The Pharmacology of Sodium Hydroxyacetate with Observations on the Toxicity of Glycine*†

By Walter F. Riker and Harry Gold

The behavior of hydroxyacetic acid (glycolic acid) in the animal organism has received some consideration in the literature (1, 2, 3, 4). Concerning the questions of whether glycolic acid is a normal product of intermediary metabolism, whether the animal organism can produce it under any conditions, and the behavior of injected material, the existing evidence is inconclusive. Observations on the effects of hydroxyacetic acid administered to animals and man are meager. Pohl (1) observed swaying of the body and drowsiness within a half-hour after the oral administration of 0.67 Gm. of sodium hydroxyacetate per Kg. in a dog. He deduced from the occurrence of these effects that it is well absorbed from the gastrointestinal tract. Milhorat and Toscani (2) did not record any symptoms in 4 patients with progressive muscular dystrophy after sodium hydroxyacetate in oral doses of the order of 0.26 to 0.4 Gm. per Kg. daily for periods up to 7 days.

The present experiments were planned to investigate further the pharmacology of hydroxyacetic acid.

EXPERIMENTAL

The experiments were performed on 35 cats, 2 dogs and 1 rabbit. The effects of the drug were studied by oral (stomach tube) and intravenous administration, after a single large dose, and after repeated oral doses for periods up to somewhat longer than 2 months.

The material was used, except when otherwise stated, in the form of a buffered mixture of hydroxyacetic acid and sodium hydroxyacetate, making a 9.8% solution with a *p*H of 7.3. It was prepared by dissolving 98 Gm. of sodium hydroxyacetate and 0.024 Gm. of hydroxyacetic acid in distilled water and diluting up to 1 L. The *p*H of the solution was checked with the Leeds-Northrup glass electrode.

DISCUSSION OF RESULTS

Some of the results on toxicity have been assembled in Table I. An oral dose of 0.1 Gm./Kg. is without effect; 0.25 Gm./Kg. usually causes some signs of poisoning; 0.5 Gm./Kg. may prove fatal; larger doses are usually fatal.

The effects resemble some of the elements of poisoning by a barbiturate, camphor, strychnine and bulbocapnine. Neuromuscular disturbances are the chief symptoms. Weakness, ataxia and anorexia are the earliest effects. The animal prefers to lie. It is easily thrown over. Its limbs sprawl. When it stands, its body sways. In the early phase of poisoning, the animal appears to be quite alert and playful, although it may be too weak to walk. The narcotic action is relatively slight. The muscular weakness progresses and the pattern of poisoning becomes complicated by signs of cerebrospinal stimulation. Catatonic disturbances appear after the larger doses. The animal assumes awkward posture with lead-pipe rigidity of the limbs. As the poisoning advances, fibrillary twitchings of the muscles appear. The gait becomes spastic. Convulsions follow at irregular intervals. At times the convulsions are camphor-like, myoclonic, without reflex hyperexcitability; at other times reflex hyperexcitability predominates and the character of the convulsions resembles more nearly those of strychnine. Convulsions are late effects; they usually developed only after many hours following the doses used and invariably terminated fatally. The pupillary reflexes remain essentially normal. The corneal reflexes are depressed in the later stages. Some analgesia is also present in more advanced poisoning. After the oral doses, signs of nausea and vomiting are very common, although they are apt to appear only after many hours. They are rare after intravenous doses. The character and severity of the symptoms depend on the doses. They are essentially similar after oral and intravenous administration.

The effects described above were substantially the same in the dog. An oral dose of 1.5 Gm./Kg. resulted in weakness, ataxia, nausea and vomiting. These effects progressed and were fairly marked 24 hrs. later, when they were greatly intensified by an intravenous injection of 1 Gm./Kg. The weakness progressed further during the next 5 days when myoclonic convulsions appeared, resulting in death on the sixth day. In another dog an intravenous dose of 1.5 Gm./Kg. caused similar symptoms but without convulsions and with recovery in about 24 hrs. There remains some question about its effect in the rabbit. An intravenous injection of 1 Gm./Kg. produced no perceptible effects on a rabbit. This requires further study.

