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A method for the determination of the hydrogen sulfide content of experimental animals has been described. Data has been compiled to support the view that hydrogen sulfide poisoning is due to paralysis of respiratory centers coupled with the inhibition of some enzyme system or other.

The following facts were established:

1. Concentrations of sulphemoglobin in the blood of animals dying of H_2S poisoning were much less than concentrations of sulphemoglobin established in experimental animals that lived.

2. There was a decrease in the CO_2 content of blood of H_2S intoxicated animals.

3. Carbon dioxide given intravenously and mixtures of CO_2 and air given through inhilation mask, increased experimental animal's tolerance for but did not abolish the toxicity of hydrogen sulfide.

4. Artificial respiration applied to animals intoxicated with H_2S prolonged life, but did not prevent death.

STUDIES IN HYDROGEN SULFIDE POISONING

by

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STUDIES IN HYDROGEN SULFIDE POISONING

PART I

DETERMINATION OF HYDROGEN SULFIDE IN STOMACH CONTENTS OF EXPERIMENTAL ANIMALS.

The problem of determining the sulfide content of solutions containing reducible or interfering substances presents difficulties. Since hydrogen sulfide is unstable and easily oxidized considerable trouble may be expected in recovering it from solution. Aeration with air, carbon dioxide, or hydrogen as well as distillation procedures have been employed (2,3,5,6) but the completeness of the removal is open to question.

Recently in connection with another investigation it was necessary to determine the hydrogen sulfide concentration in the stomach contents. Due to the nature of the material it was evident that methods based on evolution procedures were the only approach to the problem.

Preliminary experiments with various evolution procedures on pure hydrogen sulfide solutions (1-10 mg per liter) consistently gave results which varied from 50-90% of theory. Furthermore, the sulfide ion concentration rapidly diminished on standing. Other investigators (3) have reported similar observations, but the possibility of errors of this magnitude has

not been generally recognized.

In the determination of the sulfide content of metals and alloys, evolution procedures give excellent results. (1) This is probably due to the presence of nascent hydrogen which reduces any free, sulfite, or thiosulfate sulfur to sulfide ion, thus preventing any oxidation or decomposition of hydrogen sulfide. (7). This suggests that proper reducing conditions in sulfide solutions may permit quantitative removal or hydrogen sulfide.

On the basis of these considerations the following method was developed which may be useful to those confronted with a similar problem.

APPARATUS AND PROCEDURE. 1. 0.01 N sodium thiosulfate solution containing 1 volume % of amyl alcohol. 2. 12 N hydrochloric acid. 3. Cadmium chloride solution: 10 g of cadmium chloride dissolved in 400 ml of water and diluted with 600 ml of 28% ammonium hydroxide. 4. Starch solution: 2 g of soluble starch dissolved in 250 ml of boiling water and filtered. 5. Caprylic alcohol. 6. 0.01 N iodine solution, 1 ml = 6.8 mg per liter of hydrogen sulfide per 25 ml sample.

In an apparatus similar to one described by Gibbs and Clardy (4) an 8-in. test tube served as the reaction vessel and a 6-in. test tube as the receiver.

The thistle tube was equipped with a segment of rubber tubing and a screw clamp to permit the closing of the inlet tube.

The reaction vessel was charged with a 25 ml sample of material, 2-3 g of metallic zinc (S-free) and a drop or two of caprylic alcohol. The absorption tube containing 1 ml of cadmium chloride diluted to 15 ml was then placed in the system. Twenty-five ml of 12 N HCl were added to the reaction vessel, and the inlet tube was closed by means of the screw clamp. To hasten the evolution of hydrogen sulfide, the reaction vessel was warmed at the beginning. After the evolution of hydrogen was complete, the reaction vessel was brought to boiling for a minute or two, the receiver contents acidified and treated with an excess (1-2 ml) of standard iodine solution; back titration was with standard thiosulfate solution measured from a microburet.

RESULTS AND DISCUSSION. The results of several typical experiments (Table I) show that the quantitative removal of hydrogen sulfide from stomach contents can be attained by the use of nascent hydrogen as the aerating agent. When air was used instead of nascent hydrogen consistent but slightly lower results were obtained. The recovery of added amounts of hydrogen sulfide was always incomplete due probably to oxidation.

When nascent hydrogen is used for aeration of the hydrogen sulfide, other reduced forms of inorganic sulfur must be absent. (7) Pure solutions of cysteine and cystine evolved small amounts of hydrogen sulfide when treated with nascent hydrogen. When cystine, cysteine or methionine was added to stomach contents no additional hydrogen sulfide was obtained (Table I).

The stability of sulfide ion is slightly greater in stomach contents than in pure sulfide solutions; perhaps this might be expected from the nature of materials present.

