Biochemical Pharmacology, 1963, Vol. 12, pp. 465–474. Pergamon Press Ltd., Printed in Great Britain.

THE METABOLISM OF ^{35}S-(1,2-DICHLOROVINYL)-L-CYSTEINE IN THE RAT*

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(*Received* 5 *October* 1962; *accepted* 28 *December* 1962)

Abstract—The ^{35}S-labeled compound has been used to study the metabolism of S-dichlorovinyl)-L-cysteine in the rat. The major radioactive components in the urine were inorganic sulfate, one unidentified component, S-(dichlorovinyl)-L-cysteine, and its N-acetyl derivative, the last two predominating soon after administration, but inorganic sulfate being later the only detectable metabolite. The highest concentration of radioactivity was found in the kidney; heart and liver contained about one-quarter as much and other organs less than one-tenth per unit of dry weight. The major portion of the radioactivity was associated with the protein fraction of kidney, liver, and heart. In blood plasma as much as 80% of the radioactivity became bound in nondialyzable form.

IN THEIR search for the toxic factor in trichloroethylene-extracted soybean oil meal, which induces aplastic anemia in cattle,[1] McKinney *et al.*[2] synthesized a cysteine derivative which, when fed in small amounts, produces this blood dyscrasia in calves. Based on the method of synthesis and other evidence they identified their product as *S*-(*trans*-1,2-dichlorovinyl)-L-cysteine (DCVC). In this laboratory the extremely high toxicity of DCVC for calves has been amply confirmed,[3] one intravenous injection of as little as 2 mg per kg body weight being sufficient to induce fatal aplastic anemia.§ Among other species tested, the chicken§ and the rat are much more resistant. In the young rat for instance, daily intraperitoneal injections of 20 to 25 mg DCVC per kg, extending over several weeks, was required[4] to produce severe depression of growth and eventual death; aplastic anemia is not produced in this species. These striking qualitative and quantitative differences in response to DCVC suggest that the metabolism of this compound in the calf is different from that in the rat.

To study these and other properties of DCVC, we labeled the compound with ^{35}S[5] and with ^{14}C.[6] This paper summarizes our observations|| on the metabolism of ^{35}S-DCVC in the rat.

* Paper No. 4900, Scientific Journal Series, Minnesota Agricultural Experiment Station. Supported in part by funds from the U.S. Atomic Energy Commission, Contract AT(11-1)-364.

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|| Reported in part: *Fed. Proc.* **19**, Part I, 7 (1960).

EXPERIMENTAL PROCEDURES

Animals. Two groups of six male rats from a hooded strain maintained in this laboratory were used. Each rat was placed singly in a glass metabolism cage where, except while respiratory gases were being collected, it had access to water and to a ration of natural products. The experiments were started after the rats had become used to this environment and maintained constant weight.

Administration of ^{35}S-DCVC. In three separate experiments one member of each pair of rats recceived ^{35}S-DCVC by stomach tube, the other by intraperitoneal injection. In a second group of six rats intraperitoneal injections were made. The desired amount of ^{35}S-DCVC was dissolved in water and administered with a graduated 1-ml syringe. After injection the syringe, needle, and stomach tube were flushed with about 0·3 ml of water before being withdrawn from the animal. The amount of DCVC used, its radioactivity, and the duration of the experiments are listed in Table 1. The amount of DCVC administered and the internal radiation were below toxic levels.

Collection of specimens. Blood (20 mm^3) was collected periodically for counting, from the cut end of the tail of each rat. Crystalline oxyhemoglobin was isolated by the method of Drabkin[7] from the erythrocytes of the terminal blood specimen of two rats. The plasma from the same blood specimens was dialyzed at 4° for 3 days against eight changes of 20 volumes of 0·9% NaCl.

