent is converted to the corresponding sulphoxide the total acetylation probably represents 53% of the compound administered. With S-propyl-L-cysteine the maximum degree of acetylation is found; the results reported here suggest an average of 70 per cent of the dose is converted to mercapturic acid and to this should be added a further amount corresponding to the sulphoxide formed.[9] With S-butyl-L-cysteine the average amount of mercapturic acid formed is 56 per cent of the dose, and with S-pentyl-L-cysteine there is a fall in the acetylation to 6 per cent while less than 1 per cent of the dose of S-hexyl-L-cysteine is converted to the corresponding mercapturic acid.

When female rats were dosed with S-butyl-L-cysteine by stomach tube 63 per cent of the dose was excreted as mercapturic acid; female rats similarly dosed with S-pentyl-L-cysteine gave 54 per cent of the dose as mercapturic acid, and with S-hexyl-L-cysteine 6 per cent of the dose was excreted as hexylmercapturic acid. Thus the females dosed by stomach tube formed more mercapturic acid than did males dosed by injection. When female rats were injected with S-pentyl-L-cysteine the mercapturic acid formed was very slowly excreted and injected S-hexyl-L-cysteine gave only a trace of mercapturic acid. These results suggest that the injected dose of the higher S-alkylcysteines is absorbed much more slowly than that administered by stomach tube.

With S-butyl- and S-pentyl-L-cysteine trace amounts only of the mercapturic acid sulphoxides were detected; there was none from S-hexyl-L-cysteine. The metabolites which were probably hydroxyalkymercapturic acids in no case represented more than about 5 per cent of the dose of the higher S-alkylcysteines. S-Pentyl-L-cysteine gave a small amount of an unidentified metabolite clearly separated in solvent III, and after S-hexyl-L-cysteine was given by stomach tube two unidentified sulphur-containing metabolites were detected, the size of the corresponding spots on paper chromatograms indicating that each was about equal in amount to the mercapturic acid. Injected S-hexyl-L-cysteine gave only trace amounts of metabolites.

Rat liver slices showed the same degree of acetylation for S-butyl- and S-pentyl-L-cysteines with a falling off in the extent of acetylation for S-hexyl-L-cysteine.

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