EVALUATION OF RECOMMENDED TREATMENTS FOR THREE COMMON MAMMALIAN TOXICOSES

by

LAKSHMIPATY PENUMARTHY

B.V.Sc. A.P. Agricultural University,
India, 1967

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Approved by:

F. L. Cellam

Major Professor

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ABSTRACT . . . . . .

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INTRODUCTION

The enormous use of chemicals in everyday life poses a constant threat to the health of man and animals. Fortunately, biological systems have the unique ability to deal with and dispose of most of these foreign compounds in an efficient manner. However, because of species differences in biotransformation pathways, the foreign chemicals and/or their metabolites may prove toxic to certain animals. Apart from individual variations, the responses to foreign chemicals are influenced by environmental factors, such as temperature and pollutants, and the body's general physiological status[1,2].

Poisonings due to environmental and medicinal chemicals are frequently observed in veterinary practice. Three commonly used compounds responsible for poisonings in small animals are: (1) Ethylene glycol, used as antifreeze in automobiles; (2) Disophenol, an anthelminthic used for treating canine hookworm infestations; and (3) Warfarin, a rodenticide used in rat-baits.

The present study was undertaken to investigate the efficacy of recommended treatments for these three intoxications and to study possible new therapeutic procedures.

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SYSTEMATIC TREATMENT OF ETHYLENE GLYCOL TOXICOSIS IN CATS

Lakshmipaty Penumarthy, B.V.Sc., M.S.;
Frederick W. Oehme, D.V.M., Ph.D.

SUMMARY

Ethylene glycol (EG) was orally administered to 27 cats at dose levels of 4, 6, or 8 ml./kg. body weight. Treatment was initiated 4 or 8 hours after dosing and consisted of 5 ml./kg. of body weight of 20% ethanol in isotonic saline solution and 6 ml./kg. of body weight of 5% sodium bicarbonate in isotonic saline administered intraperitoneally (I.P.). This treatment was repeated every 6 or 8 hours for approximately 56 hours.

All 6 control cats and all cats receiving treatment 8 hours after dosing with 6 ml. (4 cats) or 8 ml. (4 cats) of EG/kg. of body weight died. Only 1 of 4 cats (25%)

From the Comparative Toxicology Laboratory, Department of Surgery and Medicine, Kansas State University, Manhattan, KS 66506. Reprint requests should be sent to Dr. Oehme.

The authors thank Mr. Marc Rachofsky for his help in conducting this study.

recovered in the group that received 4 ml.EG/kg. of body weight and initial treatment 8 hours later. Two of 3 cats (67%) recovered in each of the groups that received 4 ml. and 6 ml. of EG/kg. of body weight and were treated 4 hours after dosing, but only 1 of 3 cats (33%) recovered in the group that received 8 ml. of EG/kg. of body weight with treatment initiated after 4 hours. Limitations of successful treatment are high doses of EG and delays in initiating the treatment.

Ethylene glycol is used as an industrial solvent in the manufacture of dyes, lacquers, frost-proof explosives, and pharmaceutics and as a coolant in automobiles and lunar modules [14,15,30]. Deaths have been reported in humans consuming EG as commercial antifreeze, apparently as a cheap substitute for alcoholic beverages[4,6,16,21,25,30]. The poisoning in animals is accidental and is probably due to its sweet taste. Acute poisoning has been reported in dogs[3,9,10,11], cats[8,10,11], swine[18], and poultry[23].

The mechanism of EG toxicity has been the subject of controversy. Metabolic oxidation to oxalic acid has been postulated as one of the reasons for its toxicity[4,16,19]. However, since oxalic acid formation accounts for less

than 2% of the total does, this oxidation may not account fully for its toxicity[7,14,26]. Liver alcohol dehydrogenase metabolizes EG, and glycolaldehyde, glycolic acid, and glyoxylic acid are intermediates in the final oxidation of EG to formic acid, respiratory carbon dioxide, glycine, or urinary oxalic acid[6,7,14,28]. Metabolic intermediates have partly accounted for the toxicity of the parent compound[1,6,7,17,22]. Due to species differences in EG metabolism, EG itself was suggested as being toxic in some species[6,7].

The minimal lethal dose of EG in cats was observed to be 1.5 ml/kg. of body weight (16). A subcutaneous dose of 2 g. EG/kg. of body weight was also lethal[27]. Some investigations have suggested that cats do not survive doses of 1 g. EG/kg. of body weight due to cats forming more oxalic acid than other species[7].

The earliest clinical signs of EG toxicity are ataxia and depression resembling that of alcohol intoxication. Emesis, muscular incoordination, loss of reflexes leading to coma, convulsions, and death in about 64 hours are observed in acute toxicity[12]. Animals surviving the acute stages of intoxication progress into renal failure through precipitation of calcium oxalate crystals in renal tubules[2].

Consistent postmortem lesions include general congestion of all organs, petechial hemorrhages of gastric or intestinal mucosa, and pulmonary edema. However, these changes are not pathognomonic of EG toxicity. Microscopic examination of tissues reveals numerous polarizing crystals in the renal tubules[16] and in the adventitia and perivascular spaces of the blood vessels of cerebellum[12]. There is apparently no correlation between EG dose and degree of crystaline deposits in kidney or brain.

Since oxalic acid formation is at least a portion of the toxic syndrome and liver alcohol dehydrogenase is involved in EG biotransformation to oxalic acid, ethanol treatment has been suggested to competitively inhibit the metabolism and toxicity of EG[28]. Successful treatment has been reported in humans, monkeys, rats, and dogs[2,5,17,20,24,29]. Simultaneous administration of sodium bicarbonate helps correct the acidosis accompanying EG poisoning[2,3].

There is no literature on the use of ethanol treatment for EG toxicosis in cats. Because of species differences in the levels of alcohol dehydrogenase and variations in EG biotransformation[7,13], this study was undertaken to investigate the feasibility of a theoretical ethanol-bicarbonate treatment dosage and schedule for EG poisoning in cats, the EG doses at which successful antidotal therapy

could be achieved, and the maximum delay in treatment following EG poisoning which would still result in clinical recovery.

Materials and Methods

Animals. Twenty-seven mature, clinically healthy domestic shorthair cats of either sex with a mean body weight of 3.24± 0.14 (SEM) kg. were used. A commercial cat food[a] was offered twice a day and water was provided ad libitum. All the cats were stabilized for at least one week before use.

Experimental Procedure. The cats were housed in individual cages and food withheld overnight before EG administration. During the early course of investigation it was noted that some of the cats vomited after EG dosing; hence the cats were given 2 mg. triflupromazine hydrochloride[b]/kg. of body weight intramuscularly 30 minutes before EG administration. A 50% aqueous solution of EG[c] was administered via stomach tube. The cats were observed for clinical signs of toxicity.

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[a]Puss'n Boots, The Quaker Oats Company, Chicago, IL.

[b]Vetame, E.R. Squibb & Sons, N.Y.

[c]Ethylene Glycol, Analytical Reagent, Mallinckrodt Chemical Works, St. Louis, MO.

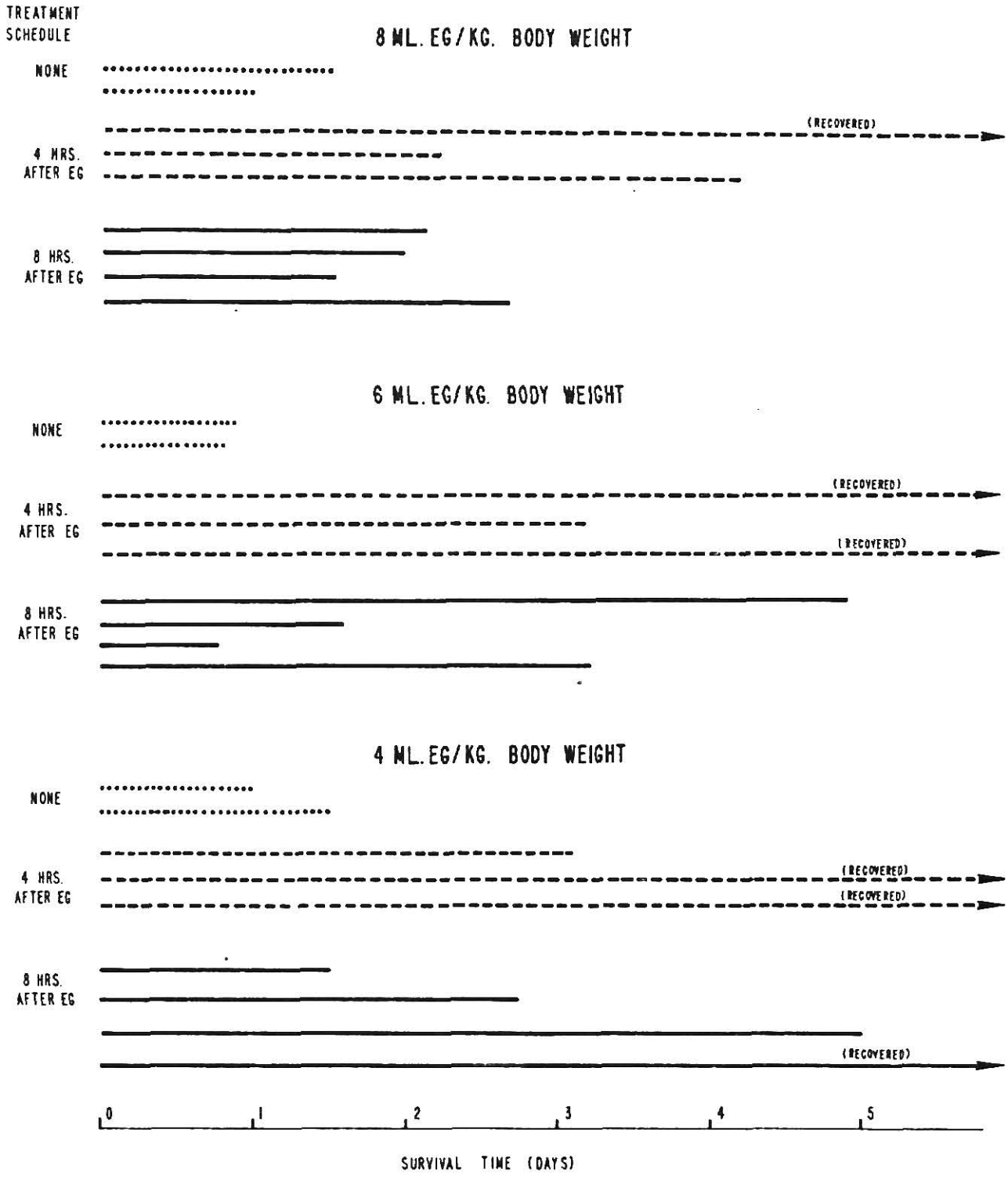
The cats were divided into 3 groups and EG was administered at doses of 4, 6, and 8 ml./kg. of body weight, respectively. In each group 2 cats served as controls, 3 cats were treated initially at 4 hours, and 4 cats were initially treated 8 hours after EG administration. The treatment consisted of 5 ml. of 20% ethanol in isotonic saline solution and 6 ml. of 5% sodium bicarbonate in isotonic saline solution/kg. of body weight I.P. After the initial treatment 4 additional treatments were given every 6 hours and another 4 treatments were provided at 8-hour intervals; the total treatment period was 56 hours. Water was provided throughout and food was offered after the last treatment was administered.

Necropsy. Cats dying during the course of the experiment were immediately necropsied, and tissues were examained for gross lesions. Tissue from both kidneys were preserved in 10% buffered neutral formalin, and sections were processed for routine histological study. Recovered cats were observed for 10 days and then euthanatized. Necropsy and collection of kidney tissues was accomplished as previous.

Results

Survival Rates. The theorized treatment doses and schedule were successful in providing satisfactory recovery

Fig. 1 -- Survival times of cats receiving Ethylene Glycol at indicated dosage rates and not treated or with treatment initiated 4 or 8 hours after dosing.


TREATMENT SCHEDULE
8 ML. EG/KG. BODY WEIGHT
NONE
4 HRS. AFTER EG
8 HRS. AFTER EG
(RECOVERED)
6 ML. EG/KG. BODY WEIGHT
NONE
4 HRS. AFTER EG
8 HRS. AFTER EG
(RECOVERED)
(RECOVERED)
4 ML. EG/KG. BODY WEIGHT
NONE
4 HRS. AFTER EG
8 HRS. AFTER EG
(RECOVERED)
(RECOVERED)
(RECOVERED)
0
1
2
3
4
5
SURVIVAL TIME (DAYS)

in cats that received 4-fold lethal doses of EG; however, survival times and recovery of cats were related to the dose of EG administered and the time of treatment initiation (Fig. 1). High doses of EG administration and long delays in initiating treatment resulted in mortality, although the survival times in treated cats were significantly increased over those of control animals.

Control cats given 4 ml. EG/kg. of body weight died in 24 and 36 hours respectively. Two of the 3 cats treated 4 hours post-dosing recovered and 1 cat died in 74 hours. Only 1 of the 4 cats treated 8 hours post-dosing survived, and the 3 cats that died had a mean survival time of 74 ± 24.3 hours.

Control cats given 6 ml. EG/kg. of body weight died in 22 and 20 hours. Two of the 3 cats treated 4 hours post-dosing recovered; 1 cat died in 76 hours. All of the 4 cats treated 8 hours post-dosing died with a mean survival time of 63.25 ± 21.85 hours.

The 2 control cats receiving 8 ml. EG/kg. of body weight died in 36 and 24 hours. One of the 3 cats treated after 4 hours recovered and the other 2 cats died in 100 and 52 hours. All of the 4 cats treated 8 hours post-dosing died with a mean survival time of 49.25 ± 5.75 hours.

Clinical Signs. Incoordination of the hind legs and mild depression were observed 1 hour post-dosing in all cats. Coffee-colored urine was observed 3-4 hours after dosing in 10 of the 27 cats. The urine returned to normal color in the treated animals after 1 or 2 treatments. Progressive incoordination, depression, loss of reflexes, and coma developed in 9-24 hours; the onset of coma was sooner in cats receiving high doses of EG. Severe convulsions of intermittent nature were observed in 2 cats during terminal stages of poisoning.

The I.P. administration of ethanol produced depression in about 10 minutes. Cats showed a slight apprehension on injection of the sodium bicarbonate solution, apparently due to its irritating effect. Ethanol-induced depression lasted for shorter time intervals with each successive treatment.

The cats that recovered from toxicosis showed improvement with each treatment, and the incoordination disappeared after 1st or 2nd treatment, after which they appeared clinically normal. Cats that did not respond to treatment had progressive deterioration.

Necropsy Findings. Congestion of all body tissues was commonly observed in cats that died during the course of study. Digestive tract hyperemia and, in acute cases, frank hemorrhage was noted in the stomach and small intestine.

The stomach contained bloody fluid in 2 cats which died early in the course of treatment. Kidneys were congested and the bladder was full of bloody urine in 2 cats. Only a slight subcutaneous reaction was observed at the site of I.P. administration. Meninges and brain had no gross lesions. Oral ulcerations were observed in 2 cats.

Microscopic Findings. Kidneys of control cats were congested; cats dying of acute poisoning had hemorrhages in the corticomedullary region. Heavy depositions of oxalate crystals (8-10 crystals/LPF) were observed in the proximal and distal convoluted tubules (Fig. 2). The amount of tubular deposition was heavier at the high dose levels.

Cats that received treatment 4 or 8 hours after EG dosing and died during the study also had oxalate crystals in the tubules (Fig. 3); however, the size and quantity of the crystals deposited appeared less than in control cats. Tubular necrosis and complete desquamation of tubular epithelium were consistent lesions in cats that died acutely (Fig. 2).

Cats treated and recovering from EG poisoning had only a few crystals (2-3/LPF) in the tubules and the tubular epithelium appeared relatively normal (Fig. 4).

Discussion

EG is rapidly absorbed from the gastrointestinal

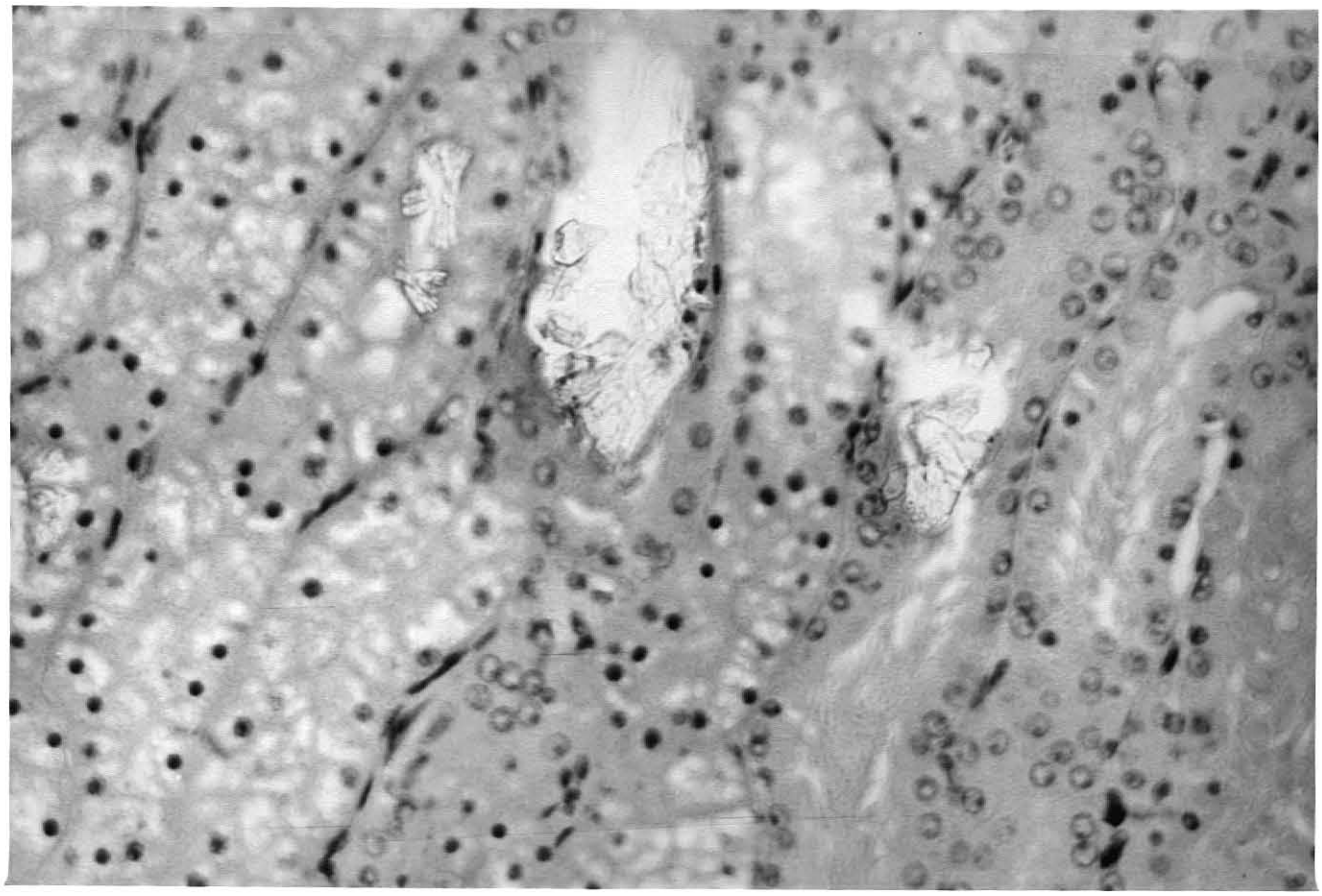


Fig. 2 -- Kidney of non-treated control cat given 6 ml. of ethylene glycol/kg. of body weight. Several oxalate crystals are seen in the tubules. Tubular necrosis and desquamation of the epithelium (especially in the area surrounding the crystals) is present. H&E stain; x 407.