SUMMARY. A simple method is described for determining hydrogen sulfide in the stomach contents of experimental animals.

TABLE I

Determination of Hydrogen Sulfide by Aeration with Hydrogen

		Stomach contents, mg H_2S/liter	Mg added/liter	Total H_2S mg/liter Found	Cale.
I.	Recovery experiments	3.7	8.6 H_2S	11.9	12.3
		4.4	8.0 H_2S	12.6	12.4
		5.1	7.8 H_2S	12.9	12.9
II.	Effect of organic sulfur on recovery of H_2S				
		2.7	*4,000 cystine	0.6-1.8	
		2.7	*4,000 cysteine	0.6-3.0	
		2.7	4,000 cystine	2.7	
			4,000 cysteine	2.7	
			4,000 methionine	2.7	
III.	Typical analyses of a given sample	3.9			
		3.8			
		3.8			
			Standard H_2S solution (made slightly alkaline) Mg H_2S/L.		
IV.	Stability of sulfide solutions	3.0		6.7	
		2.6	1 day later	5.0	
		2.7	2 " "	3.3	
		2.1	3 " "	2.2	

*With pure solutions the results varied through limits indicated.

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PART II

THE INHIBITION OF HYDROGEN SULFIDE POISONING BY CARBON DIOXIDE

Further information regarding the physiological effect of H_2S is becoming increasingly important in view of the mounting cases of poisoning which can be traced directly or indirectly to this reagent. (3, 6, 9, 11)

Some thought in this connection has been given to H_2S as an industrial hazard. (11) According to Balthazard and Valentin Sava, (1) H_2S acts as a poison of the nervous centers, in doses much smaller than those required for the characteristic spectrum to appear. Yant (10) regards the acute poisoning as due to the toxic action on the nervous system produced by the absorption and presence of H_2S in the blood, which results in unconsciousness and respiratory failure usually within a few seconds. The important reaction is paralysis of the respiratory system followed in 5-10 minutes by cardiac failure. He reports no indication of a chronic condition which confirms the observation of others.

In view of the rapidly expanding use of sulfur drugs attention is again being focussed on the question of sulfide poisoning. The effect of sulfanilamide, sulfapyridine, prontosil, on the formation of sulfhemoglobin has been reported by several investigators. (5, 8) Other drugs such as certain aniline derivatives have also been reported in this connection. (6)

Several papers have appeared in recent years accounting for the formation of sulfhemoglobin on the basis of intestinal H_2S. (2, 6, 9) According to De and Konar (2) sulfanilamide acts as a catalytic agent for the reaction of H_2S and hemoglobin. Harris and Michel (5) account for sulfhemoglobin in the course of sulfanilamide metabolism on the basis of the formation of an active substance which causes its production. Whether the pathological condition described as sulfhemoglobinemia is due to the presence of sulfhemoglobin in measurable concentrations or to other factors is not known.

In this laboratory the authors have been concerned with the role of H_2S in the symptoms and prognosis of acute tympanitis or bloat in ruminants. Dougherty (3, 4) has found a high concentration in rumens of animals suffering from bloat. He reports

a complete collapse of experimental ruminants due to the presence of H_2S in sufficient concentrations in the digestive tract.

Since apparently little is known regarding the nature of H_2S poisoning, this investigation was undertaken.

EXPERIMENTAL

Hydrogen sulfide gas introduced wither through the stomach or rectum in sublethal dosages was found to diminish the carbon dioxide content of the blood of three experimental animals (dog, sheep, and cow). Sulfhemoglobin could not be determined by spectroscopic methods (7) in the blood of these animals while in case of lethal dosages only traces of it were found. These results merely confirm the observations of others. (10)

Since the lethal dosage of hydrogen sulfide when introduced by way of the rectum of stomach is very small, it was the opinion of the authors that the toxic properties of hydrogen sulfide might be due to either its effect on some enzyme or to the failure of respiratory center (or other delicately balanced system). In an attempt to answer this question experiments were conducted in which only a part of the animal's blood was exposed to H_2S. In these experiments a considerable portion of the blood was removed,

saturated with H_2S and again returned to the animal. In two test runs using sheep, 3.4% of the hemoglobin was converted to sulfhemoglobin in case 1 and 6.9% in case 2. After 30 hours the sulfhemoglobin content was again normal. Although the urine of these animals was very highly colored during this period, the animals apparently suffered no ill effects from the experiment.

Since the CO_2 content of the blood is lowered by presence of H_2S, its effect on hydrogen sulfide poisoning was studied. The results of these experiments are presented in Table I.

