

EXPERIMENTAL DIAMIDINE POISONING DUE TO COMMONLY USED BABECIDES*

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ABSTRACT

NAUDÉ, T. W., BASSON, P. A. & PIENAAR, J. G. Experimental diamidine poisoning due to commonly used babecides. *Onderstepoort J. vet. Res.* 37 (3), 173-184 (1970).

Forty-four apparently healthy dogs and four normal cattle were given multiple therapeutic, sterilizing or higher dosages of Berenil, Phenamidine and quinoronium sulphate in order to determine the toxic effects of these drugs. The former two drugs produced severe nervous symptoms such as imbalance, rolling movements, extensor rigidity, opisthotonus, nystagmus and terminal paralysis in the majority of the dogs. Prominent haemorrhagic and malacic lesions were encountered in the brain, mainly in the cerebellum, midbrain and thalamus. Two repeated daily doses of 20 mg/kg of Phenamidine caused brain lesions in one dog after a latent period of 2 days. Only mild nervous symptoms were produced in the cattle but none of the brain lesions found in the dogs were noticed. Degenerative lesions, mainly of a fatty nature, occurred in the liver, kidneys, myocardium and skeletal muscles of some of the dogs and cattle, being more prominent in the latter species. None of the animals which received quinoronium sulphate showed similar nervous symptoms or succumbed. The results of the experiments therefore clearly indicate that the diamidines are potentially harmful drugs and that their therapeutic dosages should never be exceeded or repeated in the course of treatment of a disease.

INTRODUCTION

In about 1940, during a specific search for compounds with a trypanocidal action, a group of drugs known as the diamidines was developed. Some of them were also found to be very effective against *Babesia* spp. A new era in the treatment of babesiosis was thus ushered in with the introduction of certain members of this group such as Phenamidine + some 25 years ago and more recently Berenil ++ as apparently very safe babecides with only slight side-effects in comparison with the conventional quinoronium sulphate compounds and dyes.

The diamidines have trypanocidal, babecidal, anti-bacterial and antifungal properties but are mainly used, however, for the first two actions. Their structural formulae are given in Fig. 1.

Formula A (Fig. 1) is that of Phenamidine [di-(p-amidinophenyl) ether], schematically presented as A I. Berenil [di-(p-amidinophenyl) triazene-(N-1:3)] is represented by A II (Fig. 1). These two are the commonly used babecides and trypanocides. Propamidine + (4,4'-diamidino phenoxy propane) and its di-bromo derivative, presented as A III, are occasionally used in veterinary medicine. Pentamidine + (A-IV) is used in the treatment of trypanosomiasis and leishmaniasis in man. For comparative purposes the structure of quinoronium sulphate (Babesan + + +, Pirevan + + + + or Acaprin + + + + +) is shown as B in Fig. 1.

Several findings and events led to the present toxicity studies of the diamidines, which are commonly used in South Africa for the treatment of dogs infected with *Babesia canis* (Piana & Galli-Valerio, 1895) and also of babesiosis in cattle and horses.

Since 1967, 21 cases of suspected cerebral babesiosis and encephalitis in dogs have been submitted to the Pathology Section at Onderstepoort. These cases had

received either single or repeated doses of diamidines and death was in some cases preceded by an apparent period of recovery. Severe necrosis and haemorrhages were found in the cerebellum and basal ganglia of these

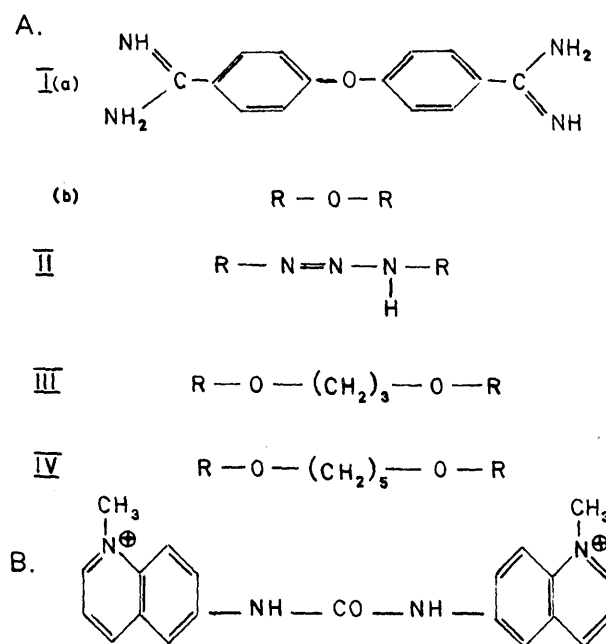


FIG. 1 Structural Formulae

- A. *The Diamidines*
 I (a) Phenamidine
 (b) Phenamidine — schematic presentation
 II Di(p-amidinophenyl) triazene — schematic presentation
 III Propamidine — schematic presentation
 IV Pentamidine — schematic presentation
- B. *Quinoronium sulphate*

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animals (Pienaar & Basson, Veterinary Research Institute, Onderstepoort, unpublished observations, 1969). The macro- and microscopic lesions in the brains closely resembled those described by Basson & Pienaar (1965) in cerebral babesiosis in dogs, the only exception being the complete or almost complete absence of parasites. In a paper presented at the 1968 Conference of the Rhodesian Veterinary Association Boyt, Lawrence & McKenzie described brain lesions in dogs as a sequel to repeated use of both Berenil and Phenamidine for their babesicidal and trypanocidal effects.

In discussing the harmful effects of these drugs veterinary textbooks rather vaguely refer mainly to liver damage. The well-known acute side-effects of vomiting and diarrhoea, histamine-like reaction and a drop in blood pressure, which accompany parenteral administration, are well documented. The effect on the central nervous system (CNS), however, has been indicated only by a few investigators.

