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METABOLIC STUDIES WITH ACETYLCYSTEINE*

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Abstract—The metabolism of acetylcysteine was studied and compared with that of L-cysteine. Incubation of acetycysteine with rat liver homogenate resulted in the rapid formation of cysteine. In the rat, 2 hr after the oral administration of acetylcysteine-^{35}S, neither acetylcysteine-^{35}S nor its disulfide could be found in the liver, but cystine^{35}S and cysteine-^{35}S were tentatively identified. An appreciable quantity of acetylcysteine-^{35}S was found in lung. Inorganic sulfate was the major urinary excretion product; however, evidence was obtained for the presence of small amounts of acetylcysteine-^{35}S and taurine-^{35}S in the urine of rats given acetylcysteine-^{35}S orally. Oral administration of cysteine or acetylcysteine to rats or dogs produced little change in blood or tissue sulfhydryl levels, and only a small increase in urinary sulfhydryl compounds.

N-ACETYL-L-CYSTEINE (acetylcysteine; Mucomyst, Mead Johnson) is an active mucolytic agent[1, 2] which has been found useful in the treatment of a variety of pulmonary disorders[3, 4] and in the liquefaction of sputum for bacteriologic examination.[5] A significant feature of both clinical and animal studies with acetylcysteine has been the absence of toxic symptoms and the appearance of remarkably few side reactions. Since mammalian tissues contain enzymes that readily remove the acetyl group of acetylated amino acids,[6] the administration of acetylcysteine to man or animals presumably is equivalent to providing a mixture of cysteine and acetate. The present study was designed to compare in the rat and dog the metabolism of cysteine with its N-acetylated derivative. In addition, owing to the importance of sulfhydryl groups in tissue metabolism, changes in blood and tissue sulfhydryl levels and the urinary excretion of sulfhydryl were measured in animals receiving cysteine and acetylcysteine.

EXPERIMENTAL AND METHODS

Materials. L-Cysteic acid (CfP) was obtained from Calbiochem; oxidized glutathione (chromatographically pure) from C. F. Boehringer & Soehne, GmbH, Mannheim, West Germany; reduced glutathione (chromatographically homogeneous) from Schwartz Laboratories, Inc., U.S.A.; L-cysteine·HCl and taurine from Mann Research Laboratories, Inc., U.S.A.; *L*-cystine from Diamalt, A. G., Munich, West Germany. N-Acetylcysteamine (b.p. 143–6° at 8–9 mm) was prepared by Dr. R. L. Evans according to the procedure of Kuhn and Quadbeck[7] and characterized by Kjeldahl, sulfur, and infrared analysis. N,N-Diacetylcystine was prepared by K. R. Bailey by oxygen aeration of a solution of N-acetyl-L-cysteine containing 1×10^{-3}M $CuSO_4$.

* Preliminary accounts of portions of this work have appeared previously in *Fedn, Proc.* **23** (2) (1964) and in *Pharmacotherapautica* **1**, 46 (1965).

† Deceased.

N-Acetyl-L-cysteine was prepared by Dr. T. A. Martin as described below for the preparation of N-acetyl-L-cysteine-^{35}S.

N-Acetyl-L-cysteine-^{35}S was synthesized by Dr. R. L. Evans according to the following procedure:[8, 9] 900 mg L-cysteine-^{35}S (Schwarz BioResearch, Inc.) was dissolved in 3·9 ml of 2 N sodium hydroxide. After the solution was cooled in an ice-salt bath, 3·0-ml portions of 2 N sodium hydroxide and 0·3-ml portions of acetic anhydride were added alternatively with stirring to a total of 18 ml and 1·8 ml respectively. The solution was then stirred for 30 min in the cold, followed by 30 min at room temperature; it was then chromatographed on a 12 × ½ in. Dowex 50-X8, H^+ cycle (200–400 mesh) column to remove sodium ions and unreacted cysteine. The column was washed with water and the eluate (N,N-diacetyl-L-cystine) was concentrated to an oil. The oil was dissolved in 4·0 ml water, and 1·4 ml glacial acetic acid and 1·2 g of powdered zinc metal were added; the mixture was then heated on a steam bath at 50–60° for 3 hr. Unreacted zinc was removed by filtration, and zinc ions in the filtrate were removed by chromatography through a second Dowex-50 column as above. Concentration of this solution under reduced pressure at room temperature gave an oil which was dissolved in a minimal quantity of water. Crystals separated on refrigeration and on recrystallization gave 310 mg of a product melting at 108–110° (the m.p. of authentic N-acetyl-L-cysteine is 109–110°). A sample of this material chromatographed on paper gave one radioactive component travelling at the same R_f as a reference sample of N-acetyl-L-cysteine.