Absorption.—The absorption of sodium hydroxyacetate from the gastrointestinal tract is fairly complete as judged by the fact that the oral and intravenous fatal doses are not far apart. It may be

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TABLE I.—TOXICITY OF SODIUM HYDROXYACETATE

Dose, Gm./Kg. Single	Dose, Gm./Kg. Total	Route	Severity of Effects (Symptoms)	Remarks
			Single Dose	
0.10		Oral	0	
0.10		Oral	0	
0.10		Oral	0	
0.10		Oral	0	
0.10		Oral	0	
0.25		Oral	0	
0.25		Oral	1+	Recovered in 2 days
0.25		Oral	1+	Recovered in 3 days
0.25		Oral	1+	Died with pneumonia
0.25		Oral	1+	Died after repeated doses in 21 days
0.50		Oral	2+	Died with pneumonia in 16 days
0.50		Oral	4+	Died in 5 days
0.50		Oral	2+	Died after repeated doses in 9 days
1.00		Oral	2+	Recovered in 1 day
1.50		Oral	4+	Died in 6 days
2.00		Oral	4+	Died in $1^1/_2$ days
2.50		Oral	5+	Died in $1^1/_2$ days
1.00		Vein	1+	Recovered in 1 day
1.00		Vein	2+	Recovered in 4 days
1.00		Vein	4+	Died in 4 days
1.00		Vein	4+	Died in 2 days
1.27		Vein	4+	Died in 3 days
1.50		Vein	4+	Died in 2 days
1.50		Vein	3+	Died after intravenous calcium
1.50		Vein	3+	Died after intravenous calcium
2.00		Vein	3+	Died in 4 days
2.40		Vein	4+	Died in 1 day
			Repeated Doses	
0.10	2.30	Oral	0	
0.10	2.30	Oral	0	
0.10	2.30	Oral	0	
0.10	3.00	Oral	0	
0.10	3.00	Oral	0	
0.25	2.25	Oral	4+	Died in 21 days
0.25	3.25	Oral	0	Marked azotemia without symptoms; dose discontinued, partial recovery
0.50	3.00	Oral	4+	Died in 9 days

seen in Table I that 2 of 4 animals survived 1 Gm./Kg. by intravenous injection, and that as little as 0.5 Gm./Kg. by oral administration may prove fatal. The absorption, however, is quite slow, as indicated by a comparison of the speed of onset of effects after oral and after intravenous administration. For example, a dose of 1 Gm. by intravenous injection produced the first effects within 26 min.; by oral administration, within 78 min. The intravenous doses produced effects as a rule within less than 30 min., whereas even fatal oral doses rarely showed effects in less than 1 hr.

Speed and Duration of Action.—There is a long latent period in the development of the effects of sodium hydroxyacetate. This is not a matter of absorption, since it also applies to intravenous doses. Its duration depends on the dose; after a small oral dose of 0.25 Gm./Kg., as long as 18 hrs. may elapse before distinct symptoms of poisoning are in evidence; with the larger intravenous doses (1 Gm./Kg. or more), effects may be in evidence as early as 10 min. after administration, although usually characteristic signs of poisoning are delayed for as long as one-half hour to an hour. The course of the poisoning is protracted, and, as seen in Table I, terminates in death or recovery after periods of days. It may require as long as 2 to 3 days for manifest signs of poisoning to subside after a dose as small as 0.25 Gm./Kg. In cases which prove fatal, the period of poisoning may be longer, up to 21 days.