Except for the first two days, when morning and evening collections were kept separate, the daily output of urine was pooled, filtered, and diluted to 25 ml with water. At sacrifice the bladder urine was collected with a syringe. The specimens were stored at 5°, with a drop of chloroform, until counted. Feces were collected twice daily, combined, dried *in vacuo* at 52° to constant weight, ground, and oxidized as described below. The expired gases were collected during intermittent 2-hr periods by drawing air from the metabolism cages through a 0·1% ethanolic solution of NaOH. The radioactivity of aliquots was measured with a liquid scintillation spectrometer. At the end of the experiments the rats were exsanguinated; specimens of tissues were removed, dried *in vacuo* to constant weight at 52°, ground, and oxidized for analysis as described below.

Tissue fractionation. A sample from the liver, both kidneys, and the heart of each rat of the second group (Table 1) was minced with a razor blade and homogenized in 5 ml cold water. The radioactivity of an aliquot of the homogenate was counted and, after cooling in an ice bath, one-fourth volume of cold 2·6 M perchloric acid (PCA) was added to the remainder and mixed. After 1 hr the precipitate was centrifuged and washed twice with 1 volume of cold 0·4 M PCA. The supernatant and washings were combined, neutralized with cold N KOH and the $KClO_4$ removed, washed with 10 ml of cold water, and the supernatant and washings were combined. The acid-insoluble residue was extracted three times at 40 to 50° with 7 ml of ethanol : ether (3 : 1) to give the lipid fraction. The lipid-free residue was suspended in 10 ml of 10% NaCl (pH 7·6) and heated for 1 hr at 100°. The protein was centrifuged and washed twice with 5 ml 10% NaCl. The NaCl solutions were combined to give the nucleic acid fraction. The protein residue was dried over P_2O_5 *in vacuo*, and samples were dissolved in formamide for counting.

Determination of radioactivity. All specimens except tissues and feces were counted with a Nuclear-Chicago, model D-47, gas-flow detector equipped with a Micromil window, under a gas mixture of 98·7% helium and 1·3% butane. The efficiency of this counting system was 30·78%. Aliquots of solutions were dried in 3-cm diameter aluminum planchets under an infrared lamp. If the residue in the planchet weighed more than 1 mg, correction was made for self-absorption as determined with a $^{35}SO_4$ standard and known weights of solids. Tissues and feces were dried at 52° *in vacuo* for 72 hr to constant weight. Their radioactivity was determined after oxidizing 50 mg or less with an equal weight of ammonium nitrate in a 500-ml Schöniger combustion flask in the presence of 1 ml N NaOH and 10 ml bromine water.[8] The solution was then evaporated to about 1 ml, transferred to a 5-ml volumetric flask and diluted to volume. A 2·70-ml aliquot was pipetted into a 20-ml counting vial which contained 1·0 g fluorescence-grade anthracene and 0·3 ml of 1 : 100 Triton GR-5 according to the procedure of Steinberg.[9] The contents were mixed, allowed to settle, and counted in a Packard Tri-Carb liquid scintillation spectrometer* at about 2°, with a photomultiplier voltage of 850 V, and at a window setting of 50–100. The efficiency of this system was 12·69%. All determinations of radioactivity were corrected for decay using the half-life value of 86·35 days[10] and the results calculated as disintegrations per minute (DPM). Electrophoretograms were examined by radioautography.

Paper chromatography. Paper chromatograms were usually made with Whatman 1 or 3MM paper and 1-butanol : acetic acid : water (12 : 3 : 5 vol/vol) as the descending developing solvent which will be referred to as the BAW solvent.