Sulfhydryl studies. Experiments were performed on overnight-fasted adult female rats of the McCollum-Wisconsin strain (Mead Johnson colony). The animals were kept in metabolism cages and given water and a 20% casein semisynthetic diet *ad libitum* for 6 days prior to the experiment. Solutions of the test compounds were given by stomach tube; control animals received an equivalent volume of 0·9% NaCl. At various time intervals after administration of the test compounds, five rats from each group were sacrificed for the measurement of sulfhydryl (–SH) levels in blood and tissues; the control group consisted of seven rats. The animals were sacrificed by cardiac puncture through the opened pleural cavity while under light ether anesthesia. Blood sulfhydryl determinations were performed immediately after sampling, whereas tissue samples were frozen on solid CO_2 and stored at −20° for subsequent analysis. Additional groups of rats were treated with the test compounds or saline, and urine excreted during the 24-hr period following treatment was collected under toluene. Sulfhydryl determinations on urine specimens were performed immediately after completion of the collection period.

The dogs used in these experiments were female mongrels confined in metabolism cages, except when removed for experimental purposes. All dogs were given Purina dog chow and water *ad libitum*; food was removed from the cages 16 hr before an experiment. Solutions of the test compounds were administered by gavage, except for three dogs given acetylcysteine (neutralized with NaOH to pH 7·3) by intravenous injection. Blood samples for sulfhydryl determination were withdrawn from the right saphenous vein 0, 30, 60, and 120 min after administration of the test compound; urine was collected by catheterization 3, 6, and 24 hr after treatment.

Total sulfur and inorganic sulfate were determined by the gravimetric method of Ogg,[10] and nonprotein sulfhydryl groups were measured by the method of Boyer.[11] The accuracy of the latter method was much improved for measuring sulfhydryl levels

in biological material by adding the sample after the other reagents; this modification was not necessary for solutions of pure thiol compounds.

Measurement of nonprotein sulfhydryl levels in tissues, blood, and urine. Representative samples of frozen tissue (or the whole organ in the case of kidney, spleen, and adrenal) were weighed and pulverized with solid CO_2 in a glass mortar. The fragmented tissue was then mixed with an equivalent weight of a 6% *m*-phosphoric acid (HPO_3) solution containing 6 M urea. The resulting slurry was allowed to thaw for 10 min before homogenization by hand with a pestle. A weighed aliquot of the homogenate was diluted tenfold (w/v) with a 3% HPO_3 solution and centrifuged at 15,000 *g* for 5 min; 5 ml of the supernatant fluid were withdrawn, adjusted to pH 4·6, and diluted with distilled water to 10 ml. One ml of this solution was used to measure nonprotein sulfhydryl by the modified method of Boyer.[11]

Blood specimens were prepared for nonprotein sulfhydryl analysis by adding 1·0 ml whole blood to an equal volume of 6% HPO_3–6 M urea solution, and the mixture was diluted to 10 ml with 3% HPO_3. Subsequent treatment of the samples was similar to that described for tissue sulfhydryl analysis.

Urea was incorporated into the HPO_3 solution on the basis of a report by Dickens and Shapiro[12] that protein binding of sulfhydryl groups was reduced by guanidine salts or high concentrations of urea. In the present study guanidine salts were not found to improve the recovery of sulfhydryl added to blood; however, the incorporation of 6 M urea into the protein precipitant solution improved considerably the recovery of sulfhydryl added to blood and tissues. With urea, the recovery of acetylcysteine added to blood was 96 per cent, liver 107 per cent, muscle 98 per cent, and urine 91 per cent. For the measurement of sulfhydryl levels in dog blood, urea was not added, and the data reported must be considered as the freely available sulfhydryl groups of blood, rather than total nonprotein sulfhydryl.

Measurements of sulfhydryl levels in urine were performed essentially as in blood. However, incorporation of urea into the *m*-phosphoric acid solution did not improve the recovery of added sulfhydryl and, therefore, it was not used for urine. The recovery of acetylcysteine added to urine averaged 91 per cent, whereas for L-cysteine—because of its rapid oxidation—the recovery averaged only 70 per cent.

Studies with acetylcysteine-^{35}S. A solution of acetylcysteine-^{35}S (8 mg/ml; sp. act., $8{\cdot}81 \times 10^4$ counts/min/mg) was prepared in normal saline and administered by stomach tube to ten fasted rats at a dose of 200 mg acetylcysteine/kg. The animals were divided into two equal groups and sacrificed either 2 or 24 hr after treatment. The urine excreted by the 24-hr group was collected under toluene. At the completion of the experiment the animals were sacrificed as previously described; the liver, kidneys, spleen, brain, adrenals, and pieces of femoral muscle were removed, weighed, frozen on solid CO_2 and stored at $-20°$ until examined; aliquots of blood were prepared immediately for measurement of ^{35}S radioactivity.