Cumulation.—A cat which received an oral dose of 0.5 Gm./Kg. in equal daily fractions during a 5-day period remained without signs of poisoning, while another cat which received a similar dose at one time developed poisoning by the following day which progressed and proved fatal at the end of 5 days. The effect of repeated doses was studied in a group of 8 animals. An oral dose was given daily during the first week and then every second day. The results of those experiments in which the blood chemistry was followed are summarized in Table II.

TABLE II.—EFFECT OF SODIUM HYDROXYACETATE ON BLOOD AND URINE IN THE CAT

No. of Cats	Dose: Single, Gm./Kg.	Dose: Total No.	Total Dose, Gm./Kg.	Experimental Day	Blood: Hb., Gm. %	Blood: R. B. C., Million per Cu. Mm.	Blood: W. B. C., per Cu. Mm.	Blood: N. P. N., Mg. %	Blood: Creatinine, Mg. %	Blood: Prothrombin, %	Urine: Sp. Gr.	Urine: Albumin and Sugar
6	Control	..	....	2	....	....	....	32	1.8	...	....	0
				8	....	....	....	35	1.4	...	1.058	0
				13	....	....	....	35	1.4	...	1.058	0
				33	....	....	....	34	1.1	...	1.042	0
				47 to 48	14.5	7.5	9,400	46	1.7	...	1.054	0
				54 to 56	11.0	7.5	6,200	...	....	100	....	...
				68	....	....	....	38	1.4	93	1.050	0
				82	....	....	....	39	1.6	87	1.070	0
				91	12.5	8.3	8,300	...	....	...	....	...
				96	....	....	....	35	1.4	94	1.050	0
				110	....	....	....	40	1.4	83	1.050	0
				120	13.0	7.8	5,500	...	....	...	....	...
				126	....	....	....	44	1.3	92	....	...
1	0.1	30	0	−10 to −8	16.0	11.7	10,400	54	1.8	100	1.032	0
			0	− 3 to −1	15.5	7.9	8,000	44	0.9	83	Q. N. S.	0
			0.6	11	....	....	....	31	1.7	100	1.054	0
			0.8	16	11.0	9.2	7,800	...	....	...	....	...
			1.1	25	....	....	....	39	1.4	75	1.050	0
			1.5	34	9.0	6.0	13,100	...	....	...	....	...
			1.7	39	....	....	....	41	1.1	100	1.048	0
			2.4	53	....	....	....	49	1.5	100	1.052	0
			2.8	63	10.0	6.0	11,200	...	....	...	....	...
			3.0	69	....	....	....	46	1.3	85	....	...
11	0.25	9	0	−10 to −8	12.0	7.8	10,600	55	1.5	...	1.060	0
			0	− 3 to −1	9.0	7.0	5,200	43	1.1	83	....	0
			1.5	11	....	....	....	106	2.5	98	1.030	0
			2.0	16	12.0	7.5	17,000	...	....	...	....	...
			2.25	19	....	....	....	197	4.2	...	....	...
10	0.50	6	0	−10 to −8	10.0	6.5	8,300	52	1.4	...	1.040	0
			0	− 3 to −1	9.0	6.8	9,100	53	1.3	78	....	0
			3.0	8	....	....	....	328	11.7	79	....	...

In the 2 cats in which the daily dose was 0.1 Gm./Kg., no effects were in evidence after 30 doses (total dose, 3 Gm./Kg. in a period of 69 days), indicating elimination of approximately 43 mg. of the agent per Kg. per day. Another cat which received a repeated oral dose of two and one-half times as much, or 0.25 Gm./Kg., developed signs of poisoning with azotemia and died in 21 days, after a total of 2.25 Gm. If the rate of elimination is a straight line function of the dose, the amount remaining after elimination during the 21 days would not be sufficient to cause poisoning in this case. The fact that the animal died indicates that some factor may have interfered with elimination. While there are other possible explanations, the indications are that the nephrotoxic action of this compound interferes with its excretion, and may explain the observation that whereas nontoxic doses when repeated show no tendency to cumulation (5 animals received 0.1 Gm./Kg. daily in Table I), recovery is slow and cumulation is marked after doses which cause toxic effects.