Synthesis of N-acetyl-S-(dichlorovinyl)-L-cysteine. Into a 50-ml Erlenmeyer flask was placed 432 mg DCVC, a magnetic stirring bar, 25 ml distilled water, and 2 drops of 1% ethanolic solution of thymolphthalein indicator. The flask was immersed in an ice bath and the DCVC dissolved with ice-cold 10% NaOH with stirring. Cold acetic anhydride was added by drops immediately, with stirring, while the pH was kept alkaline to thymolphthalein with 10% NaOH. The progress of the reaction was followed by periodically spotting some of the reaction mixture on filter paper, and drying and spraying with ninhydrin. The buffer effect which develops as the acetylation proceeds can also be used as a guide for the addition of base required. After the acetylation was completed, concentrated HCl was added, with cooling, until the solution was strongly acid, and the solution was kept cold to crystallize. The yield of crude product was 324 mg, 63%, m.p. 102° (corr.). The crude product was dissolved in absolute ethanol, decolorized with Darco G-60, and recrystallized by adding water and cooling. The yield was 243 mg, 47%, m.p. 108 to 109° (corr.), $[\alpha]_D^{24°}$: + 102 (0·33% in N NaOH). *Anal.*† Calculated for $C_7H_9Cl_2NO_3S$: C, 32·57; H, 3·51; N, 5·43; S, 12·42. Found: C, 32·66; H, 3·60; N, 5·30; S, 12·92. Colorimetric analysis with ninhydrin[11] showed no evidence of free amino nitrogen. Hydrolysis of the product with kidney acylase‡ released free amino groups, indicating that the product was indeed an acylated amino acid.

* We are indebted to Dr. F. A. Spurrell, College of Veterinary Medicine, for the use of this instrument.

† Performed by the Micro Analytical Laboratory, Dept. of Organic Chemistry, Univ. of Minnesota.

‡ Purchased from Armour Laboratories, Chicago, Ill.

Synthesis of N-acetyl-S-(dichlorovinyl)-L-cysteine-m-toluidide. This was made by an adaptation of the method of Reed *et al.*[12] To *N*-acetyl-DCVC (50 mg), dissolved in 0·14 ml N NaOH, were added redistilled *m*-toluidine (0·04 ml) and 0·08 ml of 0·2 M citrate buffer, pH 5·0, and the mixture was warmed to dissolve as much of the amine as possible. After cooling to room temperature L-cysteine hydrochloride (3 mg) in 1·0 ml of the citrate buffer was added, followed by 0·16 ml papain* extract (75 mg extracted with 0·7 ml water at room temperature for 15 min) and 0·62 ml of the citrate buffer. The mixture was shaken and kept at 37° for 3 days during which time the *m*-toluidide crystallized. The product was centrifuged, separated from the supernatant, dissolved in absolute ethanol, treated with charcoal (Darco G-60), recrystallized by addition of water. The yield was 31 mg, 47%, m.p. 150 to 151° (corr.). *Anal.* Calculated for $C_{14}H_{12}Cl_2N_2O_2S$: C, 48·42; H, 4·64; N, 8·07; S, 9·23. Found: C, 48·50; H, 4·81; N, 7·68; S, 10·01.

RESULTS

Excretion of ^{35}S

Among rats 1–6, Table 1, the mean radioactivity of the expired gases, 100, 33, and 16 DPM/hr during the first 3 days, respectively, was insignificant in comparison with the total amount administered.

TABLE 1. EXCRETION OF ^{35}S AFTER ADMINISTRATION OF ^{35}S-DCVC

	FIRST GROUP Rat number					
	1	2	3	4	5	6
Route of administration	i.p.	oral	i.p.	oral	i.p.	oral
Weight of rat when treated (g)	217	224	202	193	231	216
DCVC administered (mg)	2·56	2·56	2·65	2·65	2·67	2·67
Radioactivity administered DPM × 10^{-7}	1·94	1·94	1·97	1·97	1·75	1·75
Sacrifice and tissue removal; days after administration	20	20	3	3	3	3
^{35}S excreted in urine, to sacrifice*	37·2	21·7	12·2†	40·3	53·0	16·8†
^{35}S excreted in urine after 30·5 hr*	33·8	13·8	10·7†	39·2	51·8	14·5†
^{35}S excreted in feces, to sacrifice*	5·2	6·6	3·8	2·1	7·5	4·9

	SECOND GROUP Mean of 6 rats
Route of administration	i.p.
DCVC administered (mg)	2·0
^{35}S excreted in urine during 30·5 hr*	56·1

* Per cent of administered dose.
† Slight mechanical loss of urine.

