STUDIES ON THE TOXICITY OF DIETHYLENE GLYCOL, ELIXIR OF SULFANILAMIDE-MASSENGILL AND A SYNTHETIC ELIXIR.

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INTRODUCTION.

Shortly after the unfortunate introduction and use of the so-called "Elixir of Sulfanilamide-Massengill," with its lethal effects in nearly a hundred instances, there appeared in the literature numerous papers (1), (2), (3), (4), (5) which clearly indicated that the solvent used, diethylene glycol, was the chief toxic agent present in the "Elixir." Prior to this time the literature contained few papers dealing with the toxicity of this substance. Von Oettingen and Jirouch (6) found that subcutaneous injections of 2.5 to 5.0 cc. per Kg. of a 50% aqueous solution of diethylene glycol into rats produced severe kidney damage. Larger doses (10 cc. per Kg.) caused a marked filling of intracapsular spaces and tubules with blood, in addition to degenerative processes. Lepkovsky, Ouer and Evans (7) found that the substitution of diethylene glycol esters of fatty acids for the natural or synthetic glycerol esters in the diets of rats resulted in death of the animals after about three weeks when the glycol ester content of the diet was 60%. Effects of concentrations less than 60% were not reported. The pathology seen on microscopic examinations of organs from these animals was essentially the same as that mentioned briefly by von Oettingen and Jirouch, and recently described in more detail by Kesten, Mulinos and Pomerantz (1) and Cannon (4). Haag and Ambrose (8) found the following fatal doses in acute experiments on rats: intravenous 5 cc. per Kg. (80 % mortality), intramuscular 7 cc. (80% mortality), subcutaneous 15 cc. (60% mortality) and oral 15 cc. (100% mortality); and on rabbits: intravenous 2 cc. (60% mortality) and intramuscular 4 cc. (80% mortality). Haag and Ambrose also noted that concentrations of diethylene glycol of 1% or less caused no significant variation in rate of growth, or damage to internal organs of rats which were examined at the end of the experimental period of 100 days. Concentrations of 3% and 10% in the drinking water rapidly proved fatal. Kesten, *et al.*, obtained essentially the same results: concentrations of 1% or less produced no discernible pathology, whereas concentrations of 3% or more resulted in death of many of the animals in periods varying from one day to about two months. Not all rats were equally susceptible, as indicated by the fact that nearly half the group which received 3% diethylene glycol showed neither kidney nor liver lesions after they had received the glycol for longer periods than others in the same group which died from extensive kidney damage.

Holck (2) found that drinking water containing 5% commercial diethylene glycol was fatal within a period of 11 days to a group of five rats (average duration of life for the whole group was 8 days). In a similar experiment in which drinking water contained 4% pure diethylene glycol, three of the group of five rats died within 9 days, but the two remaining animals survived for the duration of the experiment—nine weeks. At all concentrations of pure glycol of 0.25% or more there was a definite retardation of growth. Only at a concentration of 0.125% was

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the rate of growth equal to that of the control group. Female rats were used in these experiments. Microscopic examinations of livers and kidneys from animals which received the higher concentrations (4% and 5%) showed no damage, which is in marked contrast with extensive damage seen in similar experiments reported by Kesten, *et al.*

Geiling, Coon and Schoeffel (3) confirm the determination of the oral fatal dose of diethylene glycol for rats reported by Haag and Ambrose. However, in this connection they state: "This figure, however, is no index of the toxic and possible fatal effects of the drug, if administered in small divided doses, especially since neither the fate nor the mechanism of detoxification is known." Nevertheless, these authors show in their Table I that small divided doses (2 to 4 cc.) three times daily proved fatal to all animals when a total amount of from 16 to 21 cc. per Kg. of the material had been administered. This is not greatly in excess of the single fatal dose of 15 cc. reported by Haag and Ambrose as well as by Geiling, *et al.* However, these latter authors are unquestionably correct in their contention that failure to investigate possible cumulative effects of any drug or poison may lead to serious consequences.