Total ^{35}S radioactivity in tissues was measured by the method of Jeffay *et al.*[13] in a Tri-Carb liquid scintillation spectrometer (model 314-X). In calculating the total organ content of ^{35}S, skeletal muscle was assumed to approximate 45·5 per cent of the body mass, as reported by Caster *et al.*,[14] and blood was assumed to be 9 per cent of body weight; the activity in the remaining organs was calculated from their actual weights. Correction for the radioactive decay of ^{35}S was made by counting an aliquot of the

acetylcysteine-^{35}S solution used in the experiment concurrently with the tissue samples.

To estimate the extent to which acetylcysteine-^{35}S per se and its metabolites remained in the tissues or were excreted, samples of diluted urine and 50% aqueous ethanol extracts (5% tissue, w/v) of liver, lung, and spleen were chromatographed on Whatman no. 1 filter paper in a t-butanol:acetic acid:H_2O (4:1:1, v/v) solvent system to which 0·1 g KCN and 1·0 g Na_2-EDTA/l. had been added, to reduce heavy metal oxidation of sulfhydryl groups. The chromatographed strips were dried, cut into sections, and the radioactivity of each section was measured directly in a Tri-Carb liquid scintillation spectrometer, with a counting solution consisting of 3 g 2,5-diphenyloxazole and 0·1 g 1,4-*bis*-[2-(5-phenyloxazolyl)]-benzene per liter toluene. Standard samples of the known metabolites of L-cysteine were chromatographed with the experimental samples in order to make a tentative identification of the resolved compounds. The compounds were located on the developed strips with ninhydrin (Sigma Spray, NIN-3) and/or by the I_2-NaN_3 spray of Chargaff *et al.*[15] which is specific for sulfhydryl or for groups such as disulfides or thioesters that may be reduced to sulfhydryl groups.

Studies with rat liver homogenates. Livers from fasted rats were homogenized in the cold in a Potter-Elvehjem-type homogenizer with a loose-fitting Teflon pestle; the homogenates (10%, w/v) were prepared in a pH 7·4 buffer solution containing 0·01 M phosphate, 0·15 M KCl, and 0·045 M $MgCl_2$, and were filtered through gauze before use. Each reaction vessel contained 2·0 ml liver homogenate, 0·1 ml 0·03 M ATP, 0·1 ml 0·001 M CoA, and buffer (as above) to a final volume of 2·5 ml. ATP and CoA were added in an attempt to maintain amino acid metabolism at an adequate level, although these cofactors are probably not necessary for deacetylation. Acetylation, however, requires CoA.[15] L-Cysteine or N-acetyl-L-cysteine was dissolved in distilled water at a concentration of 10 μmoles/μl just prior to the experiment and added to the tubes in place of buffer. All tubes were flushed with nitrogen and sealed before incubation. After incubation the reaction was terminated by the addition of 2·5 ml of 3% *m*-phosphoric acid. The contents of each vessel were transferred to plastic tubes, centrifuged 5 min at 10,000 *g*, and the supernatant fluid used for the measurement of cystine plus cysteine.

The hydrolysis of acetylcysteine was followed by measurement of changes in the total content of cysteine (+ cystine) in the incubation medium. Cysteine plus cystine was determined by the method of Sullivan, as modified by Neubeck and Smythe.[17] (Acetylcysteine gave no color with this procedure.) This method measures total cysteine plus cystine, but does not differentiate the two forms. However, the experiments with rat liver homogenates were performed under nitrogen to prevent oxidation of cysteine by the air, and paper chromatography showed no significant oxidation to cystine under these conditions. Since cysteine is variably adsorbed by proteins, standard curves were prepared by adding aliquots of liver homogenate to standard cysteine solutions containing protein precipitant (*m*-phosphoric acid, final acid concentration 1·5%).

Aliquots of the *m*-phosphoric acid supernatants were also chromatographed on Whatman no. 1 paper (descending) with a solvent system composed of t-butanol:acetic acid:water (4:1:1) containing 1·0 g Na_2EDTA and 0·1g KCN/l. The sulfhydryl and disulfide areas were visualized by the I_2–NaN_3 spray reagent.[15]

RESULTS

The metabolism of acetylcysteine by rat liver homogenates

The incubation of 1 μmole acetylcysteine with a rat liver homogenate for 30 min resulted in the net formation of 0·97 μmoles cysteine. Similarly, the addition of 2 μmoles acetylcysteine to the homogenate resulted in the net formation of 1·85 μmoles cysteine after 30-min incubation.