Table 1 presents a summary of the urinary and fecal excretion of ^{35}S. Large individual variations were encountered in the radioactivity excreted in the urine during the first 30 hr. Only about 5% of the total activity appeared in the feces, indicating efficient absorption of the tracer. Judged from the results obtained with calves,[13] the bile can

* Purchased from Mann Research Laboratories, New York, N.Y.

be the source of most of the radioactivity in the feces. The rats, which were kept for 20 days, continued to excrete ^{35}S in the urine and feces throughout this time.[5]

^{35}S in blood and tissues

Extrapolation from the great initial increase and subsequent rapid decline of the radioactivity of the whole blood shows that the highest activity of about 33,000 DPM/ml was reached in about 4 hr. In 6 hr the activity had decreased to about 10,000 DPM/ml after which time there was a more gradual decline to a mean of 1,700 DPM/ml in 72 hr. The distribution of the radioactivity in the blood of two rats, which had the lowest total activity in the blood, is shown in Table 2. The blood plasma contained

TABLE 2. RADIOACTIVITY OF TERMINAL BLOOD FRACTIONS

	Rat	
	5	6
	(DPM/ml)	
Whole blood	1,140	970
Plasma	710	650
Cells	325	244
Dialyzed plasma	440	535
Oxyhemoglobin (DPM/mg)*	10	22

* This activity was significantly above background at $P < 0{\cdot}01$.

over half the radioactivity, of which 60 to 80% was firmly bound in a nondialyzable form. The oxyhemoglobin also contained a small but significant amount of ^{35}S.

Among the various tissues the kidney had the highest radioactivity, as is shown in Table 3. Fractionation of liver, kidney, and heart, collected 72 hr after administration

TABLE 3. RELATIVE RADIOACTIVITY OF RAT TISSUES*

Kidney	100	Seminal vescicles	6·5
Heart	28·8	Spleen	6·3
Liver	26·4	Thymus	5·9
Cecal contents	11·6	Skeletal muscle	5·8
Bone marrow	7·7	Adrenals	5·4
Abdominal wall	7·1	Xiphoid cartilage	5·3
Turbinate bone	7·1	Lung	5·0
Small intestine	6·8	Testes	4·5
		Brain	3·7

* Using kidney as point of reference. The radioactivity in the kidney was on the average 330,000 DPM/g dry weight, 72 hr after administration of DCVC.

of DCVC-^{35}S, revealed that in these tissues from about 66 to 80% of the total radioactivity was associated with the protein fraction and only a relatively small proportion, 10 to 18%, was acid-soluble, as shown in Table 4. A considerable amount of radioactivity, of unknown nature, was associated with the lipid fraction; in the heart this amounted to 16% of the total, as much as occurred in the acid-soluble fraction and about twice the proportion found in the liver lipids. The association of radioactivity with the nucleic acid fraction, while small, may be of significance.

Nature of radioactive components in the urine—Number of metabolites

Radioactive components in the urine specimens were separated by chromatography with BAW solvent on Whatman 1 paper, and the radioactivity in 1-cm segments was measured. Typical chromatograms of rat urine, collected from rat 1, 24 and 48 hr after administration, are shown in Fig. 1. At least four different radioactive compounds were excreted in the first 24 hr; by the second day, however, only one of these could be detected in appreciable amounts. The identity of the four components and the relative amounts estimated from the proportion of the total radioactivity appearing in each fraction were: component 1: inorganic sulfate, 34%; component 2: unknown, 6%; component 3: DCVC, 18·5%; component 4: N-acetyl-DCVC, 41·5%. It must be emphasized that these estimates of the relative amounts of various components apply only to a specimen of urine which was collected 24 hr after intraperitoneal injection of DCVC.

