REFERENCES

1. K. K. Gauri and G. Malorny, *Naunyn-Schmiedeberg's Arch. Pharmak. exp. Path.* **257**, 21 (1967).
2. M. Swierkowski and D. Shugar, *J. med. Chem.* **12**, 533 (1969).
3. E. De Clercq and D. Shugar, *Biochem. Pharmac.* **24**, 1073 (1975).
4. Y.-C. Cheng, B. A. Domin, R. A. Sharma and M. Bobek, *Antimicrob. Agents Chemother.* **10**, 119 (1976).
5. E. De Clercq, J. Descamps and D. Shugar, *Antimicrob. Agents Chemother.* **13**, 545 (1978).
6. P. F. Torrence, J. W. Spencer, A. M. Bobst, J. Descamps and E. De Clercq, *J. med. Chem.* **21**, 228 (1978).
7. E. De Clercq, J. Descamps, P. F. Torrence, E. Krajewska and D. Shugar, *Current Chemotherapy*, pp. 352–354. American Society for Microbiology, Washington DC (1978).
8. E. De Clercq, J. Descamps, P. De Somer, P. J. Barr, A. S. Jones and R. T. Walker, *Proc. natn. Acad. Sci. U.S.A.* **76**, 2947 (1979).
9. E. De Clercq, J. Descamps, G. Verhelst, A. S. Jones and R. T. Walker, *Current Chemotherapy and Infectious Disease*, pp. 1372–1374. American Society for Microbiology, Washington DC (1980).
10. E. De Clercq, E. Krajewska, J. Descamps and P. F. Torrence, *Molec. Pharmac.* **13**, 980 (1977).
11. E. De Clercq, J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence and D. Shugar, *J. Infect. Dis.* **141**, 563 (1980).
12. J. A. Fyfe, P. M. Keller, P. A. Furman, R. L. Miller and G. B. Elion, *J. biol. Chem.* **253**, 8721 (1978).
13. J. Giziewicz and D. Shugar, *Acta Biochim. Polon.* **22**, 87 (1975).
14. Y.-C. Cheng, *Ann. N.Y. Acad. Sci.* **284**, 594 (1977).
15. M. S. Chen and W. H. Prusoff, *J. biol. Chem.* **253**, 1325 (1978).
16. M. S. Chen, W. P. Summers, J. Walker, W. C. Summers and W. H. Prusoff, *J. Virol.* **30**, 942 (1979).
17. W. Plunkett, L. Lapi, P. J. Ortiz and S. S. Cohen, *Proc. natn. Acad. Sci. U.S.A.* **71**, 73 (1974).
18. S. S. Cohen, *Biochem. Pharmac.* **24**, 1929 (1975).

Biochemical Pharmacology, Vol. 29, pp. 2885–2887.

0006-2952/80/1015–2885 $02.00/0

Thiol *S*-methyltransferase: suggested role in detoxication of intestinal hydrogen sulfide

(*Received* 11 *September* 1979; *accepted* 28 *April* 1980)

Hydrogen sulfide is highly toxic to higher animals, causing death in the same range of atmospheric concentrations as does hydrogen cyanide [1]. Unlike cyanide, however, hydrogen sulfide is sufficiently common in biological systems to pose a threat to the organism. The anaerobic metabolism of many bacterial species common to the colon and periodontal spaces, acting on proteins and other sulfur-containing compounds, is known to liberate hydrogen sulfide [2–4]. Indeed, H_2S has been shown to form a small but offensive component in human flatus [5] and halitosis [6]. Direct ingestion of foods subjected to bacterial spoilage [7, 8] or of mineral sulfides dissolved in water offer other routes of exposure, as does dissolved H_2S that is found in fermented beverages [9].