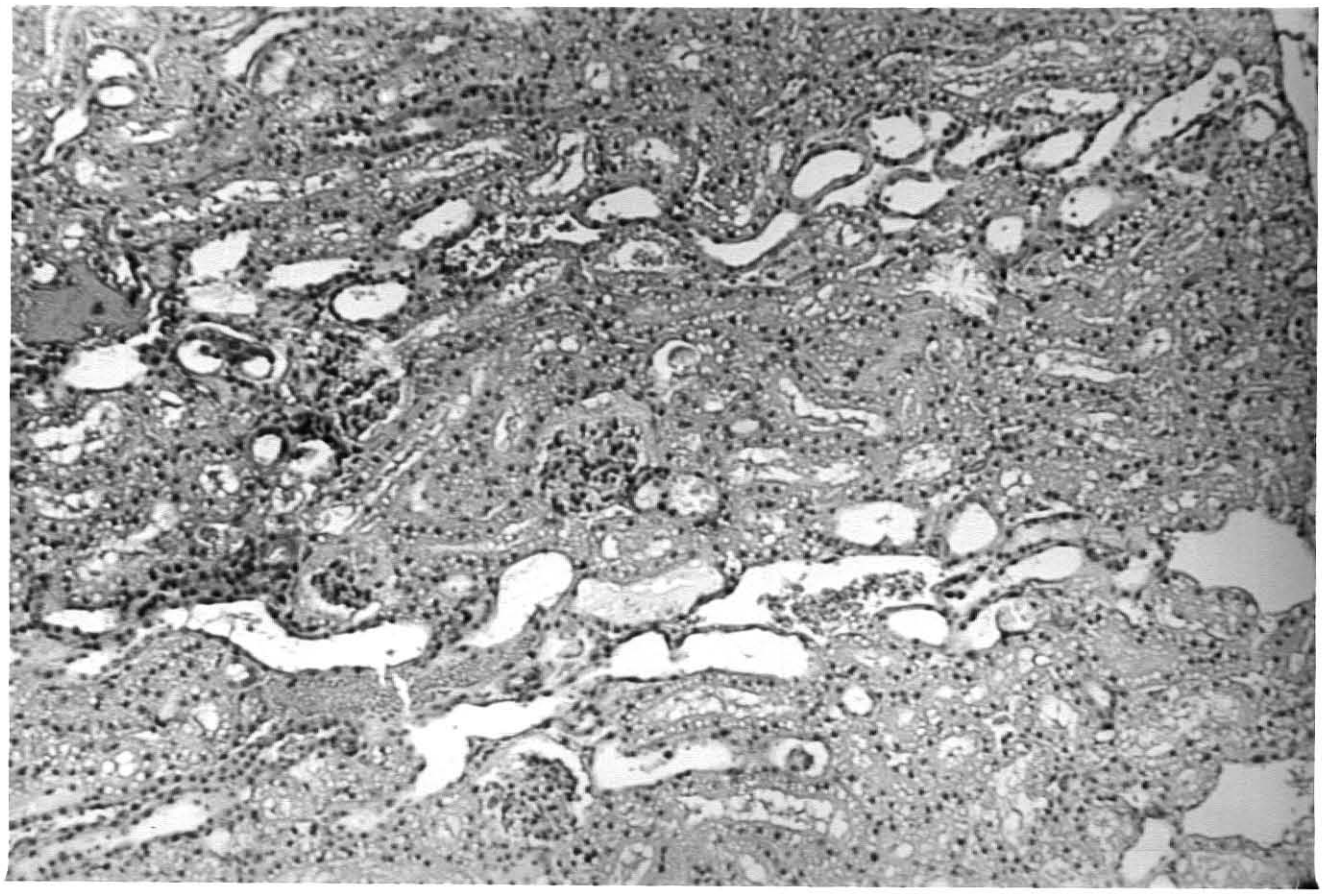


Fig. 3 -- Kidney of cat given 6 ml. of ethylene glycol/kg. of body weight and treated with ethanol-bicarbonate 4 hours later. This cat died 76 hours after the EG was given. Glomerular and tubular epithelial damage, flattened epithelium, cystic tubules, and a few oxalate crystals are seen. H&E stain; x 162.

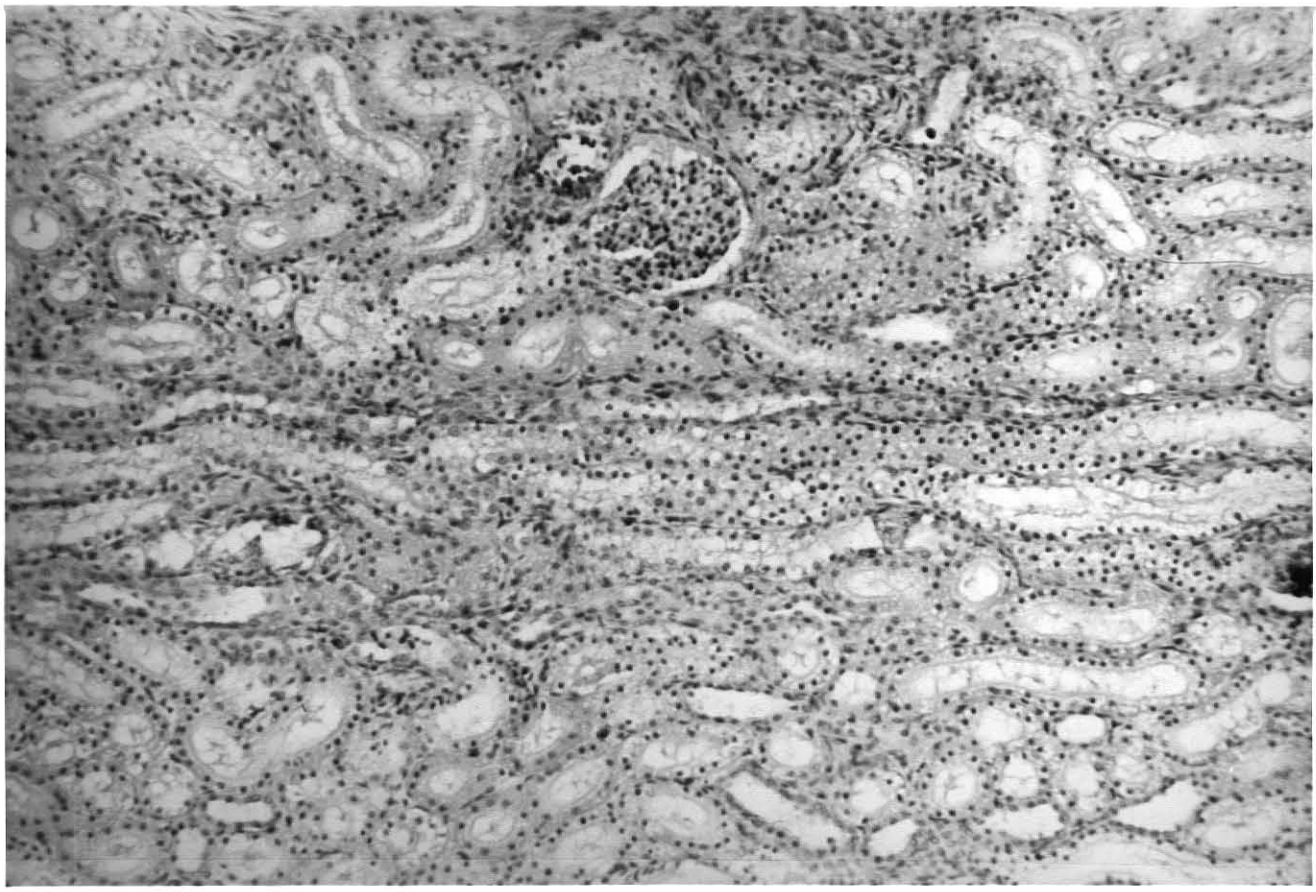


Fig. 4 -- Kidney of cat given 6 ml. of ethylene glycol/kg. of body weight, treated with ethanol-bicarbonate initially 4 hours later, and having a clinical recovery from toxicity. Tubular epithelium is essentially normal with only occasional oxalate crystals seen in the tubules. H&E stain; x 162.

and reaches plasma maximum concentration in dogs in 1 to 3 hours[17,24]. The oxidation of EG by the liver yields urinary oxalic acid and in cats results in more oxalate salts than in other laboratory animals[7]. Cats also eliminate a large amount of unchanged EG in urine; thus, toxicity in cats might be due to unchanged glycol and to oxalate formation[7].

Oxalic acid combines with calcium to form a calcium oxalate complex which crystallizes in the renal tubules, leading to tubular epithelial damage and renal failure[16,21]. Partial hepatectomy in rats increased the toxicity of the glyoxylate metabolites and decreased the toxicity of EG and the glycolate metabolite. This suggests that the toxicity of EG may be partially attributed to one of the metabolic intermediates[22].

The successful recovery of cats given several-fold lethal doses of EG indicates the effectiveness of the outlined treatment within the limitations of the EG dose and the time elapsed before initiation of the treatment. Metabolic acidosis consequent to biotransformation has been successfully controlled in dogs by the administration of sodium bicarbonate[2,24]. The presence of few oxalate crystals in the kidneys of treated cats supports the value of sodium bicarbonate in also preventing deposition of calcium oxalate in renal tubules.

The death of all control cats indicates that EG biotransformation proceeded unchallenged in these animals, as evidenced by the enormous deposition of oxalate crystals and prominent tubular epithelial damage. The 100% mortality in cats receiving 6 and 8 ml. of EG/kg. of body weight and treated 8 hours post-dosing suggests that onset of biotransformation is rapid and that treatment is of limited value with increasing time-lag in initiation of therapy. The 67% recovery in cats receiving 4 and 6 ml. EG/kg. body weight and treated 4 hours post-dosing suggests a favorable prognosis if treatment is initiated at an early stage of poisoning. The recovery of only 25% of the cats receiving 4 ml. EG/kg. of body weight and treated 8 hours post-dosing indicates the adverse results of delayed therapy. A 33% recovery in the cats receiving 8 ml. EG/kg. of body weight and treated after 4 hours suggests that both the EG dose and the relative time of initiation of treatment play significant roles in a satisfactory recovery.

Since it is usually difficult in clinical situations to determine the amount of EG consumed and the time-lag in initiating therapy, a clinical prognosis is feasible 18-24 hours after treatment initiation by observing the patient's response to the therapy. A decrease in incoordination and

a progressive increase in alertness between treatments suggests a favorable course.

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INVESTIGATION OF SOME THERAPEUTIC MEASURES FOR DISOPHENOL (D.N.P.) TOXICOSIS IN DOGS

Lakshmipaty Penumarthy, B.V.Sc., M.S.;
Frederick W. Oehme, D.V.M., Ph.D.;
Monty J. Menhusen, D.V.M.

SUMMARY

Acute disophenol toxicosis in 15 dogs, following administration of 33, 35 or 40 mg. disophenol/kg. (15, 16, or 18 mg. disophenol/lb., respectively) of body weight, was treated in 10 dogs with an antipyretic, lactated ringer's infusions, or ice baths. Rectal temperatures and estimates of hemoglobin, packed cell volume, and total white blood cell count and differential were recorded. Higher hemogram values were observed for dogs dying of the toxicosis. One of 5 dogs (20%) receiving no treatment recovered. Two of 3 dogs (67%) treated with the anti-pyretic recovered, 0 of 2 dogs (0%) receiving lactated ringer's infusion recovered, and 2 of 5 dogs (40%) treated with ice baths recovered.

From the Comparative Toxicology Laboratory, Department of Surgery and Medicine, Kansas State University, Manhattan, KS 66506. Reprint requests should be sent to Dr. Oehme.

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the possible value of the antipyretic and ice baths in treating disophenol toxicosis should be studied in future investigations.

Disophenol[a] (2,6-diiodo-4-nitrophenol) is an effective systemic anthelminthic for the treatment of hookworm infestations in dogs and cats. Successful treatment of canine ancylostomiasis is reported with 0.1 ml. of the commercial product/lb. of body weight (10 mg. disophenol/kg. of body weight) given by the oral, intramuscular (I.M.), or subcutaneous (s.c.) route[1,15,16].

The lethal dose of disophenol following single I.M. or s.c. injection was estimated as 36 mg./kg. of body weight[15,16]. Deaths occurred in dogs 5-10 hours after s.c. administration of disophenol at dosages greater than 15 mg./kg. of body weight[13]. The acute intravenous (I.V.) dose is 60 mg. disophenol/kg. of body weight[5]. The minimal oral lethal dose is between 100 and 200 mg./kg. of body weight[16]. Signs of acute toxicity were observed in an Irish wolfhound on administration of the recommended dose of 10 mg. disophenol/kg. of body weight. High ambient temperature and vigorous

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[a]D.N.P., 4.5% solution of disophenol, American Cyanamid Company, Princeton, N.J.

muscular exercise were probable factors in the precipitation of this acute syndrome[7].

Sublethal doses of disophenol may also induce subacute toxicosis. Death in dogs followed a daily oral dosing of 12.5 mg. disophenol/kg. of body weight for 5-6 days, while another dog survived 14 days on identical doses of 2,4-dinitrophenol (2,4-DNP), a compound that elicits similar clinical signs but produces greater toxicity than disophenol (6). Disophenol is more slowly eliminated than 2,4-DNP and produces a toxic body burden.

Distribution, biotransformation, and mechanism of action of disophenol have not been documented. Since clinical signs of disophenol toxicity resemble those produced by 2,4-DNP[6,11], the mechanism by which the latter produces toxicosis may apply to disophenol toxicity. 2,4-DNP causes fever by increasing tissue metabolism through a peripheral mechanism[11]. Uncoupling of oxidative phosphorylation also occurs in cell free homogenates[8]; consequently the energy requiring-endergonic processes are inhibited and much of the free energy is dissipated as heat[10]. Respiration rates and glycolysis are stimulated. Death in acute 2,4-DNP poisoning may result from direct circulatory depression, hyperpyrexia, or acidosis and anoxemia[11]. High ambient temperatures increased the toxicity of dinitrophenol manyfold[3]. Pyrexia

of 2,4-DNP toxicity was not mediated through the central nervous system (including heat regulating centers), the neuroreceptors, the adrenal glands, or the thyroid glands[11].

Clinical signs of acute toxicity include panting, increased respiration rate, temperature and heart rate, vomiting, hyperemia and edema of visible mucous membranes, and an almost immediate rigor mortis[6,7,13,16]. Single oral doses of 50 mg. disophenol/kg. of body weight or lethal (36-40 mg. disophenol/kg. of body weight) I.M. doses produced emesis in dogs[16]. Following I.V. infusion of 0.5mg. disophenol/kg. of body weight/minute, shivering, profuse salivation, increased temperature of the skin and nose, bright red gums and tongue, emesis with greenish-yellow vomitus, convulsions, and death occurred in about 2 hours. Rectal temperatures continued to rise for 10 minutes after cardiac arrest[6].

Transient cataracts of varying severity were observed following administration of recommended doses of disophenol by several routes. Young puppies were especially susceptible[9]. No significant alterations were observed in hemograms of dogs with acute intoxication[6,15]. High packed cell volume, neutrophilia, and lymphopenia occurred in a clinical case of disophenol intoxication in a dog[7]. Congestion and hydropic degeneration of the lungs and liver, and fatty degeneration of the myocardium, were reported in dogs with acute disophenol toxicosis[13].

Treatment of disophenol toxicosis has been symptomatic and limited[7]. Successful recovery in one dog has been reported using ethanol sprays, ice packs, and infusions of buffered lactated ringer's solution[7]. Whole blood transfusions, use of oxygen, and cooling baths have also been suggested[15]. Symptomatic measures, such as administration of fluids to avoid hemoconcentration and to assure adequate blood volume and topical application of cool water have antagonized the febrile response and fatality associated with 2,4-DNP toxicosis[2,12].

This study was undertaken to determine the effectiveness of an antipyretic, infusion of fluids, and ice baths in reducing the fever and/or mortality in dogs receiving toxic doses of disophenol.

Materials and Methods

Animals. Fifteen mature, healthy Beagle and mixed-Beagle dogs of either sex and weighing an average of 11.3± 0.88 (SEM) kg. were used. The animals were housed in individual cages with an ambient temperature of 67-78F (20-25c). All animals were stablized for at least one week and given a commercial dog food[b] and water ad libitum. Food was

[b]Ken-L Ration, The Quaker Oats Company, Chicago, IL.

withheld for 12 hours prior to disophenol administration, but water was offered continuously; feeding resumed 24 hours after the disophenol was administered.