In the medical textbook by Goodman & Gilman (1965) the following passage appears: "The diamidines have prominent effects on the CNS of dogs but not of other animals. Maximum tolerated doses produce vomiting, defaecation and tremors followed in some cases by footdrop, spastic paresis, decerebrate type of rigidity, mania and coma. Chronic administration of doses within the therapeutic range (0.8 to 2.0 mg/kg) produces pathological changes in the brain and meninges". No specific reference was given, and in an extensive study of the available literature on Phenamidine no specific reference to brain damage in dogs was found. Lourie & Yorke (1939), when investigating the trypanocidal action of certain aromatic diamidines, found that with large doses, bordering on the lethal, the acute immediate effects in dogs were often accompanied by collapse. If the animal recovered from the immediate effects a peculiar spastic paresis, which invariably ended fatally, developed 3 to 4 days later. Greer & Banks (1950) and Koutz (1952) reported nervous symptoms in dogs when repeated doses of Phenamidine were employed in attempted treatment of demodectic mange in dogs. However, no gross or microscopic lesions were described in the CNS in any of the above-mentioned reports. In cattle, delayed toxicity with extensive liver damage was reported by Randall & Laws (1947).

Similarly, in the case of Berenil, Fussgänger & Bauer (1958) mentioned the fact that a dose of more than 10 mg/kg could in some cases cause toxic disturbance of the CNS in dogs. Again no description of any pathology in the CNS was given.

MATERIALS AND METHODS

Experimental animals

In pilot experiments marked individual variations in susceptibility were encountered. It was, therefore, decided to use three male dogs and three bitches for each of the various dosage levels in the main experiment. Due to difficulties in obtaining suitable experimental dogs a rather heterogeneous group of animals was used. The ages varied from 6 months to 7 years and the breed from purebred Alsatians to nondescript mongrels. As far as could be ascertained none of these animals had been treated with diamidines for a period of at least 6 months before the commencement of the experiment. The cattle consisted of two 18-month old Afrikaner oxen (Ox 2 and 4), one 10-month old Jersey

ox (Ox 1) and one 4-month old Jersey-Friesland cross (Ox 3). The shortage of experimental animals necessitated the use of certain dogs in more than one experiment.

Drugs

The drugs employed were the following commercially obtained products:

(1) *Phenamidine isethionate* (Tables 1, 2, 3 and 7): Phenamidine, 5 per cent (w/v), mainly of Batches 81 and 71, and 40 per cent (w/v) of Batch 75 were used in the dogs and cattle respectively. The drug was administered subcutaneously at different sites anterior and posterior to the scapula on both sides, in such a sequence that the injections were repeated on approximately the same site only every fourth time.

The following dosages were administered to various groups of animals:—

Group 1: Dogs 1 to 6: Multiple doses of 15 mg/kg/day

Repeated daily doses of the recommended therapeutic dosage (15 mg/kg) were given to each of three males and three females until nervous symptoms appeared.

Group 2: Dogs 7 to 12: Multiple doses of 20 mg/kg/day

Repeated daily doses of 20 mg/kg were administered to each of three males and three females until nervous symptoms appeared. According to Malherbe (Faculty of Veterinary Science, Onderstepoort, personal communication, 1970) 20 mg/kg is the optimal effective dosage for the prevention of the development of Phenamidine-resistant strains of *B. canis*. This dosage has been used at the Onderstepoort clinic for the past 25 years.

Group 3: Dogs 13 and 14: Multiple doses of 20 mg/kg/day combined with thiamine therapy

Two bitches were each given 10 times the recommended therapeutic dosage (10 mg/kg) of thiamine hydrochloride + by intramuscular route, followed the next day and on subsequent days by 20 mg Phenamidine/kg until the development of nervous symptoms. Thiamine was tried in conjunction with Phenamidine because of the similarity of the brain lesions in Phenamidine poisoning to those of thiamine deficiency. The latter occurs in carnivores which are fed raw fish that contains thiaminases (Edwin, Lewis & Allcroft, 1968). Cerebrocortical necrosis has also been produced in pre-ruminant and ruminant lambs and pre-ruminant calves by the feeding of massive doses of amprolium, which appears to be a competitive inhibitor of thiamine (Edwin *et al.*, 1968).

Group 4: Dogs 15 to 24: 20 mg/kg/day for 2 days

Because of the frequent practice of treating dogs suffering from babesiosis twice with Phenamidine, five dogs and five bitches were given single doses of 20 mg/kg on 2 successive days.

Group 5: Dogs 16 to 25: 20 mg/kg followed by 3.5 mg/kg Berenil

Because of the shortage of experimental animals Dogs 16 to 24 from Group 4 which did not develop any symptoms, were rested for 9 weeks and subsequently with Dog 25 used for two successive daily administrations of 20 mg/kg Phenamidine and 3.5 mg/kg Berenil. The latter was given intramuscularly.

Group 6: Dog 26: 50 mg/kg

One dog was given a single dose of 50 mg/kg.

+Propan Pharmaceuticals, Germiston

Group 7: Cattle: 12 mg/kg/day and 24 mg/kg/day

Ox 1 received 12 mg/kg/day for 5 days and Ox 2 24 mg/kg/day for 2 days, while Ox 3 received 24 mg/kg for 3 days, followed by an interval of 7 days before the administration of two additional similar doses on successive days.

(2) *Berenil* (Tables 4, 5 and 7): Berenil soluble powder, Batch 134U062, was used. The solutions were prepared according to the manufacturer's instructions and stored in a refrigerator at 4°C. Fresh solutions were prepared every 7 days. It was administered intramuscularly in both front and hind limbs of both sides, following the same sequence of administration as described above for Phenamidine. The following dosages were used:

Group 8: Dogs 16 to 24: Recommended therapeutic dosage (3.5 mg/kg) on 2 successive days

Nine of the dogs from Group 5, which did not show any symptoms, were rested for 12 days before they were given 3.5 mg/kg/day on 2 successive days.