Since the alimentary tract is the major portal of entry in each instance, it follows that the gut mucosa, in particular, may benefit from a mechanism for the detoxication of hydrogen sulfide. We propose this function for thiol *S*-methyltransferase, an enzyme present in the microsomes of many mammalian tissues [10]. The enzyme catalyzes

$$\text{RSH} + \textit{S}\text{-adenosyl-L-methionine} \rightarrow \text{RS–CH}_3 + \textit{S}\text{-adenosyl-L-homocysteine} \quad (1)$$

Reaction 1 in which R may be one of a large number of diverse groups as represented by thiophenols [11], aliphatic mercaptans [10–12], thiopyrimidines and thiopurines [13–16], dithiocarbamates [17, 18] and H_2S [10, 11]. We have described elsewhere the solubilization and purification to homogeneity of the enzyme from rat liver using 2-thioacetanilide as the assay substrate [11]. Here we examine the activity of the enzyme with specific attention to H_2S and methanethiol as potential physiologic substrates and to the tissue distribution of the enzyme in the rat. These data are in accord with a role for thiol *S*-methyltransferase in the detoxication of intestinal hydrogen sulfide.

Solutions of sodium sulfide and methanethiol were prepared in 20 mM KOH containing 10 μM EDTA; the concentration of each substrate was confirmed with *N,N*-dimethyl-*p*-phenylenediamine for H_2S [19] and by the method of Ellman [20] for methanethiol. *S*-Adenosyl-L-[methyl-^{3}H]methionine (9–15 Ci/mmole) was obtained from Amersham/Searle (Arlington Heights, IL) and diluted, when necessary, in 10 mM sulfuric acid.

The standard assay for thiol methyltransferase activity with 2-thioacetanalide and *S*-adenosylmethione has been described [11]. Because of the volatility of H_2S and its products at physiologic pH values, however, assays for these compounds required modification to minimize substrate or product losses. Small test tubes with an inside diameter of 4 mm (Kimax No. 45048) were cut to 30 mm in length so that they would hold slightly more than 0.4 ml. Hydrogen sulfide was added to each tube as an alkaline solution of sodium sulfide and was followed by a mixture containing enzyme and radiolabeled *S*-adenosylmethionine in a Triton X-100 containing buffer. The assay mixture contained, in a final volume of 0.4 ml, 0.25 μM *S*-adenosyl-[methyl-^{3}H]methionine, 0.2 mM dithiothreitol, 0.5% Triton X-100, 0.1 M potassium phosphate (pH 7.9), 1 mM EDTA and 10 mM sodium sulfide. The amount of enzyme was chosen to produce between 1 and 5 per cent conversion of added *S*-adenosylmethionine. Under these conditions, the reaction was a linear function of the amount of enzyme added.

After mixing the contents by suction and release with a 200 μl Eppendorf pipette, the tubes were sealed with Parafilm and incubated in a water bath at the desired temperature. Assays were terminated by transferring the

tube contents to a test tube containing 1 ml of ice-cold 1 N HCl. After addition of 6 ml of toluene, the tubes were stoppered and shaken vigorously for 10 sec. Following brief centrifugation, 5 ml of the toluene phase, containing the extracted radioactive product, was counted by liquid scintillation [11]. For the study of kinetic parameters, only the steady-state rate of the enzyme [11] was examined. This was accomplished by incubating duplicate assay mixtures at 30° and terminating the reaction at 2 and 12 min, respectively; the difference in product formation was taken as the steady-state rate for subsequent calculations.

The partition coefficient for hydrogen sulfide between air and a saline buffer [140 mM NaCl, 10 mM potassium phosphate (pH 7.4) and 0.1 mM EDTA] was determined by adding 50 ml of the buffer, 10 μM in H_2S, to a tightly stoppered 500-ml flask that was incubated at 37° and shaken periodically for 1 hr; the liquid phase was assayed for H_2S by the method of Siegel [19]. In each trial, approximately 50 per cent of the measurable H_2S was lost. Inclusion of 0.1 mM dithiothreitol in the buffer to minimize oxidation of hydrogen sulfide had no effect on this value and no loss occurred if the incubation was performed in a completely full and stoppered 50-ml flask. From these data we estimated a partition coefficient of 10 in favor of the liquid phase.

