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Toxicological Studies of 2-Mercaptoethanol

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Abstract □ The toxicity of 2-mercaptoethanol, a potential reaction product between residual ethylene oxide and sulfides in rubber medical devices sterilized by ethylene oxide, was investigated. This report includes LD_{50} determinations for mice by both the intraperitoneal and oral routes of administration, as well as subchronic dosage studies. Both ethanol and sodium pentobarbital significantly decreased the acute toxicity potential of 2-mercaptoethanol, although pretreatment with atropine and with a combination of metal ions had no beneficial effects. 2-Mercaptoethanol was found to be more toxic to all tissues than ethanol but showed a marked decrease in such activity upon dilution.

Keyphrases □ 2-Mercaptoethanol—toxicity profile □ Toxicity profie—2-mercaptoethanol □ Sterilization residues, toxic—profile of 2-mercaptoethanol as potential reaction product between ethylene oxide and sulfides □ Ethylene oxide sterilization—toxicity profile of 2-mercaptoethanol as potential residue

The contemporary procedure of sterilization by ethylene oxide of rubber and plastic medical devices has provoked a number of pertinent questions as to potential health hazards such devices might propound. Bronsted *et al.* (1) demonstrated that ethylene oxide will react with a wide variety of nucleophilic agents, thus introducing the likelihood of production of reaction products from chemical contaminants in the sterilized articles. One such reaction product, the extremely toxic chlorohydrin 2-chloroethanol, was investigated by Guess (2). Guess and O'Leary (3) found another reaction product of ethylene oxide sterilization, 2-(2-hydroxyethylmercapto)benzothiazole, to be quite toxic to cells in culture and to mice.

A number of rubber devices, including tracheotomy tubes, in-dwelling catheters, multidose vial stoppers, bottle cap liners, and gloves are commonly sterilized with ethylene oxide. During the vulcanization of rubber with sulfur, it is likely that sulfides, including hydrogen sulfide, are formed. The formation of 2-mercaptoethanol by the mechanism proposed by Bronsted *et al.* (1) is then possible. The aforementioned medical materials come into both direct and indirect contact with various body tissues, including mucous membranes, epithelial and endothelial cells, muscle, and ocular tissues. Therefore, if these devices are to be used with safety, the toxic potential of any possible contaminants must be determined.

A search of the literature revealed a paucity of information on the systemic and specific tissue toxicity of 2-mercaptoethanol. Finley and Carlson (4) speculated that the LD_{50} by intraperitoneal injection in mice was approximately 195 mg./kg. A patent authored by Utrumi

Table I—LD_{50} Values for 2-Mercaptoethanol in Mice

Route	LD_{50}, mg./kg.	95% Confidence Interval
Intraperitoneal	322.0	296.5–350.9
Oral	344.8	317.7–374.3

et al. (5) included an LD_{50} value of 0.48 mg./g. on intravenous administration to mice. Smyth and Carpenter (6) investigated the toxicity of 2-mercaptoethanol, although the studies were intended only as rapid, range-finding assessments of toxicity potential. These investigators determined the oral range-finding LD_{50} for rats to be 300 mg./kg., the topical range-finding LD_{50} for guinea pigs to be 0.3 ml./kg., a rabbit epidermal irritation similar to morpholine, and a rabbit corneal toxicity equivalent to butyl alcohol.

2-Mercaptoethanol has enjoyed widespread academic interest in the biological and biochemical fields. The presence of a sulfhydryl group in the molecule portends a number of potential *in vivo* reactivities. According to Coombs *et al.* (7), 2-mercaptoethanol may inhibit zinc carboxypeptidase activity *via* complexation with zinc ions vital to enzymatic functionality. Erdös and Wohler (8) postulated a potentiation of action of bradykinin and kallidin by such a complexation mechanism, with resultant inhibition of the specific catabolizing carboxypeptidase. A number of investigators (9–11) reported the activation of enzymes *via* reduction of enzymatic sulfhydryl groups by 2-mercaptoethanol. However, as suggested by Edman *et al.* (9), higher concentrations of 2-mercaptoethanol may inhibit these same enzymes by disruption of their tertiary protein structure.