TABLE 4. DISTRIBUTION OF ^{35}S IN RAT TISSUES

Fraction	Per cent of total radioactivity		
	Liver	Kidney	Heart
Acid-soluble	10·3	18·0	16·3
Lipid	7·3	10·3	16·0
Nucleic acid	2·0	4·5	3·7
Protein	80·2	67·4	64·9

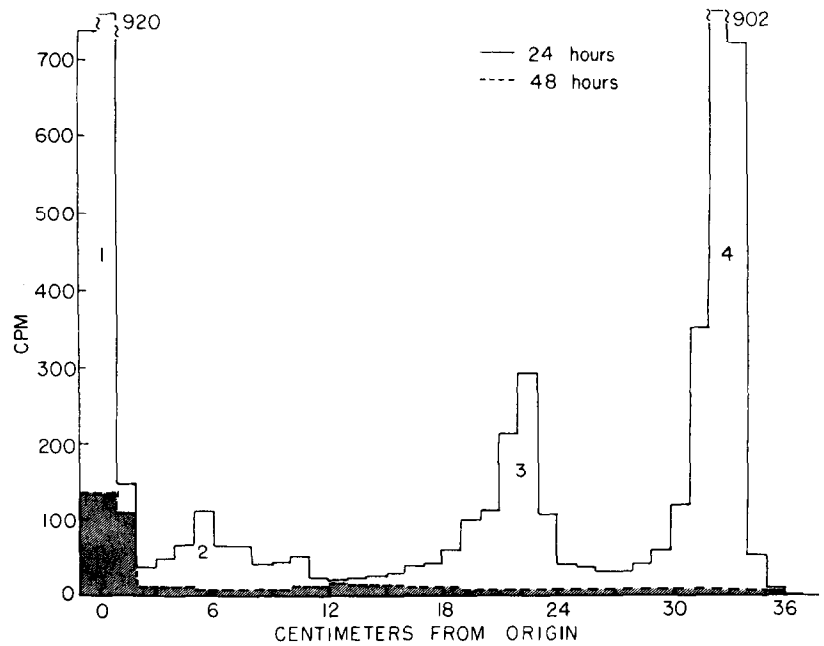

FIG. 1. Chromatogram of rat urine. Specimens representing excretion between 0 and 24 hr; and 24 to 48 hr after injection of ^{35}S-DCVC.

Electrophoresis of this urine specimen on Whatman 3 MM paper at 500 v with a pyridine : acetic acid : water buffer (1 : 10 : 89 vol/vol),[14] of pH 3·6, also revealed

three major and one minor component after radioautography. Electrophoresis in veronal–acetate buffer, pH 8·6, ionic strength 0·1,[15] also showed three major but two minor components upon radioautography.

Separation of a different specimen of the urine by ion-exchange chromatography on Dowex 50-X5[16] revealed three major and four minor radioactive components. The elution pattern of three minor components corresponded to components B, C, and E; and the fourth was between A and B of the elution pattern of calf urine.[16]

Identity of major components

Component 1: ^{35}*S-inorganic sulfate.* The identification of component 1 as inorganic sulfate was based upon the following evidence. (1) Chromatography: The Rf of a standard ^{35}S-sulfate solution on Whatman 1 paper, with BAW as developing solvent was 0·01, the same as that of component 1, located by scanning of radioactivity. (2) Electrophoresis: The mobility of component 1 upon electrophoresis on Whatman 3MM paper at 500 V with both pyridine : acetic acid : water buffer, pH 3·6,[14] or veronal–acetate buffer, pH 8·6,[15] was the same as that of a ^{35}S-sulfate standard as shown by radioautography; and the same as sodium sulfate at pH 8·6 located by the rhodizonic acid procedure of Burma.[17] Component 1 was radiochemically pure as far as detection by radioautography could determine. (3) Barium sulfate precipitation: Urine was acidified to pH 2 with HCl and extracted with 1-butanol. Carrier inorganic sulfate was added to the water eluate of component 1 from Whatman 3MM chromatograms of the 1-butanol-extracted urine, and barium sulfate was precipitated by addition in drops of 5% $BaCl_2$ over a period of 2 hr without shaking. The mixture was kept cold overnight and the $BaSO_4$ collected on a tared Whatman 52 paper, washed with acetone, dried *in vacuo* over P_2O_5 for 1 hr, weighed, and counted. The barium sulfate precipitate accounted for 89% of the radioactivity in the eluate. (4) Benzidine sulfate precipitation: To a portion of the same eluate used for the barium sulfate precipitation above, carrier inorganic sulfate was added and benzidine sulfate precipitated, according to the procedure of Hawkins and Young.[18] The precipitate was centrifuged, washed twice with absolute ethanol, dissolved in 88% formic acid, and an aliquot counted. The precipitate of benzidine sulfate contained 94% of the radioactivity present in the eluate.