Group 9: Dogs 27 to 32: Multiple doses of 3.5 mg/kg/day

Three dogs and three bitches were given repeated daily doses of 3.5 mg/kg until nervous symptoms developed. Dog 32 which did not react was re-used in Group 10.

Group 10: Dogs 32 to 37: Multiple doses of 10.5 mg/kg/day

The recommended sterilizing dosage for babesiosis, 10.5 mg/kg/day, was administered repeatedly to three dogs and three bitches until nervous symptoms developed.

Group 11: Ox 4: Multiple doses of 7 mg/kg/day
Ox 4 received 7 mg/kg daily for 15 days.

(3) *Quinoronium sulphate* (Table 6): For comparative purposes this non-diamidine babicide was repeatedly administered at therapeutic (0.25 mg/kg) and four times therapeutic (1.0 mg/kg) levels. Babesan, 0.5 per cent (w/v), Batch HP 25 SA, as well as a 0.5 per cent (w/v) solution prepared weekly from Acaprin 5 per cent (w/v), Batch FF22, were used. They were stored in a refrigerator at 4°C and administered subcutaneously following the same method as described for Phenamidine.

Group 12: Dogs 38 to 44: Multiple doses of 0.25 mg/kg/day and 1.0 mg/kg/day

Repeated doses of 0.25 mg/kg or 1.0 mg/kg were administered for periods varying from 5 to 15 days.

Chemical-pathological tests

Chemical pathological tests using standard methods were done on the blood of all animals before the administration of the various drugs in order to obtain normal values. These were repeated periodically on blood obtained during the course of the experiment and just before death or euthanasia. The tests included determinations for sedimentation rate, haemoglobin percentage and serum glutamic oxalacetic and pyruvic transaminases (GOT and GPT), total bilirubin, blood urea nitrogen (BUN), blood glucose, total plasma protein, calcium, sodium, potassium and bicarbonate. All animals were examined clinically every day.

Euthanasia was performed on some of the animals in order to prevent unnecessary suffering by giving an overdose of pentobarbitone (Euthatal⁺) intravenously. In Tables 1 to 3 this is indicated by an asterisk next to

the figure in the column "Day of death". Autopsies were done on all the animals that died naturally as well as those that were destroyed. The entire brain and various tissue specimens were collected and placed in 10 per cent buffered formalin. After fixation, serial coronal or sagittal sections of the brain, approximately 3 mm in thickness, were prepared and studied for lesions. Suitable areas from these slices and blocks of other tissues were cut and embedded in paraffin wax. Sections, 3 μ in thickness, were cut and stained with haematoxylin and eosin. Frozen sections of the livers, kidneys, myocardium and muscles were stained with oil red O (ORO).

RESULTS

A. Dogs

(1) *Phenamidine*

The results of these experiments are summarized in Tables 1, 2 and 3.

Symptoms: The well-known acute symptoms of nausea, vomiting and depression occurred within 30 to 60 minutes after injection and became progressively more pronounced and of longer duration with successive administrations, irrespective of the dosage rate. This led to severe anorexia and, in those cases which lived for more than approximately 7 days, secondary complications like dehydration and loss in weight were consequently encountered. The membrana nictitans became prolapsed and became fixed over the eyeball, even covering the pupil in severe cases. Initially this condition reverted to normal some time after injection but terminally it persisted until death. In several instances severe cardiac arrhythmia was encountered. The effect on the CNS was manifested by a variety of symptoms which occurred singly, in combination with others or in sequence, the one syndrome being followed by another. These nervous symptoms may be summarized as follows:

(a) *Behavioural changes:* These included mild to severe depression and stupor, altered facial expression, apparent deafness, disturbed vision, excessive barking for no particular reason (hallucination), continuous whining and, exceptionally, even severe hypersensitivity and convulsions on stimulation.

(b) *Imbalance:* This varied from slight ataxia to complete loss of balance and even compulsive, continuous rolling or circling movements, which occasionally resulted in self-inflicted trauma. Paresis and paralysis were often seen terminally.

(c) *Spasticity:* The degree of extensor rigidity of the limbs varied from extended front limbs, which could still be bent easily, to severe rigid, simultaneous extension of all four legs and even complete opisthotonus reminiscent of tetanus or decerebrate rigidity.

(d) *Nystagmus:* Both horizontal and vertical nystagmus were often encountered, especially terminally.

(e) *Coma:* Several dogs became comatose after the preceding nervous symptoms. Dog 5 showed no symptoms at all and was found comatose shortly after the seventh administration of the drug.

Chemical pathology: Moderate to severe increases in GOT, GPT and BUN were encountered in those cases that survived for several days. Values of up to 100 King units per ml were regarded as normal for both GOT and GPT and values between 100 to 150, 151 to 200 and more than 200 units per ml, are indicated in the

⁺May & Baker Ltd., England

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TABLE 1 Dogs: Group 1: 15 mg/kg/day Phenamidine

No.	Sex	No. of Doses	Symptoms					Day of death	Chem. path.			Post mortem: lesions					
			Behav.	Imbal.	Spast.	Nyst.	Coma		GOT	GPT	BUN	Brain	Liver	Kidney	Lung	Myocard.	Muscle
1	♂	6		+				8	-	+	+	+	-	-	-	-	-
2	♂	8		?				17*	+	+++	+++	+	-	Nephritis	-	-	-
3	♂	13	?	?				18*	-	+++	+++	-	++	Nephritis	-	-	-
4	♀	6		+				7	+	+	-	+	-	Nephritis±	-	-	-
5	♀	7						8	-	++	-	+	?	-	-	-	-
6	♀	9	+		+		+	10*	++	+++	+++	-	++	+	-	-	+

* indicates euthanasia
 Behav. = Behavioural changes
 Imbal. = Imbalance
 Spast. = Spasticity
 Nyst. = Nystagmus