In addition to the chemical measurement of cysteine, aliquots of the incubation mixtures were subjected to paper chromatographic analysis (Fig. 1). Paper strip 1 is

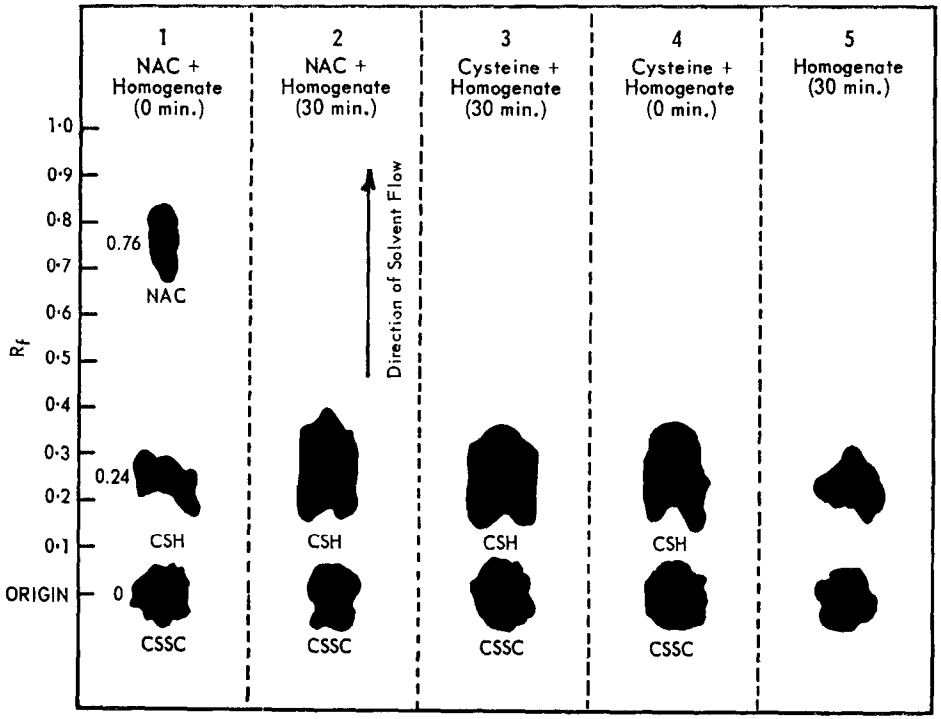

FIG. 1. Formation of cysteine from acetylcysteine by a rat liver homogenate. Strip 1, chromatogram of a nonincubated homogenate to which acetylcysteine was added. Strip 2, homogenate following 30-min incubation with acetylcysteine. Strip 3, homogenate incubated with cysteine for 30 min. Strip 4, homogenate plus cysteine without incubation. Strip 5, homogenate control. Abbreviations are as follows: cysteine, CSH; cystine, CSSC; acetylcysteine, NAC.

a traced chromatogram of a homogenate to which acetylcysteine (R_f 0·76) had been added, but which was not incubated. After 30-min incubation of this homogenate (strip 2), the spot representing acetylcysteine was gone, and there was a concomitant increase in the size of the cysteine area (R_f 0·24).

Incubation of replicate samples of liver homogenate with cysteine rather than acetylcysteine (strip 3) resulted in little, if any, loss of cysteine (cf. strip 4), indicating that under the conditions used, the rate of formation of cysteine from acetylcysteine was much faster than the subsequent metabolism of cysteine.

Tissue distribution and urinary excretion of ^{35}S *compounds after the oral administration of acetylcysteine-*^{35}S *to rats*

Two hours after the administration of acetylcysteine-^{35}S, appreciable radioactivity was present in all tissues examined (Table 1). The highest concentration of ^{35}S was in kidney and liver, followed in descending order by adrenal, lung, spleen, blood,

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN THE TISSUES, BLOOD, AND URINE OF RATS FED ACETYLCYSTEINE-^{35}S

Tissue or fluid	2 hr		24 hr	
	Specific activity (cpm/mg[2])	Total activity (counts/min)	Specific activity (cpm/mg)	Total activity[1] (counts/min)
Kidney	74·4 ± 6·8	122,400	23·3 ± 3·4	30,400
Liver	71·2 ± 4·6	425,500	18·8 ± 1·5	109,500
Adrenal	28·5 ± 0·9	1700	20·6 ± 0·5	1000
Lung	15·8 ± 0·8	18,200	15·0 ± 0·3	15,400
Spleen	12·3 ± 0·2	7900	15·4 ± 0·5	8200
Blood	10·2 ± 0·4	175,000	5·5 ± 0·2	98,200
Muscle	3·5 ± 0·2	302,800	2·6 ± 0·2	229,700
Brain	2·6 ± 0·2	3500	2·8 ± 0·1	4100
Urine				1,960,000

[1] Total activity administered was $3{\cdot}48 \times 10^6$ counts/min.