Experimental Procedures. Disophenol[a] was administered s.c. in the neck region at dosages of 33, 35 or 40 mg./kg. of body weight (15, 16 or 18 mg./lb, respectively). Rectal temperature was recorded in all dogs before disophenol administration and successive temperatures were recorded at periodic intervals. A 5 ml. sample of whole blood in heparin[c] was collected from the external jugular vein before administering the disophenol and thereafter at 2 or 4 hour intervals. The samples were examined for percent hemoglobin by the cyanmethemoglobin method, packed cell volume by microhematocrit, and total white blood cell count by use of Coulter counter[d] and differential counts by differentiating 100 cells on a blood smear stained with Wright's stain.

Treatment Procedures. The dogs were closely watched for signs of toxicity after disophenol administration and treatment procedures were initiated as follows.

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[c]Heparin (Ammonium salt), Scientific Products, Evanston, IL.

[d]Coulter Electronics, Inc., 11748-46 W. 86th Terrace, Lenexa, KS.

In the 33 mg. disophenol/kg. of body weight group, 2 dogs served as controls and 1 dog received treatment with an antipyretic, dipyrone[e]. Of the 10 dogs receiving 35 mg. disophenol/kg. of body weight, 2 served as controls, 2 were treated with dipyrone, 2 received lactated ringer's solution[f], and 4 dogs were treated with ice baths. In the 40 mg./kg. of body weight group, 1 dog served as control and 1 dog received ice bath treatment.

The dipyrone was administered I.V. at a dose of 27.6 mg./kg. (12.5 mg./lb.) of body weight when a 2 F (1.1 C) rise in rectal temperature was noted. No more than 2 injections were given over a 12-hour period. Dogs receiving lactated ringer's solution were given 100 ml. I.V. or s.c. 2 hours after disophenol administration; this was repeated every 2 hours. The ice bath treatment consisted of keeping the dog's body immersed in ice water for at least 10 minutes whenever the 1.1 C rise in rectal temperature occurred.

Necropsy. Dogs that died during the experimental period were necropsied within 2 hours of death and examined for gross lesions. Dogs that recovered from disophenol

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[e]Novin, Haver-Lockhart Laboratories, Shawnee, KS. 66201, USA

[f]Cutter Laboratories, Inc., Berkeley, Calif. 94710, USA

toxicity were observed for a period of 10 days and then euthanatized with gross necropsy examination following immediately.

Results

Survival Rates. The survival times were not related to the dosage of disophenol administered (Table 1). Successful control of pyrexia by various treatments did not prevent deaths due to acute disophenol toxicosis.

Clinical Signs. The clinical signs consistently observed in all dogs were increased body temperature, elevated heart and respiration rate, and hyperventilation. The rise in body temperature was usually noticed within 1 hour following disophenol administration. In 4 dogs the increase in body temperature was not observed until 5-6 hours after administration of the disophenol. Hyperventilation was slight in the initial stages, but after 3-4 hours it was moderate to heavy and continuous. Rapid and shallow respirations and heavy panting were observed at the peak of pyrexia. The hour at which maximum body temperature occurred and the peak temperature in each animal is given in Table 2. In recovering dogs the pyrexia and hyperventilation returned to normal after approximately 40 hours.

TABLE 1. Sex and Survival Times (Hours) of Dogs Receiving Disophenol and Various Treatments

Treatment	Disophenol dose (mg./kg.)		
	33	35	40
None (Control)	M , 4	M , 25	M , 8
	F , Recovered	F , 6	
Dipyrone	F , Recovered	M , 10	.....
		M , Recovered	
Lactated Ringer's Infusions	.....	M , 6	.....
		M , 9	
Ice Baths	.....	F , Recovered	M , 7
		F , 5.5	
		F , 10.5	
		M , Recovered	

TABLE 2. Time (Hours) Required to Reach Maximum Body Temperature and the Temperature in Dogs Receiving Disophenol and Various Treatments

Treatment	Disophenol dose (mg./kg.)		
	33	35	40
None (Control)	2 , 104.8	24 , 106	5 , 104.8
	12 , 104	4 , 103.8	
Dipyrone	12 , 102.7	8 , 105.8	.....
		6.5 , 103.8	
Lactated Ringer's Infusions	.....	6 , 106	.....
		8 , 104.5	
Ice Baths	.....	16 , 104.3	4 , 103.8
		5.5 , 103	
		8.5 , 106.4	
		7 , 103.2	

Emesis was noticed in 4 dogs, and the vomitus was yellowish and of mucoid consistency. Dehydration was slight to moderate. Urination occurred in several dogs; the urine was of normal color and consistency. The mucous membranes of conjunctiva and gums were frequently hyperemic.

Death occurred at variable body temperatures. The peak body temperatures were not related to the dosage employed (Table 2). Death ensued in one dog at a body temperature of 103.0 F (40 C). Death was sudden and rigor mortis occurred immediately following.

Hemograms. The hemograms of some dogs revealed a high packed cell volume, an increase in percent hemoglobin, and leucocytosis, neutrophilia, and lymphopenia (Tables 3 and 4). However, these findings were not totally consistent. Blood pH studies in 1 dog did not reveal significant changes in blood pH.

Necropsy. Hemorrhages of the gastrointestinal tract (especially stomach and duodenum), hyperemia of the omentum, mesentery, and urinary bladder, and hemorrhages in the thymus were the most frequent lesions observed. Congestion of the lungs and kidneys and bloody urine were observed in occasional dogs. A mild tissue reaction was observed at the s.c. site of injection. The recovered dogs did not have gross pathology on postmortem examination.

TABLE 3. Mean Hemograms of 10 Dogs Dying From Disophenol Overdosage

Hours after admin.	White Blood Cells ($x10^3$)	Total Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Hemoglobin (%)	Packed Cell Volume
0	10.2	64.3	24.0	5.4	6.3	14.3	48.4
2	9.8	62.8	27.9	4.9	4.4	14.0	51.9
4	10.2	71.6	18.3	4.4	5.7	16.3	54.2
6	13.5	77.4	19.4	2.6	0.6	18.8	54.8
8	15.7	86.3	11.3	2.0	0.4	19.0	58.1

TABLE 4 - Mean Hemogram of 4 Dogs Recovered From Disophenol Overdosage

Hours after admin.	White Total Blood Cels ($x10^3$)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Hemoglobin (%)	Packed Cell Volume
0	10.6	57.0	30.8	6.2	6.0	16.4	47.8
2	10.1	64.3	28.3	5.0	2.4	16.5	47.3
4	9.4	59.8	30.0	6.8	3.4	17.3	50.5
6	11.9	67.0	26.0	3.7	3.3	18.3	53.7
8	13.3	69.0	25.0	3.7	2.3	17.8	53.5
10	11.2	70.0	24.0	5.0	1.0	17.1	51.2
12	12.1	71.8	21.0	5.5	1.7	17.0	52.0
14	12.2	72.3	24.0	3.7	...	16.4	48.6

Discussion

Administration of fluids and applications of cool water to the skin have been suggested for the treatment of dinitrophenol toxicosis[12]. Successful recovery of a dog from acute disophenol toxicosis occurred following treatment with ice packs, ethyl alcohol sprays, and buffered lactate ringer's solution[7].

The 2 control dogs receiving 33 mg. disophenol/kg. of body weight were studied at an ambient temperature of 78 F (26 C). Survival of one of the 2 control dogs at this dose suggests that this relatively low ambient temperature does not influence mortality and that the response is an individual variable. Successful recovery of 2 of the 4 (50%) dogs receiving 35 mg. disophenol/kg. of body weight and treated with ice baths suggests that ice bath treatment is of value in counteracting disophenol toxicity. Recovery of 1 of 2 (50%) dogs treated with dipyrone suggests the value of this antipyretic. Infusions of lactated Ringer's solution neither prevented mortality nor checked the febrile response.

Pyrexia progressed unchecked in dogs that did not respond to treatment with dipyrone or lactated Ringer's solution. Ice baths, however, did significantly reduce the pyrexia, but the death of some dogs treated with ice baths

suggests that toxicity from disophenol may be due to factors other than hyperpyrexia. Biotransformation and excretion studies with disophenol may lead to insight into the mechanism of its toxic action. Further studies are also needed to determine the efficacy of antipyretics, ice baths, and a combination of these as a treatment aid for disophenol toxicosis.

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TREATMENT AND PROTHROMBIN RESPONSES DURING WARFARIN TOXICOSIS IN RATS AND MICE

Lakshmipaty Penumarthy, B.V.Sc., M.S.;
Frederick W. Oehme, D.V.M., Ph.D.

SUMMARY

Efficacy of whole dog blood or vitamin K_3 treatment during warfarin feeding was investigated in rats and mice. Prolonged prothrombin times were observed in mice after 9-12 hours of warfarin feeding. Prothrombin times greater than 300 seconds were consistently observed in mice on continuous warfarin feeding irrespective of treatment. Withdrawal of warfarin resulted in normal prothrombin times after 96, 48-72, or 48 hours in mice receiving no treatment, whole bood, or 72 mg. vitamin K_3/kg. of body weight/day, respectively. Mortality was consistently higher in mice given warfarin continually and in those receiving the greater number of treatments. Frequent handling appears to aggravate warfarin toxicosis.

From the Comparative Toxicology Laboratory, Department of Surgery and Medicine, Kansas State University, Manhattan, KS 66506. Reprint requests should be sent to Dr. Oehme.

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Warfarin (3-[alpha-acetonylbenzyl]-4 hydroxycoumarin) is an effective oral anticoagulant rodenticide; commercial baits are prepared with corn meal, rice meal, or other grain material, and contain approximately 0.025% warfarin by weight [16,21]. Sodium warfarin is employed as a therapeutic anticoagulant in man[10,14,15].

Warfarin poisoning is a common clinical problem in veterinary practice[2]. Episodes of acute poisoning have been reported in animals, primarily dogs, cats and pigs, due to either accidental or malicious feeding of warfarin-containing rodent baits[2,5,7,19,24,26,27]. Single doses of warfarin, unless very large, do not exert a lethal action. If repeatedly ingested in small amounts, however, warfarin may prove fatal.

The single mean lethal dose in common laboratory animals ranges between 200 and 400 mg. warfarin/kg. of body weight[14]. Rats may withstand single oral doses of 50 mg. warfarin/kg. of body weight, but are killed by repeated dosing for 5 successive days with 1 mg. warfarin/kg. of body weight. Consumption of a total of 2.87 mg. warfarin/kg. of body weight over a 14-day period caused 100% mortality in rats[16,21]. Single intraperitoneal (I.P.) doses up to 100 mg. warfarin/kg. of body weight produced only 20% mortality; however, 83% mortality occurred following I.P. administration of a total warfarin dose of 2.5 mg./kg. of body weight over a 7-day

period. Similar results were also observed when warfarin was administered orally[25].

Following oral administration in rats, warfarin is rapidly absorbed and reaches detectable liver concentrations within 24 hours[13]. In man, maximal plasma concentration occurred 2-12 hours after oral dosing, and the mean plasma half-life of warfarin was 42 hours. The plasma warfarin level was identical following oral and intravenous (I.V.) administration. Warfarin was found highly bound to human plasma albumin[10,15,22,28].

In studies involving the metabolism of 4-^{14}C warfarin sodium in rats, unchanged warfarin, several hydroxylated derivatives including a glucuronide, and an intramolecular condensation product of warfarin were found in urine and feces. Urine and fecal excretion accounted for 2/3 and 1/3, respectively, of the total dose. Biliary excretion of the metabolites, followed by partial reabsorption and excretion via urine, was assumed to explain similarities in urinary and fecal metabolites[3].

Warfarin in vivo primarily effects blood coagulation. It inhibits the action of fat-soluble vitamin K and thereby depresses hepatic synthesis of prothrombin and other plasma clotting factors dependent on vitamin K (factors VII, IX and X). The nature of the interaction between vitamin K and

warfarin is not definitively established. It was earlier assumed that warfarin acts through competitive inhibition with vitamin K for a specific site on a regulator protein. Subsequent studies, however, suggested that the site of action may be an enzyme regulating the metabolism of vitamin K[10]. Recent studies in rats suggested that warfarin inhibits prothrombin synthesis by increasing the liver ratio of phylloquinone oxide (an inhibitor of vitiman K_1) to vitamin K_1[4].

Ultrastructural studies of capillaries from warfarin-treated rats revealed the loss of cytoplasmic ground substance of endothelial cells[18]. Fatal hemorrhage seems to result from interference with liver production of the prothrombin complex and consequent lengthening of blood clotting time. Due to capillary damage, the traumas of normal life seem to be sufficient to produce fatal hemorrhage[21,23]. Direct toxicity or indirect effects via a reaction involving vitamin K have also been suggested as possible mechanisms of warfarin action[18].

Clinical signs of warfarin poisoning are variable and include: pallor of the mucous membranes; weakness; subcutaneous hemorrhages and/or swelling due to hematomata visible externally, especially on the appendages; oral or rectal bleeding; loss of body weight, especially in animals with

long survival times; and lameness due to hemorrhage in joints and over long prominences of the limbs. Some animals die without premonitory signs. The clinical appearance and post mortem findings suggest that death is caused by shock[16,21].

Warfarin produces a typical pathology. General pallor of all organs, multiple hemorrhages throughout the body musculature (particularly over the bony prominences of the limbs and ribs), subcutaneous hemorrhages, and extensive internal hemorrhages are the major post mortem lesions. Less frequent are hemorrhages of the genital organs, bleeding in the pelvic and lumbar region, and small circumscribed hemorrhages of the lungs[16,21].

Since vitamin K is involved in the liver synthesis of the prothrombin complex, administration of vitamin K is the physiological antidote for warfarin toxicosis. Varying results have been reported on the efficacy of vitamin K_1 (naturally-occurring vitamin K) and the other vitamin K analogues (synthetic compounds with lesser vitamin K activities). Several authors suggest administration of vitamin K_1 as the only therapeutic choice and state that the vitamin K analogues, such as menadione and menadione sodium bisulfite, are of no value in combating the excessive anticoagulant-induced hypoprothrombinemia[1,8,11,12,17,27]. However, others report

successful recovery of warfarin-poisoned animals following treatment with blood transfusions and/or vitamin K analogues [5,6,9,20].

I.P. administration of 5 mg. vitamin K_1/kg. of body weight/day offered some protection in rats against daily low doses of warfarin, but when warfarin was administered at the rate of 5 mg./ kg. of body weight/day, no further protection was observed with vitamin K_1 even though the daily dose was raised to 10 mg. vitamin K/kg. of body weight/day[25]. Administration of Koagamin, a commercial solution of oxalic and malonic acids, and saline-glucose solutions have also been suggested as therapy[19].

Since species differences exist in the metabolism of warfarin, the present investigation tested the effect of whole blood or vitamin K analogue, menadione sodium bisulfite, therapy when administered at different stages of warfarin toxicosis in mice and rats. Information was sought on the effect of repeated handling and the time-course prothrombin response following consumption of warfarin bait.

Materials and Methods

Housing and Maintenance of the Experimental Animals.-- Sprague-Dawley rats or groups of 5 White Swiss mice each, separated as to male and female, were housed in individual

cages with separate watering devices. Fresh bedding of soft wood shavings was provided daily. The animals were stabilised before experimentation for at least 1 week by feeding a dry pelleted ration[a]. The average weight of the mice and rats at initiation of the toxicity trial was approximately 30 and 450 G., respectively.

Warfarin Feeding.--A commercial warfarin bait feed[b] containing 0.029% warfarin by weight was provided in individual feed cups to each cage on a no-choice basis. The number of days the bait feed was provided varied with the trial.

Pilot Trial.--Fifty mice and 6 rats (equal numbers of male and female) were used to estimate the parameters to be employed in subsequent treatment procedures. With daily consumption of the warfarin bait, initial clinical signs and/or death occurred in mice and rats at 2 days (48 hours) and 4 days (96 hours) respectively. Daily consumption of warfarin bait for 8-10 days resulted in 100% mortality in both mice and rats. Repeated I.P. administration of whole dog blood to mice and rats was totally compatible and no untoward signs were

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[a]Purina Laboratory Chow, Ralston Purina Co., Checkerboard Square, St. Louis, Missouri.

[b]Gordon Corporation, 300 S. 3rd. St., Kansas City, Kansas.

noticed. The I.P. route was also satisfactory for administering vitamin K_3 in physiological saline.

Based on these observations it was decided to test the efficacy of I.P. administration of whole dog blood or vitamin K_3 in counteracting warfarin toxicosis, the time-course effect of warfarin consumption on prothrombin time, and the effect of frequent handling of the animals on the rate of mortality. Each trial was conducted for 8-10 days.

Treatments Employed.--A commercial solution of vitamin K_3 (menadione sodium bisulfite)[c] was administered undiluted or diluted with physiological saline. Normal whole dog blood was obtained from the jugular vein of clinically healthy dogs using a minimum amount of heparin as an anticoagulant. The I.P. route of administration was employed in all treatments. Physiological saline solution was used for sham injections.

Effect of Warfarin on Prothrombin Time.--Warfarin bait feed was given to 20 mice (equal numbers of male and female) and the prothrombin time estimated at periodic intervals for 24 hours. One randomly selected mouse was anesthetised with ether at each predetermined time and the thoracic cavity exposed. A 0.45 ml. volume of blood was drawn from

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[c]Hykinone, Abbot Laboratories, North Chicago, Illinois, 60064, USA.

the right ventricle into a tuberculin syringe containing 0.05 ml. of 0.1M sodium oxalate. The contents of syringe were thoroughly mixed and centrifuged for 5 minutes at 3,000 rpm. Plasma prothrombin time was estimated by the Lab-Tek Prothrombin System[d]. Each mouse was sacrificed after withdrawing the blood sample.

Studies in Rats.--Forty rats (equal numbers of male and female) were randomly assigned into 4 groups of 10 each. The warfarin bait was given to each of the 4 groups on a no-choice basis. Feed consumption, body weights, and mortality were recorded daily. Treatment procedures initiated 96 hours after beginning the warfarin feeding were as follows:

Group 1 -- no treatment.

Group 2 -- 1.5 ml. blood at the 96th hour.