In view of the lack of concrete data concerning animal toxicity of 2-mercaptoethanol, an evaluation of various toxicity parameters was carried out. When pharmacological effects were observed, efforts were made to assess the cause through tests designed to block specific actions or symptoms. These various tests were intended to elucidate the toxicity profile of the compound and to determine its mechanism of toxic action.

MATERIALS AND METHODS

Purity Determination—2-Mercaptoethanol, with a boiling range of 69.5–70.5° (157–159°F.), was obtained from a commercial source[1]. Purity of the compound was ascertained by IR analysis and GC. The pH of aqueous solutions of the chemical was monitored with a pH meter[2]. All dilutions were prepared with physiological saline immediately prior to administration.

Animal Care—Rabbits (New Zealand albino males) and mice (Swiss-Webster albino males, 20–25 g.) were the animals of choice for this series of experiments. All subjects were maintained under constant environmental conditions, with laboratory chow[3] and water available *ad libitum*, except as noted in subsequent sections of this paper.

LD_{50} Determination—Both oral and intraperitoneal LD_{50} values for mice were determined by use of the Cornfield–Mantel (12) modification of the Karber method. Food was withheld from the oral intubation group for approximately 24 hr. before testing. The mice were divided into groups of 10 per dosage level, each animal receiving the specified dose in a constant volume of 0.5 ml./20 g. body weight. All subjects were observed closely during the post-injection period of 24 hr. for toxic symptoms and for 14 days for fatalities. Survivors were also observed periodically during this time for delayed or progressive toxic manifestations.

Subacute Studies—To study the effects of repeated exposure to sublethal doses of 2-mercaptoethanol, three groups of 15 mice each were administered the chemical intraperitoneally daily for 5 consecutive days in each of 4 weeks. The first group received one-half the LD_{50} dose, the second one-fourth the LD_{50} dose, and the third normal saline as a control. A daily record was kept of toxic symptoms and body weights. Several subjects from each group exhibiting extreme toxic manifestations were sacrificed by cervical fracture, and the kidneys, spleen, liver, lungs, heart, adrenals, pancreas, stomach, intestine, and brain of each were removed and weighed to yield organ–body weight ratios. Specimens of each organ were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological examination.

Primary Skin Irritation—The method of Draize *et al.* (13), with certain modifications by Guess (2), was employed to evaluate irritation by 2-mercaptoethanol upon topical application. Both abraded and unabraded sites of exposure on the shaved rabbit back were scored for extent of erythema and edema at 24 and 72 hr. after exposure.

Intradermal Irritation—Evaluation of the intradermal irritation potential of 2-mercaptoethanol was accomplished by the technique utilized by Guess (2). The test solution (0.2 ml.) was injected intracutaneously into two sites on the shaved backs of two rabbits. Ethanol (20%) was used as a positive control, while normal saline served as a negative control. Toxic reactions were graded after 1 hr. and at 24-hr. intervals thereafter on the basis of a system ranging from a value of +4 for extreme irritation with necrosis to a value of 0 for no reaction.

Ocular Irritation—Ocular irritation was evaluated according to the modification of the Draize technique published in the Federal Register (14), utilizing the regulations and color charts included therein. The irritation scores of six subjects per concentration level were averaged to yield a primary irritation number. Evaluations of reaction severity were performed 1, 24, 48, and 72 hr. following exposure to the test solution.

Mucosal Irritation—The irritation potential of 2-mercaptoethanol to penile mucosa was evaluated by the method outlined by Guess (2), using penile tissue of anesthetized rabbits. The test solution (0.2 ml.) was instilled into a well formed with peripenile tissue and was allowed to remain in contact with the mucosal tissue for 1 min. Adhering solution was not removed upon release of the well.

RESULTS AND DISCUSSION

The results of the LD_{50} determination by oral and intraperitoneal routes are given in Table I. Previous determinations of LD_{50} values by other workers resulted in the publication of varying estimates. Smyth and Carpenter (6), as previously stated, found the oral (range-finding) LD_{50} for rats to be 0.3 g./kg. However, no mention was made of whether or not the test animals were fasted prior to treatment. Finley and Carlson (4), estimating the LD_{50} by intraperitoneal injection in mice to be approximately 195 mg./kg., used a peanut oil vehicle with a standard injection volume of 0.2–0.3 ml. The disparity in procedures of intraperitoneal administration between the present investigation and that conducted by Finley and Carlson (4) may account for the difference in reported LD_{50} values. The technique utilized for the present study dictated a standard injection volume of 0.5 ml./20 g. body weight. It was felt that this larger injection volume would minimize errors in accurately measuring amounts of active principle to be administered. Also, the influence a larger volume should have in increasing the effective area of drug absorption must be considered. One would expect that such an enlarged area would enhance both time of onset and degree of toxic manifestations. Normal saline was selected instead of an oil to rule out inhibition of systemic absorption of toxicant by the vehicle.