Chem. path. = Chemical Pathology
 Myocard. = Myocardium
 +, ++, +++ = increasing order of severity
 Only oedema was recorded as lung lesion

TABLE 2 Dogs: Group 2: 20 mg/kg/day Phenamidine

No.	Sex	No. of Doses	Symptoms					Day of death	Chem. path.			Post mortem: lesions					
			Behav.	Imbal.	Spast.	Nyst.	Coma		GOT	GPT	BUN	Brain	Liver	Kidney	Lung	Myocard.	Muscle
7	♂	3	+					4	-	-	-	+	-	+	±	-	-
8	♂	4	+		+			4	-	-	-	+	-	-	+	-	-
9	♂	5		+	+	+		7	-	+	-	+	-	-	-	+	-
10	♀	3		+	+	+		3	-	-	-	+	-	?	-	-	-
11	♀	3						4				+	-	-	-	-	-
12	♀	3						4				+	-	-	-	-	-

TABLE 3 Dogs: Groups 3 to 6: Phenamidine, Phenamidine-Thiamine, Phenamidine-Berenil

No.	Sex	No. of doses	Symptoms					Day of death	Chem. path.			Post mortem: lesions						
			Behav.	Imbal.	Spast.	Nyst.	Coma		GOT	GPT	BUN	Brain	Liver	Kidney	Lung	Myocard.	Muscle	
Group 3: 20 mg/kg/day + 10 mg Thiamine/kg/day																		
13	♂	4						6*	-	-	-	+	+	Nephritis±	-	+	-	
14	♀	7		+			+	11*	-	++	+++	-	++	Nephritis	-	-	-	
Group 4: 20 mg/kg Twice																		
15	♂	2	+	+	+	+		6*	-	±	-	+	-	Nephritis±	-	-	++	
16-19	♂	2																
20-24	♀	2																
Group 5: 20 mg/kg + 3.5 mg/kg Berenil																		
16-19	♂	1																
20-24	♂	1																
25	♂	1																
Group 6: 50 mg/kg																		
26	♂	1	+	+	+	+		4*	-	+++	+++	+	?	+	-	+	-	

TABLE 4 Dogs: Groups 8 and 9: 3.5 mg/kg/day Berenil

No.	Sex	No. of doses	Symptoms					Day of death	Chem. path.			Post mortem: lesions						
			Behav.	Imbal.	Spast.	Nyst.	Coma		GOT	GPT	BUN	Brain	Liver	Kidney	Lung	Myocard.	Muscle	
Group 8																		
16-19	♂	2																
25	♂	2																
20-24	♀	2																
Group 9																		
27	♂	6		+	+	+		8*	-	++	-	+	-	-	-	-	-	
28	♂	7		+	+		+	10*	-	-	-	+	?	-	-	-	+	
29	♂	15							-	-	-							
30	♀	6	+	+	+			8*	++	++	-	+	-	?	-	-	-	
31	♀	9		+			+	10*	+	+++	-	+	-	?	-	-	-	
32	♀	30							-	+	-							

TABLE 5 Dogs: Group 10: 10.5 mg/kg/day Berenil

No.	Sex	No. of doses	Symptoms					Day of death	Chem. path.			Post mortem: lesions					
			Behav.	Imbal.	Spast.	Nyst.	Coma		GOT	GPT	BUN	Brain	Liver	Kidney	Lung	Myocard.	Muscle
32	♀	4			+		+	5	-	+	-	+	-	Nephritis±	+	-	-
33	♀	2		+	+		+	3	-	+	-	+	?	?	-	-	-
34	♂	3		+	+		+	3	-	+	-	+	-	-	-	-	-
35	♂	3	+	+	+		+	3	-	+	-	+	-	-	-	-	-
36	♀	3	+	+	+		+	4	-	+	-	+	-	?	-	-	-
37	♀	3	+	+	+		+	4*	+	++	-	+	-	Nephritis±	±	-	-

tables as elevations with symbols of +, ++ and +++ respectively. Similarly, BUN values of less than 20 mg per cent were regarded as normal and elevations of 20 to 30, 31 to 50 and more than 50 mg per cent are symbolized as +, ++ and +++ respectively.

Group 1 (Table 1): The therapeutic dose was administered 6 to 13 times on consecutive days before the onset of nervous symptoms and the animals lived 7 to 18 days, which is considerably longer than those in the next group. Consequently more severe liver and kidney changes were encountered chemically-pathologically and at autopsy. In Dogs 2, 3 and 6 severe dehydration and loss of weight were encountered. In Dog 2, administration of the drug was terminated on Day 8 after the onset of ataxia. The ataxia proved to be of a transient nature and the dog recovered completely. Euthanasia was performed on the 17th day. In Dog 3 it was difficult to decide whether the locomotor disturbances were due to disturbed balance or mere weakness. Of all the experimental animals, Dog 6 showed the most severe hypersensitivity and convulsions. The symptoms closely resembled strychnine poisoning.

Group 2 (Table 2): Three to four administrations were required to provoke nervous symptoms and the animals died 3 to 7 days after the first dose. Dogs 11 and 12 died suddenly without any premonitory signs.

Group 3 (Table 3): Both dogs developed typical symptoms after 4 and 7 doses respectively.

Group 4 (Table 3): Dog 15 developed typical symptoms on the 5th day after a latent period of 2 days following the termination of the administration of the drug. This animal was euthanized on Day 6. Typical macroscopic brain lesions were encountered. The other nine dogs showed no untoward reactions.

Group 5 (Table 3): These dogs did not show any clinical signs of intoxication.

Group 6 (Table 3): This dog showed severe nausea and depression. Anorexia was in evidence until the nervous symptoms appeared. In addition it developed a severe haemorrhagic enteritis.