Group 3 -- 0.06 mg. vitamin K_3/kg. of body weight at the 96th hour.

Group 4 -- 0.06 mg. vitamin K_3/kg. at the 96th and 168th hour.

Studies in Mice.--Trial A - Eighty mice (equal numbers of male and female) were randomly assigned into 8 groups of 10 mice each. Groups 1 to 7 were given warfarin bait feed all the time and Group 8 received the warfarin bait for only 48

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[d]Lab-Tek Instrument Co., 39 East Burlington Street, Westmont, Illinois 60559.

hours with normal feed thereafter. The treatments instituted in the various groups were as follows:

Group 1 -- no treatment.

Group 2 -- 0.4 ml. physiological saline daily after 48 hours.

Group 3 -- 0.4 ml. blood daily after 48 hours of feeding.

Group 4 -- 0.4 ml. blood at 0, 72 and 144 hours of feeding.

Group 5 -- 0.12 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. of body weight daily from day 0 of feeding.

Group 6 -- 0.12 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. daily after 48 hours of feeding.

Group 7 -- 0.12 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. at 0, 72, 144 hours of feeding.

Group 8 -- 0.12 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. daily from day 0 of feeding.

Trail B - One hundred mice (equal numbers of male and female) were randomly assigned to 10 treatment groups of 10 mice each. Groups 1-6 were given warfarin feed for 2 days and normal feed thereafter. Groups 7-10 received warfarin feed continually. The treatment procedures instituted in the various groups were as follows:

Group 1 -- no treatment.

Group 2 -- 0.4 ml. physiological saline daily after 48 hours of feeding.

Group 3 -- 0.4 ml. blood daily after 48 hours of feeding.

Group 4 -- 5 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. daily after 48 hours of feeding.

Group 5 -- 0.4 ml. blood at 48 and 120 hours of feeding.

Group 6 -- 5 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. at 48 and 120 hours of feeding.

Group 7 -- 0.4 ml. blood daily from day 0 of feeding.

Group 8 -- 5 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. daily from day 0 of feeding.

Group 9 -- 5 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. daily after 48 hours of feeding.

Group 10-- 5 mg. vitamin K_3 in 0.4 ml. physiological saline/kg. at 48 and 120 hours of feeding.

Trail C. - Forty mice (equal numbers of male and female) were assigned randomly among 4 treatment groups of 10 mice each. Groups 1 and 2 were given warfarin feed for the first 48 hours and normal feed thereafter; groups 3 and 4 were given warfarin feed continually. The treatment procedures were as follows:

Group 1 -- 72 mg. vitamin K_3/kg. daily from day 0 of feeding.

Group 2 -- 72 mg. vitamin K_3/kg. daily after 48 hours of feeding.

Group 3 -- 72 mg. vitamin K_3/kg. daily from day 0 of feeding.

Group 4 -- 72 mg. vitamin K_3/kg. daily after 48 hours of feeding.

Other Observations.--The animals were observed for clinical signs of warfarin toxicosis. Feed consumption, body weights, and mortality were recorded every day in each trial group. An additional 10 mice were kept in each trial group for prothrombin estimation, and 1 mouse was sacrificed every day for estimation of prothrombin time. Animals dying during the course of investigation were examined for gross lesions. The surviving animals were sacrificed at the end of the investigational period and necropsy was performed.

Results

Effect of Warfarin on Prothrombin Time in Mice.--The warfarin bait feed consumption and the resulting changes in prothrombin time during a 24-hour period were approximately equal in both sexes. The averages for all mice are represented in Table 1. The normal prothrombin time for mice ranged from 9.5-12 seconds. A significant change in prothrombin time occurred within 6 hours of warfarin feeding. With increase in feed consumption, the prothrombin time increased. A dramatic depression in clotting ability, as evidenced by prothrombin time, occurred after 18 hours following consumption of 3.92 G. of warfarin bait (equivalent to 1.14 mg. warfarin).

TABLE 1--Effect of Feeding Warfarin on Prothrombin Time

Hours after feeding	Average cumulative feed consumption (G.)	Mean prothrombin time (seconds)
0	0	10.9
2	0.57	10.4
4	1.03	11.6
6	1.50	15.0
9	2.25	21.2
12	2.93	40.4
15	3.14	51.1
18	3.92	>300
24	5.05	>300

Studies in Rats.--The mortality of rats in the 4 treatment groups is represented in Table 2. Treatment with the indicated doses of blood or vitamin K_3 did not prevent warfarin toxicosis. All the rats in group 4 died before receiving a second treatment of vitamin K_3.

Average values of feed consumption and body weights in the 4 groups are presented in Tables 3 and 4. Groups 3 and 4 were combined and average values represented, since the two groups received identical treatments due to all group 4 rats dying before the scheduled 168-hour treatment was given. With daily feeding of warfarin bait, the feed consumption and body weights decreased with time in all groups. Warfarin consumption over the 7-day period ranged from 24.5 to 29.8 mg./rat and resulted in 100% mortality.

Studies in Mice. Trial A--The mortality of warfarin-fed mice receiving various treatments is presented in Table 5. At the levels employed, the treatments did not afford protection against warfarin toxicosis. Further, the lower mortality in groups 1 and 8 suggest that frequent handling and repeated trauma from injections were factors responsible for the higher mortality in groups 2-7. The prothrombin times in groups 1-7 (receiving warfarin bait continually) were greater than 300 seconds throughout the feeding period. Group 8 (receiving warfarin bait for 48 hours) showed prothrombin times greater

TABLE 2--Mortality in Rats Given Warfarin Bait Feed and Receiving Different Treatments

Day	Group (N=10)			
	1*	2**	3†	4‡
0 ↓ 3	0	0	0	0
4	3	3	4	3
5	4	3	1	2
6	3	2	5	5
7	⏦	2	⏦	⏦

* No treatment.

** Received 1.5 ml. blood at the 96th hour.

† Received 0.06 mg. vitamin K_3/kg. of body weight at the 96th hour.

‡ Received 0.06 mg. vitamin K_3/kg. at the 96th and 168th hour.

⏦ All rats in group dead.

TABLE 3--Average Daily Warfarin Bait Feed Consumption (G.) in Rats Receiving Different Treatments. Values in Parentheses are mg. of Warfarin Received in Feed.

Day	Group (N = 10)		
	1*	2**	3&4†
1	23.3 (6.76)	29.0 (8.41)	24.2 (7.02)
2	21.6 (6.26)	26.1 (7.57)	23.9 (6.93)
3	19.9 (5.76)	25.6 (7.42)	18.3 (5.31)
4	15.2 (4.41)	14.6 (4.23)	14.2 (4.12)
5	7.7 (2.22)	5.4 (1.57)	3.1 (0.90)
6	2.0 (0.58)	1.6 (0.46)	0.7 (0.21)
7	‡	0.5 (0.15)	‡
Total mg. warfarin consumed.	25.99	29.81	24.49

* No treatment.

** Received 1.5 ml. blood at the 96th hour.

† Received 0.06 mg. vitamin K_3/kg. of body weight at the 96th hour.

‡ All rats in group dead.

TABLE 4--Average Daily Body Weight (G.) of Rats Given Warfarin Bait Feed and Treated With Various Procedures

Day	Group (N = 10)		
	1*	2**	3&4†
0	448.8	472.3	446.0
1	441.0	464.0	441.3
2	441.2	465.1	442.0
3	437.8	461.2	436.3
4	431.2	453.1	429.0
5	427.8	440.4	425.0
6	414.3	412.8	405.8
7	‡	423.4	‡

* No treatment.

** Received 1.5 ml. blood at the 96th hour.

† Received 0.06 mg. vitamin K_3/kg. of body weight at the 96th hour.

‡ All rats in group dead.

TABLE 5--Mortality in Mice (Trial A) Given Warfarin Bait Feed and Treated With Various Procedures*.

Day	Group (N=10)							
	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	1
2	1	0	1	0	1	0	0	0
3	2	1	1	0	3	1	2	0
4	2	4	7	5	3	5	6	5
5	0	1	1	0	2	1	0	1
6	1	3	**	2	1	1	0	0
7	1	0	**	1	**	0	2	0
8	0	0	**	0	**	0	**	0
No. mice surviving	3	1	0	2	0	2	0	3

* Treatment procedure for each group in this trial is outlined in Materials and Methods.

** All mice in group dead.

than 300 seconds on the first 3 days, almost normal time (15 seconds) on 4th day, and normal time (11 seconds) on days 7 and 8. Decreases in feed consumption and body weights were also observed.

Trial B - Mortality, feed consumption, and prothrombin times are presented in Tables 6-8. Despite the treatments, 100% mortality occurred in the mice receiving warfarin continually (groups 7-10). When given warfarin bait for only 48 hours, lower mortality was observed in mice receiving no treatment or only a few treatments. Decreases in feed consumption and body weights and prothrombin times greater than 300 seconds were observed in mice receiving warfarin continually. Most of the mice died during the 3rd and 4th day. Since the prothrombin times of the mice receiving warfarin for 48 hours did not return to normal until the 5th day, repeated injections and handling may have contributed to the deaths of mice in groups 1-6 after removal from warfarin.

Trial C - The data on mortality rate, feed consumption, and prothrombin times of mice receiving warfarin and high doses of vitamin K_3 are given in Tables 9-11. In mice removed from warfarin, most of the mortality occurred on day 3. Mice on continual warfarin feeding had decreased feed intake and reduced body weights. Administration of 72 mg. vitamin K_3/kg. did not return the prothrombin times to normal values

TABLE 6--Mortality in Mice (Trial B) Given Warfarin Feed and Receiving Different Treatments*. Groups 1-6 Received Warfarin Feed for the First 48 Hours; Groups 7-10 Received Warfarin Feed Continually.

Day	Group (N = 10)									
	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	1	0	3	0	0	0
3	2	1	4	4	4	4	3	4	6	2
4	2	7	4	3	1	1	3	4	3	2
5	2	0	1	1	2	0	1	2	1	2
6	0	0	0	0	0	0	**	**	**	3
7	0	0	1	0	0	0	**	**	**	0
8	0	0	**	0	0	0	**	**	**	0
No. mice surviving	4	2	0	2	2	5	0	0	0	1

* Treatment procedure for each group in this trial is outlined in Materials and Methods.

** All mice in group dead.

TABLE 7--Average Daily Warfarin Bait Feed Consumption (G.) in Mice (Trial B) Receiving Different Treatments*. Groups 1-6 Received Warfarin Feed for the First 48 Hours; Groups 7-10 Received Warfarin Continually.

Day	Group (N=10)									
	1	2	3	4	5	6	7	8	9	10
1	4.5	5.9	4.5	5.5	4.9	5.5	3.3	5.2	5.0	4.9
2	4.4	5.2	4.2	4.3	4.8	4.0	2.5	3.5	5.6	3.7
3	.....	.....	.....	.....	.....	.....	1.9	2.3	2.3	3.1
4	.....	.....	.....	.....	.....	.....	0.6	0.7	0.4	0.9
5	.....	.....	.....	.....	.....	.....	1.2	0.2	0.0	0.3
6	.....	.....	.....	.....	.....	.....	**	**	**	0.2
Total feed consumed (G.)	8.9	11.2	8.7	9.7	9.7	9.4	9.5	11.8	13.3	13.2
Total warfarin (mg.)	2.58	3.24	2.53	2.82	2.81	2.74	2.76	3.43	3.87	3.83

* Treatment procedure for each group in this trial is outlined in Materials and Methods.

** All mice in group dead.

TABLE 8--Daily Prothrombin Times (seconds) in Mice (Trial B) Given Warfarin Feed and Receiving Different Treatments*. Groups 1-6 Received Warfarin for the First 48 Hours; Groups 7-10 Received Warfarin Continually.

Day	Group (N=10)									
	1	2	3	4	5	6	7	8	9	10
1	45	104	.....	.....	.....	.....	.....	102	.....	.....
2	>300	>300	>300	>300	.....	.....	>300	>300	>300	>300
3	>300	.....	100	>300	100	>300	.....	>300	>300	>300
4	>300	.....	18	19	91	.....	.....	>300	>300	.....
5	33	.....	.....	.....	12.4	11.5	**	**	**	**
6	.....	.....	.....	.....	10.8	11.6	**	**	**	**
7	10.3	10.3	.....	11	10.2	11.0	**	**	**	**
8	10.6	11	11.2	.....	10.4	10.8	**	**	**	**

*Treatment procedure for each group in this trial is outlined in Materials and Methods

**All mice in group dead.

TABLE 9--Mortality in Mice (Trial C) Given Warfarin Feed and Receiving 72 mg. Vitamin K_3/kg. I.P. by Different Methods. Groups 1 and 2 Received Warfarin Feed for the First 48 Hours; Groups 3 and 4 Received Warfarin Feed Continually.

Day	Group (N=10)			
	1*	2**	3†	4‡
1	0	0	0	0
2	1	0	0	0
3	5	3	3	2
4	1	2	5	4
5	1	0	1	4
6	0	0	1	≈
7	0	0	≈	≈
8	0	0	≈	≈
No. mice surviving.	2	5	0	0

* Received vitamin K_3 daily from day 0 of warfarin feeding.

** Received vitamin K_3 daily after 48 hours of warfarin feeding.

† Received vitamin K_3 daily from day 0 of warfarin feeding.

‡ Received vitamin K_3 daily after 48 hours of warfarin feeding.

≈ All mice in group dead.

TABLE 10--Average Daily Warfarin Bait Feed Consumption (G.) in Mice Treated with 72 mg. Vitamin K_3/kg. Body Weight I.P. by Different Methods. Groups 1 and 2 Received Warfarin Feed for the First 48 Hours; Groups 3 and 4 Received Warfarin Continually.

Day	Group			
	1*	2**	3†	4‡
1	4.8	6.8	4.9	6.8
2	1.6	2.1	4.4	6.1
3	.....	.....	1.6	3.0
4	.....	.....	0.5	1.5
5	.....	.....	0.6	0.3
6	.....	.....	0.0	☡
Total feed consumed (G.)	6.4	8.9	12.0	17.7
Total warfarin consumed (mg.)	1.85	2.59	3.50	5.13

* Received vitamin K_3 daily from day 0 of warfarin feeding.

** Received vitamin K_3 daily after 48 hours of warfarin feeding.

† Received vitamin K_3 daily from day 0 of warfarin feeding.

‡ Received vitamin K_3 daily after 48 hours of warfarin feeding.

☡ All mice in group dead.

TABLE 11--Prothrombin Times (seconds) in Mice (Trial C) Given Warfarin Feed and Receiving 72 mg. Vitamin K_3/kg. Body Weight I.P. by Different Procedures. Groups 1 and 2 Received Warfarin Feed for the First 48 Hours; Groups 3 and 4 Received Warfarin Feed Continually.

Day	Group (N=10)			
	1*	2**	3†	4‡
1	96	129	>300	>300
2	>300	>300	>300	>300
3	>300	>300	>300	>300
4	13.0	13.5	>300	≀
5	10.3	12.4	≀	≀
6	11.2	10.0	≀	≀
7	10.4	10.4	≀	≀

* Received vitamin K_3 daily from day 0 of warfarin feeding.

** Received vitamin K_3 daily after 48 hours of warfarin feeding.

† Received vitamin K_3 daily from day 0 of warfarin feeding.

‡ Received vitamin K_3 daily after 48 hours of warfarin feeding.

≀ All mice in group dead.

if warfarin was fed continually. This prolonged prothrombin time and associated repeated injections and handling probably aggravated the mortality rate.

Clinical Signs.--Animals dying within 2 days of warfarin feeding did not have consistent clinical signs. Delayed deaths were characterised by lamenesses, especially in hind legs, and subcutaneous swellings over the head, face, shoulder, thoracic and abdominal walls, and thigh regions. Bleeding from the tail, toes or the nostrils, disinclination to move, and labored respirations were also observed. Cannibalism was sometimes noticed in mice and was probably due to the occurrence of external bleeding.

Necropsy Findings.--Characteristic hemorrhagic lesions were not detected in the mice that died in less than 48 hours of warfarin bait consumption. Localised or diffuse subcutaneous hemorrhages were observed in various areas of the body of those animals dying after 48 hours of warfarin feed consumption. Extensive subcutaneous hemorrhages at the injection site were noticed in most of the treated animals. Hemorrhages in the musculature and under the skin (Fig. 1 and 2), thoracic and abdominal cavities (Fig. 3 and 4), brain, gastro-intestinal tract, thymus, testes and liver were other consistent lesions.