Both routes of administration yielded essentially the same LD_{50} values, but the nature and onset of symptomatology varied with the dosage administered. Doses of 480 mg./kg. and greater killed within 1–2 hr. Within 30 min. of such treatment, the subjects exhibited intermittent tremors and difficulty in coordinated muscular

[1] J. T. Baker Chemical Co.
[2] Corning.
[3] Purina.

Table II—Average Scores for Primary Irritation in the Rabbit

Compound	Dilution	Hour[b]	Rabbit Skin[a] Unabraded Erythema	Unabraded Edema	Abraded Erythema	Abraded Edema
2-Mercaptoethanol	Undiluted	24	3.3	2.0	3.3	1.0
		72	0.3	0.0	3.3	0.7
	1:5	24	1.0	0.3	2.7	1.0
		72	0.3	0.0	1.0	0.0
	1:10	24	1.0	0.0	1.7	0.3
		72	0.7	0.0	1.0	0.0
Alcohol USP	1:5	24	0.0	0.0	1.0	0.3
		72	0.0	0.0	0.0	0.0
Saline control	—	24	0.0	0.0	0.3	0.0
		72	0.0	0.0	0.0	0.0

[a] Score (each score represents an average of six animals): 1, barely perceptible erythema or edema; 2, well-defined erythema or edema; 3, moderate erythema or edema; and 4, severe (beet red) erythema or edema. [b] The time after treatment at which the evaluation was made.

activities. Severe clonic convulsions of long duration, accompanied by salivation and urination, were experienced by all subjects immediately preceding death. Death apparently resulted from convulsive seizures with subsequent respiratory failure. Histopathological examination of specimens of brain, lung, heart, liver, kidney, spleen, adrenals, pancreas, intestine, and stomach from these mice revealed no degenerative alterations.

With doses of less than 480 mg./kg., death was not so rapid. Rather, the mice slowly entered a state of deepening depression, terminating in coma and death in 1–3 days. During this progressive state, there were few voluntary movements, respiration was slowed and deepened, and response to externally applied pain stimuli was minimal. Histopathological studies of organs removed 24 hr. after dosing mice intraperitoneally with 322 mg./kg. revealed minimal changes in the liver and kidneys, with no alteration of other tissues. Slight vacuolation of scattered hepatocytes and deposition of eosinophilic, amorphous material in some renal tubular lumens was observed. Specimens excised after 48 hr. from mice receiving 322 mg./kg. i.p. exhibited similar toxic manifestations, although vacuolation of hepatocytes was slightly more pronounced. An additional finding was a marked increase in hyperchromacity and mitotic activity of hepatocyte nuclei.

Judging from the rapid onset and the nature of toxic responses elicited by higher doses of 2-mercaptoethanol, there appeared to be important involvement of the CNS. Since salivation and urination were commonly observed at the higher dosage levels, investigation of a possible cholinergic reaction mediated by the autonomic nervous system was initiated. Three groups of 10 mice each were injected intraperitoneally with 0.5 mg./kg. of atropine sulfate. Thirty minutes later, the first group received 500 mg./kg. of 2-mercaptoethanol intraperitoneally, the second 322 mg./kg. of 2-mercaptoethanol intraperitoneally, and the third 500 mg./kg. of sodium chloride intraperitoneally. No alteration in toxicity was evident in the first group, because each mouse exhibited extreme CNS stimulation with salivation, followed by convulsions terminating in death within 1.5 hr. Six mice in the second group died within 24 hr., while all animals in the third group lived and remained symptom free. These results suggested that some toxic mechanism other than a cholinergic action be suspect.