Heart, Circulation and Respiration.—The effects were observed in 6 cats. The mean blood pressure was recorded from the carotid artery with a mercury manometer on a smoked drum, the respiration from a pleural cannula. Electrocardiographic tracings were taken for the effect on the heart.

Three cats under dial anesthesia received intravenous injections of hydroxyacetic acid as the acid in a 5% solution (*p*H 3.4). These produced marked hyperpnea with fall of the blood pressure and death with circulatory collapse and depression of respiration after total doses of 178, 225 and 312 mg. per Kg., respectively. Undoubtedly, nonspecific acid effects were obtained. The buffered mixture was used in the subsequent experiments. One cat received an intravenous injection of 1.35 Gm./Kg. in the form of a 5% solution. This produced no effect on the respiration or the blood pressure within a period of 15 min. In another cat the experiment was performed during local anesthesia with procaine. A dose of 0.4 Gm./Kg. was administered at intervals of 5 to 14 min., a total of 2.4 Gm. in a period of 55 min. There was no effect on the respiration. The blood pressure declined from an initial level of 190 mm. of mercury to 150 mm., while the heart rate accelerated somewhat. The following day the animal was moribund, and at this time the heart rate had fallen to 40/min. In the case of another cat, a blood pressure record was taken during the period of advanced symptoms of poisoning with marked azotemia. This animal had received repeated oral doses of 0.25 Gm./Kg. during 21 days (total dose, 2.25 Gm./Kg.). At the end of this period the N. P. N. was 197 mg. % and creatinine, 4.2 mg. %, and the mean blood pressure was 135 mm. of mercury.

It is clear from these experiments that when *p*H disturbances are excluded, hydroxyacetic acid exerts relatively insignificant direct effects on the blood pressure and respiration. Even during severe poisoning, the blood pressure, though lower than the average blood pressure for a normal cat, was fairly well sustained.

Electrocardiograms were taken at intervals in 4 cats which received repeated oral doses of 0.1 and 0.25 Gm. per Kg. No significant changes in the tracings were observed even during the stage of severe toxic symptoms.

Changes in Urine and Blood Chemistry.—The urine was examined for albumin (heat and acetic acid) and for sugar (Benedict's solution). The blood was examined for N. P. N. (Folin and Denis (5)), creatinine (Folin and Wu (6)), and prothrombin (Warner, Brinkhous and Smith (7)). Typical results are summarized in Table II.

Three cats were studied as controls of the effect of the laboratory conditions and diet on the blood and the urine for 4 months. They remained normal in every respect. A constant diet of dog food ration, chopped meat and fresh liver was used in the control as well as the treated animals.

Each of 5 cats received daily oral doses of sodium hydroxyacetate varying from 0.1 to 0.5 Gm. per Kg. As previously described, the larger doses caused symptoms of poisoning and death. The urine remained negative for albumin and sugar. The specific gravity of the urine remained essentially unchanged. This was also true in those cases in which signs of renal insufficiency developed as shown by the azotemia. There were no consistent changes in the number of red blood cells, white blood cells, or the amount of hemoglobin. The prothrombin time remained unchanged. Doses of 0.1 Gm./Kg. repeated daily caused no change in the N. P. N. or creatinine. The larger doses, 0.25 Gm. and 0.5 Gm. per Kg. repeated daily, however, caused marked elevation of the N. P. N. and creatinine. In one animal it was observed that this change is partially reversible; the N. P. N. rose from 41 to 123 mg. %, and the creatinine from 1.5 to 2.4 mg. % during the use of the 0.25 Gm./Kg. dose. The drug was then discontinued, and both the N. P. N. and creatinine fell off, the creatinine to the normal level, the N. P. N. showing only partial recovery, namely, 67 mg. %. In this animal sodium hydroxyacetate produced a marked nephrotoxic action with azotemia, although the appearance and behavior of the animal remained normal during the period of observation.