Poe and Witt (5) found the fatal dose of diethylene glycol for young white rats to be about the same, 12.5 Gm. per Kg., whether administered orally or intraperitoneally. They also confirmed the degree of toxicity of a synthetic "elixir" reported by Geiling, *et al.*

Authors who have investigated the question are agreed that the presence of sulfanilamide in either the "Elixir of Sulfanilamide-Massengill" or in a synthetic "elixir" prepared on the basis of chemical analyses of the Massengill product had but little part in determining the toxicity of the mixture, the entire clinical and pathological pictures having been reproduced in laboratory animals by equivalent quantities of diethylene glycol alone.

As previously mentioned, the nature of the emergency created by the "Elixir of Sulfanilamide-Massengill" necessitated prompt and concerted action by various research groups. The immediate question was obviously answered in a satisfactory manner; but there still remains the question of sub-acute and chronic effects developing from the repeated ingestion of small amounts of diethylene glycol, as mentioned by Geiling, *et al.* There also arises the question of differences in susceptibility between the sexes, such as have been reported for a variety of drugs. Some of the experiments to be described in the following pages were designed to provide additional information as to possible cumulative and chronic effects; others were performed for the purpose of further substantiating results reported previously.

EXPERIMENTAL METHODS AND RESULTS.

The diethylene glycol used in these experiments was the commercial grade obtained from the Carbide and Carbon Chemicals Corporation. The synthetic "elixir" contained 90 mg. sulfanilamide per cc. dissolved in 70% diethylene glycol; no coloring or flavoring agents were added. The "Elixir of Sulfanilamide-Massengill" was taken from the original container, previously unopened.

The experiments may be divided conveniently into three groups on the basis of quantities of diethylene glycol administered; (*a*) those in which a single dose of fatal or near fatal proportions was administered; (*b*) those in which approximately half the Average Fatal Dose previously reported in the literature was administered daily, either as a single dose, or as one third that amount given three times daily; and (*c*) those in which relatively small quantities were ingested

daily mixed with the drinking water. These three groups will be subsequently referred to as acute, subacute and chronic experiments respectively, although it is recognized that such a classification must be purely arbitrary.

Acute Experiments.—In this group of experiments diethylene glycol was administered orally and intravenously to rats, and orally to dogs. Rats from the Wistar Institute strain were used throughout these experiments, and except where otherwise noted males weighing from 175 to 225 grams were selected. The dogs were female mongrels, and weighed from 5 to 8 Kg. The Average Fatal Dose (50% mortality) of diethylene glycol for rats was found to be 27 cc. per Kg. on oral administration, and 5.8 cc. on intravenous administration. Haag and Ambrose reported 100% fatalities among five rats which received 15 cc. per Kg. orally. Geiling, *et al.*, also reported 100% fatalities from 15 cc. doses, although the number of animals in the series was not mentioned. In the present studies 11 rats received 15 cc. per Kg. orally with 2 fatalities (18%), one of five died from 25 cc., and all of five died from 30 cc. Two dogs which received 15 cc. per Kg. orally died in 3 and 4 days respectively; one which received 10 cc. died in 26 days, and another survived the same dose for a period of eight months, after which observation was discontinued.

Organs of rats in the acute experimental group were not examined microscopically. Of the four dogs in this group, No. 10, which died 26 days after a single dose of 10 cc. per Kg. of diethylene glycol, showed few pathological changes. There were no vacuolic changes in liver and kidney, such as characterize acute poisoning from this substance. The kidney showed a few interstitial scars, possibly indicating previous damage from diethylene glycol. Dogs Nos. 11 and 12, which died 3 and 4 days respectively after oral doses of 15 cc. per Kg., showed extensive vacuolic degeneration of livers and kidneys.

