Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

A. G. DuMez, Editor, Baltimore, Maryland

Volume XXIX | *JULY, 1940* | Number 7 Consecutive No. 13

Studies on Colloidal Sulfur—Polysulphide Mixture. I.—Toxicity*

By Harry Greengard, Ph.D., M.D., and Jean Rea Woolley, M.D.

INTRODUCTION

An investigation has been made of a colloidal sulfur preparation which is a concentrate of a naturally occurring spring water[1] and differs from the native water mainly in that it is quite alkaline (p_H of 10.1) and contains a large amount of sulfur (5 per cent). Much of the sulfur is in the colloidal state, since the Tyndall phenomenon is demonstrable, and due to the alkalinity of the solution, there is also some polysulphide present. The proportion of polysulphide sulfur cannot be determined, and there is reason to believe that for practical purposes it is equivalent to colloidal sulfur. The solution is transparent, orange-red in color, has a very pronounced sulfurous odor and taste, and undergoes precipitation on acidification or on heating to above 90° C.

The activity of sulfur in the organism appears to be a function of the degree to which it may be converted to H_2S. Various investigators (1–6) have demonstrated that such a reaction occurs when living organic matter acts on sulfur; hence it follows that the toxic manifestations of sulfur absorption are those of sulphide intoxication. The stigmata of such intoxication have been reported (7), (8) to be narcosis, convulsions, central motor depression, pulmonary edema and hyperemia and cardiac irregularities with terminal arrest; and several workers (9–15) have noted that the results when colloidal sulfur was given in toxic doses were identical with H_2S or sulphide poisoning. The more recent work (13), (14) has shown that the degree of toxicity of colloidal sulfur depends upon the route of administration, the degree of dispersion of the colloidal particles and the speed of injection when the material is given intravenously. The ability of the organism to survive a given dose of sulfur depends, therefore, on whether the resultant H_2S can be cleared from the system before it accumulates to a fatal level.

Toxic manifestations from sulfur administration have been reported clinically. Burmeister (16) described a case of violent poisoning after use of a sulfur-containing salve for scabies, evidenced by a marked burning sensation of the skin, followed by shock and syncope with recovery after

* From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

[1] The source of this water is Graham Springs, Harrodsburg, Kentucky.

vigorous stimulation. Hesse (17) noted intoxications and febrile reactions occasionally when cases of scabies or pediculosis were treated with sulfur. Basch (18) had several fatalities in infants treated for scabies with a 10 per cent sulfur ointment; the autopsy findings were those of chronic H_2S intoxication. He stated that the absorbability of sulfur in this form depended on the degree of injury to the skin and that the intact skin is almost impervious, and he was able to duplicate this intoxication experimentally in animals (19).

In the present work, the toxicity of the preparation under investigation was studied with regard to its acute and chronic effects by various routes of administration. The minimal lethal dose was determined by the intravenous route in dogs, rats and rabbits; by the intraperitoneal route in guinea pigs; and by the oral route in rabbits. The effect on circulation and respiration of the anesthetized dog was studied. Finally, the material was given orally to a series of rats, and orally and intravenously to rabbits, with autopsy studies after an appropriate interval. It was also given to human subjects in large doses for seven days, after completion of the animal experiments.

EXPERIMENTAL

Minimal Lethal Dose Determinations.—In the light of the knowledge gained by other investigators regarding the effect of rate of injection on the toxicity of the material, it was considered necessary to standardize the intravenous injection rate for all species used. Therefore, the solution was diluted with distilled water to a concentration of 0.5 per cent and the injection was made over a period of exactly one minute at as constant a rate as possible. The rats were prepared by ether anesthesia and exposure of the external jugular vein through a small incision; after injection the wound was closed and recovery allowed to take place.

Effect on Circulation and Respiration in the Anesthetized Dog.—The dogs were anesthetized with sodium pentobarbital and tracings were made of carotid blood pressure and respiration in the usual manner. Gross and microscopic examinations of the lungs were made after death of the animals.

Chronic Toxicity.—This problem was investigated on rabbits and rats. Six rabbits were given a daily ration of 10 Gm. of alfalfa hay, 10 Gm. of sucrose and 150 cc. of milk. Three of them received, respectively, 0.5, 1.0 and 2.0 mg. per Kg. of colloidal sulfur daily intravenously, and the others 3–5, 10 and 20 mg. per Kg. per day orally. After a period of 20 days they were sacrificed and pathological studies were made of the kidneys, livers, stomach and lungs. The sulfur excretion of these animals was followed daily, and the number of analyses required made it impossible to use a larger series.

The rats were given a diet of the following composition:

Casein—18%
Dextrin—37%
Sucrose—15%
Salt mixture (Osborne-Mendel)—4%
Agar—2%
Cod liver oil—5%
Lard—19%

This diet and water were given *ad lib.* and supplemented with 400 mg. of yeast daily in the form of pills. After a preliminary 20-day period to permit the animals to demonstrate their ability to grow normally, daily doses of 2, 5, 10 and 15 mg. of colloidal sulfur were given; the sulfur was incorporated into the pill, and the animals maintained on this régime for 100 days.