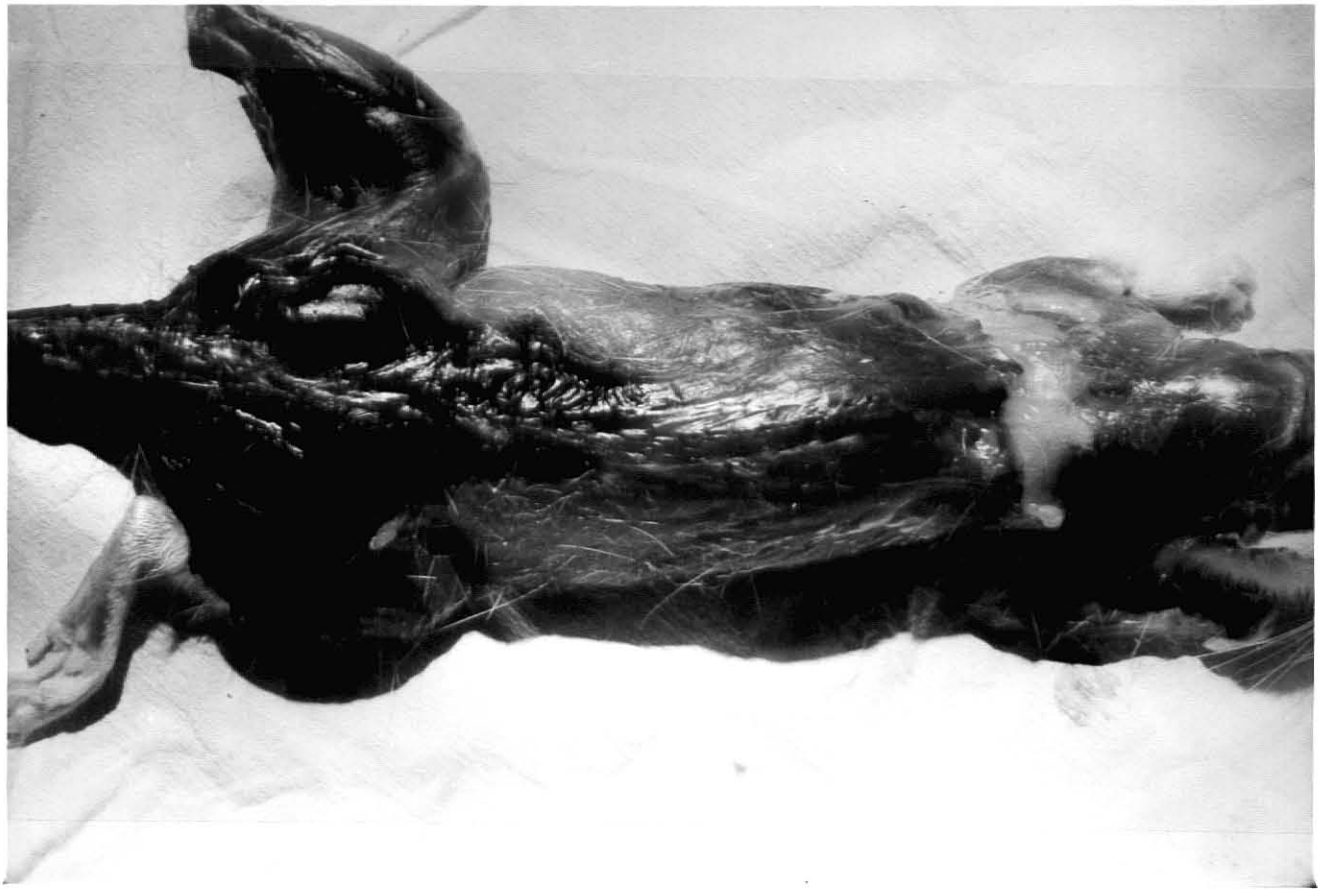

Fig. 1 -- Subcutaneous and intramusculature hemorrhage in a rat dying after warfarin consumption for 4 days.

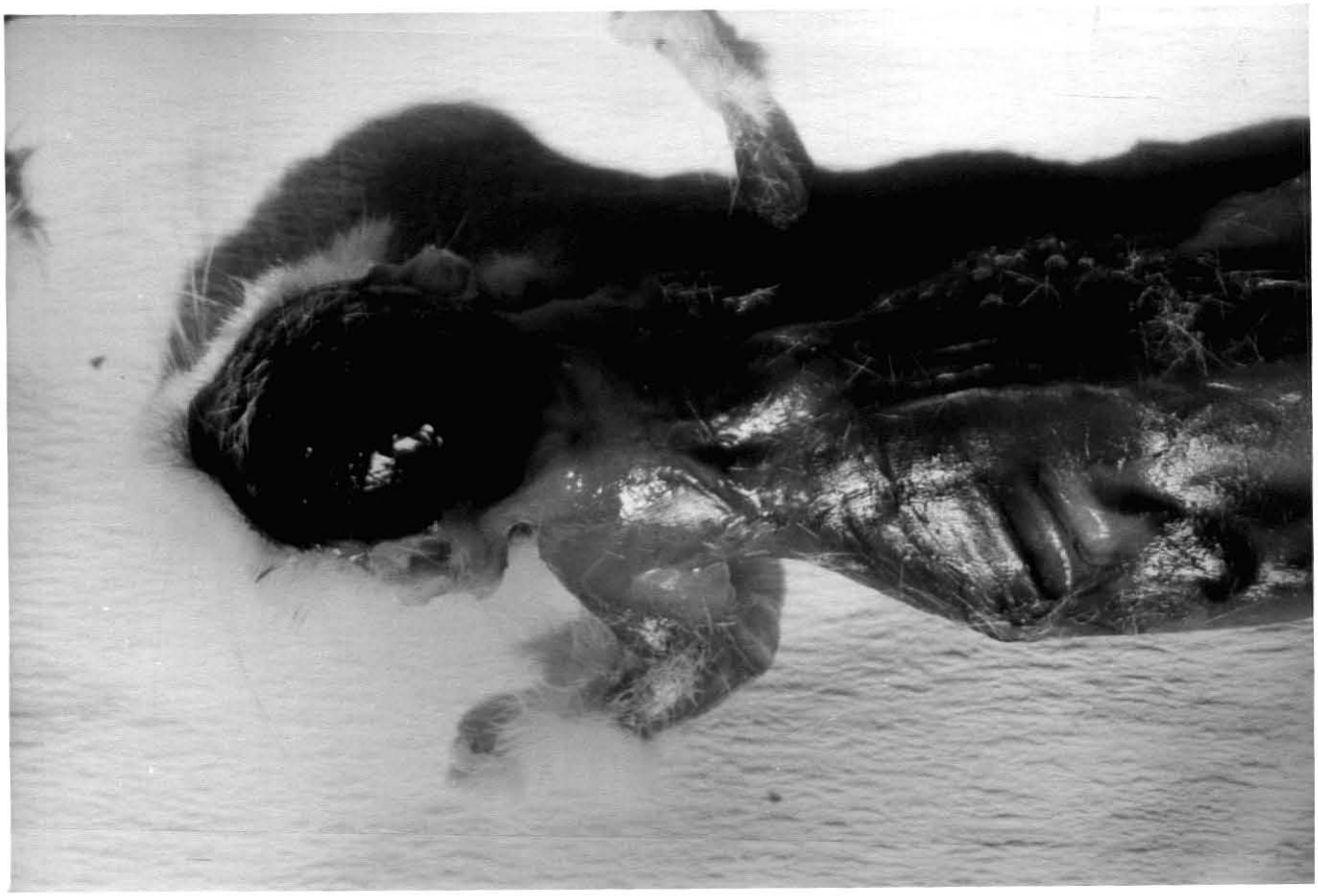

Fig. 2 -- Subcutaneous hemorrhage over the skull of a rat dying after 5 days of warfarin consumption.

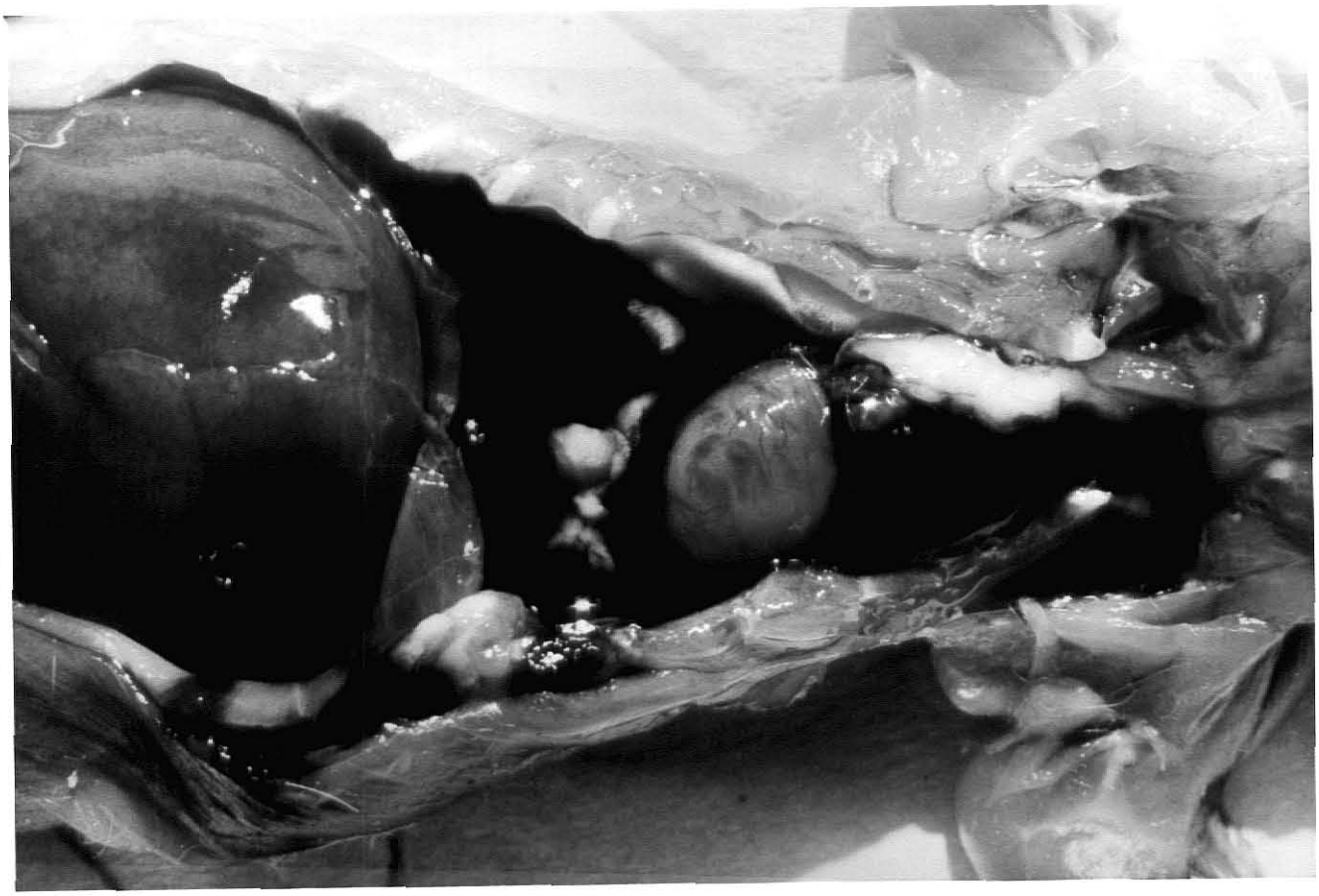

Fig. 3 -- Massive hemorrhage in the thoracic cavity of a mouse dying after 3 days of warfarin consumption.

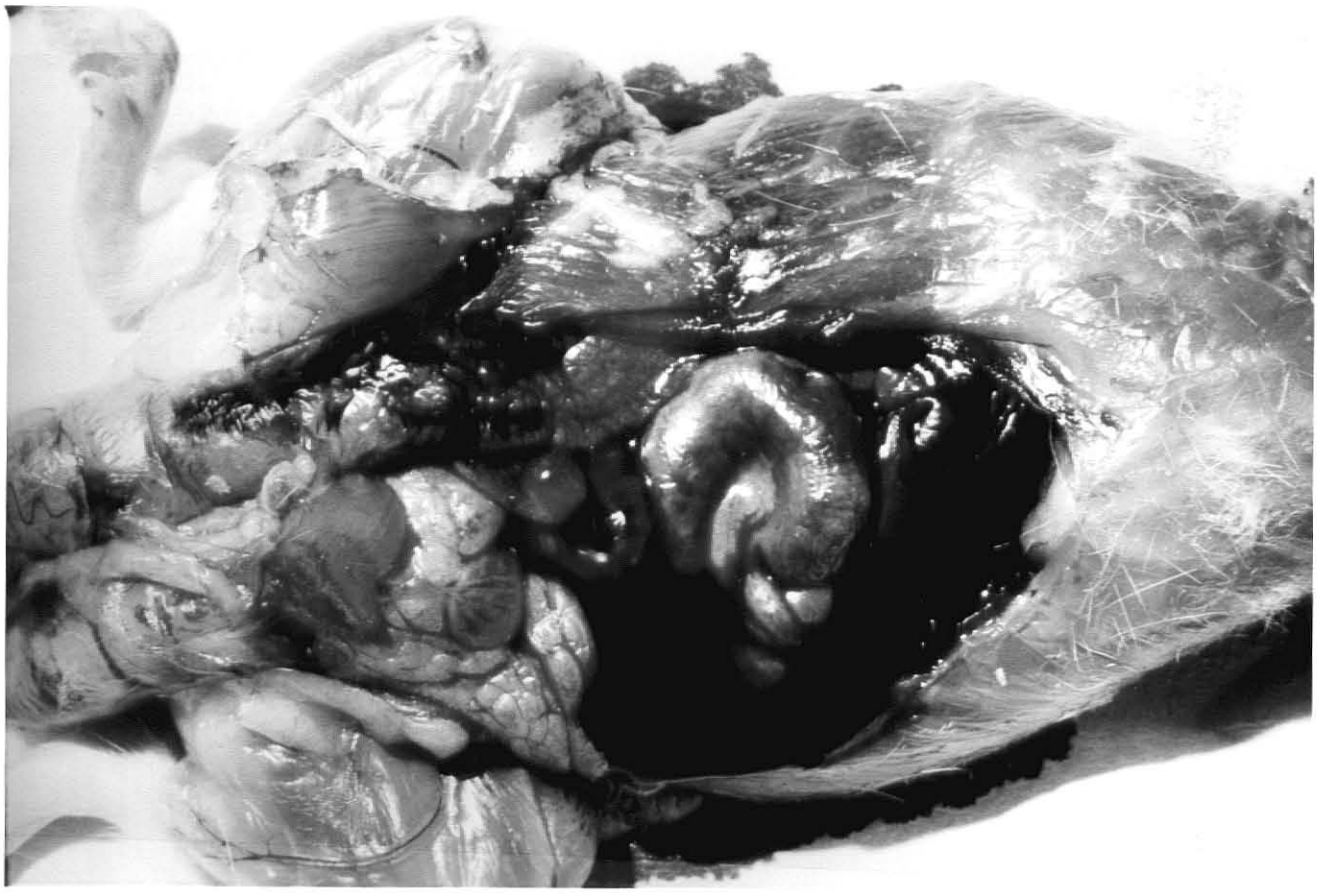

Fig. 4 -- Massive hemorrhage in the abdominal cavity of a mouse dying after warfarin consumption for 3 days.

Discussion

The dramatic depression of prothrombin complex observed in the mice within 9-12 hours of warfarin bait consumption (19.5-25.5 mg. warfarin/kg. of body weight) suggests that the reserves and/or the half-lives of the clotting factors are low in this species. Mice given warfarin feed for 48 hours and not receiving any treatment had their prothrombin times return to normal 96 hours after warfarin withdrawal.

Rats administered ^{14}C-labelled warfarin excreted most of the radioactivity during the first 2 days after drug administration[3]. The prothrombin values observed in this investigation indicate that the excretion of warfarin in mice might closely follow that of rats. The consistently prolonged prothrombin times in mice given warfarin bait continually and receiving no treatment suggest that plasma warfarin is maintained at concentrations sufficient to depress the prothrombin synthesis throughout the experimental period.

Transfusion of whole blood serves as a temporary and immediate source of prothrombin and therefore should alleviate the hypoprothrombinemia induced by warfarin[14]. However, whole blood, at the levels employed, did not protect the mice and rats from warfarin toxicosis. Return of prothrombin times to normal values 48-72 hours after warfarin bait withdrawal in mice receiving single or repeated treatments with whole blood (as

compared to the return of normal values 96 hours after withdrawal in mice given no treatment) suggests that the treatment was at least partially effective in counteracting the warfarin-induced hypoprothrombinemia.

Unlike warfarin, heparin does not block prothrombin synthesis in liver. Prevention of thrombin formation is its primary coagulant effect and it has a short half-life[6,15]. Since prothrombin times in mice were estimated 24 hours after blood therapy, the minute amounts of heparin used to obtain blood from dogs did not influence the potential efficacy of this treatment.

Vitamin K_3 did not protect rats in this study from warfarin toxicosis. Since vitamin K_3 is considered less active than vitamin K_1[11], the dose employed was possibly insufficient to counteract the warfarin toxicity. However, synthetic vitamin K_3 has been suggested for the treatment of warfarin-induced hypoprothrombinemia[5,6,9,20].

Doses of 72 mg. vitamin K_3/kg. body weight did not prevent mortality in mice given warfarin continually. Mice given warfarin feed for 48 hours and treated with 72 mg. vitamin K_3/kg./day had normal prothrombin values within 48 hours of warfarin withdrawal; mice that received no treatment had normal values only 96 hours after warfarin withdrawal. This suggests that the higher doses of vitamin K_3 were able to

counteract the warfarin-induced hypoprothrombinemia but not the mortality. Non-return of prothrombin values to normal in mice on continuous warfarin feed and receiving 72 mg. vitamin K_3/kg./day further suggests that high doses of vitamin K_3 are not effective in counteracting hypoprothrombinemia after critical dose levels of warfarin are reached.

The survival rates were consistently higher in mice receiving no treatment or only few treatments. In addition to the evident hypoprothrombinemia and capillary damage, repeated injections and frequent handling (even in the sham injected mice) seemed to have precipitated higher mortality rates in all the treatment groups.

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APPENDICES

APPENDIX I

Literature Review for the Systematic Treatment of Ethylene Glycol Toxicosis in Cats

LITERATURE REVIEW

Chemistry

Ethylene glycol (EG) has the chemical formula $HO-CH_2-CH_2-OH$. It is a colorless, odorless, viscous hygroscopic liquid with a bitter-sweet taste. It has a specific gravity of 1.113, a boiling point of 197.6 C , and a freezing point of -13 C . It is miscible with water, lower aliphatic alcohols, aldehydes, and ketones. As a solvent it assumes an intermediate position between glycerine on one hand and alcohol and acetone on the other. It is commercially prepared by the hydration of ethylene oxide[8,22,30,35,43].

Uses

EG is used as an antifreeze in automobiles, owing to its capacity of lowering the freezing point of water. Recently it has also been employed as a circulant coolant in the lunar modules[21]. It is used as a solvent in the paint and plastic industries and in the synthesis of safety explosives[22].

It has been used as a preservative of fruit juices and alcohol-free drinks and as a solvent in the pharmaceutical industry. Medically, EG has been employed as a substitute for glycerine[43]. The internal use of EG is banned in

the United States. A topical concentration exceeding 5% is considered a health hazard[13,30].

Toxicity

Numerous acute deaths have been reported in humans owing to consumption of EG as a substitute for alcoholic beverages[4,7,23,31,37,43]. This compound appears to be more toxic to man than to laboratory animals[12]. The minimum lethal dose for man is about 1.6 G./kg. body weight[12]. Contact with the skin and eyes is most likely to occur in industry, but its low volatility at ordinary temperatures eliminates inhalation risk. The minimum allowable concentration of EG in the environment has not been established[8,22,30,35].