Component 2: Unknown. Component 2 was not identified. However, by isotopic dilution analysis, it was shown not to be taurine or the sulfoxide of DCVC,[19] both of which have similar Rf values in the solvent system used.

Component 3: DCVC. Component 3 was identified as DCVC as follows: (1) Paper chromatography: When DCVC and rat urine were chromatographed side by side on Whatman 1 paper with BAW, the Rf of component 3 and of DCVC was the same. Furthermore, when DCVC was added to the urine a new ninhydrin-positive spot appeared which coincided with component 3; and when ^{35}S-DCVC was added, its radioactivity and that of component 3 were superimposed. The amount of DCVC on the chromatograms of rat urine was not sufficient to give a color with ninhydrin. (2) Paper electrophoresis: One of the radioactive compounds in urine had the same mobility as ^{35}S-DCVC upon electrophoresis at pH 8·6 in veronal–acetate buffer.[15] (3) Isotopic dilution analysis: When urine was acidified and extracted with 1-butanol, most of component 3 appeared in the butanol. After chromatography of the butanol

extract on Whatman 3MM paper with the BAW solvent, component 3 was eluted and DCVC added to an aliquot of the eluate. The ^{35}S-DCVC, crystallized from 50% ethanol, contained 81·5% of the activity of the eluate. The 2,4-dinitrophenyl derivative[19] of the isolated ^{35}S-DCVC contained 83·5% of the expected activity and was identical with an authentic specimen with respect to mixed melting point (160°), chromatography on Whatman 1 paper with *tert*-amyl alcohol-potassium acid phthalate (0·05 M, pH 6·0), and ultraviolet absorption spectrum.

Component 4: N-acetyl-DCVC. The possibility that N-acetyl-DCVC might be one of the urinary metabolites was considered because mercapturic acids are excreted when various *S*-arylcysteines such as *S*-phenylcysteine, *S*-(*p*-bromophenyl)-cysteine and *S*-benzylcysteine are administered to rats,[20] or when the cytotoxic alkylating agent, ethyl methanesulfonate, is fed to rats.[21] The identification of component 4 was contingent on the synthesis of the N-acetyl derivative of DCVC and the following evidence. (1) Paper chromatography: The Rf of synthetic N-acetyl-DCVC on Whatman 1 paper with BAW solvent and detected with the potassium dichromate-silver nitrate reagent of Knight and Young[22] was the same as that of component 4. (2) Paper electrophoresis: A component of rat urine and authentic N-acetyl-DCVC during electrophoresis on Whatman 3MM paper with veronal–acetate buffer, pH 8·6, at 500 V had the same mobility. (3) Hydrolysis with kidney acylase: One ml rat urine was incubated with 1 mg kidney acylase dissolved in 1 ml 0·1 M phosphate buffer, pH 7·0, at 37° for 48 hr in the presence of toluene. After incubation the solution was passed through an ultrafilter[23] to remove the protein, and then chromatographed on Whatman 1 paper with BAW solvent side by side with an equivalent amount of untreated urine. Counting of radioactivity showed that in the N-acetyl DCVC area it had decreased, whereas in the DCVC area it had increased, while remaining unchanged in the other two components. (4) Isotopic dilution analysis: A 1-butanol extract of urine was chromatographed on Whatman 3MM paper with BAW as developing solvent. Component 4 was eluted with warm absolute ethanol, filtered, and an aliquot counted. Synthetic N-acetyl-DCVC (50 mg) was added, dissolved, and then crystallized by the addition of water and cooling. The crystallized product contained 95% of the original activity and was converted to the *m*-toluidide, as previously outlined. The specific activity of the *m*-toluidide was 75% of the theoretical and was constant upon recrystallization (m.p. 150°).