Table 1. Distribution of thiol methyltransferase in homogenates of rat tissue*

Tissue	Specific activity† (fmoles·mg^{-1}·min^{-1})
Cecal mucosa	127 ± 10
Colonic mucosa	125 ± 4
Liver	88 ± 12
Lung	64 ± 11
Kidney	63 ± 6
Jejunal mucosa	34 ± 3
Ileal mucosa	33 ± 5
Duodenal mucosa	31 ± 6
Stomach mucosa	27 ± 5
Ileal muscularis	17 ± 3
Jejunal muscularis	14 ± 1
Spleen	13 ± 1
Heart	6 ± 1
Skeletal muscle	2 ± 1
Erythrocyte	1 ± 1
Large bowel contents	0

* Tissues were obtained by rapid excision from exsanguinated, male, 300 g Sprague–Dawley rats. Tissues were washed with 140 mM NaCl–10 mM potassium phosphate (pH 7.4) at 0°, and minced with a razor blade. After addition of 4 volumes of a solution of 0.1 M potassium phosphate and 1 mM EDTA (pH 7.9), the suspension was homogenized with 100 strokes of a 1 ml-size Dounce homogenizer, type B. Aliquots (20 μl) of each homogenate were assayed without further preparation. Mucosa was obtained from bowel by rinsing segments with buffered saline at 0–4°, opening them longitudinally with scissors, and scraping them with the edge of a glass microscope slide on a glass plate maintained at 4°. To obtain small bowel muscularis essentially free of mucosa, a 2-cm length of the open bowel, after scraping, was thoroughly scrubbed between thumb and forefinger in iced saline until only the glossy muscle layer remained. After fine mincing with a razor blade, the muscularis was homogenized as noted above. All protein assays were by the method of Lowry *et al.* [21], standardized with bovine serum albumin.

† Values represent specific activity ± S.D. and are derived from triplicate determinations on each tissue, with 2-thioacetanalide as methyl group acceptor.

As shown in Table 1, enzyme activity was widely distributed. The highest concentration of the enzyme was found in large bowel mucosa, with successively declining levels in small bowel, duodenum and stomach. Liver, lung and kidney, all organs involved in detoxication, also had relatively high amounts. Despite thorough removal of the mucosa prior to assay, jejunal and ileal muscularis had enzyme concentrations that were 42 and 52 per cent of those of their respective mucosae, whereas other muscular tissues tested had relatively low levels. To evaluate the possibility that gut flora contributed to measured enzyme, rat feces were assayed and found to be free of detectable activity.

The methylation of hydrogen sulfide (Reaction 2) leads to methanethiol, which is also a substrate for methylation (Reaction 3) by the same enzyme.

$$H_2S + \textit{S}\text{-adenosylmethionine} \rightarrow CH_3SH + \textit{S}\text{-adenosylhomocysteine} \quad (2)$$

$$CH_3SH + \textit{S}\text{-adenosylmethionine} \rightarrow CH_3SCH_3 + \textit{S}\text{-adenosylhomocysteine} \quad (3)$$

In establishing the actual product of the enzyme-catalyzed reaction, therefore, it was necessary to find a means for differentiating methanethiol from dimethylsulfide. Because methanethiol has a free reactive sulfhydryl group, but dimethylsulfide does not, the two sequential products could be distinguished by differential extraction from alkaline solution with toluene following reaction with an organic mercurial. Therefore, a standard enzyme reaction mixture containing hydrogen sulfide was treated with a 3-fold excess of *p*-chloromercuriphenylsulfonic acid at pH 7.9 after 10 min of incubation with the enzyme. After an additional 10 min, the mixture was extracted with 6 ml of toluene. Less than 5 per cent of the radioactive products were extractable into the organic solvent. Thus, the major product of the reaction in the presence of excess H_2S appears to be methanethiol, resulting from a single methylation step.