(2) *Berenil*

The results of these experiments are reflected in Tables 4 and 5.

Symptoms and Chemical Pathology: The symptoms encountered following the Berenil administration were identical to those observed with Phenamidine except that the acute symptoms of nausea and vomiting were only encountered in two of the six dogs at the 10.5 mg/kg/day dosage rate on the third day just prior to death. Comparable chemical-pathological changes were

encountered as for Phenamidine but were not as pronounced.

Group 8 (Table 4): No clinical signs were encountered but raised serum transaminase levels persisted and were in evidence in several dogs for as long as 3 weeks after the administration of the drug.

Group 9 (Table 4): One dog (29) and one bitch (32) were completely resistant to repeated therapeutic dosages for 15 and 30 administrations respectively. The others showed typical symptoms after 6 to 9 daily administrations and all of them had to be destroyed on Days 8 to 10.

Group 10 (Table 5): Two or three repetitions at the sterilizing dosage level were rapidly fatal for all the dogs except Dog 32. The impression was gained that the symptoms were often precipitated within a few hours by the last administration of the drug. Dog 32 was an extremely resistant individual which had also been used in the previous group.

(3) *Quinoronium sulphate*

Group 12 (Table 6): Occasional vomiting, diarrhoea and anorexia occurred in most of the dogs during the course of the experiment. Apart from these symptoms no other untoward reactions were recorded and none died. Slightly or prominently raised serum transaminase levels developed in all the animals except Dog 41. Dog 44 was anaemic (Hb 9.5 g per cent) and rather emaciated before it was subjected to repeated doses of 1 mg/kg/day. It reacted severely, showing nausea, vomiting and diarrhoea and pronounced anorexia during the 5 days of administration. Because of severe dehydration administration of the drug was terminated. It made an uneventful recovery.

B. Cattle

Only limited pilot experiments were performed with cattle. The results are listed in Table 7.

(1) *Phenamidine*

Group 7: Symptoms: Ox 1 developed slight muscle tremors on Day 4 and listlessness, depression, ataxia and anorexia became evident on Day 5. On Day 7 it was recumbent and showed hypersensitivity as well as cardiac arrhythmia. It was killed *in extremis* on Day 7. Ox 2 was found *in extremis* on the morning of Day 3 and it died on the same day. No symptoms were seen during the preceding 2 days. No symptoms were seen in Ox 3 except on Day 4 and 5 when slight ataxia and an increase in the respiratory rate were recorded. The animal was found dead on the morning of Day 13.

TABLE 6 Dogs: Group 12: Quinoronium Sulphate

No.	Sex	No. of doses	Symptoms			Chem. Path.		
			Vomition	Diarrhoea	Anorexia	GOT	GPT	BUN
<i>Acaprin 1.0 mg/kg/day</i>								
38	♂	15	+	—	+	+	++	—
39	♀	15	++	—	+	+++	+++	—
<i>Babesan 0.25 mg/kg/day</i>								
40	♂	15	—	+	—	—	+	—
41	♂	15	+	+	—	—	—	—
42	♀	15	—	—	—	—	+	—
43	♀	15	+	—	—	—	+	—
<i>Babesan 1.0 mg/kg/day</i>								
44	♂	5	++	++	+	—	+++	—

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TABLE 7 Cattle: Groups 7 and 11: Phenamidine or Berenil

No.	Sex	No. of doses	Day of death	Chem. path.			Post mortem: lesions			
				GOT	GPT	BUN	Liver	Kidney	Myocard.	Lung
Group 7: Phenamidine 12 mg/kg/day		5	7*	+++	+++	+++	++	+	+	—
1	ox									
Phenamidine 24 mg/kg/day		2	3	—	—	—	±	±	+	—
2	ox									
Phenamidine 24 mg/kg/day		5	13	—	—	+++	+	±	±	—
3	ox									
Group 11: Berenil 7 mg/kg/day		15	18	+++	+++	+++	++	—	±	+
4	ox									

Chemical pathology: From Day 5 increases in GOT, GPT and BUN levels occurred in Ox 1. No chemical-pathological changes were found in Ox 2. The only significant change noticed in Ox 3 was a severe increase of bilirubin on Day 9 which decreased after Day 11. The various degrees of elevation of GOT values were 180 to 250(+), 250 to 350(++) and more than 350(+++) units/ml; those of GPT 111 to 150(+), 151 to 250(++) and more than 250(+++) units/ml and those for BUN 28 to 37(+), 38 to 47(++) and more than 47(+++) mg per cent.

(2) Berenil

Group II: Symptoms: From Day 14 listlessness, slight ataxia and muscle tremors were seen in Ox 4. Ruminal stasis developed on Day 16 and on Day 17 the animal was recumbent and refused to rise. It died on Day 18.

Chemical Pathology

A progressive increase in the GOT and GPT levels occurred from Day 3. A terminal increase of BUN was also recorded.

PATHOLOGY

Macroscopic Findings

Dogs: The number of dogs that died or that had to be destroyed and the main lesions encountered are summarized in the various tables. The most significant lesions were localized areas of haemorrhage and malacia in the CNS [Plate 1(1)]. These lesions occurred in both the Phenamidine and Berenil groups and at both low and high levels of multiple administration. Their distribution was as a rule bilateral, but unilateral lesions were present in a few cases. The different anatomical areas of the CNS which were affected macroscopically and the percentage of cases in which these particular areas contained lesions are summarized in Table 8.