The indications are that sodium hydroxyacetate is less nephrotoxic than tartrate, although we do not have sufficient data for a precise comparison. Previously (8), it was found that 0.3 Gm. of tartrate per Kg. caused severe renal damage. In a cat of the present study, 0.25 Gm. of sodium hydroxyacetate per Kg. repeated at intervals of 24 to 48 hrs. during a period of 11 days (total dose, 1.5 Gm./Kg.) produced no azotemia, although the continued use of this dose resulted in marked renal damage with azotemia.

It should be noted, however, that the renal damage is not the cause of most of the early toxic symptoms, since an animal may show severe poisoning within an hour or two after the dose, while the cat with bilateral nephrectomy goes without serious effects for more than 24 hrs. Furthermore, after sodium hydroxyacetate, the cat may develop severe tubular nephritis without general signs of poisoning.

Pathological Changes.—Gross autopsy findings in the case of animals that died from the drug or were sacrificed after doses of the drug which did not prove fatal were, in the main, negative. No significant changes were observed in the heart, lungs, liver, kidneys, spleen, adrenals or gastrointestinal tract. After the large intravenous doses, small pulmonary hemorrhages were seen, but these are probably nonspecific, since large intravenous injections of sodium chloride also produce them. The brain was examined in gross sections in 2 cases, and the findings were also negative. Microscopic sections of the kidney and liver were made in the case of 5 animals which received daily oral doses as follows: 0.1, 0.25 and 0.5 Gm. per Kg. (total dose, 2.25, 3.0 and 3.25 Gm. per Kg.). The liver showed no changes. The kidneys showed tubular degeneration in all but one case (0.1 Gm./Kg.). It was slight in 1 cat which received the dose of 0.1 Gm./Kg. In this cat the blood chemistry remained normal. The tubular degeneration was more pronounced after the larger doses, and corresponded to the azotemia. The histological changes in the kidneys are similar to those observed after the tartrates.

Mechanism of Action.—The anorexia, and the neuromuscular disturbances with weakness and convulsive symptoms, suggested several possible mechanisms of action: a curare-like action, acidosis, disturbed calcium metabolism (since the possibility of oxalate formation from hydroxyacetic acid has been suggested), disturbance in sugar metabolism, interference with metabolism of thiamine. These possibilities were tested chiefly by attempts to counteract the toxic effects of sodium hydroxyacetate. The measures used failed to influence them, and hence indicate that the foregoing factors are probably not involved.

A cat received an oral dose of 1 Gm./Kg.; and when the poisoning was pronounced, 0.24 mg. of physostigmine salicylate per Kg. was injected intramuscularly. There was no effect on the neuromuscular weakness. In 2 cats the phrenic and femoral nerves were stimulated with the Harvard inductorium during severe poisoning with intravenous doses of 1.3 and 5 Gm. per Kg., respectively. The response of the diaphragm and the leg muscles appeared to be normal. In 2 additional cats, the tension developed by the gastrocnemius muscle was measured by means of an isometric lever with the technique described by Wolff and Cattell (9), after intravenous doses of 1 and 1.6 Gm. per Kg., respectively. The drug produced no change in tension developed by the muscle during contraction. These types of experiments exclude a curare-like action.

