The supplement of α-tocopherol under the circumstances of this experiment did not measurably counteract the damage from the heated oil diets. The just significantly greater number of survivors at 21 days in the vitamin E group as compared to the number receiving no vitamin E is possibly explained on the basis of variability between tests, since all the animals receiving vitamin E were fed at one time and those receiving no vitamin E at a different time.

The proportion of young surviving or retained at 7 days, which were eventually weaned, is rather poor in the case of the rats receiving the unheated oil diet. We are inclined not to attribute this to any nutritional defect of the oil, since linseed oil included in the diet at levels of up to 30% has been observed to give rates of gain and feed efficiency comparable with those given by other edible oils when included in the diet at the same levels (*1*).

It is, however, quite clearly evident that heating the oil damaged its nutritive value. The reproductive and lactation responses of the female rat and her litter may offer a more critical test of thermal damage to oils intended for dietary use than does the growth of the weaned rat.

References

1. CRAMPTON, E. W., and LANGERMAN, H. L. The Effect of Heat Treatment on the Nutritional Value of Some Vegetable Oils. Presented at the Gordon Research Conference, New London, N. H. (Aug. 1949).
2. CRAMPTON, E. W., FARMER, F. A., and BERRYHILL, F. M. *J. Nutrition* (in press).

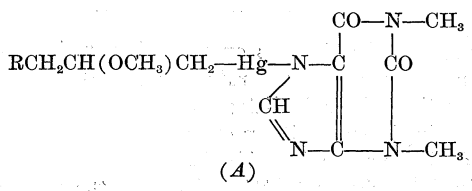
Reactions of Mercurial Diuretics with Mono- and Dithiols

J. F. Lehman, L. P. Barrack,[1] and R. A. Lehman[1]

Department of Therapeutics,
New York University-Bellevue Medical Center, New York

A recent review on renal transport mechanisms summarizes as follows: "There can be little doubt that the kidney is the principal site of the diuretic effect of the mercurial agents. . . . In general, mercurial agents combine with sulfhydryl groups, and this is responsible for their inhibitory effect on a number of essential cellular dehydrogenases. That the diuretic effect of mercury is attributable to the inhibition of such enzymes seems likely . . ." (*1*). The purpose of this communication is to describe certain reactions that occur *in vitro* between mercurial diuretics and thiols and to discuss their physiologic significance.

Prior to 1949 the mercurial diuretics used in medicine could be grouped under the general structure

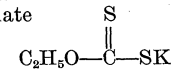
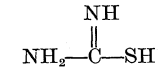
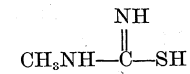
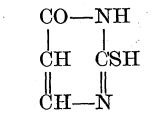
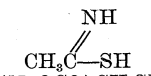
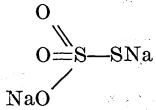


(*A*)

[1] Present address: Research Laboratories, Campbell Pharmaceutical Co., New York.

TABLE 1

RECOVERY OF THEOPHYLLINE FROM THE REACTION BETWEEN MERCUZANTHIN AND VARIOUS SULFHYDRYL COMPOUNDS

Monothiols		Mols theophylline recovered per mol mercury
Potassium ethyl xanthate		1.12
Thiourea		1.06
N-methyl thiourea		0.89
Thiouracil		1.20
Sodium thiosalicylate	$(NaOCO)C_6H_4SH$	1.17
Thioacetamide		1.23
Sodium thioglycollate	$(NaOCO)CH_2SH$	1.06
Sodium salt of cysteine	$(NaOCO)CH(NH_2)CH_2SH$	1.08
Sodium thiosulfate		1.06
Thio phenol	C_6H_5SH	1.12

where R is usually the sodium salt of a carboxylic acid residue to which the three-carbon side chain is attached through the nitrogen atom of a carbamyl group. It has been found that these drugs, represented by Mercuzanthin (Mercurophylline, U.S.P.), Salyrgantheophylline (Mersalyl with theophylline, U.S.P.) and Meruhydrin (Meralluride, N.N.R.), will react immediately at room temperature with a wide variety of monothiols according to the following equation:

$$RCH_2CH(OCH_3)CH_2Hg(\text{theophylline}) + R'S^- + H^+ \rightarrow RCH_2CH(OCH_3)CH_2HgSR' + (\textit{theophylline}) \quad (1)$$

where R'SH may be any simple sulfhydryl compound. If the reaction is carried out in concentrated solution and the rise in pH caused by removal of hydrogen ions is prevented by buffering with CO_2, the theophylline precipitates quantitatively.

To 10 ml of 0.2 *M* Mercuzanthin solution was added a mol equivalent of the various sulfhydryl compounds listed in Table 1. The solution was then saturated with CO_2, and the precipitated theophylline monohydrate was filtered off on a sintered glass filter, washed with a small volume of ice water, dried in a desiccator, and weighed. From the

TABLE 2

ESTIMATION OF UNBOUND SULFHYDRYL GROUPS IN THE REACTION OF MERCUZANTHIN WITH EXCESS THIOGLYCOLLATE

mM of mercury taken as Mercuzanthin	mM of sodium thioglycollate added	mM of –SH recovered	Mols of –SH bound per atom of mercury
0.193	0.214	0.025	0.98
.193	.214	.023	0.99
.193	.333	.138	1.01
.193	.416	.222	1.02
.193	.428	.230	1.03
.187	.669	.477	1.03
0.187	0.669	0.476	1.03

weight of theophylline found (corrected for the solubility of theophylline in the reaction mixture) it was possible to calculate the approximate mols of theophylline precipitated per mol of mercury which appear in the table.[2]

However, this reaction also goes to completion in dilute solution at physiologic pH without the separation of theophylline. This was shown as follows:

To one ml of a 0.2 *M* solution of Mercuzanthin in 10 ml of phosphate buffer, pH 7.4, were added varying amounts of an approximately 0.2 *M* solution of sodium thioglycollate, and the mixture was immediately titrated with 0.1 *M* potassium ferricyanide until a pale-green color appeared which persisted for 10 sec. The thioglycollate solution was standardized iodimetrically; the results agreed with those obtained by ferricyanide titration. Furthermore, the total –SH in the reaction mixture can be titrated iodimetrically in the presence of mercury. The difference between the mols of thioglycollate recovered and added is equivalent to the mols of mercury bound as mercaptide. The data in Table 2 are also in agreement with equation (1).

Most tissues and fluids in the body contain glutathione and cysteine as well as proteins bearing thiol groups which are readily accessible to mercury. In fact Milnor (*2*) has shown that Mercuhydrin, a mercurial of structure (*A*), is partially bound *in vitro* by plasma protein. Hence it may be considered almost inevitable that reaction (1) takes place *in vivo* and that the xanthine-bearing diuretics circulate in the blood as mercaptides formed from simple thiols or plasma albumin.

Recently the mercurial diuretic Thiomerin (Mercaptomerin, N.N.R.) has been shown to be at least as potent a diuretic as the drugs of structure (*A*) (*3, 4*). It has also been shown that the time of onset and course of excretion of mercury, water, and chloride after administration of Thiomerin and Mercuzanthin are virtually identical (*5*). It was anticipated that Thiomerin might not have diuretic properties at all since it is a mercaptide of the structure

$$RCH_2CH(OCH_3)CH_2HgSCH_2COONa.$$

(*B*)

[2] Acetamide and urea do not precipitate theophylline under these conditions in contrast to their thio analogs, which behave like true sulfhydryl compounds.