Accidental poisoning has been reported in dogs[3,15,16,17], cats[14,16,17], poultry[33], and swine[26]. Rabbits did not show signs of toxicity on the subcutaneous administration of 5 ml. EG/kg. body weight, while a dose of 9 or 10 ml. EG/kg. body weight resulted in death in 12 to 24 hours[27].

In monkeys, the oral LD50 was estimated to be less than 2.5 ml. EG/kg. body weight[29]. Roberts et al.[34] stated that the toxic dose of EG in old world monkeys (Maccaca mulatta and M. irus) is probably similar to the 1.6 G./kg. body weight established earlier for man.

In dogs, death was observed on oral administration of 4.2 and 4.4 ml. EG/kg. body weight[2,16]. Kersting et al.[18] reported an oral minimum lethal dose of 6.6 ml. EG/kg. body weight, although some dogs required a dose of 13.2 ml. EG/kg.. This variation was explained on the basis of individual tolerance.

In cats, the minimum oral lethal dose of EG was estimated to be 1.5 ml./kg. body weight[23]. Spector[38] reported a subcutaneous lethal dose of 2 G. EG/kg. body weight. A dose of 1 G. EG/kg. was also observed as fatal to cats within 48 hours[12]. This was explained on the basis that cats form more oxalic acid than other species[12,28]. However Milles[23] observed recovery in a cat that received 1 ml. of EG/kg. body weight orally after showing slight signs of toxicity.

In poultry, the median lethal dose of EG was estimated as 67.5 ml./L. of drinking water. Birds receiving 25 ml. or more of EG/L. had oxalate crystals in their renal tubules[33].

In chronic studies, Blood et al.[5] observed no toxic effects in the Rhesus monkey when EG was given as 0.2% and 0.5% of the diet over a 3-year period. One dog was given 1,036 ml. of EG over 6 months without apparent untoward effects. The estimated dose in this case was 13.2 ml./kg.

body weight, but the dog was an erratic eater throughout the experiment[18]. In their studies on the effect of chronic administration of EG in drinking water at concentrations of 0.25 to 10% to several macaque species, Roberts et al.[34] reported calcium oxalate crystals within the proximal tubules and tubular degeneration when the monkeys received 15 ml. or more/kg. body weight. In animals receiving less than 15 ml./kg., oxalate crystals were not observed in the renal tubules, but glomerular damage and azotemia were present. It was suggested that a toxic effect of EG, apart from its conversion to oxalic acid, was responsible for the observed pathological changes.

Clinical Signs

The clinical signs of EG poisoning are well documented in humans and domestic animals[2,8,9,10,13,14,15,16,17,18,23,25,30,31,41,42]. The toxicity usually runs a short course, and there is frequently insufficient time for a thorough clinical examination as the patient is generally brought to the hospital in a coma. However, the clinical syndrome of EG poisoning has been divided into three stages.

The first stage is that of central nervous system involvement and develops 30-60 minutes after ingestion. Nausea, vomiting, signs resembling those of ethanol intoxication, somnolence, occasional diarrhea, ataxia, abnormal

placing reflexes, abdominal pain, subnormal temperature, polyuria, hematuria, proteinuria and crystalluria may be seen. After 4-6 hours this stage progresses with severe acidosis to paralysis and coma. Sometimes convulsions develop late in this stage.

An indistinct second stage is that of cardiopulmonary signs. Hyperpnea, tachycardia and moderate tachypnea are consistent early findings, and bradicardia is associated with the terminal stages in fatally poisoned animals. The patient may last 2-12 hours while in a coma. Death results from pulmonary edema and/or cardiac failure. Death usually occurs 8-36 hours post-ingestion.

Should the patient survive the first two stages, the third one is that of renal failure due to the precipitation of oxalate salts in the renal tubules. Anuria, uremia, coma and death occur in 5-14 days.

Post Mortem Lesions

Acute poisoning is associated with congestion of all body tissues. Pulmonary edema, swollen kidneys, hyperemia of gastro intestinal mucosa, and hyperemia of the brain are commonly observed. Generalized edema has been reported in humans[31]. Delayed deaths are characterized by dehydration, uremic ulcers in the mouth, and a hemorrhagic gastritis[18,34].

Microscopically, the kidneys are the organs most commonly affected in EG poisoning. Birefringent crystals of light-yellow color are found primarily in the proximal convoluted tubules[15,17,18]. Histochemical examination and differential staining identifies the crystals as salts of oxalic acid, principally calcium oxalate[2,15]. The crystals appear brilliant and double refractile when examined under polarized light[15,18].

In humans renal deposition of oxalate crystals is known to occur in (1) EG poisoning, and (2) primary oxalosis, a congenital disease of childhood due to an inborn error in the metabolism of glycine. Since primary oxalosis has not been reported to occur in animals, the presence of oxalate crystals in the renal tubules can be considered pathognomonic of EG poisoning in small animals[2,15,17,18]. Hydropic degeneration, cystic tubules, and acidophilic casts are constant findings. The damage to the renal tubular epithelium increases with the duration of the illness.

Lungs are hyperemic and have a varied degree of edema. These changes, seen early in the toxicity, are attributed to the direct effect of EG[10].

In the central nervous system, hyperemia of the brain, cerebellum, and a focal loss of purkinje fibers was observed[10,34]. Acute encephalitis and meningitis has also been reported[31]. Small crystals have been reported,

principally in the blood vessels of brain in humans, dogs, monkeys, and rats[10,16,17,18,34,37].

The liver is greatly distended with blood[10,16,17,18,34]. Central hydropic degeneration and hepatic fatty change with focal necrosis are common findings in human EG poisoning[10,31]. Moderate edema and centrolobular hemorrhage have also been reported in dogs[18].

Hyperemia of the gastrointestinal mucosa has been cited as a direct toxic effect of EG on the endothelial cells[17]. Cardiac enlargement and myocarditis has been reported in humans[10]. Concentric dilatation of the heart has been reported in experimental EG poisoning in a cat[23]. The toxic effects on skeletal muscle that were reported in human beings include interstitial myositis[10]. No such reports exist in veterinary literature.

Physiopathology

The mechanism of EG toxicity has not been definitely established. The species variation involved in the biotransformation of EG probably explains the controversy on why certain of the animal species show relatively greater sensitivity to this compound.

Ethylene glycol is readily absorbed from the digestive tract of experimental animals and is rapidly and uniformly distributed throughout the blood and body tissues[1].

In dogs, maximum blood EG concentration has been observed in 3-4 hours following oral administration[24,36]. In the early 1940's rat liver preparations containing liver alcohol dehydrogenase were found to oxidise EG to oxalic acid[44]. Since then, several studies have shown a series of metabolites resulting from EG biotransformation[11,12,28,29,40,44].

Gessner et al.[11,12] developed the complete series of metabolites resulting from the biotransformation of EG. In order of sequence, these metabolites include glycolaldehyde, glycolic acid, and glyoxylic acid. Glyoxylic acid is finally degraded into two minor metabolites, glycine and oxalic acid, and two major metabolites, formic acid and carbon dioxide. It was also observed that the major end-product of EG biotransformation was respiratory carbon dioxide and the main metabolite in the urine was unchanged EG. Renal clearance studies in dogs suggest that glomerular filtration and passive reabsorption are the main mechanisms involved in urinary excretion of EG[39].

There is great variation in the degree of biotransformation of EG in the various species. It was suggested that EG is least toxic to species which are able to oxidise it to carbon dioxide and most toxic to species which primarily excrete oxalate salts and the unchanged glycol in the urine[28].

At low doses (0.1-2.0 G./kg.) of ^{14}C-labelled EG, rabbits and rats excreted 17-28% of the dose as unchanged EG in the urine and about 60% as carbon dioxide in the expired air. At doses of 2.5 and 5 G./kg., nearly 50% of the administered radioactivity was recovered in the urine. However, oxalic acid output was no more than 0.37% of the administered dose. Based on these results, it was suggested that the toxicity of EG may be partly due to the unchanged glycol[12].

For many years oxalic acid was considered the only metabolite responsible for EG toxicity, and there was little doubt that it played a direct role in the nephrotoxicity[10, 23,42]. The amount of EG converted to oxalic acid varies with the dose and the species of animals[12,28]. For man oxalic acid formation has been established to be less than 2%; for rabbits it is no more than 0.37%; in guinea pigs, little or no oxalate is excreted (approximate values are 0 to 0.5%); and in cats 0.7 to 3.7% of the dose is excreted as oxalic acid. The toxicity of EG is higher for cats than for the other species mentioned.

McChesney *et al.*[21] observed glycolic acid as the most important urinary metabolite, after carbon dioxide and unchanged EG, in both monkey and rat. The output of oxalic acid in both species was quite small, approximately 0.3% of

the dose for monkeys and 2.5% for rats. Monkeys excreted 0.1% of the dose as hippuric acid. Glucuronic acid conjugation of EG has not been found to occur in man or in experimental animals[11].

Renal tubular oxalosis followed the administration of glycolaldehyde, glycolic acid, and glyoxylic acid in rats[7], supporting the sequential degradation scheme of EG proposed by Gessner et al.[12]. The toxicity of each of the three compounds used was significantly greater than that of the EG itself.

In a study on the effect of partial hepatectomy on the toxicity of EG and its metabolites in rats, Richardson[32] reported an increased toxicity for glyoxylate and a decreased toxicity for EG and glycolate. Partial hepatectomy also increased the concentration of urinary oxalates from rats fed glyoxylate but not from rats fed EG, glycine, and glycolate. The order for increased oxalate synthesis was glycine, EG, glycolate, and glyoxylate. He suggested that the toxicity of EG and glycolate is due to the formation of metabolic products such as glyoxylate or oxalate.

The subcellular effects of EG and some of its major metabolites have been studied using mitochondria isolated from liver, kidney, and brain of dogs, rats, and monkeys (Maccaca mulatta)[1]. Tissue respiration, oxidative phosphorylation, and citric acid activities are unaffected by

EG, while substrate-level phosphorylation was inhibited at 10 mM and higher concentrations. However, reduced mitochondrial respiration and uncoupling of oxidative phosphorylation were observed with 1 mM or higher concentrations of glyoxylic acid. Glyoxylate at 10 mM or higher concentrations also inhibited citric-acid cycle enzymes (isocitrate and alpha-ketoglutarate dehydrogenase). It was concluded that at low levels of exposure, adverse effects of EG are most likely attributable to the formation of glyoxylate and the interaction of this metabolite with citric-acid intermediates, such as oxaloacetate.

Proposed Treatments

Ethylene glycol poisoning was treated for years as an oxalic acid intoxication[23]. Induction of emesis, gastric lavages, and calcium therapy were primarily used[8,13,30]. However, treatments were almost universally unsuccessful due to the short course of the poisoning and the variable length of time between ingestion and initiation of treatment. In the few attempts at treatment reported in the early veterinary literature, only supportive measures were used and death occurred in all cases[8,15].

When peritoneal dialysis became available, they became the treatments of choice for EG poisoning. These procedures were used to reduce the blood and tissue levels

of EG and its metabolites and also to correct the accompanying severe acidosis. Several reports followed documenting recovery after the ingestion of lethal doses of EG with several days of oliguria and coma[9,10,37].

Intensive studies on the biochemical properties of the liver enzyme alcohol dehydrogenase[12,19,20,40], lead to attempts at a rational competitive antidotal therapy using ethanol, the natural substrate of the enzyme. Peterson *et al.*[29] found that the LD_{50} of EG for rats treated simultaneously with ethanol was almost twice that for untreated rats. In addition, squirrel monkeys (*Saimiri sciurea*) given toxic doses of EG all died, whereas monkeys receiving ethanol within 30 minutes after the same dose of EG survived. The animals given EG and ethanol excreted an average of 75% of the administered EG unchanged in the urine. The animals given EG alone developed oxaluria and excreted less than 10% of the dose as the unoxidized EG.

Wacker *et al.*[42] successfully used ethanol in 2 men that ingested lethal doses of EG and displayed severe toxic manifestations. Both patients also were treated with sodium bicarbonate to correct acidosis and received repeated peritoneal dialysis. It was suggested that successful therapy must be initiated promptly after EG ingestion and preferably within 8 hours after ingestion.

Borden[6] tested the value of an alkalinizing agent, such as sodium bicarbonate, in the treatment of EG toxicity. He showed that ethanol and sodium bicarbonate each individually decreased toxicity in rats. However, the highest survival rate and the least tissue toxicity occurred when ethanol and sodium bicarbonate were given together. Sodium bicarbonate was shown to increase the amount of sodium and citrate excreted in the urine. It also produced urinary alkalosis, thus enhancing the combination of citrate with calcium and reducing the amount of calcium available for combination with oxalate[2,6].

In experiments using 10 dogs receiving 4.4 ml. EG/kg. body weight in a dry commercial dog food, 100% recovery was reported when treatment occurred within 30 minutes after ingestion[2]. One and one-tenth ml. absolute ethanol and 0.44 Gm. sodium bicarbonate/kg. body weight were given intraperitoneally every 6 hours for 6 treatments. All the 10 control animals died.

Complete recovery was reported in 5 dogs that received 11 ml. EG/kg. body weight orally via a dose syringe[24]. Treatment occurred within 1 hour after dosing. A 50% ethanol solution was given intravenously until the dogs were comatosed and could not be aroused by pinching a forelimb digit. The treatment was repeated whenever the dogs responded to

that stimulus. The length of treatment was not mentioned. All 5 control animals died.

In another study[36] dogs were given oral doses of 10, 8, or 6 ml. EG/kg. body weight and were treated with an intravenous dose of 5.5 ml. of 20% ethanol in physiological saline/kg. body weight and 8 ml. of sodium bicarbonate in physiological saline/kg. body weight intraperitoneally. Treatments were repeated every 4 to 6 hours for 40 hours. All the control dogs and dogs receiving 10 ml. EG/kg. and treated 1 or 4 hours post-dosing died. A 60% recovery was observed in dogs receiving 8 ml. EG/kg. and treated 2 hours post-dosing. A 75% recovery was obtained in dogs receiving 6 ml. EG/kg. and treated 4 hours post-dosing. A 50% recovery was observed in dogs receiving 6 ml. EG/kg. and treated 6 hours post-dosing. High dosages of EG and long delays in the initiation of treatment have been suggested as limitations in the successful treatment of EG toxicosis.

Allogenic renal transplant has been used for the treatment of EG poisoning in 1 dog[3]. However, the patient died 11 days postsurgery from a generalized mycotic infection.

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APPENDIX II

Literature Review for the Investigation of Some Therapeutic Measures for Disophenol (D.N.P.) Toxicosis in Dogs.

LITERATURE REVIEW

Chemistry

The generic designation of disophenol is 2,6-diiodo-4-nitrophenol. It has a molecular weight of 391 and is relatively insoluble in water. Addition of sodium hydroxide increases its solubility in aqueous formulations. Solvents, such as a 1:1 water and polyethylene glycol, 0.1 N sodium hydroxide and distilled water, or water-polymethylene, are used for parenteral administration of disophenol[5,7,21].

Uses

Disophenol is an effective systemic anthelminthic used for treatment of hookworm infestation in dogs and cats. It is suitable for dogs of all ages including day-old puppies and pregnant bitches[8,21]. Commercially, it is available as a 4.5% solution* and the recommended dose is 0.1 ml./lb. of body weight (equivalent to 10 mg. disophenol/kg.). Administration of 7.5-10 mg. disophenol/kg. when given by oral, subcutaneous or intramuscular routes was highly effective against Ancylostoma caninum, A. braziliensi, and Uncinaria stenocephala[2,20,21]. Moderate efficacy was also observed against spirocercosis

*D.N.P., American Cyanamid Company, Princeton, N. J.

in dogs[2]. Since, the drug is primarily effective against adult hookworms, a second treatment 2-3 weeks after the initial treatment is recommended for complete elimination of the hookworm infestation. A single oral dose of 3.5 mg. disophenol/kg. body weight, or a daily dose of 3.5 mg. disophenol/kg. body weight in the feed, removed 90-93% of the immature forms of syngamus trachea in turkey poults[1]. In dogs and cats, disophenol has the greatest anthelminthic effect when administered intramuscularly, but it produces transitory pain at the site of injection[5,21].

Toxicity

Toxicosis is not usually observed in dogs at the recommended dose of 10 mg. disophenol/kg. body weight. Single intramuscular or subcutaneous doses of 15-30 mg. disophenol/kg. were also found safe in adult dogs, pups, and pregnant bitches[5,20,21]. However, pups given a subcutaneous dose of 20-30 mg./kg. showed signs of prostration and sleepiness on the day of medication, but none died. These signs were attributed to high ambient temperature and severe anemia of the pups at the time of medication[20]. Signs of acute toxicity were observed in an Irish wolfhound following subcutaneous administration of the recommended dose of 10 mg. disophenol/kg., but the dog responded to treatment. High ambient

temperature (90F, 32C) and vigorous muscular exercise were the probable precipitating factors of acute syndrome in this dog[9].

The lethal dose of disophenol in dogs, when given by single intramuscular or subcutaneous route, was estimated to be 36 mg./kg. body weight[20,21]. However, dosages of 15 mg. disophenol/kg. or higher, administered subcutaneously, caused deaths in dogs after 5-10 hours[18]. The minimal oral lethal dose in dogs was estimated between 100 and 200 mg. disophenol/kg. body weight[21]. The acute intravenous dose in dogs was observed to be 60 mg./kg.[6]. In dogs, disophenol was less toxic than 2,4-dinitrophenol (2,4-DNP), following continuous intravenous infusion and the lethal doses of disophenol and 2,4-DNP were 63.5 mg./kg. and 36 mg./kg. respectively[7]. Disophenol was 50% less toxic than 2,4-DNP on a mg./kg. basis, when given to mice and rats in single doses. However, when, compared on a molecular-weight basis, the LD_{50}'s of the two drugs were similar. In rats and mice disophenol was less toxic orally than when given by other routes. The toxicity of dinitrophenol remained the same irrespective of the route of administration[7].