Six human subjects were given the material daily for 10 days in a dosage of 500 to 750 mg. per individual, or 10 to 15 mg. per Kg. of body weight.

RESULTS

1. Minimal Lethal Dose Determinations.—The results are tabulated below.

Table I.—Minimum Lethal Dose

Species	Route of Administration	Dosage mg./Kg.	Lived	Died
Dog	Intravenous	10	16	14
Rabbit	Intravenous	5	8	11
Rat	Intravenous	8	12	9
Guinea pig	Intraperitoneal	55	10	12
Rabbit	Oral	175	12	13

Convulsions, syncope and an H_2S odor of the breath were the manifestations noted.

2. Effect on Circulation and Respiration.—There was uniformly a fall in blood pressure immediately after injection with bradycardia and an initial stimulation of respiration followed by arrest; the severity of these manifestations depended on the amount of material injected and on the rate of injection. There was invariably an accumulation of serous fluid in air passages, so marked when the dose was large as to pour out of the trachea in large amounts. Examination of the lungs revealed them to be boggy and very hemorrhagic; the bronchial tree was filled with a frothy fluid; and microscopically there was evident a pronounced edema of the alveolar walls. In order to establish that these phenomena were due to the sulfur some of the material was desulfurized by acidification and filtration followed by alkalinization to the original p_H. Injection of the desulfurized material in large amounts was without effect on the animals.

3. Chronic Toxicity.—Autopsy of the three rabbits which were given colloidal sulfur intra-

venously revealed thickening and fibrosis of the alveolar walls of the lungs. There were no abnormalities in the kidneys, livers or stomachs. In the case of the rabbits receiving the material orally in much larger doses there were no pathological changes in any of the organs.

The series of rats which were given sulfur at various levels for 120 days grew normally (Table II) and on autopsy no abnormalities were found. A second series of rats were also studied. At maturity these rats were mated, and it was found that both control and sulfur-fed rats gave birth to healthy litters which they nursed successfully.

The human subjects given the sulfur solution reported no unpleasant subjective symptoms from the ingestion.

Table II.—Effect of Oral Administration

Rat No.	Sulfur Daily	Initial Wt.	Final Wt.	Average Daily Gain	Average Daily Food Consumption
1	Control	40	245	1.7	7.8
2	Control	37	267	1.9	7.5
3	2 mg.	37	180	1.2	7.5
4	2 mg.	44	187	1.2	7.3
5	2 mg.	32	181	1.2	7.3
6	2 mg.	47	252	1.7	8.3
7	5 mg.	61	183	1.0	8.0
8	5 mg.	49	238	1.6	8.3
10	5 mg.	42	173	1.1	7.8
11	10 mg.	43	232	1.5	8.0
12	10 mg.	37	164	1.0	6.8
13	10 mg.	27	175	1.2	7.0
14	10 mg.	39	269	1.9	9.0
15	15 mg.	36	195	1.3	7.8
16	15 mg.	39	270	1.9	8.5
17	15 mg.	36	142	0.9	6.0
18	15 mg.	28	289	2.2	8.8
19	5 mg.	51	303	2.1	9.3

DISCUSSION

The findings reported above confirm the evidence in the literature to the effect that colloidal sulfur is highly toxic when given intravenously and that it owes its toxicity to rapid conversion to hydrogen sulphide. This gas is excreted for the most part, if not entirely, through the expired air; and in its passage through the lung it produces inflammatory changes as reflected acutely by hemorrhage and edema and chronically by fibrosis. The work of Wheeldon and Main (14) was also confirmed in that the material was much less toxic when injected intraperitoneally in guinea pigs, the M. L. D. as determined by them with the technic was 40 mg. per Kg. whereas in the product used in this work it was 55 mg. per Kg. It is obvious that the absorption of H_2S into the circulation is much slower in this case, so that considerably larger amounts may be tolerated.

No definite evidence exists in the literature that colloidal sulfur is toxic when given orally; and it was found in this work that 35 times the intravenous minimal lethal dose was tolerated without the production of deleterious effects by half of the animals to whom it was given by stomach tube. This may be explained either by the fact that the sulfur undergoes precipitation when it comes in contact with the acid gastric contents and is thereby rendered inert, or else that it is competely absorbed and so modified in its passage through the liver as to be rendered nontoxic unless the dose is overwhelming. Evidence given in another paper (20) indicates the latter view to be the correct one. The effect of oral sulfur administration continued over long periods of time in the rats, some of which received over 100 mg. per Kg. per day for over three months, shows that colloidal sulfur when given orally is in no way incompatible with normal growth and function. The administration of colloidal sulfur is not incompatible with pregnancy and lactation in the rat.