Sub-Acute Experiments.—In these experiments dogs were given orally the following substances in the doses indicated: 70% diethylene glycol, 7.5 cc. per Kg. daily; synthetic "elixir" of sulfanilamide containing 70% diethylene glycol and 90 mg. sulfanilamide per cc., 7.5 cc. per Kg. daily; a suspension of 90 mg. sulfanilamide per cc. in mucilage of acacia, 7.5 cc. per Kg. daily. Rats were given orally the following substances in the doses indicated: 98+% diethylene glycol, 2.5 cc. per Kg. three times daily; "Elixir of Sulfanilamide-Massengill," 7.5 cc. per Kg. daily; sulfanilamide in mucilage of acacia as above, 7.5 cc. per Kg. daily; 98+% diethylene glycol, 7.5 cc. per Kg. daily. Table I shows the significant data obtained from these experiments. These

TABLE I.—TOXICITY OF DIETHYLENE GLYCOL, "ELIXIR OF SULFANILAMIDE-MASSENGILL," A SYNTHETIC "ELIXIR" AND SULFANILAMIDE IN MUCILAGE OF ACACIA FOR DOGS AND RATS.

Animal.	Animal No.	Substance Administered.	Dose.	Total Glycol.	Fate.	Liver and Kidney Damage.
Dogs	1	Diethylene	7.5 cc.	36.75 cc.	Died 7 days	Present
	2	glycol 70%	daily	66.25	Died 13 days	Present
	3			21.00	Died 4 days	Present
	4	Synthetic	7.5 cc.	94.50	Died 18 days	Present
	5	"elixir"	daily	42.00	Died 8 days	Present
	6	Sulfanilamide	7.5 cc.	...	Died 15 days	Slight*
	7	in mucilage	daily	...	Killed 18 days	Slight*
	8	of acacia		...	Killed 18 days	Slight*
Rats	1	Diethylene	2.5 cc.	322.5	Died 43 days	Present
	2	glycol	three	337.5	Killed 45 days	Absent
	3	98 + %	times	150.0	Died 20 days	Present
	4		daily	300.0	Died 40 days	Present
	5			30.0	Died 4 days	Present
	6	"Elixir"	7.5 cc.	230.6	Killed 41 days	Absent
	7	Massengill	daily	230.6	Killed 41 days	Absent
	8			230.6	Killed 41 days	Absent
	9			230.6	Killed 41 days	Absent
	10	Sulfanilamide	7.5 cc.	...	Killed 44 days	Absent
	11	in mucilage	daily	...	Killed 44 days	Absent

12	of acacia			Killed 44 days	Absent
13				Killed 44 days	Absent
14				Killed 44 days	Absent
15	Diethylene	7.5 cc.	120.0	Died 16 days	Present
16	glycol	daily	82.5	Died 11 days	Present
17	98 + %		450.0	Killed 60 days	Absent
18			382.5	Died 51 days	Absent
19			195.0	Died 26 days	Present

* These changes were limited to the liver, and were different from those observed following diethylene glycol poisoning. See text.

data illustrate two important facts; *first*, that there were remarkably great variations in susceptibility, even within the same species; and *second*, that many of the animals (most of the rats and some of the dogs) exhibited a degree of tolerance not suggested by the researches of other investigators. When this great tolerance first became evident it was thought that perhaps the hygroscopic property of the glycol had resulted in the absorption of sufficient water from the atmosphere to so dilute it that the animals were actually receiving much less of the glycol than was intended, this in spite of the fact that it had been obtained from the manufacturer only recently. However, measurements of specific gravity, distillation range, and index of refraction indicated a product of better than 98% purity. Also, the "Elixir of Sulfanilamide-Massengill" was obtained from a previously unopened container. As a further check on the toxicity of the glycol as well as on the relative susceptibility of the rats used in these experiments, Dr. R. C. Neale of the Biochemical Research Foundation of the Franklin Institute very kindly consented to ascertain the toxicity of our glycol on rats from their colony, which likewise were derived from the Wistar strain. The authors gratefully acknowledge their indebtedness to Dr. Neale for the following data: of 20 rats fed the same diet and of approximately the same weight as those used by the authors, 4 died after the second daily dose of 10 cc. per Kg. of 72% diethylene glycol administered orally, 9 died after the third dose, 1 after the fourth and the remaining 6 after the fifth dose. These data agree satisfactorily with those previously reported by other authors. It is difficult to explain these differences in the light of our present knowledge other than to attribute them to some obscure and uncontrolled factor such as diet previous to that used during the experiments, state of nutrition, health, climate or something else equally intangible. Concerning the diet, all animals used in these experiments, as well as the rats used by Dr. Neale, were fed Purina dog chow.