With homogeneous thiol *S*-methyltransferase from rat liver [11], double reciprocal plots of substrate concentration against product formed allow the calculation of an apparent K_m of 64 μM for H_2S and of 240 μM for methanethiol under otherwise standard [11] assay conditions. The calculated maximal velocity for H_2S as substrate, 7.8 nmoles·mg^{-1}·min^{-1}, was considerably higher than that for methanethiol, 0.9 nmole·mg^{-1}·min^{-1}. On this basis, the conversion of methanethiol to dimethylsulfide *in vivo* would be expected to proceed more slowly than the initial conversion of H_2S to methylthiol.

Fractions enriched with mucosal villus and crypt cells were obtained, for which the gradients of phosphatase and thymidine kinase activity indicate adequate separation of the cell types. Figure 1, however, shows that thiol methyltransferase activity is only slightly higher in the extracts from crypt cells than in those from villus cells. It was established that this result was not due to loss of enzyme activity during the different periods of washing required to obtain the two cell types because less than 15 per cent of the observed transferase activity was lost by an additional hour of incubation of eluted cells at 37°.

We have shown that thiol *S*-methyltransferase is distributed in a manner that is consistent with a role of the enzyme in the detoxication of the mercaptans adsorbed by the bowel. Since glutathione and cysteine, hydrophilic mercaptans normally found in cells, are not substrates, it seems likely that the major function of the enzyme is in its action on foreign mercaptans, many of which are highly toxic.

As with other enzymes active in detoxication [24], thiol *S*-methyltransferase is characterized by a broad specificity for substrates, among which is hydrogen sulfide. The high solubility of hydrogen sulfide in physiological fluids suggests that, at equilibrium between flatus and the bowel mucosa,

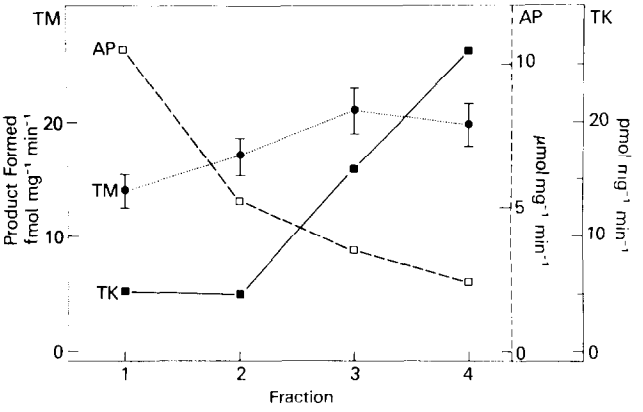

Fig. 1. Distribution of thiol methyltransferase (TM), thymidine kinase (TK), and alkaline phosphatase (AP) in mucosal cells prepared by the Weiser procedure [22]. The first fraction was enriched with villous cells and the last with crypt cells. Rat jejunal mucosal cells were serially released by the procedure of Weiser [22]. The fractions obtained were identified by standard assays for thymidine kinase [23], a marker for the crypt cells, and alkaline phosphatase [22], a marker for villous cells. Approximately 10% (v/v) cell suspensions in a solution of 0.1 M potassium phosphate and 1 mM EDTA (pH 7.9) were disrupted with 100 strokes of a 1 ml-size Dounce homogenizer, type B, followed by centrifugation at 15,000 *g* for 30 min. Thiol methyltransferase with 2-thioacetanalide as acceptor and the activity of the two marker enzymes were determined in the resulting supernatant fluid.

much higher concentrations will be present in the latter and that potentially toxic amounts can diffuse into the portal circulation. The product of the enzyme catalyzed reaction with hydrogen sulfide is methanethiol, which is slightly less toxic than H_2S [25] and can itself serve as a substrate for a second methylation to dimethylsulfide. Thus, the mucosal enzyme may serve as a primary defense against the effect of locally produced hydrogen sulfide. Because the K_m and V_{max} for the second methylation are less favorable, much of the methanethiol, as well as any overload of hydrogen sulfide, would enter the portal system. The liver, with its greater mass and relatively high enzyme concentration, would encounter these substrates and would be capable of more extensive methylation. Both methylated products are also sufficiently volatile to be excreted in the breath. In human hepatic failure, accumulation of the least reactive of the substrates, i.e. methanethiol, has been observed in the systemic blood [26, 27] and may contribute to the genesis of hepatic coma [28].