That it is a diuretic can best be explained on the basis of an exchange reaction at the tubular cell at which site it may be expected to attain a relatively high concentration.

$$RCH_2CH(OCH_3)CH_2HgSCH_2COONa + R'SH \rightarrow RCH_2CH(OCH_3)CH_2HgSR' + HSCH_2COONa. \quad (2)$$

where R'SH may represent an enzyme bearing one or more sulfhydryl groups. Webb, Bhatia, Corwin, and Sharp (*6*) have shown that such reactions proceed at physiologic temperature and pH. Alternative mechanisms are, of course, possible.

TABLE 3

IMMEDIATE REACTION BETWEEN BAL AND MERCUZANTHIN

mM of mercury taken as Mercuzanthin	mM of –SH added as BAL	mM of –SH recovered	Mols of –SH bound per atom of mercury
0.094	0.430	0.342	0.95
.094	.434	.347	1.04
.094	.434	.343	0.98
.097	.430	.333	1.00
.155	.430	.280	0.97
.187	.430	.265	.88
.187	.434	.260	.93
0.194	0.430	0.248	0.94

On the other hand, it has been demonstrated, both in animals and in man, that administration of British antilewisite during mercurial diuresis resulting from compounds of structure (*A*) (*7–9*) or from Thiomerin (*10*) will rapidly but transiently suppress the diuresis. Although this phenomenon has been attributed to stimulation of production of the antidiuretic hormone by BAL (*9*), it nevertheless seemed desirable to determine the nature of any reactions which might occur between BAL and the mercurial diuretics.

Immediate reaction with BAL. One half to one ml of a 0.2 *M* solution of Mercuzanthin was mixed with 10 ml of phosphate buffer, pH 7.4, and a stream of nitrogen was run into the reaction flask and maintained throughout the experiment to prevent air-oxidation. One ml of an ethyl alcohol solution of BAL, approximately 0.43 *N* with respect to –SH, was then added, and the mixture immediately titrated with 0.1 *M* potassium ferricyanide solution to the same end point as before. The reaction was carried out at room temperature.

The values for recovered –SH in Table 3 indicate the binding of one –SH group for each atom of mercury present. The titer of BAL determined by ferricyanide titration agreed closely with that obtained iodimetrically.

Progressive reactions with BAL. One hundred ml of phosphate buffer, pH 7.4, 9 ml of Mercuzanthin, and 11 ml of an alcoholic solution of BAL were mixed under nitrogen and maintained in a water bath at 37.5° C. The final solution was such that each 10-ml sample contained 0.143 mM of mercury and 0.687 milliequivalents of –SH. At timed intervals 10-ml aliquots were removed from the reaction mixture and titrated with 0.1 *M* potassium fer-

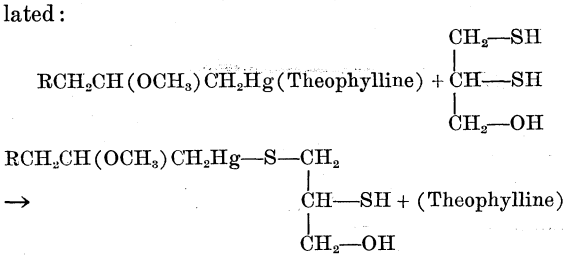
TABLE 4

PROGRESSIVE REACTIONS BETWEEN BAL AND MERCUZANTHIN

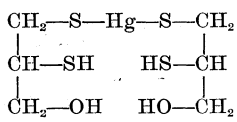
Time after mixing (min)	Mols of – SH bound per atom of mercury
5	1.17
15	1.31
30	1.73
45	2.01
60	2.27
75	2.55
90	2.67
120	2.67

ricyanide. One tenth ml of a 0.5% copper sulfate solution added to each titration flask was found to give a sharper end point. An aliquot removed after 85 min was titrated iodimetrically and showed no loss in total – SH. The drop in potassium ferricyanide titer is then assumed to be due to the formation of mercaptides. The results are given in Table 4.