[2] Mean activity per wet weight of tissue (5 animals/group) ± S.E.

muscle, and brain. Twenty-four hours after the administration of acetylcysteine-^{35}S, the radioactivity in the tissues had diminished as follows: kidney 70 per cent, liver 74 per cent, adrenals 28 per cent, and blood 56 per cent. In lung, spleen, muscle, and brain tissue, the radioactivities found after 2 and 24 hr were not appreciably different. The urinary excretion of ^{35}S during the 24-hr period following the ingestion of acetylcysteine-^{35}S amounted to 56 per cent of the ingested dose.

Metabolites of acetylcysteine-S^{35} *in tissues*

Chromatograms of liver extracts (Fig. 2) did not indicate the presence of acetylcysteine (R_f 0·75) or of its disulfide, N,N′-diacetylcystine (R_f 0·64) in either the 2- or 24-hr samples. Compounds with the R_f values of cysteine (R_f 0·24), cystine (R_f 0·03), and the mixed disulfide of acetylcysteine and cysteine (R_f 0·14) were found in appreciable quantities in the 2-hr specimens. Liver extracts from rats sacrificed 24 hr after receiving acetylcysteine-^{35}S contained relatively little radioactivity. However, the ^{35}S content of the alcoholic extracts accounted for essentially all the radioactivity in the livers from rats sacrificed both 2 and 24 hr after having received acetylcysteine-^{35}S. Thus, little if any of the sulfur from acetylcysteine was incorporated into liver proteins under the conditions of this experiment.

Liver extracts (from rats which received acetylcysteine-^{35}S 2 hr before being sacrificed) were chemically reduced by treatment with an excess of N-acetylcysteamine, thus converting disulfides to free sulfhydryl. This treatment resulted in a marked diminution of ^{35}S compounds in the R_f range of cystine and an increase in a compound with the R_f value of cysteine, indicating the presence of cystine, some of which may

have been formed from cysteine during the preparation of the extracts. A slight increase in the acetylcysteine-^{35}S peak also occurred after reduction with N-acetylcysteamine, suggesting that a mixed disulfide of acetylcysteine with cysteine had been present in the original extract. A small ^{35}S peak remained at the origin after treatment of the liver extracts with N-acetylcysteamine. Thus, some organic and/or inorganic sulfates may

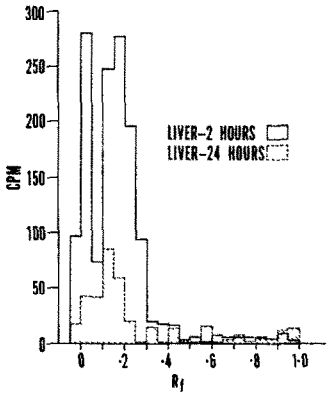

FIG. 2. Compounds containing ^{35}S, in the livers of rats receiving an oral dose of acetylcysteine-^{35}S. Ethanol extracts of the livers were prepared and chromatographed by single-dimensional paper chromatography as described under Methods. The area under the solid line represents data from animals sacrificed 2 hr after receiving acetylcysteine-^{35}S, and the area under the dotted line represents data from animals sacrificed after 24 hr.

also have been present, since these compounds are not reduced by N-acetylcysteamine. The results indicate that cysteine and cystine are the major metabolites of acetylcysteine in the liver 2 hr after its oral administration.

Contrary to the results obtained with liver, chromatograms of lung were found to contain 1/3 to 1/4 of the lung radioactivity in the acetylcysteine fraction. Appreciable quantities of cysteine also appeared to be present in the lung.

Extracts of spleen chromatographed on paper showed essentially no radioactivity, indicating that almost none of the nonprotein organic metabolites of acetylcysteine remained in that tissue 2 hr after the administration of acetylcysteine-^{35}S.

Metabolites of acetylcysteine-^{35}S in urine

Five rats given acetylcysteine-^{35}S were kept in metabolism cages and their urine collected for 24 hr. The urine samples were subjected to paper chromatographic separation, and the results are shown in Fig. 3. Most of the radioactivity was concentrated near the origin and could represent any of a number of substances such as inorganic sulfate (0·00), cystine (0·03), cysteic acid (0·04), oxidized glutathione (0·02), reduced glutathione (0·13), taurine (0·15), and mixed disulfides.

Treatment of this urine with the reducing agent N-acetylcysteamine resulted in essentially no change in the major peak and only a small increase in the peak corresponding to acetylcysteine (R_f 0·69 in this experiment). The major peak, being nonreducible by N-acetylcysteamine, would thus consist of cysteic acid or inorganic sulfate. Although these two components were not separated, the results support those previously obtained by a gravimetric procedure,[7] which indicated that the sulfur in these

urine samples was present almost entirely as inorganic sulfate. The gravimetric procedure does not measure cysteic acid; thus, cysteic acid could be present only in negligible quantities. The slight increase in acetylcysteine-^{35}S after reduction of the sample is probably due to very small quantities of mixed disulfides present in the original urine.