TABLE III.—TOXICITY OF SEVERAL ORGANIC ACIDS

No. of Cats	Drug	Dose	Systemic Poisoning	Remarks
1	Sodium fumarate	5 Gm./Kg., oral, 15% soln.	None during 6 days	Vomiting and diarrhea, vomitus returned
1	Sodium citrate	2 Gm./Kg., oral, 20% soln.	None during 9 days	Repeated vomiting, about 1/2 of dose lost
1	Sodium citrate	1 Gm./Kg., oral, 5% soln.	None during 5 days	
4	Sodium acetate in buffered mixture with acetic acid	1 to 2.5 Gm./Kg., oral, 20% soln.	None	
1	Sodium acetate in buffered mixture with acetic acid	1 Gm./Kg., vein, 20% soln.	None	
1	Glycine[a]	3 Gm./Kg., oral, 20% soln.	None in 50 min.	After 50 min., next dose given
		2.5 Gm./Kg., oral, 20% soln.	None during 4 days	Vomited in 75 min.
1	Glycine	6 Gm./Kg., oral, 20% soln.	Profuse drooling for 3 hrs., otherwise none during 5 days	Vomited most of dose in 10 min.
1	Glycine	6 Gm./Kg., oral, 10% soln.	Weakness, swaying, awkward posture in 25 min.; convulsions in 75 min.; pupils dilated and do not react to light	Partial recovery over 5-day period
1	Glycine	1 Gm./Kg., vein, 10% soln.	Drooling, muscular weakness and reflex hyperexcitability in 25 min.; pupils dilated and do not react to light	Recovery complete in 4 hrs.
1	Glycine	2 Gm./Kg., vein, 10% soln.	Same as after 1 Gm., but more marked; also vomiting	Recovery complete in 24 hrs.
2	Glycine	3 Gm./Kg., vein, 10% soln.	Same as after 2 Gm., but more marked; convulsions	One died in 1 hr., 45 min.; the other in 10 hrs.

[a] Effects the same when given as the acid as when neutralized with sodium bicarbonate.

In one cat which received an intravenous dose of 1 Gm./Kg., a control sample of blood taken from the jugular vein showed a carbon dioxide combining power of 44 vols. % (manometric method of Van Slyke) and sugar, 135 mg. %. A sample taken 2 hrs. after the dose, when symptoms of poisoning were pronounced, showed a carbon dioxide combining power of 47, and blood sugar, 143. The effects of sodium hydroxyacetate, therefore, appear not to be related to acidosis or disturbance in blood sugar level.

In one cat which received an intravenous dose of 1.5 Gm./Kg., repeated intravenous injections of a 10% solution of calcium chloride (total, 160 mg./Kg.) had no effect on the neuromuscular disturbance. In this animal an intravenous injection of 0.25 mg. of thiamine hydrochloride per Kg. was also without effect on the muscular weakness.

In one cat which received an intravenous injection of 1 Gm./Kg., and developed marked symptoms of poisoning, the intravenous injection of a 10% solution of glucose in a total dose of 3.7 Gm./Kg. was without influence on the course of the symptoms.

While, therefore, certain possibilities regarding the mechanism of the toxic effect of sodium hydroxyacetate have been excluded, the mechanism of this action remains uncertain.

Comparison with Some Other Organic Acids.—The toxicity of sodium hydroxyacetate was compared with acetate, fumarate and citrate in a series of cats, the results of which are presented in Table III. The latter three compounds produced no systemic toxic effects in doses varying from 4 to 20 times the toxic dose of sodium hydroxyacetate.

Aminoacetic acid (glycine) presents a problem of special interest in relation to the toxicity of sodium hydroxyacetate, since it has been suggested that glycine may be a precursor of hydroxyacetic acid in the synthesis of creatine (Davenport, *et al.* (3)), or that hydroxyacetic acid is an intermediary in the conversion of glycine into glucose (Lusk (4), or, inversely, that hydroxyacetic acid may be a precursor of glycine (Milhorat and Toscani (2)). The toxicity of glycine has received little attention in the literature. Ni (10) found glycine toxic to guinea pigs in oral doses of the order of 2 Gm./Kg., with symptoms of muscular weakness and nervous disturbances. Lewis (11), who injected glycine intravenously in dogs in a study of the rate of metabolism of glycine, recorded the incidental observation that one dog developed vomiting, diarrhea and convulsions, after what appears to have been a total of about 1.5 Gm./Kg. He stated that these symptoms disappeared when the injection was stopped.