Microscopic examination of the internal organs from all animals in the sub-acute experimental group showed varying degrees of vacuolic degeneration of livers and kidneys of all the dogs and some of the rats which received diethylene glycol. In dogs Nos. 6, 7 and 8, which received sulfanilamide in mucilage of acacia, the kidneys were entirely normal, but the liver cells in Nos. 7 and 8 were swollen, and the cytoplasm contained varying numbers of minute vacuoles. These were entirely different in appearance from those seen in diethylene glycol poisoning. Special stains indicated the absence of fat in these vacuoles. Autolytic changes in dog No. 6 masked all but a few scattered patches of similar vacuoles. Further work is necessary before these changes can be interpreted properly. Shortly after the beginning of the experiment, dog No. 6 began having diarrhea and convulsive seizures, and became severely emaciated. Its illness and death after 15 days probably was not related to the experimental conditions. Those rats which received dairly oral doses of 7.5 cc. "Elixir of Sulfanilamide-Massengill" showed none of the characteristic liver and kidney lesions, although this dosage was continued for 41 days. This was equivalent to a total glycol intake of 230 cc. per Kg. (assuming the elixir to have contained 75% glycol). The tolerance shown by these rats is in contrast to the death in 8 and 18 days respectively of two dogs which received 7.5 cc. per Kg. daily of a synthetic "elixir" which contained 70% glycol. One might assume that some other constituent of the Massengill product afforded marked protection for rats against diethylene glycol were it not for the fact that other investigators (Geiling, *et al.*) found the "Elixir" to be of the same order of toxicity as diethylene glycol alone in equal doses.

Chronic Experiments.—The effect of small quantities of diethylene glycol ingested daily for an extended period of time, and possible sex differences in this respect, were investigated in the

following manner. Young rats of approximately fifty Gm. weight were divided into groups of ten animals each, three groups each of males and females. One group of each received 1.0% diethylene glycol in the drinking water, another of each received 0.3%, and the two remaining groups received distilled water as controls. Food in the form of Purina dog chow was allowed all groups *ad libitum*. Individual animal weights, food consumption for each group, and water consumption for each group were recorded at five day intervals during the course of the experiment. During the first 100 days one animal from each of the groups which received glycol died. Of these, two were almost completely consumed by other animals in the cages, and therefore were not available for microscopic examination. The other two animals were included. At the end of this period of 100 days five animals from each group were sacrificed and the internal organs examined microscopically. These examinations showed no definite pathological changes which could be assigned to the effect of diethylene glycol. Occasional changes of doubtful significance were observed in all groups, and these no less frequently in the control groups. Because of the constantly changing weights of the animals it was impossible to make exact calculations of the

TABLE II.—AVERAGE FOOD, WATER AND GLYCOL INTAKE, AND FINAL AVERAGE WEIGHT OF RATS WHICH RECEIVED VARIOUS QUANTITIES OF DIETHYLENE GLYCOL IN THE DRINKING WATER.