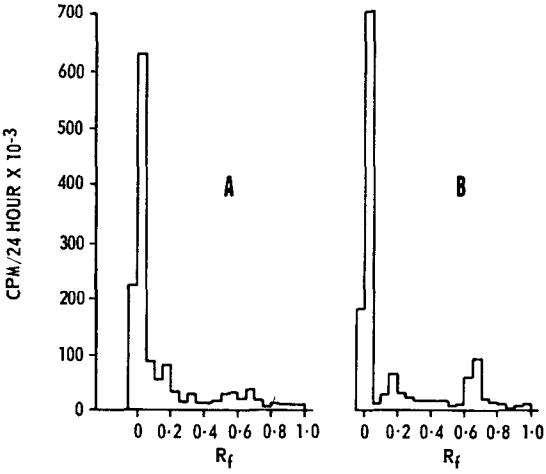

FIG. 3. Compounds containing ^{35}S, in the urine of rats during the 24-hr period following the administration of an oral dose of acetylcysteine-^{35}S. The diluted urine was chromatographed by one-dimensional chromatography as described under Methods. Graph A represents the distribution obtained with untreated urine; Graph B is the distribution following treatment of the urine with N-acetylcysteamine.

The cysteine (R_f 0·24) and reduced glutathione (R_f 0·13) peaks were not increased by treating the urine with the reducing agent, indicating that the disulfide form of these compounds was not present in the original specimen. Thus, there is no evidence to suggest that the urinary excretion of cysteine and glutathione are appreciably increased by the oral administration of acetylcysteine. The ^{35}S peak with an R_f of 0·15 is probably taurine.

Tissue distribution and excretion of sulfhydryl compounds after the administration of acetylcysteine and cysteine

The functionality of many enzyme systems, both in man and animals, may be impaired by high levels of sulfhydryl compounds. Consequently, the effect of large doses of acetylcysteine, as well as cysteine, on the sulfhydryl levels of blood and tissues, and on the rate of sulfhydryl excretion, was studied in the rat and dog.

Blood nonprotein (free) sulfhydryl levels increased by 35 per cent 1 hr after the oral administration of L-cysteine to rats at a dose of 1650 μmoles (200 mg/kg) (Table 2); but this increase was not statistically significant. Animals sacrificed 4 hr after treatment had blood levels similar to those of the controls.

The sulfhydryl level in lung doubled within 1 hr after the administration of L-cysteine ($P < 0{\cdot}01$), but returned to control levels by the fourth hour. In muscle, the changes were variable and not signifiicantly different from the pre-treatment level.

The sulfhydryl levels appeared to decrease in liver and kidney and to increase in spleen during the first 2 hr after the administration of cysteine; however, the values for these tissues were obtained from only two animals at each time interval, and therefore must be accepted with reservation.

TABLE 2. BLOOD AND TISSUE NON-PROTEIN SULFHYDRYL LEVELS OF RATS RECEIVING A SINGLE ORAL DOSE OF L-CYSTEINE*

Tissue	Sulfhydryl concentration				
	Control	1 hr	2 hr	4 hr	24 hr
			(μmoles/g tissue)		
Blood	1·7 ± 0·3	2·3 ± 0·2	2·1 ± 0·1	1·7 ± 0·1	2·1 ± 0·1
Lung	1·1 ± 0·1	2·2* ± 0·1	2·0* ± 0·1	0·9 ± 0·2	1·7 ± 0·1
Muscle	0·8 ± 0·2	1·1 ± 0·1	0·4 ± 0·1	1·3 ± 0·1	0·4 ± 0·1
Liver	2·8 ± 0·2	2·9	1·2	2·9	2·1
Kidney	2·3 ± 0·6	1·1	1·0	2·2	1·6
Spleen	5·0 ± 0·3	5·7	6·5	4·7	3·6

* The dose was 1650 μmoles/kg body weight. Values are expressed per grams wet weight of tissue or per milliliter blood (± S.E.). An asterisk indicates $P < 0.01$ compared to the control value. Where standard errors are not given the data represent the average values from two animals.

TABLE 3. BLOOD AND TISSUE NONPROTEIN SULFHYDRYL LEVELS OF RATS RECEIVING A SINGLE ORAL DOSE OF ACETYLCYSTEINE*

Tissues	Sulfhydryl concentration				
	Control	1 hr	2 hr	4 hr	24 hr
			(μmoles/g tissue)		
Blood	1·7 ± 0·3	1·8 ± 0·1	1·9 ± 0·1	1·7 ± 0·1	1·2 ± 0·2
Lung	1·1 ± 0·1	1·3 ± 0·2	1·3 ± 0·2	1·4 ± 0·1	1·4 ± 0·1
Muscle	0·8 ± 0·2	1·0 ± 0·2	0·8 ± 0·2	1·1 ± 0·2	0·8 ± 0·1
Liver	2·8 ± 0·2	3·7 ± 0·6	3·0 ± 1·1	3·7 ± 1·2	4·0 ± 0·1
Kidney	2·3 ± 0·6	1·0 ± 0·6	1·9 ± 0·3	1·2 ± 0·4	1·1 ± 0·3
Spleen	5·0 ± 0·3	5·4	4·8	4·7	3·2
Adrenal	3·0	5·0	4·0	8·2	4·6

* The dose was 1950 μmoles/kg body weight. Values are expressed per gram wet weight tissue or per milliliter blood (± S.E.). The values for spleen (except for the control) are the average values from two animals. The adrenal values were obtained by a single analysis of a composite sample of ten adrenals from five animals.