Section of Enzymes and Cellular Biochemistry,
National Institute of Arthritis, Metabolism and Digestive Diseases,
National Institutes of Health, Bethesda, MD 20205, *and*
†*Department of Medicine, Nassau County Medical Center, East Meadow, NY* 11554, *U.S.A.*

RICHARD A. WEISIGER*
LAWRENCE M. PINKUS†
WILLIAM B. JAKOBY‡

REFERENCES

1. C. L. Evans, *Q. Jl. Physiol.* **52**, 231 (1967).
2. H. Kadota and Y. Ishida, *A. Rev. Microbiol.* **26**, 127 (1972).
3. H. Soggard, *Acta vet. scand.* **16**, 31 (1975).
4. J. Tonzetich, *J. Periodont.* **48**, 13 (1977).
5. E. Kirk, *Gastroenterology* **12**, 782 (1949).
6. J. Tonzetich, *Archs oral Biol.* **16**, 587 (1971).
7. L. R. Freeman, G. T. Silverman, P. Angelini, C. Memitt, Jr. and W. B. Esselen, *Appl. envir. Microbiol.* **32**, 222 (1976).
8. D. L. Nichol, M. K. Shaw and D. A. Ledword, *Appl. Microbiol.* **19**, 937 (1970).
9. P. Stetten, *Nahrung* **15**, 71 (1971).
10. J. Bremer and D. M. Greenberg, *Biochim. biophys. Acta* **46**, 217 (1961).
11. R. A. Weisiger and W. B. Jakoby, *Archs Biochem. Biophys.* **196**, 631 (1979).
12. R. T. Borchardt and C. F. Cheng, *Biochim. biophys. Acta* **522**, 340 (1978).
13. C. N. Remy, *J. biol. Chem.* **238**, 1078 (1963).
14. E. J. Sarcione and J. E. Sokal, *J. biol. Chem.* **231**, 605 (1958).
15. R. H. Lindsay, B. S. Hulsay and H. Y. Aboul-Enein, *Biochem. Pharmac.* **24**, 263 (1975).
16. L. L. Bennett, Jr. and P. W. Allan, *Cancer Res.* **31**, 152 (1971).
17. M. Jakabowski and T. Gessner, *Biochem. Pharmac.* **21**, 3073 (1972).
18. T. Gessner and M. Jakabowski, *Biochem. Pharmac.* **21**, 219 (1972).
19. L. M. Siegel, *Analyt. Biochem.* **11**, 126 (1965).
20. G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70 (1959).
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
22. M. M. Weiser, *J. biol. Chem.* **248**, 2536 (1973).
23. T. R. Breitman, *Biochim. biophys. Acta* **67**, 153 (1963).
24. W. B. Jakoby and J. H. Keen, *Trends biochem. Sci.* **2**, 299 (1977).
25. National Research Committee on Medical and Biologic Effects of Environmental Pollutants, *Hydrogen Sulfide*, p. 44. University Park Press, Baltimore (1979).
26. W. M. Doizaki and L. Zieve, *J. Lab. clin. Med.* **89**, 849 (1977).
27. S. Chen, L. Zieve and V. Mahadevan, *J. Lab. clin. Med.* **75**, 628 (1970).
28. L. Zieve, W. M. Diozaki and F. J. Zieve, *J. Lab. clin. Med.* **83**, 16 (1974).

* Present address: Department of Medicine, University of California, San Francisco, CA 94143, U.S.A.

‡ Address correspondence to: William B. Jakoby, Building 10/Room 9N-119, National Institutes of Health, Bethesda, MD 20205, U.S.A.