CONCLUSION

A colloidal sulfur-polysulphide preparation has been tested for toxicity by the intravenous, intraperitoneal and oral routes of administration. It was highly toxic when given intravenously and yielded an M. L. D. of 5 to 10 mg. of sulfur per Kg. of body weight, depending on the species used, when injection is made in one minute's time. The toxicity depends on the prompt conversion of the sulfur to H_2S, and death takes place from H_2S poisoning. Pathological findings in the lungs are edema and hemorrhage acutely, and fibrous tissue proliferation chronically. The same acute symptoms are noted with toxic doses by the intraperitoneal and oral routes, but much larger amounts are tolerated here, especially in the case of oral administration. Unless the dose is extreme, large amounts may be taken by mouth over a long period of time. The human subject easily tolerates up to 750 mg. of colloidal sulfur daily when given orally.

REFERENCES

(1) Heffter, *Arch. exp. Path. Pharmakol.*, 51 (1904), 175.

(2) Taegan, *Arch. exp. Path. Pharmakol.*, 69 (1913), 263.
(3) Andrews, *J. Biol. Chem.*, 122 (1938),687.
(4) Stigler, *Münch. med. Wchschr.*, 76 (1929), 1795.
(5) De Rey-Poilhade, *Bull. soc. chim. biol.*, 11 (1929), 1143.
(6) Rostelli and Casazza, *Bull. Soc. Ital. Biol. Sper.*, 5 (1930), 939.
(7) Pohl, *Arch. exp. Path. Pharmakol.*, 22 (1877), 1.
(8) Lehmann, *Arch. Hyg. Bakt.*, 14 (1892), 835.
(9) Sabbatini, *Pathologica*, 5 (1913), 410.
(10) Piery, Bonamour, Milhaud and Guigonet, *C. r. soc. biol.*, 91 (1924), 681.
(11) Cluzet, Piery, Bonamour, Milhaud and Kofman, *Ibid.*, 94 (1926), 51.
(12) Piery, Bonamour and Milhaud, *Ibid.*, 94 (1926), 69.
(13) Messini, *Arch. exp. Path. Pharmakol.*, 127 (1925), 368.
(14) Wheeldon and Main, *J. Bone and Joint Surg.*, 15 (1933), 94.
(15) Kirsner, *J. Lab. and Clin. Med.*, 22 (1937), 1026.
(16) Burmeister, *Dermatol. Wchschr.*, 58 (1901), 389.
(17) Hesse, *Münch. med. Wchschr.*, 62 (1915), 1236.
(18) Basch, *Monatsschr. Kinderheilk.*, 32 (1926), 239.
(19) Basch, *Arch. exp. Path. Pharmakol.*, 11 (1936), 126.
(20) Greengard and Wooley, *J. Biol. Chem.*, 132 (1940), 83.

Use of Sodium Pentobarbital for Repeated Anesthesia in the Rabbit

*By V. Everett Kinsey**

The need in this laboratory for an anesthetic which could be safely administered to small laboratory animals over periods of approximately one year gave rise to the present investigation. It was desired to find an anesthetic which could be easily given, required no further attention, and especially one which would be relatively safe and leave the animal in an apparently normal physiological state even though it be repeated every other day over a period of months.

Of the many anesthetics available it appeared that one of the medium to short-acting barbiturate derivatives would come nearest to meeting all of the above requirements. Many previous studies on the action of this series of compounds have been concerned with detoxification, elimination, the effect of single doses on various organs of the body and, particularly, with the hypnotic and minimal lethal dose. Tatum (1) has written a review of the status of the whole barbiturate problem. Investigations dealing with repeated administration of various barbiturates are adequately referred to here, by Masuda, *et al.* (2), and by others, some of whose papers will be discussed in more detail later. Most workers concur in the opinion that several barbiturates produce appreciable tolerance after administration for relatively short periods of time (days, not weeks) as measured by a lessening of sleeping time. Few of the workers, however, have subjected the animals to weeks or months of treatment and little stress has been placed on the important question of survival, either at the time of injection or later, although deaths presumably due directly to the anesthetic are recorded in several instances.

The choice of barbiturate appeared to be limited to those compounds which would produce sleep in small laboratory animals for periods not greatly in excess of two hours when given every other day. Several preliminary experiments confirmed this and showed that when, after repeated anesthesia, animals were kept unconscious even for two hours following a single injection of the barbiturate, long periods of depression followed. The debilitating effect of the latter apparently led to the extraordinarily high mortality which was observed. Because of these discouraging results, a shorter acting compound, sodium pentobarbital (Nembutal)[1] was chosen for this investigation, even though two or sometimes more separate injections were required to produce anesthesia for two-hour periods.

It is the purpose of the present study to review the whole question of repeated intraperitoneal injections of sodium pentobarbital in the rabbit from the viewpoint of the in-

* Department of Physiological Chemistry, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

[1] Kindly furnished by the Abbott Laboratories, North Chicago, Ill.