When acetylcysteine was administered at the same dose level as cysteine, only a slight increase in the blood nonprotein sulfhydryl levels was observed (Table 3). In lung, muscle, and liver, the sulfhydryl level also appeared to increase slightly. The nonprotein sulfhydryl level in kidney appeared to fluctuate widely, and the changes observed after treatment were not significantly different from the pretreatment level. No changes in sulfhydryl content occurred in spleen. In the adrenal the sulfhydryl level appeared to increase; however, the values were based on a composite sample from five animals, and therefore the changes are not meaningful.

Acetylcysteine was also administered by stomach tube to rats for 68 days at dose levels of 250 and 500 mg/kg per day, corresponding to 17·5 and 35 g acetylcysteine/day for a 70-kg man. It was found that the total non-protein sulfhydryl levels of blood plasma, lung, liver, and kidney were essentially the same in the treated animals as in the controls.

The blood levels of nonprotein sulfhydryl in dogs which received an oral dose (1650 μmoles/kg) of L-cysteine or acetylcysteine are presented in Table 4. The sulfhydryl level was 14 per cent higher (not a statistically significant difference) than the pretreatment level 30 min after the administration of L-cysteine, and had returned to the

TABLE 4. THE SULFHYDRYL LEVEL IN THE BLOOD OF DOGS RECEIVING AN ORAL DOSE OF L-CYSTEINE AND ACETYLCYSTEINE

Treatment	Dose of SH (μmoles/kg)	Blood sulfhydryl levels (μmoles/100 ml)			
		Control	30 min	60 min	120 min
Control (4),* 0·9% NaCl		131 ± 4†	117 ± 5	120 ± 6	122 ± 8
L-Cysteine (4)	1650	114 ± 8	130 ± 18	118 ± 13	125 ± 14
Acetylcysteine (6)	1650	106 ± 8	123 ± 4	128 ± 7	133* ± 7

* The figures in parenthese indicate the number of animals in each group.
† Standard error of the mean. An asterisk indicates $P < 0.05$ in comparison with the pretreatment value.

TABLE 5. THE URINARY EXCRETION OF SULFHYDRYL GROUPS BY RATS RECEIVING A SINGLE ORAL DOSE OF L-CYSTEINE AND ACETYLCYSTEINE

Compound administered	Urinary sulfhydryl (μmoles/kg/24 hr)	
	Rat	Dog
Control, 0·9% NaCl	46* ± 4 (7)	4 ± 0·6 (4)
L-Cysteine, 1650 μmoles/kg	54 ± 2 (7)	14 ± 3* (4)
Acetylcysteine, 1650 μmoles/kg	58 ± 7 (7)	28 ± 4** (6)

* Mean ± S.E. The figures in parentheses indicate the number of animals in each group. Asterisks indicate that the value is significantly different from the control value: * $P < 0.05$, ** $P < 0.01$.

control level in 60 min. In the case of acetylcysteine, the increase in sulfhydryl level was of longer duration, and 2 hr after the compound was administered the level was still 29% higher ($P < 0.05$) than the control.

When the same dose of acetylcysteine was given intravenously to three dogs, the blood sulfhydryl concentrations were almost double the pretreatment level at 15 and 30 min after administration. By the end of 2 hr the concentrations had returned to pretreatment levels.

The urinary excretion of sulfhydryl compounds was not significantly increased after the oral administration of acetylcysteine or L-cysteine to rats (Table 5). In addition,

after the administration of acetylcysteine to rats for 68 days at levels of 250 and 500 mg/kg per day, the sulfhydryl excretion was similar to that following one dose. In dogs (Table 5), there was a significant increase in urinary sulfhydryl levels after the oral administration of both acetylcysteine and L-cysteine. However, with both compounds the amount of sulfhydryl excreted was only 1·0 to 1·5 per cent of the dose. After the intravenous administration of acetylcysteine (1650 μmoles/kg) to three dogs, approximately 3·5 per cent of the dose was excreted in the urine.