The possibility of mediation of action *via* the cervical sympathetic ganglion was investigated. The mice were given 20 mg./kg. of sodium pentobarbital intraperitoneally, 15 min. prior to administration of 500 mg./kg. of 2-mercaptoethanol by the same route. A second group received 500 mg./kg. of 2-mercaptoethanol but no pentobarbital, while the third group received only 20 mg./kg. pentobarbital. All subjects receiving either pentobarbital-mercaptoethanol or pentobarbital alone exhibited a decreased level of activity, with some members appearing markedly depressed. No subject in either of these groups experienced enhanced salivation and urination or convulsions or died within 12 hr. However, seven of the mice receiving pentobarbital-mercaptoethanol died within 24 hr. In contrast, mice receiving only 2-mercaptoethanol experienced the typical CNS stimulatory effects, each member dying within 2 hr.

Since several investigators (7, 8) proposed that 2-mercaptoethanol complexes with metal ions critical to various enzyme systems, an attempt was made to block toxic effects mediated by such a mechanism. Twenty mice were each administered intraperitoneally the following dose of salts in 0.5 ml. distilled water: 4.0 mg. sodium chloride, 0.1 mg. potassium chloride, 0.1 mg. calcium chloride, 0.1 mg. zinc chloride, 0.05 mg. magnesium chloride, and 0.1 mg. ferrous sulfate. Thirty minutes later, 10 of the mice were administered 322 mg./kg. of 2-mercaptoethanol intraperitoneally. A third group of 10 mice received only 322 mg./kg. of 2-mercaptoethanol intraperitoneally. All subjects were then observed for 24 hr. No mice receiving only the solution of metal ions exhibited toxic symptoms or died. However, members of each group treated with 2-mercaptoethanol showed marked depression within an hour. Six subjects pretreated with metal ions died, while five animals in the group given only 2-mercaptoethanol died. These results suggest that the toxic effects of an LD_{50} dose of 2-mercaptoethanol in mice are not dependent upon complexation of sodium, potassium, calcium, zinc, magnesium, or iron ions.

The likelihood of metabolism of 2-mercaptoethanol to toxic metabolites must be considered. From a study of the metabolism of 2-chloroethanol, an analogous compound to 2-mercaptoethanol, Johnson (15) suggested that the toxicity of 2-chloroethanol was due to oxidation to chloroacetaldehyde in target organs. Peterson *et al.* (16) reported that ethanol exerted a protective action against the lethal effects of 2-chloroethanol and 2-fluoroethanol in rats and monkeys. The LD_{50} of 2-chloroethanol in unprotected rats was found to be four times less than that for a group dosed previously with ethanol. These investigators concluded that, at present, there is no reason to propose the existence of more than one alcohol dehydrogenase in liver.

Based on the results of such studies, it was decided to investigate the protection potential of ethanol in 2-mercaptoethanol poisoning. One group of 10 mice was given 322 mg./kg. of 2-mercaptoethanol intraperitoneally. Ten other mice were pretreated with 500 mg./kg. of ethanol, 15 min. before administration of the 322 mg./kg. of 2-mercaptoethanol. Animals exhibiting excessive depression in the latter group were given a subsequent intraperitoneal injection of 500 mg./kg. of ethanol during the next 12 hr. Twenty-four hours after dosing with 2-mercaptoethanol, six mice had died in the unprotected group while all but one animal given ethanol-mercapto-

Table III—Intradermal Irritation in the Rabbit[a]

Chemical	Dilution	1 hr.	24 hr.	48 hr.	72 hr.
2-Mercapto-ethanol	Undiluted	4[b]	4[b]	4[b]	4[b]
	1:5	4	4[b]	4[b]	4[b]
	1:10	4	4[b]	4[b]	4[b]
	1:50	4	4[b]	3.7[c]	3.7[c]
	1:100	0	2	1	0
	1:200	0	0	0	0
Alcohol USP	Undiluted	4	4[b]	4[b]	4[b]
	1:5	0	4	4	4[b]
	1:10	0	2	1	0
Saline control	—	0	0	0	0

[a] Score (each score represents an average of two injection sites in two rabbits): 0, no visible reaction; 1, barely perceptible erythema; 2, well-defined erythema; 3, moderate erythema; and 4, severe (beet red) erythema with a blanched center. [b] Necrosis at the site of injection. [c] Necrosis at the site of injection in only one animal.