TABLE 5. COMPOSITION OF URINE EXCRETED

Time after administration (hr)	Component				
	Sulfate	2	DCVC	N-acetyl-DCVC	Total ^{35}S excreted*
	Per cent of total activity excreted†				
1·75	9·2	2·1	4·7	83·9	25·7
6·25	40·9	12·7	10·9	35·5	36·3
12·50	61·2	2·5	8·6	27·9	45·8
23·75	100	0	0	0	49·4
30·50	100	0	0	0	50·9

* Per cent of total ^{35}S activity administered intraperitoneally, cumulative.
† Per cent distribution of radioactivity in urine among various major radioactive components.

Time course of excretion of urinary metabolites

Aliquots of the urine specimens excreted by the second group of rats (Table 1) were examined by paper chromatography on Whatman 1 paper with BAW as solvent and scanned for radioactivity. After administration of DCVC, the sooner the urine was excreted, the greater the percentage of N-acetyl-DCVC in the specimen; after 24 hr only sulfate was detected by the procedures used. The change in composition of the urine excreted with time for one rat is shown in Table 5. Other rats had a similar pattern of excretion, including those given DCVC orally.

DISCUSSION

In about 30 hr after the oral or intraperitoneal administration of DCVC, rats excreted on the average 56% (up to 69%) of the sulfur of which one-half to two-thirds could be accounted for by DCVC and its *N*-acetyl-derivative. Acetylation of DCVC in the rat occurs rapidly; over 80% of the ^{35}S excreted within about 100 min was in this form. Subsequently the proportion of the actylated derivative which was excreted became less, and it was replaced by inorganic sulfate as the major product of metabolism of sulfur in DCVC. Acetylation of DCVC may be an important mechanism through which the rat can protect itself against toxic effects of DCVC and it may account, in part at least, for the difference in susceptibility to DCVC which is so striking in the rat and the calf. In the urine of the latter, no evidence could be found for the presence of N-acetyl-DCVC after i.v. administration of a similar dose of DCVC (mg/kg).[13, 24]

Whether or not N-acetyl-DCVC is completely excreted and thus escapes metabolic degradation, or is subject to the same or different reactions, through which inorganic sulfate is formed from DCVC, is not known.

Kuchinskas demonstrated that when ^{35}S-methyl-L-cysteine is metabolized by the rat, ^{35}S-sulfate, ^{35}S-methylcysteine, N-acetyl-^{35}S-methylcysteine, and ^{35}S-methylcysteine sulfoxide are excreted in the urine.[25] DCVC appears to be handled in the same way except that no evidence was found for its oxidation to the sulfoxide by the rat. Binkley[26] demonstrated that *S*-methylcysteine was cleaved to methanethiol *in vitro* by a rat liver enzyme preparation and that some other *S*-alkylcysteine derivatives are also cleaved to the corresponding thiols. Armstrong and Lewis[27] showed that the same compounds were not able to serve as precursors of L-cysteine in young rats. If DCVC were cleaved in the rat to yield cysteine or one of its oxidation products, the urine of our rats should have contained both $^{35}SO_4$ and ^{35}S-taurine, inasmuch as both are formed from a common precursor, cysteine sulfinic acid,[28] and taurine is a normal component of rat urine.[29] In spite of careful search, including dilution analyses, no evidence for the excretion of radioactive taurine could be found in this work. It appears, therefore, that the metabolism of DCVC in the rat does not involve the formation of L-cysteine; more likely the molecule is cleaved with the formation of a *S*-dichlorovinyl moiety, which, in turn could give rise to inorganic sulfate or react with other components of biological systems.

The chemical nature of the radioactivity which was found in the tissues is not known. The occurrence of such "bound" radioactivity after administration of DCVC to an animal is not necessarily the result of binding of unaltered DCVC. In view of the rapid

metabolism of DCVC it is not unlikely that one or more of its fragments combines with proteins or other components of tissues and can thus initiate the chain of events through which the toxicity of DCVC manifests itself in various biological systems. These possibilities are now under investigation in this laboratory.

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