Measurements were also made of the retention of sulfur after the oral administration of cysteine and acetylcysteine to rats and dogs. The quantities of total sulfur excreted in the urine in 24 hr by rats receiving an oral dose of 1650 μmoles (200 mg) cysteine/kg or an equivalent amount of acetylcysteine were similar, representing 34 and 38 per cent respectively, of the amount given. However, in dogs, 71 per cent of the acetylcysteine sulfur and only 20 per cent of the cysteine sulfur was excreted in the urine within 24 hr. With both compounds, most of the sulfur excreted was in the form of inorganic sulfate.

DISCUSSION

Cavallini *et al.*[18] recently identified the major metabolites of L-cystine in extracts of tissue and blood of rats injected intravenously with DL-cysteine-^{35}S. In extracts of liver obtained 2 hr after the injection of DL-cystine-^{35}S, only taurine and hypotaurine could be identified.

In the present study, 2 hr after the oral administration of acetylcysteine-^{35}S, acetylcysteine was no longer found in liver; cysteine-^{35}S and cystine-^{35}S were the major metabolites present. Mixed disulfides of acetylcysteine, as well as taurine, were probably also present, but no attempt was made to identify these compounds further, since it has been well established that taurine is a metabolic degradation product of L-cysteine, and mixed disulfides may have resulted during the preparation of the samples. No effort was made to identify the small amounts of glutathione which were probably formed from acetylcysteine-^{35}S (and cysteine-^{35}S), since it has already been shown that there is an increase in hepatic glutathione following the feeding of cysteine to rats.[19]

The major urinary excretory product of acetylcysteine metabolism revealed by the present study was inorganic sulfate, as measured chemically. The experiment with acetylcysteine-^{35}S also indicated that sulfate was the primary excretory product, but that, in addition, some taurine was probably present. Pirie and Hele[20] reported that when acetylcysteine was fed to dogs, 48 per cent of the ingested sulfur was excreted as sulfate and 32 per cent as neutral sulfur within 48 hr, whereas with cysteine, 70 per cent was excreted as sulfate and only 4 per cent was neutral sulfur. In the present experiment, whether cysteine or acetylcysteine was administered to dogs, almost all the sulfur was excreted as sulfate.

The excretion of total sulfur in the urine of rats was essentially the same whether the animals recieved acetylcysteine or L-cysteine. However, a considerably larger quantity of urinary sulfur was excreted by dogs receiving acetylcysteine than by those given L-cysteine, suggesting that in dogs the N-acetyl derivatives was more rapidly metabolized than cysteine itself. Pirie and Hele[20] have reported that the percentage of orally administered acetylcysteine or cysteine sulfur excreted by dogs in 48 hr is approximately the same, although the percentage excreted as sulfate is somewhat

different. It is likely that the retention of sulfur, after the ingestion of sulfur-containing amino acids, varies widely and is influenced by the state of nutrition of the animals.

In the present work the urinary excretion of sulfhydryl groups under fasting conditions was found to be quite low, and after the oral administration of relatively large doses of L-cysteine or acetylcysteine the excretion above the fasting level was only about 1 per cent of the dose. Similar results were obtained by Eldjarn and Nygaard,[21] who administered cysteamine-^{35}S and cystamine-^{35}S to rats and found only 1 per cent of the dose was excreted unchanged in the urine.

The blood sulfhydryl levels of both rats and dogs were increased only slightly after they had been fed L-cysteine or acetylcysteine. In the dog the increase with acetylcysteine (but not with cysteine) was statistically significant. In this respect, Kirnberger[22] did not observe an increase in blood sulfhydryl levels after the oral administration of L-cysteine to rats for 28 days. Also, Eldjarn and Pihl[23] reported that cystamine and cysteamine administered to mice rapidly became incorporated into the blood proteins, apparently by a sulfhydryl–disulfide exchange reaction.

Nonprotein sulfhydryl levels in the tissues studied were generally changed very little after the administration of either of the thiol compounds to rats. Only in lung, after treatment of the animals with L-cysteine, was the level of sulfhydryl significantly increased. Similar to the results obtained in this study, with both L-cysteine and acetylcysteine, Eden *et al.*[19] could find no accumulation of cysteine in the liver after the compound was administered to rats at a dose of 30 mg per animal. In order to demonstrate an increase in liver cysteine, these investigators found it necessary to give the compound intraperitoneally at a level of 150 mg per animal.

Kirnberger[22] found a 42 per cent increase in the liver sulfhydryl levels of rats fed a normal diet supplemented with 0·25% L-cysteine for 28 days; and Garattini and Mussini[24] found that feeding cysteine to rats, either as a single dose or over a period of time, considerably augmented the sulfhydryl content of liver. However, in the present study, the administration of acetylcysteine daily for 68 days at a dose of 500 mg/kg produced no significant change in the tissue nonprotein sulfhydryl levels. It has also been observed that the administration of acetylcysteine to rats, 1600 mg/kg per day for 6 weeks, produces no evidence of toxicity or pathological changes in tissues.*

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