The immediate reaction which occurs with the disappearance of 1 SH/atom of mercury may be formulated:



This reaction proceeds further with the disappearance of 2–3 mols of SH per atom of mercury. Presumably the C—Hg bond is ruptured with the formation of some such complex as that suggested by Gilman and co-workers (*11*).



(*C*)

It is also of interest that these reactions are progressively accelerated as the pH is reduced below 7.

In summary, it is felt that a mercurial diuretic, whether of the xanthine or mercaptide type, circulates in the blood as a mercaptide derived either from a simple monothiol or a protein. At the site of mercurial diuresis a reaction similar to (2) may occur between the mercurial and an enzyme bearing essential sulfhydryl groups. If there is more than one SH group present on the same molecule the mercury may be removed from the parent drug and temporarily bound. Rapid reactivation of the enzyme and excretion of the mercury undoubtedly occur, however, since no permanent damage is done to the kidney even on frequent administration over long periods. Removal of mercury from such an enzyme-mercury complex by BAL with suppression of diuresis would be anticipated and resumption of diuresis could occur from fresh mercurial presented to the kidney.

References

1. BEYER, K. H. *J. Pharmacol. Exptl. Therap.*, **99**, 227 (1950).
2. MILNOR, P. J. *Proc. Soc. Exptl. Biol. Med.*, **75**, 63 (1950).
3. GROSSMAN, J., *et al. Circulation*, **1**, 508 (1950).
4. BATTERMAN, R. C., UNTERMAN, D., and DEGRAFF, A. C. *J. Am. Med. Assoc.*, **140**, 1268 (1949).
5. LEHMAN, R. A., KING, E. E., and TAUBE, H. *J. Pharmacol. Exptl. Therap.*, **99**, 149 (1950).
6. WEBB, J. L. A., *et al. J. Am. Chem. Soc.*, **72**, 91 (1950).
7. FARAH, A., and MARESH, G. *J. Pharmacol. Exptl. Therap.*, **92**, 73 (1948).
8. SUSSMAN, R. M., and SCHACK, J. A. *Proc. Soc. Exptl. Biol. Med.*, **66**, 247 (1947).
9. EARLE, D. P., JR., and BERLINER, R. W. *Am. J. Physiol.*, **151**, 215 (1947).
10. BATTERMAN, R. C., and ROSE, O. A. Unpublished communication.
11. GILMAN, A., *et al. J. Clin. Invest.*, **25**, 549 (1946).

Sedimentation Cylinder for Particle Size Analysis

Carlton J. Leith

U. S. Engineers, South Pacific Division Laboratory, Sausalito, California

Among the simplest of the techniques for separation of fine-grained sediments into fractions based on particle dimensions is gravity settling followed by decantation, involving the settling of sediments in liquids in accordance with Stokes' law. In a recent analysis of a suite of samples it became necessary for the writer to isolate the 1/32–1/16 mm grade size for further study, and for this purpose a settling cylinder was devised, incorporating the best features of the Kühn settling tube (*1*) and the Atterberg sedimentation cylinder (*2*). A side opening was drilled into an ungraduated liter cylinder, far enough above the base of the cylinder so that the streamlines created by flow through the opening would not affect the sediment that had come to rest on the bottom of the cylinder or on the surface of the base of the stirring rod (in this case a rubber stopper attached to a glass rod). Flow through the side opening of the cylinder was regulated by means of a stopcock in a length of glass tubing, held in the opening by insertion of the tube through a hole in a rubber stopper (Fig. 1). Water was allowed to drain through the side outlet, and when drainage ceased, heights of 5 cm, 10 cm, and 20 cm above the level of the water remaining in the cylinder were marked on the cylinder walls, and these levels were etched into the glass of the cylinder. In Fig. 1 the etched lines are marked with wax pencil. and the levels just described are represented by the upper line of each pair of black lines.

The procedure in the analysis for which the apparatus was devised consists of the introduction of a sediment suspension (from which oversized material has been removed by wet sieving through a U. S. Standard No. 230 sieve) into the cylinder and the