General congestion and cyanosis were prominent features in both euthanized animals as well as those that died naturally. Congestion was most conspicuous in the liver and lungs. The livers of some of the animals which survived the largest number of multiple doses of Phenamidine (Groups 1 and 3) revealed marked fatty changes. Pulmonary oedema was mild and infrequent, being present only in Dogs 7 and 8 of Group 2. Mode-

TABLE 8 Dogs: Distribution of brain lesions

Area Affected	% Positive		
	Phenamidine (16 cases)	Berenil (10 cases)	
<i>Medulla oblongata</i>	Vestibular area	12.5(19.0)	70
	Other areas	31.0(44.0)	60
<i>Cerebellum</i>	Roof nuclear area	56 (75.0)	80(100.0)
	Cortex (Uvula)	—	20
	Peduncles	—	10(20.0)
<i>Midbrain</i>	Colliculus aboralis	50 (56.0)	40(50.0)
	Colliculus oralis	25 (31.0)	30
	Tegmentum	6 (19.0)	60
<i>Thalamus</i>		25 (37.5)	50
<i>Hypothalamus</i>		25	—
<i>Basal ganglia</i>	Caudate	19 (37.5)	20
	Putamen	6 (12.5)	—
	Clastrum	6	—
	Globus pallidus	19	20
	Internal capsule	— (12.5)	10
<i>Olfactory System</i>	<i>Limbic System</i>		
	(i) Hippocampus	19	—
	(ii) Cingulate gyrus	19	—
	(iii) Frontal lobe	31 (37.5)	30(40.0)
	<i>Rbinal System II</i>	19	10

Wherever additional lesions were found microscopically parenthesised figures are used to indicate the total incidence

rate nephrosis was encountered in Dogs 6, 7 and 26 of Groups 1, 2 and 6 respectively. However, suspected mild changes were also noticed in a few other dogs. Varying degrees of chronic nephritis, which was unrelated to diamidine poisoning, occurred in a small number of animals. Localized myocardial degeneration was observed in the septum of Dog 9 (Group 2) and one papillary muscle of Dog 13 (Group 3). Subepicardial, subendocardial and myocardial haemorrhages were uncommon. Bilateral localized areas of muscular degeneration were found in Dogs 6, 15 and 28 of Groups 1, 4 and 9 respectively. These areas were more diffuse and prominent in Dog 15. The longissimus dorsi, quadriceps group, semimembranosus, gastrocnemius, adductor, triceps, latissimus dorsi, supraspinatus and the infraspinatus muscles were affected. In Dog 28 the longissimus dorsi, quadriceps group and the flexors of the hind limb contained lesions. The vastus medialis and subscapularis were bilaterally affected and the semitendinosus and gluteus medius only unilaterally in Dog 6. Intussusception of the small intestine was found in Dog 2 (Group 1).

Cattle: On gross examination of the CNS no lesions were found except very marked congestion in Ox 2. The livers of all four cases revealed varying degrees of fatty changes, the most marked being in Oxen 1 and 4. Similar mild or very mild changes were also present in the kidneys of Oxen 1, 2 and 3. Severe diffuse degeneration of the myocardium was observed in Oxen 1 and 2, the entire organ being diffusely parboiled in appearance. In the other two animals myocardial degeneration was mild and more localized. Moderate subepicardial haemorrhages occurred in Ox 3.

Constipation was seen in Ox 1, mild ruminal stasis and oedema of the gall bladder in Ox 4. The lungs of Oxen 1 and 2 were markedly congested, but oedema was only encountered in Ox 4. Localized areas of cellulitis and myositis occurred at the injection sites.

Microscopic Findings

With some exceptions the lesions encountered in dogs were usually very similar, irrespective of the type and dosage level of diamidines used. A few of these exceptions are the variation of the degree of fatty changes in various tissues, the absence of lesions in Dog 14 (20 mg/kg Phenamidine + thiamine) and the presence of very mild brain lesions in Dogs 3 and 6 (20 mg/kg Phenamidine). However, in spite of these differences, the lesions encountered in the dogs in the various groups can be dealt with simultaneously. The severity of the lesions in cattle differed considerably from those in dogs.

Brain

Dogs: With the exception of Dog 14, which had received thiamine injections, all the animals used in the various Phenamidine and Berenil groups which died, revealed microscopic brain lesions. These lesions had essentially the same microscopic features, the only variation being those of a progressive nature associated with the duration of the disease. In those animals that died within a day or two after the onset of the nervous symptoms, localized areas of severe haemorrhages [Plate 2(2)] accompanied by marked oedema and malacia were seen. Apart from the haemorrhages, mildly eosinophilic proteinaceous fluid occurred in the perivascular spaces and as pools in the neuropil [Plate 2(3 and 4)]. The latter appeared rarefied and microcavitated [Plate 2(3)]. Some of the vessels around the affected

areas were also congested and surrounded by oedematous fluid.

Swollen glial cells, mainly astrocytes, characterized by enlarged vesicular nuclei and increased cytoplasm as well as the appearance of eosinophilic intracytoplasmic granules and globules of varying sizes (granular glia) were regular features from Day 2 onwards [Plate 2(6)]. The nuclei of these granular glia were frequently pyknotic and karyorrhectic and many of the eosinophilic globules were dispersed in the neuropil and present in the perivascular spaces. These changes, however, were only confined to the haemorrhagic, malacic lesions and the adjacent areas.

Neutrophil infiltration between Day 2 and 5 was another very prominent feature of the lesions [Plate 2(5)]. They seemed to diminish from Day 5 and were gradually substituted by comparatively smaller numbers of mononuclears. Some of these cells, however, appeared almost simultaneously with the neutrophils, but usually in smaller numbers and only perivascularly. Many of the neutrophils eventually became necrotic, their nuclei revealing pyknosis and karyorrhexis. Between Day 2 and 5 necrosis of the blood vessels within and immediately adjacent to the lesions was evident. The walls of these vessels were swollen, stained more intensely eosinophilic and contained karyorrhectic material [Plate 2(4 and 5)]. Fibrinoid thrombi occurred in some of the affected vessels of seven dogs (27 per cent), all of which survived 2 or more days after the onset of nervous symptoms. Hypertrophy and hyperplasia of the endothelial cells were detectable from Day 4 and seemed to be more pronounced in those animals which received the largest number of doses.