Table IV—Average Scores for Ocular Irritation in the Rabbit[a]

Compound	Dilution	Hours after Administration	Cornea	Iris	Conjunctiva	
					Chemosis	Redness
2-Mercapto-ethanol	Undiluted	1	2	3	3	3
		24	1	2	3	3
		48	1	2	3	3
		72	1	0	0	1
	1:5	1	0	1	0.4	0.6
		24	0	0.4	0	0.4
		48	0	0	0	0.2
		72	0	0	0	0
Alcohol USP[b]	Undiluted		—	—	—	—
		24	0	1	1.5	1.1
		48	0	0.6	1.5	0.6
		72	0	0.5	0.8	0.6
	1:5	1	—	—	—	—
		24	0	0	0	0
		48	0	0	0	0
		72	0	0	0	0
Saline control		1	0	0	0	0
		24	0	0	0	0

[a] Score (each score represents an average from six animals); scoring was graded according to photographs and description in the Federal Register (14). [b] Results previously reported by Guess (2) were used here since the series of compounds was evaluated at the same time.

ethanol survived. Such results imply that ethanol may competitively inhibit the oxidation of 2-mercaptoethanol to toxic metabolites.

The dependence of symptomatology upon dosage level lends support to the premise of a dual mechanism of toxicity of 2-mercaptoethanol. Toxic responses to higher concentrations of the compound are indicative of a strong CNS stimulation, possibly initiated by the irritating effect of the nonmetabolized alcohol. The onset of toxic reactions following injection of a high concentration of the toxicant was too rapid to allow for its significant metabolism. Doses of 2-mercaptoethanol near and below its LD_{50} value produced strikingly different symptomatology, characteristic of CNS depression. Such toxicity appeared cumulative, evidenced in almost all subjects by deepening depression with eventual coma. These phenomena suggest that as long as a critically high dose of 2-mercaptoethanol is not administered acutely, the characteristic CNS stimulation manifestations will not occur. Rather, the subject will steadily degrade the alcohol to toxic metabolites which elicit depression of bodily functions. This premise is supported by the finding that sodium pentobarbital blocked the rapidly fatal convulsive effects of 2-mercaptoethanol but not the delayed lethal actions.

Subacute toxicity studies, as outlined previously, were conducted to ascertain the effect of prolonged exposure to 2-mercaptoethanol. Seven animals died after 8 days of treatment in the group receiving one-half the LD_{50} dose, while only two subjects receiving one-fourth the LD_{50} dose died. All mice in each group, however, succumbed within the 4-week period. Only one death was recorded among the saline control animals. Symptomatology was similar to that noted during the low dose, acute experiments. The mice exhibited progressive weakness and depression with curtailed activity, decreased response to pain stimuli, and loss of body weight. Gross observation of several vital organs of these animals revealed no significant alterations. Histopathological examination of specimens of brain, heart, lung, liver, kidney, spleen, adrenals, pancreas, intestine, and stomach revealed only small, scattered foci of hepatic necrosis. Organ–body weight ratios of control and test animals showed no significant differences.

It can be seen from Table II that undiluted 2-mercaptoethanol was moderately irritating on topical application to both abraded and normal skin of rabbits. Although the irritation to normal skin was transient, that to abraded areas was quite long lasting. Furthermore, primary reactions of a similar magnitude to ethanol healed much more quickly than those to 2-mercaptoethanol. Dilutions of 1:5 and 1:10 of 2-mercaptoethanol produced a moderate, transitory irritation in abraded skin and only a very slight reaction in normal skin. Edema was of little significance at any concentration level below 100%. It is apparent from Table II that 2-mercaptoethanol is considerably more toxic when applied topically than is its congener, ethanol.

As may be noted in Table III, a very severe reaction resulted from intracutaneous injection of undiluted 2-mercaptoethanol. Within 5 min. following such treatment, it became evident that a marked necrotic reaction would ensue. Therefore, only one rabbit was administered this concentration. Injection of dilutions of the toxicant of 1:5, 1:10, and 1:50 also produced marked inflammation with subsequent necrosis. After 72 hr., the slightest tendency of recovery was present only with the 1:50 dilution. A concentration of 1% 2-mercaptoethanol produced a well-defined irritation only after 24 hr. of exposure. At 72 hr. postinjection, the reaction was essentially negative. All higher dilutions of 2-mercaptoethanol elicited no inflammation. No systemic toxic effects were observed from this route of administration, although the test animal given the pure compound received a total dose of approximately 800 mg./kg.