Subacute toxicosis results from the repeated administration of sublethal doses of disophenol. A 60-day regimen

of 5 mg./kg. orally once daily in 4 dogs produced only a slight retardation in weight gain. No toxicosis was observed when twice the recommended dose of disophenol was given subcutaneously once a week for 8 weeks[21]. One dog, receiving a daily oral dose of 5 mg./kg., had emesis and died after 13 days. This dog was considered hypersensitive[7]. Two dogs receiving a daily oral dose of 9 mg. disophenol/kg. died after 7 and 16 days respectively[21]. Deaths occurred in dogs in 5-6 days on a daily oral dose of 12.5 mg. disophenol/kg.; while another dog on an identical dose of 2,4-DNP survived even after 14 days. Death ensued much earlier on daily administration of higher doses of disophenol. Death due to disophenol results from slower elimination of the compound and toxic buildup in the body[7,21]. Rats, fed a diet containing 0.05% disophenol, showed a decreased food intake, a marked depression of body weight gain, and death within 9 days; another group of rats subsisting on a diet containing an identical percentage of dinitrophenol survived and had a food intake and weight gain similar to control rats[7]. Cats tolerate disophenol well, even though they are known to be very sensitive to phenol compounds[5].

Clinical Signs

Clinical signs of acute toxicity in dogs include: panting; increases in respiration rate, temperature and heart

rate; dehydration; vomiting; hyperemia and edema of visible mucous membranes; and an extremely quick rigor mortis[7,9,18,21]. Similar toxicologic signs have been reported in 2,4-DNP toxicosis[16]. A single oral administration of 50 mg. disophenol/kg. or lethal (36-40 mg. disophenol/kg.) intramuscular doses produced emesis in dogs[21]. Following a continuous intravenous infusion of disophenol at the rate of 0.5 mg./kg./minute, the signs produced included shivering, profuse salivation, increased temperature of the skin and nose, bright red gums and tongue, emesis with greenish-yellow vomitus, finally leading to convulsions and death in about 2 hours. Rectal temperature continued to rise for another 10 minutes after cardiac arrest[7].

Subacute toxicity in dogs, on repeated daily oral administration of 12.5 mg. disophenol/kg. or higher doses, was characterised by elevated heart rate, respiratory rate, and body temperature. A decrease in spontaneous motor activity was observed one to several days prior to death[7].

Signs of acute toxicity in rodents include tremors, prostration, increase in respiratory rate, tonic convulsions, and rigidity of limbs prior to or immediately after death[7].

Transient cataracts of varying severity were observed following administration of disophenol in excess of the recommended dose by several routes. Young pups were

especially susceptible[11]. No significant alterations were observed in the blood picture of dogs with acute intoxication[7, 20]. High packed cell volume, neutrophilia, and lymphopenia were reported in a clinical case of disophenol toxicosis in a dog[9]. Two-fold increases in BUN over predose values were recorded in 2 dogs administered 5 mg. disophenol/kg./day after 35 days of oral dosing. An extremely high bromsulfalein retention value was observed in a dog receiving an oral dose of 25 mg. disophenol/kg./day for 3 days. Electro-cardiogram revealed a diphasic T-wave, T-wave reversal, elevation or depression of S-T segment, and reduction in amplitude of R-wave[7]. Methemoglobin content 4 to 5 hours after disophenol administration was not significantly greater than the control value[20].

Post Mortem Lesions

Congestion and hydropic degeneration of the lungs and liver, and fatty degeneration of the myocardium were reported in dogs that died of acute disophenol toxicosis[18]. In another investigation, significant tissue alterations were not observed, except for the characteristic early onset of rigor mortis[20]. In one dog with high bromsulfalein retention value, the liver was congested and revealed central lobular hemorrhagic necrosis[7].

Mechanism of Toxicosis

Disophenol is rapidly absorbed in dogs by oral and parenteral routes[5,7]. Following oral administration, both disophenol and 2,4-DNP are rapidly absorbed and reach detectable plasma concentrations in 30 minutes. With disophenol, however, a constant increase in plasma concentration was observed. Increase in plasma concentration together with prolonged blood levels indicate the tendency of disophenol to accumulate in the body tissues. When dogs received a daily oral dose of 5 mg. disophenol or 2,4-DNP/kg. for 13 days, plasma concentrations of 102 and 1 microgram/ml. respectively were observed on the 13th day. Urinary excretion of disophenol during the first 24 hours was slight, while 1771 µg. of dinitrophenol was recovered in a 24-hour urine sample. Binding, movement to extravascular sites, or other mechanisms were suggested for this condition[7].

The biotransformation and mechanism of disophenol toxicosis have not been elucidated. Since clinical signs of acute disophenol toxicity resemble those of 2,4-DNP[7], the mechanism by which the latter brings about toxicosis in man and animals may also apply to disophenol toxicity.

2,4-DNP, a by-product in the manufacture of nitrated explosives, increases the basal metabolism when administered to man and animals. At one time the drug was used in the treatment of obesity, but the practice was discouraged because

of the inherent toxicity of this compound[16,19]. Even to date the mechanism underlying the toxicity of dinitrophenol has not been thoroughly explained. Initial studies have shown that 2,4-DNP causes fever by increasing tissue metabolism through a purely peripheral mechanism and that the metabolic increase occurs primarily at the expense of carbohydrates[16]. Further work, however, demonstrated the uncoupling of oxidative phosphorylation in cell-free homogenates[10]. 2,4-DNP does not stimulate the breakdown of the soluble high-energy intermediates of oxidative phosphorylation, but once the intermediates are broken down by other reactions, the coupling enzyme is prevented from reassociation with the electron transport particles[12]. In addition to the uncoupling of oxidative phosphorylation, 2,4-DNP also elicits adenosinetriphosphatase activity of normal hepatic mitochondria, but also inhibits the enzyme in injured mitochondria[19]. With increasing concentrations of 2,4-DNP both respiration rates and glycolysis are stimulated. Although stimulation of rate of respiration implies a stimulation of glycolysis, it has been shown that the primary action of 2,4-DNP is to stimulate glycolysis rather than oxidative metabolism as such[13]. Inhibition of phosphate esterification will cause an equivalent inhibition of energy-requiring processes since energy-rich phosphate is

the chief agent by which energy is transferred from exergonic to endergonic reactions. Energy is released by inefficient pathways, resulting in liberation of much heat, excess oxygen consumption, and fever production[13,19]. Pyrexia, characteristic of acute toxicity, may be due to rapid respiration and dissipation of much of the free energy of the hexose molecule as heat[13]. Dinitrophenol is effective in raising body temperature if the temperature of the environment is 22C or more. High ambient temperatures increased the toxicity of 2,4-DNP manyfold[4,14]. Through carefully planned experiments, Tainter and Cutting[16] have shown that pyrexia was not mediated through: (a) the central nervous system, including the heat regulating centers; (b) neuroreceptors; (c) the adrenal glands; or (d) the thyroid gland. Dinitrophenol exerts a direct action on the cerebrum and lower brain centers, causing stimulation followed by depression[13]. Death in acute 2,4-DNP poisoning may result from: (a) direct circulatory depression; (b) hyperpyrexia; or (c) acidosis and anoxemia[3,16].

Dinitrophenol is temporarily stored in the liver, but is excreted by the kidneys within 2 or 3 days[19]. In dogs, 2,4-DNP is reduced to 2-amino-4-nitrophenol and 4-amino-2-nitrophenol and excreted unchanged or as glucuronide conjugates [15]. Nothing is known regarding the biotransformation of disophenol.

Treatment

Since little is known regarding the biochemical basis of the mechanism of disophenol action, the therapeutic measures to counteract disophenol toxicosis have been limited and primarily symptomatic. Successful recovery has been reported in an Irish wolfhound treated with ethyl alcohol sprays, icepacks, and buffered lactated ringer's solution[9]. Use of oxygen, whole blood transfusions, and a cooling bath have been suggested to prevent death in overdosed dogs[20].

Since clinical and, to a certain extent, pharmacological similarities exist between disophenol and 2,4-DNP toxicosis[7], the antidotal measures employed in the latter case are worth trying for disophenol. Symptomatic measures, such as: (1) administration of fluids to avoid over-concentration of blood and to provide an adequate blood volume for vital organs, and (2) application of cool water to the skin to increase heat loss, have proven of value to antagonise the febrile response and fatality associated with 2,4-DNP toxicosis[3,17]. Central and circulatory stimulants (caffein, digitaloids) in cases of collapse and oxygen inhalation for cyanosis have also been suggested[17,19]. Antipyretics were not effective in reducing the body temperature of 2,4-DNP toxicity[3,17].

Treatment of a pup suspected of dying from methemoglobin formation was unsuccessful using intravenous 1% methylene blue in physiologic salt solution given at 0.44 mg./kg. body weight[20].

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APPENDIX III

Results of Individual Experimental Dogs from the Investigation of Some Therapeutic Measures for Disophenol (D.N.P.) Toxicosis in Dogs

Treatment: None

Disophenol Dose: Varied

Dog No. 1

Breed: Mixed Beagle

Ambient Temperature: 67F (20C)

Body Weight: 22.5 lbs.

Disophenol Dose: 35 mg./kg.

Sex: Male

Survival Time: 25 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutro-phils (%)	Lymph-ocytes (%)	Mono-cytes (%)	Eosin-ophils (%)
0	101.5	--	48	--	68	19	6	7
2	103.3	--	55	--	--	--	--	--
4	103.7	--	--	--	--	--	--	--
6	104.2	--	57	--	--	--	--	--
8	104.2	--	--	--	--	--	--	--
10	103.6	--	--	--	--	--	--	--
14	105.0	--	--	--	--	--	--	--
16	104.4	--	--	--	--	--	--	--
24	106.0	--	56	--	--	--	--	--

Dog No. 2

Breed: Mixed Beagle — Ambient Temperature: 70F (21C)

Body Weight: 21 lbs. — Disophenol Dose: 40 mg./kg.

Sex: Male — Survival Time: 8 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	101.2	--	50.0	--	--	--	--	--
1	101.8	--	--	--	--	--	--	--
2	101.7	--	55.0	--	--	--	--	--
4	104.0	--	52.5	--	--	--	--	--
5	104.8	--	--	--	--	--	--	--
6	104.8	--	52.5	--	--	--	--	--
8	--	--	59.5	--	--	--	--	--

Dog No. 4

Breed: Beagle — Ambient Temperature: 78F (26C)

Body Weight: 26 lbs. — Disophenol Dose: 35 mg./kg.

Sex: Female — Survival Time: 6 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	101.4	15.4	--	9500	65	19	7	9
2	101.5	12.0	50	8200	65	25	6	4
4	103.8	10.8	53	9500	65	26	5	4

Dog No. 5

Breed: Beagle — Ambient Temperature: 78F (26C)

Body Weight: 18 lbs. — Disophenol Dose: 33 mg./kg.

Sex: Male — Survival Time: 4 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.7	14.2	--	16,600	75	16	4	5
2	104.8	11.6	51	17,600	78	15	5	2

Dog. No. 15

Breed: Mixed Beagle — Ambient Temperature: 78F (26C)

Body Weight: 31 lbs. — Disophenol Dose: 33 mg./kg.

Sex: Female — Survival time: Recovered

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.8	17.4	48.0	10,700	75	17	3	5
2	102.1	18.0	52.0	12,700	74	17	4	5
4	103.2	18.6	54.0	8,200	67	19	5	9
6	103.1	19.4	59.0	10,900	80	15	2	3
8	103.7	19.4	60.0	15,600	76	15	3	6
10	103.7	18.6	57.0	8,100	74	19	3	4
12	104.0	17.6	57.0	8,800	76	19	4	1
14	103.4	17.8	53.5	7,000	75	21	4	--
17	103.8	17.6	55.0	10,500	69	23	6	2
21	103.0	17.6	51.0	8,700	71	21	7	1
26	103.0	16.6	47.0	8,600	75	19	4	2
34	102.8	16.2	48.0	7,400	74	20	6	--

Treatment: Dipyrone, 27.6 mg./kg. intravenously, whenever a 2F rise in body temperature occurred, but not more than two injections every 12 hours.

Disophenol Dose: Varied

Dog No. 3

Breed: Beagle

Ambient Temperature: 68F (20C)

Body Weight: 27 lbs.

Disophenol Dose: 33 mg./kg.

Sex: Female

Survival time: Recovered

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	101.2	---	---	---	---	---	---	---
2	101.2	---	---	---	---	---	---	---
4	---	---	---	---	---	---	---	---
6	102.3	---	---	---	---	---	---	---
7	102.6	---	---	---	---	---	---	---
10	102.4	---	---	---	---	---	---	---
12	102.7	---	---	---	---	---	---	---
14	102.3	---	---	---	---	---	---	---
15	102.3	---	---	---	---	---	---	---
18	101.3	---	---	---	---	---	---	---
22	100.8	---	---	---	---	---	---	---
25	101.0	---	---	---	---	---	---	---

Dog No. 6

Breed: Beagle

Ambient Temperature: 78F (26C)

Body Weight: 43 lbs.

Disophenol Dose: 35 mg./kg.

Sex: Male

Survival time: 10 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	101.4	16.2	---	10,800	71	19	7	3
2	101.6	---	---	---	---	---	---	---
4	102.6	13.0	52.5	10,500	61	24	9	6
6	104.2	19.2	53.0	18,000	65	28	7	---
8	105.8	18.6	58.0	20,200	86	11	2	1

Dog No. 14

Breed: Mixed Beagle

Ambient Temperature: 78F (26C)

Body Weight: 30 lbs.

Disophenol Dose: 35 mg./kg.

Sex: Male

Survival time: Recovered

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.6	16.8	50	13,300	57	38	4	1
2	102.0	16.8	46	9,400	56	39	5	---
4.5	102.4	17.4	49	10,700	62	33	5	---
6.5	103.8	17.4	50	14,900	53	40	6	1
8.5	103.4	17.6	50	12,800	56	39	5	---
10.5	103.4	16.4	49	12,700	59	37	4	---
12.5	102.6	16.6	48	12,800	56	38	6	---
14.5	102.6	16.2	47	12,800	54	41	5	---
18	102.8	16.2	47	9,900	59	37	4	---
22	102.0	16.2	47	12,600	67	28	5	
27	102.4	16.6	48	12,400	65	30	4	1
35	103.0	14.8	42	9,400	64	26	8	2

Treatment: Lactated Ringer's infusion, 100 ml. intravenously or subcutaneously, 2 hours after disophenol administered and repeated every 2 hours thereafter.

Disophenol Dose: Varied

Dog No. 7

Breed: Mixed Beagle

Ambient Temperature: 78F (26C)

Body Weight: 19 lbs.

Disophenol Dose: 35 mg./kg.

Sex: Male

Survival Time: 6 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	101.0	12.6	53.0	9,300	43	51	5	1
2	102.2	12.6	54.5	9,700	42	52	3	3
4	102.4	18.4	55.0	12,890	86	9	3	2
6	106.0	---	---	---	---	---	---	---

Dog No. 8

Breed: Mixed Beagle

Ambient Temperature: 68F (20C)

Body Weight: 20 lbs.

Disophenol Dose: 35 mg./kg.

Sex: Male

Survival time: 9 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.2	13.0	54	9,200	68	15	3	14
2	102.6	13.0	55	9,500	69	24	5	2
4	103.4	21.4	60	11,300	76	10	5	9
6	104.0	22.8	61	14,500	90	8	1	1
8	104.5	21.8	62	18,900	93	6	1	---

Treatment: Ice bath for 10-15 minutes, whenever a 2 F rise over the predisophenol rectal temperature was noted.

Disophenol Dose: Varied

Dog No. 9

Breed: Mixed Beagle — Ambient Temperature: 78 F (26 C)

Body Weight: 29 lbs. — Disophenol Dose: 35 mg./kg.

Sex: Female — Survival time: Recovered

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.5	14.6	44	6,950	47	31	5	17
2	102.0	14.8	44	8,160	63	29	6	2
4	103.0	16.5	51	9,150	61	30	6	3
6	103.4	18.1	52	9,800	68	23	3	6
8	103.0	17.6	52	11,500	75	21	3	1
10	104.0	16.6	47	10,460	76	18	6	---
12	103.7	17.0	50	10,880	72	21	7	---
14	103.9	16.6	48	10,565	75	21	4	---
16	104.3	16.8	49	9,150	68	25	6	1
19	103.5	15.2	46	8,200	68	24	6	2
24	104.0	15.8	46	7,175	70	27	1	2
28	103.4	16.6	47	8,100	66	27	5	2
31	103.5	16.2	46	8,400	86	11	3	---
34	103.8	15.2	45	8,470	78	14	6	2
40	104.0	15.2	44	6,400	85	11	4	---
50	102.8	14.6	42	7,700	80	18	2	---
56	102.8	14.2	41	4,450	64	26	8	2
60	102.9	14.2	41	5,060	74	20	6	---

Dog No. 10

Breed: Mixed Beagle

Ambient Temperature: 78 F (26 C)

Body Weight: 15 lbs.

Disophenol Dose: 40 mg./kg.

Sex: Male

Survival time: 7 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.4	13.6	41	12,940	64	24	4	8
2	103.3	16.6	49	13,025	62	30	6	2
4	103.8	16.6	50	16,640	70	18	2	10
6	103.2	15.8	47	24,100	84	13	2	1

Dog No. 11

Breed: Mixed Beagle — Ambient Temperature: 68 F (20 C)

Body Weight: 15 lbs. — Disophenol Doses: 35 mg./kg.

Sex: Female — Survival time: 5.5 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.4	15.3	48	8,950	65	26	7	2
2	101.6	16.2	51	6,500	59	26	4	11
3	102.6	---	---	---	---	---	---	---
4	100.8	16.6	57	5,960	57	34	5	4
5.5	103.0	18.6	58	8,900	65	33	1	1

Dog No. 12

Breed: Mixed Beagle — Ambient Temperature: 78 F (26 C)

Body Weight: 27 lbs. — Disophenol Dose: 35 mg./kg.

Sex: Female — Survival time: 10.5 hours

Hours after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	103.2	14.2	45	4,734	60	27	6	7
2	102.8	15.8	47	4,226	65	23	5	7
3	104.0	---	---	---	---	---	---	---
4	104.1	17.0	54	4,870	86	7	2	5
6	104.6	17.6	55	4,960	83	15	2	---
7	105.4	---	---	---	---	---	---	---
8.5	106.4	16.6	53	8,056	80	17	3	---
9.5	105.2	17	51	9,412	83	14	3	---
10	101.6	---	---	---	---	---	---	---

Dog No. 13

Breed: Mixed Beagle — Ambient Temperature: 68 F (20C)

Body Weight: 32 lbs. — Disophenol Dose: 35 mg./kg.