The haemorrhagic and malacic lesions were encountered in both white and grey matter. Chromatolysis, swelling and necrosis of the neurones were present within and adjacent to these lesions. From the third day after the commencement of symptoms swollen axis cylinders (Wallerian degeneration) were frequently seen, mainly in the peripheral zone of the lesions. Foci of gliosis and perivascular gliosis in the vicinity of the lesions were noticeable in Dogs 3, 6, 27 and 37. All these animals received 6 to 13 doses of the specific diamidines before the nervous symptoms appeared. Scattered gutter cells and proliferation of the capillaries were found in the malacic lesions of Dog 2 which had eight doses of Phenamidine and survived 10 days after the last dose. Dog 13 had localized areas of neutrophilic meningitis close to the affected areas.

The primary malacic and haemorrhagic lesions were encountered in all the animals except in Dogs 3, 6 and 14. Glial swelling, endothelial hyperplasia, lymphocytic vasculitis and microcavitation were some of the lesions noticed in the former two dogs.

The lesions were only confined to the brain and none were noticeable in the spinal cord. A microscopic survey of brain sections revealed additional lesions which were not detectable grossly (Table 8).

Cattle: In contrast to the severe brain lesions of dogs, only very mild and limited changes were found in the brains of cattle. Malacia, oedema and severe haemorrhages were never encountered.

Vacuolation of a few neurones in the midbrain as well as neuronal swelling and chromatolysis were observed in Ox 1. The nuclei of the astrocytes appeared enlarged and vesicular. The brain of Ox 2 was prominently congested and had a few small haemorrhages in the anterior portion of the medulla oblongata. The cytoplasm of some glial cells seemed swollen and some

EXPERIMENTAL DIAMIDINE POISONING DUE TO COMMONLY USED BABECIDES

contained eosinophilic globules. Many neurones were chromatolytic and swollen.

A small area of microcavitation occurred in the thalamus and glial swelling in the cerebellum and corpus striatum of Ox 3, whereas mild swelling of the thalamic glia was the only finding in the brain of Ox 4.

Liver

Most of the livers were prominently congested. This was most marked in those animals that died naturally. Oedema, as indicated by the presence of a very mild eosinophilic substance in the spaces of Disse, occurred in 15 per cent of the dogs only. Mild cloudy swelling was seen in 23 per cent, but very mild to moderate fatty changes were noticed in the majority of dogs, being most prominent in the more advanced cases. There appeared to be no correlation between either the dosage level or the number of doses of the two diamidines used and the degree of fatty changes present. These lesions, however, were very marked in all the cattle. In those animals treated with Phenamidine, the periphery of the lobules was more severely affected than the centrilobular area [Plate 2(7)], but in the Berenil groups, either the entire lobule was diffusely vesicular or the centrilobular area was mainly involved.

Lungs

Various degrees of congestion were present in most of the animals, but oedema, which was never severe, occurred only in 39 per cent of the dogs and seemed to be associated frequently with the presence of myocardial lesions. The lungs of only two cattle were examined, one being negative for lesions and the other one revealing mild congestion and oedema.

Kidneys

Congestion was present in approximately 50 per cent of the dogs, but was absent in the bovine kidneys. Mild cloudy swelling or hydropic degeneration and mild fatty changes occurred in 27 per cent and 30 per cent of the dogs respectively. However, in the cattle, congestion was never observed but the fatty changes were very marked. Mild focal disseminated subacute or chronic nephritis of undetermined non-specific aetiology was seen in seven dogs (2, 3, 4, 8, 9, 14 and 36). Mild localized hyaline degeneration was present in Dogs 14 and 36. This change was probably related to the nephritis and not to the diamidines.

Myocardium

Very mild to moderate cloudy swelling, hydropic and/or fatty changes were noticed in 39 per cent of the dogs, but localized Zenker's degeneration occurred in only 15 per cent. In the cattle, on the other hand, all the animals had either mild or marked hydropic and fatty changes. The fibres were rarefied in appearance, somewhat finely vesicular with some swollen mitochondria.

Skeletal muscle

Mild lesions similar to those noticed in the myocardium were found in some of the skeletal muscles of a small number of dogs. Twenty-three per cent revealed hydropic and fatty changes and 15 per cent localized Zenker's degeneration. Dogs 6 and 15 had more severe lesions involving the longissimus dorsi, triceps, gastrocnemius, semimembranosus and adductor muscles. These lesions were constant in cattle and more severe than in the dogs. Even the diaphragm of one case (Ox 1) which was studied, proved to be affected. However, in general, the fatty changes were invariably

milder than those in the myocardium. In some of the animals mild purulent myositis occurred in the areas where the diamidines had been administered.

Spleen and Lymph nodes

Most of the bovine spleens were prominently congested, but this was rarely so in dogs. The peripheral lymph nodes, however, were more frequently congested and mildly oedematous. Mild purulent lymphadenitis, evidently due to the repeated injections, was found in two cattle.

Adrenal

Congestion was noticed in exceptional cases. Eosinophilic intracytoplasmic globules occurred in the zona glomerulosa and medulla of Ox 4.

DISCUSSION

The typical history of diamidine poisoning in dogs is usually either one of fatal, repeated, therapeutic administrations or one of recovery after treatment for babesiosis, followed by a latent period of a few days before a sudden onset of nervous symptoms. In the present study, excessively large doses and/or repeated therapeutic doses were used in order to produce similar symptoms. No attempt was made to produce a latent period either in treated, diseased animals or with a single therapeutic dose in normal dogs. The almost impractical feasibility of such experiments as well as the limited number of available experimental animals made such studies impossible. A further complicating factor was the marked individual variation in susceptibility of the animals that was encountered. This can probably be ascribed to the heterogeneity of the experimental animals. In Groups 4, 5 and 8 where only two doses were administered, only one animal from Group 4 developed nervous symptoms and was destroyed after a latent period of 2 days. Unfortunately all the unaffected nine animals from this group had to be re-used in Groups 5 and 8. Consequently, the variation of the susceptibility factor does not allow one to determine the incidence in all three groups. Fewer repetitions of the dosages administered in the other groups, however, probably would have caused typical symptoms and lesions in a large percentage of dogs.