Only in undiluted form was 2-mercaptoethanol markedly toxic to ocular tissue and surrounding mucosa. Iritis, redness, and chemosis were tabulated in Table IV as severe, while corneal opacity was moderate in severity although prolonged in duration. Scars and opacity of the cornea were apparent 4 months after treatment of the single rabbit with the pure chemical. In addition to those toxic manifestations included in Table IV, enhanced ocular secretory activity and depilation were manifest. Two days after treatment with undiluted 2-mercaptoethanol, there was complete loss of hair in the periocular area.

Pure 2-mercaptoethanol was observed to be at least twice as potent an ocular toxicant as 95% ethanol. However, upon dilution, the toxic potential of each compound decreased drastically. A dilution of 1:5 of 2-mercaptoethanol was only slightly irritating, while the same concentration of ethanol elicited no inflammatory response. Further dilutions of 2-mercaptoethanol produced no observable injurious effects.

Reaction of penile mucosal tissue to 100% 2-mercaptoethanol was intense. Severe erythema, moderate edema, and eschar formation were manifest 24 hr. after exposure. Due to the application technique, some of the pure chemical contacted the scrotum and was hindered from evaporating normally. Necrotic lesions developed in these regions within 24 hr. As in the ocular mucosal study, a 1:5 dilution produced only transitory erythema and edema; lesser concentrations caused no apparent irritation. 2-Mercaptoethanol-induced injuries to penile mucosal and scrotal epithelium healed more promptly than analogous lesions in intradermal and ocular tissues. No manifestations of toxicity were visible 10 days following application of 100% 2-mercaptoethanol to penile and scrotal tissue.

2-Mercaptoethanol, when present in a high enough concentration, actively denatures cellular protein and solvates lipid. Such actions of alcohols have a profound effect on the structural and functional integrity of both tissues and cellular organelles (17, 18). Necrotic foci caused by 2-mercaptoethanol in intradermal and in ocular tissues were slow to heal. Complete repair, as ascertained by visual examination, generally took over 1 month. This phenomenon of delayed healing might be anticipated because several investiga-

tors reported (19–21) mitotic inhibition by 2-mercaptoethanol. 2-Mercaptoethanol is believed to exert this inhibitory effect by interference with the formation of intermolecular protein linkages involving sulfhydryl moieties, *via* either reduction of disulfide bonds and/or competition with protein —SH for sites at which intermolecular bonds are formed.

SUMMARY

This study delineates the toxicity profile of 2-mercaptoethanol in a variety of exposure situations. The major findings include:

1. LD_{50} values for mice by both oral and intraperitoneal routes of administration were determined and found to be 322.0 mg./kg. (i.p.) and 344.8 mg./kg. (oral).

2. 2-Mercaptoethanol appeared to exert its toxic effects *via* a dual mechanism, dependent upon dosage level. Higher doses produced severe toxic effects within minutes, characteristic of CNS stimulation. However, doses near and below the LD_{50} elicited marked depression, possibly resulting from metabolism to toxic metabolites.

3. Although atropine sulfate did not inhibit the CNS stimulatory effects of 2-mercaptoethanol, sodium pentobarbital was quite effective in this respect.

4. The systemic toxicity of 2-mercaptoethanol did not appear to result from complexation of potassium, calcium, zinc, magnesium, or iron ions.

5. Concurrent administration of ethanol and 2-mercaptoethanol significantly reduced the toxicity of the latter, lending credence to the concepts of metabolism of each *via* the same enzymatic pathway and to metabolism of 2-mercaptoethanol to toxic metabolites.

6. Exposure to low levels of 2-mercaptoethanol for 4 weeks produced progressive loss of body weight and deepening depression followed by death.

7. 2-Mercaptoethanol was only slightly irritating upon topical application to normal skin but elicited quite severe reactions on exposure to abraded and intracutaneous tissues.

8. Mucous membranes were extremely sensitive to the irritant effects of undiluted 2-mercaptoethanol, although a rapid decline in toxicity occurred upon dilution of the alcohol.

9. 2-Mercaptoethanol was, in every instance, more toxic to tissues than was ethanol.

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