In the present study 7 cats received glycine orally and intravenously in doses ranging from 1 to 6 Gm. per Kg. The results are also summarized in Table III. Glycine, in doses larger than those commonly used, is toxic both by oral and intravenous administration. The indications are that it possesses about one-sixth to one-third of the toxicity of

sodium hydroxyacetate. About 3 Gm./Kg. by intravenous injection may prove fatal, and about 6 Gm./Kg. by oral administration may be survived. The symptoms of glycine poisoning are in some respects similar to, but not identical with those of sodium hydroxyacetate. Both cause nausea and vomiting, muscular weakness, ataxia and convulsions. Glycine, however, produces profuse drooling and a pupillary change (complete dilatation of the pupil which may fail to react to light) which we have not seen with sodium hydroxyacetate. In one dog an oral dose of 6 Gm./Kg. caused similar symptoms with convulsions and death in 4 hrs.

Metabolism of Hydroxyacetic Acid.—It has been shown in several studies (1, 2) that the conversion of hydroxyacetic acid into oxalic acid which takes place *in vitro* does not occur in animals or man. In several of the cats of the present study, specimens of urine reduced Benedict's solution after sodium hydroxyacetate. Since the suggestion has been made that hydroxyacetic acid might be a source of glucose (Lusk (4)), blood sugar determinations were made over a period of 5 to 7 hrs. in a group of 6 cats after intravenous doses of 1 Gm./Kg. No elevation of the blood sugar resulted. The urine in these animals also frequently showed a positive reaction with sodium nitroprusside. The possibility of the conversion of sodium hydroxyacetate into acetone bodies was considered. In 4 of the foregoing 6 animals, total acetone bodies in the blood were determined by the salicylaldehyde method of Behre (12). The total acetone bodies was not elevated.

A comparison of the toxic effects of glycine and sodium hydroxyacetate throws some light on the question of conversion of one into the other. If the conversion were quantitative and took place fairly rapidly, a gram of glycine should produce the toxic effects of approximately a gram of sodium hydroxyacetate. However, the present experiments show that sodium hydroxyacetate is about 3 to 6 times as toxic as glycine. Furthermore, the character of the symptoms in glycine poisoning are in some respects different. The evidence obtained in the present study indicates, therefore, that the conversion of glycine into hydroxyacetic acid, if it occurs at all, takes place to a very small extent.

Toxicity of Sodium Hydroxyacetate in Man.—We have made no experiments in humans bearing on this matter. On the basis of body weight, the oral dose of sodium hydroxyacetate which could be repeated daily for a long period of time with little danger of poisoning in cats (0.1 Gm./Kg.) would correspond to a daily oral dose of about 7 Gm. for the average man. A dose twice as large (14 Gm. daily for a man) corresponds to a dose which caused poisoning in cats. The indications seem to be that sodium hydroxyacetate by oral administration may be more toxic for the cat than for man, since doses larger than those toxic for cats were given to 4 patients by Milhorat and Toscani (2) without apparent toxic effects. This matter requires further study.

SUMMARY AND CONCLUSIONS

1. Sodium hydroxyacetate is toxic for cats and dogs, and produces similar effects in both species.

2. An oral dose of 0.1 Gm./Kg. rarely causes toxic effects. An oral dose of 0.25 Gm./Kg. is toxic, although not fatal. An oral dose of 0.5 Gm./Kg., corresponding to about 35 Gm. for a man, may prove fatal.

3. The absorption of sodium hydroxyacetate from the gastrointestinal tract appears to be rather slow, but indications are that it is fairly complete.

4. The onset of effects of sodium hydroxyacetate is slow even after intravenous injection. The length of the latent period varies inversely with the dose. The course of action is protracted. In the typical case, effects appear after about 30 min., progress in intensity during the next 24 hrs., and either subside gradually during the subsequent several days or increase in intensity and prove fatal in several days.