TABLE I

Animal	Method admin. H_2S	% hemoglobin converted to sulfhemoglobin	Ml's H_2S	Ml's/min.	Remarks
I Animals Breathing Air					
dog	per rectum		175	3.6	lived
"	"		175	5.8	died
"	"		225	4.1	died
sheep	"	1.2	300	10.0	died
II Animals breathing mixture containing 90% oxygen - 10% Carbon dioxide					
dog	per rectum	.7	650	22	lived
"	"	1.9	500	26	lived 24 hrs.
"	"	5.5	850	40	died
"	"		400	8	lived
III CO_2 introduced intravenously					
sheep	per rectum	1.2	2800		died
"	"	10.4	3000	33	lived 100 hrs.
"	"	.3	1500	17	died

From these experiments it is evident that CO_2 has a marked effect both on the amount and rate of hydrogen sulfide dosage.

In order to detect a possible interference with the brain metabolism, the respiration of brain slices in the presence of various substrates was determined by means of the Warburg manometric technique.

Large guinea pigs were selected for the experiment. The control animal in each case was killed by breaking its neck and cutting off its head. The other animal was killed by placing it under a large jar in which hydrogen sulfide was passed, and its head was likewise immediately cut off. The brain of each animal was cut out and bathed in a 0.16 N. sodium chloride solution. Tissue slices were prepared according to the method of Wong from the cerebral cortex, and enough of the slices from each animal were placed in three different Warburg flasks so that the total dry weight of tissue in each flask was from 8 to 20 mg. The flasks had been previously charged with 2.8 cc. of 6 M sodium hydroxide on a roll of filter paper in the center well. The flasks were immediately greased onto the manometers, flushed with oxygen and placed in the constant temperature bath.

When glucose (final concentration 250 mg. per cent) was used as the substrate, no difference could be detected between the normal and H_2S treated animals, the Q_O varying between 11.0 and 12.9 for all six experiments. When no substrate was used in the flasks, thus determining the extent of sutolysis in the tissue, no difference could be detected between the normal and poisoned animals, the Q_O varying between 3.0 and 4.5.

Since catalase is very sensitive to H_2S, a set of determinations was run in which o.2 cc. of an H_2O_2 solution equivalent to 320 cmm. of liberated O_2 were tipped in from the sidearm after the manometers had come to equilibrium. Close agreement was obtained between the brain tissue from normal and an H_2S-killed animal as to the rate at which the hydrogen peroxide was broken down. The only conclusion that can be drawn from the latter experiment is that the amounts of H_2S reaching the brain cortex did not inhibit the action of catalase in this tissue.

DISCUSSION

The fact that sulfhemoglobin is found in relatively small amounts in the blood of animals dying of acute hydrogen sulfide poisoning indicated that its presence has little or no bearing on the cause of death. The evidence is especially strong when a

comparison is made of the amounts found in acute poisoning and the concentrations tolerated by experimental animals transfused with blood containing high concentrations of sulfhemoglobin.

During the course of the experiments it was noted that sulfhemoglobin is much more stable in vitro than vivo. Blood samples were taken just before or immediately after the heart stopped beating, and from animals that lived at the height of the H_2S intoxication, and sulfhemoglobin determinations started very soon afterwards. It is felt that the final determinations are representative, since the blood sample handling time was much less than the time required for noticeable breakdown of sulfhemoglobin in vivo.

The results of these experiments indicate that there is no satisfactory test or series of tests for H_2S poisoning. The finding of small amounts of sulfhemoglobin might be indicative, but would not be conclusive.

The hypothesis advanced, that H_2S inactivates or inhibits some enzyme system, seems most logical. In support of this view is experimental evidence showing that relatively small amounts of H_2S are required to cause death, the extreme rapidity with which intoxication develops, the high tolerance when only part of the blood was saturated with H_2S and the rapidity with

which animals recover even when prostrated. The latter fact suggests that the reaction is readily reversible. In the case of insects, poisoned with low concentrations of H_2S reversible paralysis is reported to occur.

The lowered CO_2 content of the blood and the early depression of the respiratory center during H_2S intoxication suggested the use of CO_2 as a physiological antidote. Carbon dioxide or a mixture of carbon dioxide and oxygen administered intravenously or by inhalation increases the animals' tolerance for H_2S, but does not prevent death when sufficient quantities of the toxic gas are administered. It is apparent that respiratory center collapse is due to some changes affecting the cells of the center, rather than to insufficient concentrations of CO_2 in the blood.

CONCLUSIONS

Concentrations of sulfhemoglobin in the blood of animals dying of H_2S poisoning were much less than concentrations of sulfhemoglobin established experimentally in animals that lived.

There was a decrease in the CO_2 content of blood of H_2S-intoxicated animals.

Carbon dioxide given intravenously and a mixture of oxygen (90%) and carbon dioxide (10%) given through an inhalation mask, increased experimental animals' tolerance for, but did not abolish, the toxicity of hydrogen sulfide.

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