Group No.	Avg. Food Intake. Gm./Kg./Day.	Avg. Water Intake. Gm./Kg./Day.	Avg. Glycol Intake. Gm./Kg./Day.	Final Average Weight.
1 Males	78	235	2.35	307
2 Males	78	220	0.66	314
3 Males	79	204	..	341
4 Females	77	194	1.94	212
5 Females	82	197	0.59	203
6 Females	88	218	..	210

food, water and glycol consumption per Kg. of body weight for the entire period. However, by obtaining the average consumption for each group for the first, tenth and twentieth five-day periods, data were obtained which are comparable for these various groups. It will be seen in Table II that food and water consumption varied within relatively narrow limits. Rates of growth were reasonably close to those published for the Wistar strain, and even closer to those observed for the rat colony in this institution. The remaining rats in each group not sacrificed at the end of 100 days were continued on the same diet and glycol intake for an additional 75 days, after which they too were sacrificed and examined microscopically. These examinations likewise were essentially negative. Therefore, the results obtained from chronic toxicity studies are in accord with those reported previously by Haag and Ambrose and Kesten, *et al.* Concentrations of diethylene glycol of 1.0% and 0.3% in the drinking water produced no characteristic changes in any of the animals. No difference in susceptibility between males and females was observed. There was a slight inhibition of growth of males of questionable significance; but females grew equally as well as control animals. This is in contrast to the definite inhibition of growth observed by Holck in his experiments on female rats.

SUMMARY.

1. When administered in large single doses to dogs diethylene glycol was found to be of the same order of toxicity as previously reported by other authors. The most important pathological lesions consisted of vacuolic degeneration of hepatic cells in general, and the convoluted tubule epithelium of the kidney. Small doses administered daily (5.25 to 7.5 cc. per Kg.) produced essentially the same results.

2. Rats exhibited an extremely wide variation in susceptibility to diethylene glycol. The two extremes were represented by death in 4 days from 2.5 cc. per Kg. three times daily, and survival for 60 days of daily doses of 7.5 cc. per Kg. In general, those rats which died following the administration of the glycol were found to have suffered extensive liver and kidney damage; whereas, those which survived were found to be normal when sacrificed for microscopic examination. Furthermore microscopic examinations of organs from four rats which received daily doses of 7.5 cc. per Kg. of "Elixir of Sulfanilamide-Massengill" for a period of 41 days showed no pathological changes.

3. The ingestion of 1.0% and 0.3% solutions of diethylene glycol in the drinking water for a period of 175 days had no apparent deleterious effect on growing male and female rats.

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STUDIES OF NATIONAL FORMULARY DRUGS.*

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LAPPA.

The purpose of this investigation was to examine the botanical and pharmacognostical portions of the monograph on Lappa in the sixth edition of the National Formulary in order to ascertain whether the facts therein set forth were scientifically correct and to make recommendations to the Revision Committee wherever changes were found necessary.

History.—Lappa or Burdock Root was first introduced into the U. S. P. in 1850 where it appeared in the secondary list under the title of Lappa, and was there defined as "The root of *Lappa minor*, De Candolle." (*Lappa minor* is now known as *Arctium minus*, Bernh.). It remained in the same list with the same definition in the pharmacopœias of 1860 and 1870. In the sixth revision of the pharmacopœia of 1880 it was transferred to the primary list and defined as "The root of *Lappa officinalis*, Allioni (nat. ord. *Compositæ*)." (Allioni's concept for this species now includes *Arctium minus*, Bernh. and *A. Lappa*, L.). The seventh revision of the U. S. P. of 1890 defines it as the root of *Arctium Lappa*, L. and of some other species of *Arctium* (nat. ord. *Compositæ*). In the eighth revision of the U. S. P. (1900) the definition was changed to "The dried root of *Arctium Lappa*, Linné or of other species of *Arctium* (Fam. *Compositæ*) collected from plants of first year's growth."

Lappa was dropped from the ninth revision of the U. S. P. (1910) and admitted into the fourth edition of the National Formulary (1916) with the same definition as appeared in the

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