5. The cat eliminates nontoxic doses of sodium hydroxyacetate within 24 hrs. or less. The recovery from toxic doses, however, is so slow as to suggest some impairment of elimination or an injury which progresses independent of the elimination of the drug.

6. The symptoms of sodium hydroxyacetate toxicity are anorexia, nausea and vomiting, neuromuscular disturbances, with weakness, ataxia, muscle twitching and convulsions.

7. Sodium hydroxyacetate exerts a nephrotoxic action resulting in tubular degeneration and marked elevation of the blood N. P. N. and creatinine.

8. Limited comparisons with other organic acids were made. It is more toxic than fumaric acid, citric acid, acetic acid and aminoacetic acid.

9. Glycine is toxic to cats and dogs, producing symptoms resembling in many respects those of sodium hydroxyacetate, but not identical with the latter.

10. Observations concerning the mechanism of action and metabolism of sodium hydroxyacetate are discussed.

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Estimation of Magnesium in Solution of Citrate of Magnesia*

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Since the work of Berg (1) in 1927, the use of 8-hydroxyquinoline, or oxine, as a quantitative precipitant for certain metallic ions, has become definitely established among quantitative analytical procedures. This reagent is especially suitable for the determination of magnesium and the method offers distinct advantages over the classical phosphate procedure.

For the assay of magnesium oxide in Solution of Citrate of Magnesia the Pharmacopœia employs the phosphate method (2). It is believed that this method, even in the considerably simplified form which the Pharmacopœia prescribes, is entirely adequate in accuracy. The liberal limits for the magnesium oxide content (1.6–1.9%) are well within the therapeutic range so that the assay may be regarded to a large extent as a control of the stability of the final preparation. The present study was undertaken to determine the availability of the oxine method for this assay with the realization that the presence of relatively large amounts of sugar and citrate ion would constitute a somewhat special application of the method.

For our preliminary studies we have not attempted to prepare quantitative solutions of the official preparation. We have avoided the obvious difficulties involved in making such preparations by using a simplified solution containing the desired amount of magnesium oxide as magnesium sulfate, sugar and citric acid. Two of these simplified solutions were prepared representing the limits of magnesium oxide set by the Pharmacopœia, *i. e.*, 1.6% and 1.9%. The sugar and citric acid content of both solutions was the same and in accord with the values prescribed by the Pharmacopœia.

Two methods of procedure for the oxine precipitation were examined. In the first method (3), the slightly acid solution is treated with the required amount of oxine dissolved in acetic acid. The magnesium oxyquinolate is then precipitated by the addition of ammonia. Using this method we found it very difficult to obtain constant weight in the drying of the precipitate.

In the second method (4), the strongly ammoniacal solution, with ammonium chloride added to prevent the precipitation of the magnesium, is treated with the required amount of an alcoholic solution of the oxine. In the subsequent drying of the precipitate, constant weight could be obtained satisfactorily, and this method is therefore the one that was adopted.

For the preparation of the simplified test solutions a reagent grade of the heptahydrate of magnesium sulfate was used and was assayed in the following manner. Approximately 0.5 Gm. of the salt, accurately weighed, was dissolved in 150 cc. of distilled water previously heated to 70° to 80° C. After adding 5 cc. of a 2 *N* solution of ammonium chloride and 3 cc. of stronger ammonia, 14 cc. of a 5% solution of oxine in alcohol was added slowly with stirring. The mixture was allowed to stand for about 30 min. and then the supernatant liquid was decanted through a sintered glass filter crucible. The precipitate was washed four times by decantation with 25-cc. portions of 1% ammonia and then completely transferred to the filter crucible and washed thoroughly with distilled water. The filter crucible and contents were then dried to constant weight at 100–105° C. The results of three determinations gave values of 100.4%, 100.3% and 100.5%, respectively, corresponding to an average value of 100.4% $MgSO_4.7H_2O$.

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