Sex: Male — Survival time: Recovered

Hour after admin.	Temp. (F)	Hb (%)	PCV	Total WBC	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
0	102.7	16.6	49	11,450	49	37	13	1
2	102.4	---	---	---	---	---	---	---
3	102.6	15.8	48	10,300	41	51	8	---
4.5	102.4	16.6	48	9,620	49	38	11	2
6	102.3	---	---	---	---	---	---	---
7	103.2	16.2	49	11,510	50	35	12	3
8	102.0	16.8	52	---	---	---	---	---
9	102.7	---	---	---	---	---	---	---
10	102.8	16.8	52	13,420	71	22	7	---
12	103.2	16.6	53	15,880	83	6	5	6
13	103.2	---	---	---	---	---	---	---
14	102.6	15	46	18,480	85	13	2	---
16	103.2	16.6	50	14,590	73	23	4	---
17	102.4	---	---	---	---	---	---	---
22	103.2	---	---	---	---	---	---	---
24	102.4	---	---	---	---	---	---	---
36	103.2	---	---	---	---	---	---	---

APPENDIX IV

Literature Review for the Treatment and Prothrombin Responses During Warfarin Toxicosis in Rats and Mice.

LITERATURE REVIEW

Chemistry

Warfarin (3-[alpha-Acetonylbenzyl]-4 hydroxycoumarin) is a colorless, odorless, tasteless compound prepared by the condensation of 4-hydroxycoumarin with benzylacetone. It is freely soluble in acetone, dioxane, moderately soluble in alcohols, and insoluble in water, benzene and cyclohexane. Warfarin has a molecular weight of 308.32 and crystals of warfarin obtained from alcohol have a melting point of 161 C [28,29]. It forms a water-soluble sodium salt on treatment with aqueous solution of sodium hydroxide.

Uses

Warfarin is used as an oral, anticoagulant rodenticide and is one of the most efficient rat poisons presently available. Unlike dicoumarol (a coumarin compound present in improperly cured sweet clover hay and also employed in rat control), warfarin does not produce acquired bait refusal. Commercial rat baits are prepared with cereal grains and contain approximately 0.025% warfarin by weight. Under field or laboratory conditions, warfarin bait kills the common rat in 5-8 days[10,20,27,28]. Sodium warfarin is frequently employed as a therapeutic anticoagulant in man[4,18,19].

Toxicity

Single doses of warfarin, unless very large, do not exert a lethal action. If repeatedly ingested in small amounts, however, warfarin may prove fatal. Rats withstand single oral doses of 50 mg. warfarin/kg. of body weight, but are killed by repeated dosing for 5 successive days with 1 mg./kg. body weight. Deaths were also observed in rats on consumption of a total of 2.87 mg./kg. over a 14-day period. However, warfarin was found slightly less effective when given on alternate days[20,28]. Single intraperitoneal doses of up to 100 mg. warfarin/kg. produced only 20% mortality; however, 80% mortality occurred following intraperitoneal administration of 2.5 mg./kg. over a 7-day period. Similar results occurred when warfarin was administered by oral route[36].

Warfarin poisoning is a common clinical problem in veterinary practice[2]. Episodes of acute poisoning have been reported in dogs, cats and pigs due to either accidental or malicious feeding of warfarin-containing rodent-bait[6,8,24,35,41,43]. Secondary poisoning may occur if dogs or cats eat several warfarin-poisoned rats over a period of 4-10 days[10,20,27,28]. Deaths from secondary poisoning were observed in mink and mongrel dogs on feeding meat from warfarin-killed nutria[14]. In another study, doses of 0.2 mg.

warfarin/kg./day was found lethal in dogs; however, dogs were found unaffected by levels of 15 mg. warfarin/day (2.5mg./kg./day) when this was supplied as mice which have eaten warfarin bait[32].

The toxicity levels of warfarin for dogs are variable, but daily doses of 4 mg. or higher/kg. for 4 days are fatal. Some dogs survived doses of 25mg./kg./day for 4 days[28]. Doses of 1 mg./kg. have been reported to kill cats and dogs if repeated daily for about one week[27]. Poultry and calves appear very resistant to the quantities likely to be consumed accidentally. Sheep and cattle withstand single doses of up to 50 mg./kg. body weight. Administration of 50 mg./kg./day for 10 days to a calf did not produce any clinical signs other than delayed clotting time; it was not until the dose rate was increased to 200 mg./kg. on the 11th day and continued for another 12 days that the animal became ill[27,28]. However, following ingestion of a daily dose of 17.6 gm. warfarin-containing rodent bait (equivalent to 40-120 mg. warfarin/cow), 8 of 23 pregnant cattle aborted during the following 2 days. A 20% decrease in normal prothrombin value was observed on daily oral dosing of 0.25 mg. warfarin/kg. for 10 days[33]. Pigs are susceptible to warfarin poisoning and deaths occurred by repeated doses of as little as 0.4 mg./kg./day for 6 days. Castration resulted in extensive

hemorrhage and deaths of piglets that consumed a total of approximately 30 mg. warfarin[8,27,28]. Poultry appear to be resistant to repeated doses of as much as 10 mg./kg. body weight[27]. The threshhold limit value of warfarin dust in the air is 0.1 mg. per cu.M. and the estimated oral lethal dose in man is 0.5 G.[39].

Clinical Findings

Since warfarin poisoning can develop following ingestion of small quantities of the compound over a period of days, the sudden onset of clinical signs (dyspnea, severe anemia and hemoptysis) may not be readily associated with warfarin toxicosis. Prolonged prothrombin times and the existence of certain other clinical signs will aid in a definite diagnosis.

Clinical signs of warfarin poisoning commonly observed in all animals include: pallor of the mucous membranes; weakness; subcutaneous hemorrhages and/or swelling due to hematomata visible externally, especially on the appendages; oral or rectal bleeding; loss of body weight, especially in animals with longer survival times; dehydration; severe anemia; and lameness due to hemorrhages over long prominences of the limbs or in the joints. More commonly the hematomata develop in those areas subject to bruising during

handling or fighting. Some animals may die without any premonitory signs[20,27,28,34]. In dogs, radiological examination revealed evidence of massive hemorrhage into the lung Parenchyma and pleural cavity[41]. Very large doses of warfarin may cause rapid vasodilation, with a consequent drop in blood pressure. This vascular collapse may prove fatal[10].

Abnormal changes were not detected in the liver function (bilirubin, alkaline phosphatase, SGOT, and SGPT) in warfarin-poisoned dogs. A rise in blood urea nitrogen was observed at the point of maximum poisoning and was probably due to dehydration[16].

Post Mortem Lesions

Animals dying acutely without any premonitory signs show acute internal hemorrhage at necropsy. The typical lesions of warfarin poisoning include: multiple hemorrhages throughout the body musculature, particularly the bony prominences of the limbs and ribs; subcutaneous hemorrhages; and extensive internal hemorrhages with considerable quantities of unclotted blood in the chest and abdominal cavities. Diarrhea is frequently present and may be bloody. In cattle, hemorrhages were noticed in the wall of rumen, omasum, wall of the large intestine, and on the surface of liver. In some dogs, fluid accumulation in the pleural cavity and hemorrhages in the meninges, and cerebral, inguinal and femoral vessels

were noticed. Less frequent are hemorrhages of the genital organs, pelvic and lumbar region, and small circumscribed hemorrhages of the lungs[20,27,28,34].

Histological examination of the kidneys of dogs did not reveal any significant changes that can be attributable to the toxic action of warfarin[16]. Ultrastructural studies of capillaries from warfarin-treated rats showed loss of ground substance of the endothelial cells[23]. In rabbits administered warfarin from early pregnancy until term, all of the fetuses were still-born with widespread hemorrhages[21]. Central liver necrosis has been reported in rats given large doses of dicumarol[22].

Absorption, Biotransformation, and Excretion

Warfarin is completely absorbed by passive diffusion in the upper gastrointestinal tract and reaches peak plasma concentration in 3-9 hours in man[31,44]. All courmarin compounds are highly lipophilic and since they exist in an unionized state in the stomach (PKa 4.8-5.8), they are rapidly absorbed[44]. Because of its more predictable absorption from gastrointestinal tract, warfarin is preferred over dicoumarol as a therapeutic anticoagulant in man. Rapid and complete absorption of warfarin takes place following its administration by the parenteral routes as well[4,42].

Warfarin can also be absorbed by the skin in toxic amounts. It was also shown to cross the maternal placenta in rabbits and was also secreted in milk[21,39]. Following oral administration in rats, it reaches detectable liver concentrations within 24 hours[17]. Oral administration of vitamin K_1 (the physiological antidote for warfarin toxicosis) does not influence the rate of warfarin absorption or its biotransformation[31].

Within the circulation, warfarin is almost entirely (98%) bound to serum albumin and has a half-life of about 40 hours in man[31,39]. The high percentage of binding accounts in part for the slow rate of degradation and for the negligible renal excretion of warfarin. Warfarin and other coumarin anticoagulants accumulate primarily in lungs, liver, spleen and kidney. Appreciable amounts are found in erythrocytes, but none is found in cerebrospinal fluid. Drugs (such as acetylsalicylic acid, phenylbutazone, and widespectrum antibiotics) compete for a binding site on serum albumin and tend to potentiate the effect of warfarin[13,39,44].

Warfarin is hydroxylated to inactive compounds by enzymes of the endoplasmic reticulum. In man, the drug appears in urine almost entirely as metabolites and virtually no unchanged urinary warfarin could be detected[19,26,31]. In studies involving the metabolism of 4-^{14}C labelled

warfarin sodium in rats, the metabolites detected in urine and feces were: unchanged warfarin (6.6% of the urinary radioactivity); several hydroxylated warfarin derivatives, 7-hydroxywarfarin (35%), 4'-hyroxywarfarin (21%), 6-hydroxywarfarin (15.4%), 8-hydroxywarfarin (8.9%), and a glucuronide of 7-hydroxywarfarin (3.9%); and an intramolecular condensation product, 2,3-dihydro-2-methyl-4-phenyl-5-oxopyrano (3,C-C)(1) benzopyran (6.6%). Urinary and fecal excretion accounted for 2/3 and 1/3 respectively of the total dose. Biliary excretion of the metabolites, followed by partial reabsorption and excretion via urine, was assumed to explain similarities in urinary and fecal metabolites[3]. Of these metabolites, 4'-hydroxycoumarin is an active metabolite, being one-fourth as potent as the parent compound. This warfarin metabolite has not been identified in man[44].

Because of the high degree of albumin binding, glomerular filtration of warfarin is minimal. Furthermore, any coumarin filtered is largely unionized at the usual urinary pH range and is passively reabsorbed during concentration of the filtrate in the renal tubules. No active transport of the coumarins has been demonstrated[44]. Compounds which induce the metabolic hepatic enzymes (such as alcohol and barbiturates) cause a more rapid biotransformation and excretion of warfarin[11,37,39]. In chronic alcoholics,

the half-life of warfarin is 25 hours compared to the normal value of 40 hours[39].

Mechanism of Action

Warfarin and other coumarin anticoagulants primarily effect the blood coagulation mechanism. They inhibit hepatic synthesis of vitamin K dependent plasma clotting factors--II (prothrombin), VII, IX and X. They effect the rate of synthesis of the prothrombin complex, but do not alter the catabolism of the clotting factors. Single doses of warfarin larger than those required to stop clotting factor synthesis will not speed the development of hypoprothrombinemia, but will merely prolong its duration[12,13,16,18,19,31,38,42].

The nature of interaction between vitamin K and warfarin is not definitively established. The coumarin anticoagulants are thought to decrease the transport of vitamin K to its site of action rather than to inhibit protein synthetic mechanisms. It was earlier assumed that warfarin acts through competitive inhibition with vitamin K for a specific site on a regulator protein[12,19]. Subsequent studies, however, suggested that the site of action of warfarin may be an enzyme regulating the metabolism of vitamin K rather than a binding site for the vitamin[12]. It was observed that in the presence of dicoumarol there is a specific failure to incorporate carbohydrate into prothrombin[30]. Recent studies in

rats suggested that warfarin inhibits prothrombin synthesis by increasing the ratio of phylloquinone oxide (a metabolite of vitamin K_1 which also acts as an inhibitor of vitamin K_1) to vitamin K_1 in the liver[5].

Prolonged prothrombin times and increased capillary fragility have been assumed to produce fatal hemorrhage under the stresses of normal life[27,34]. Direct toxicity or indirect effects via a reaction involving vitamin K have also been suggested as possibilities for warfarin mechanism of action[16,23]. Thyrotoxicosis was observed to enhance the warfarin-induced hypoprothrombinemia in man[40].

Proposed Treatments

The treatment of hemorrhage associated with warfarin poisoning first involves immediate withdrawal of the drug. In acute single dose consumption of warfarin, gastric lavage has been suggested to remove the unabsorbed warfarin from gastrointestinal tract[18,19].

Administration of vitamin K is considered the physiological antidote for warfarin toxicosis. Administration of vitamin K_1 (the naturally-occurring vitamin K) is suggested as the only therapeutic choice and synthetic vitamin K analogues, such as menadione and menadione sodium bisulfite, are said to be of little value in combating the excessive anticoagulant-induced hypoprothrombinemia[1,9,15,16,43]. Immediate

intravenous administration of 10-25 mg. of vitamin K_1 emulsion twice daily has been recommended in man for a rapid return of prothrombin to safe levels[18,42]. Intravenous administration of 100 mg. vitamin K_1 was observed to rapidly reverse the action of warfarin in dogs[16]. Intraperitoneal administration of 5 mg. vitamin K_1/kg./day offered some protection in rats against low daily doses of warfarin, but when warfarin was administered at 5 mg./kg./day, no further protection was observed with vitamin K_1, even though the daily dose of the latter compound was raised to 10 mg./kg./day[36].

Several authors suggested the use of synthetic vitamin K analogues in counteracting warfarin toxicosis[7,10]. Hysterectomy, intramuscular administration of menaphthone, and withdrawal from warfarin bait have also been indicated as probable factors responsible for the successful recovery of a warfarin-poisoned Collie bitch[6].

Transfusion of fresh whole blood as a temporary source of prothrombin and red blood cells has been advocated. Reconstituted lyophilised plasma is also effective, but stored plasma should not be used[18,19]. Successful recovery has been reported in dogs treated with 20 ml. of citrated whole blood/kg. body weight[25]. However, on infusion of 300 ml. fresh dog plasma, no significant prothrombin response was observed when given to treat warfarin poisoning in a dog[16].

Klausman and Brown[24] reported successful treatment of 47 dogs with 2 ml. of Koagamin (a commercial coagulant solution containing 5% oxalic and malonic acids) intravenously or 2 ml. Koagamin in 200-300 ml. glucose saline solution intravenously and vitamin K intramuscularly. However, the type of vitamin K employed in therapy was not indicated.

Walker[41] suggested the importance of chest radiographs in dogs to assess the extent of pulmonary involvement, in addition to correcting the anemia and disordered blood coagulation. When hemothorax is present, he recommended immediate aspiration of the blood to allow lung expansion and to prevent the dangers of clotted hemothorax and permanent lung collapse.

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EVALUATION OF RECOMMENDED TREATMENTS FOR THREE COMMON MAMMALIAN TOXICOSES

by

LAKSHMIPATY PENUMARTHY

B.V.Sc. A.P. Agricultural University,

India, 1967

AN ABSTRACT OF A MASTER'S THESIS

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Three common compounds causing poisonings in small animals are: (1) ethylene glycol (EG), used as antifreeze in automobiles; (2) disophenol, an anthelminthic for canine hookworms; and (3) warfarin, a rodenticide. The present study was undertaken to investigate new treatment procedures and the efficacy of reported treatment recommendations for these three intoxications.

Treatment of EG poisoning was studied in 27 cats dosed with 4 ml., 6 ml., or 8 ml. EG/kg. body weight. In each dosage group 2 cats served as controls, 3 cats received initial treatment 4 hours after dosing, and 4 cats received first treatment 8 hours after dosing with EG. Treatment consisted of 5 ml. 20% ethanol solution and 6 ml. 5% sodium bicarbonate solution/kg. body weight intraperitoneally. Treatment was repeated every 6 or 8 hours for a total period of 56 hours. All control cats and cats receiving treatment 8 hours after dosing with 6 ml. or 8 ml. EG died. One of 4 cats survived in the 4 ml. EG group that received initial treatment only 8 hours after dosing. Two of 3 cats recovered in each of the 4 ml. and 6 ml. groups treated 4 hours after dosing, while only 1 of 3 recovered in the 8 ml. group. The recovery rate suggests the effectiveness of the proposed treatment.

Disophenol toxicosis was investigated in 15 dogs receiving 33, 35, or 40 mg. disophenol/kg. (15, 16, or 18 mg.

disophenol/lb., respectively) body weight and treatment with an antipyretic, lactated ringer's infusion, or ice bath. Rectal temperatures and estimations of percent hemoglobin, packed cell volume, total white blood cell count, and differential white blood cell count were monitored. Higher hemogram values were observed for dogs dying of acute toxicosis. One of 5 dogs receiving no treatment recovered. Two of 3 dogs treated with the antipyretic recovered, 0 of 2 dogs receiving lactated ringer's infusion recovered, and 2 of 5 dogs treated with ice baths recovered.

Effects of warfarin feeding and blood or vitamin K_3 treatment was investigated in rats and mice. Prolonged prothrombin times occurred after 9-12 hours of warfarin feeding. Irrespective of treatment, prothrombin times greater than 300 seconds were consistently observed with continuous warfarin feeding. Withdrawal of warfarin resulted in normal prothrombin times after an additional 96, 48-72, or 48 hours in mice receiving no treatment, whole blood, or 72 mg. vitamin K_3/kg./day, respectively. Mortality was consistently higher in mice consuming warfarin continually and in those receiving a large number of treatments. Frequent handling seemed to aggravate the toxicosis.