The nervous symptoms which have been reported by several workers in Berenil and Phenamidine poisoning in dogs were confirmed in the present series of experiments. Furthermore, very marked haemorrhagic and malacic lesions in the brain which accounted for these symptoms were demonstrated in affected dogs. These were present particularly in certain areas such as the roof nuclear area of the cerebellum, the midbrain, medulla oblongata and the thalamus, of which the first mentioned area was most constantly affected, the incidence being 75 and 100 per cent in the Phenamidine and Berenil groups respectively (Table 8). These lesions did not occur in the cattle. There was also some variation in the distribution of the lesions produced by these two drugs in dogs. In the Berenil groups, the medulla oblongata and tegmentum of the midbrain contained lesions far more frequently than in the Phenamidine groups. The variation in the number of animals, doses and dosages, however, leaves some doubt as to the statistical significance of these findings. They should consequently be verified by the use of specific lethal dosages in a large and equal number of animals for each drug.

The early histopathological features of the brain lesions in dogs were congestion, haemorrhages, oedema,

rarefaction and malacia. These changes were followed by an influx of neutrophils, neuronal changes and Wallerian degeneration. As the lesions were studied mainly at a terminal stage of the disease, an attempt to explain its pathogenesis would be somewhat premature. However, the well-known histamine-like action which leads to increased vascular permeability and the drop in blood pressure caused by these diamidines, could account for some of these lesions. A more pronounced effect on certain tissues such as the vessels of the brain, especially in view of the vascular necrosis in this organ, should also be considered. Launoy, Guillot & Jonchère (1960) reported that no detoxication of the diamidines occurs in the body and that they accumulate mainly in the liver, kidney and brain. Although only small quantities are taken up by the brain it is almost permanently fixed in this organ. These findings could explain why these organs were most constantly affected in the present series of experiments. The peripheral distribution of the fatty changes in the liver, furthermore, may be indicative of a direct, and eventually toxic, effect on the hepatocytes. It is also of interest to note that the myocardium and even skeletal muscles are markedly affected in some animals, especially in cattle. Similarly, the liver and kidneys revealed more severe lesions in cattle. Thrombosis in the affected areas of the brain was only seen in seven dogs and is evidently secondary to the vascular necrosis. The eosinophilic granules and globules in the perivascular spaces, neuropil and glial cells were identical to those described by Pienaar, Basson & van der Merwe (1966) in heartwater in ruminants. Occurrence of these structures coincided with the regressive changes which are associated with brain swelling and oedema.

The close resemblance of the brain lesions in diamidine poisoning in dogs to those of canine cerebral babesiosis (Basson & Pienaar, 1965) and thiamine deficiency in certain carnivores (Evans, Carlson & Green, 1942; Edwin *et al.*, 1968) is significant. In the former, bilateral malacia and haemorrhages were described in the region of the cerebral cortex, caudate nucleus and in the region of the cerebellar roof nuclei (Basson & Pienaar, 1965). However, subsequent observations on four additional untreated cases of this disease (Pienaar & Basson, Veterinary Research Institute, Onderstepoort, unpublished observations, 1969) indicated that the lesions were confined either to the caudate nucleus and cerebral cortex or to the latter only. Softening and haemorrhages in the cerebral cortex extended into the depth of the sulci and affected the cortical grey matter of opposing gyri. On the other hand, in diamidine poisoning, lesions were seldom found in the cerebral cortex and when present lacked the characteristic association with the sulci. Furthermore, areas such as the roof nuclear area and the mid-brain were constantly affected. It is also interesting to note that the typical haemorrhagic and malacic lesions were not produced in cattle by repeated doses of these diamidines. Even in cerebral babesiosis in the bovine, similar lesions have never been encountered, the disease being characterized mainly by very severe sludging of parasitized erythrocytes, congestion, the above-mentioned glial changes of brain swelling and some oedema (Pienaar & Basson, unpublished observations, 1969).

In thiamine deficiency the lesions were reported mainly in the medulla oblongata, midbrain, cerebellum, basal ganglia and cerebral cortex, in decreasing order of frequency (Evans *et al.*, 1942).

Lourie & York (1939) encountered intussusception in young puppies treated with the aromatic diamidines. They suggested that this condition was apparently due to intestinal contractions produced by these compounds. Intussusception was seen in only one case in the present study in an adult dog which had received eight consecutive doses of 15 mg/kg/day of Phenamidine.

Both dogs and bitches seemed to be about equally susceptible to diamidine poisoning and the prophylactic use of very large dosages of thiamine hydrochloride did not significantly reduce their susceptibility, although the brain of one of the two dogs that received such prophylactic therapy did not reveal any lesions. The possible beneficial effect of thiamine therapy consequently should be re-evaluated in a more extensive and critical experiment.

On a weight basis, Berenil was much more toxic for dogs than Phenamidine, but the therapeutic indices appeared to be approximately of the same order. In the apparently healthy experimental dogs, Phenamidine was much more toxic at 20 mg/kg than at 15 mg/kg. The possible increased toxic effect of similar dosages on anaemic, febrile patients suffering from babesiosis, with possible concomitant malfunction of the liver and kidneys and a lowered resistance, was not determined experimentally, but can be anticipated. The absolute safety of a single therapeutic dose in diseased animals cannot be claimed without exception because of the variance in susceptibility. Cases have also been encountered in practice, especially in heavier breeds, where a single correctly calculated dosage resulted in typical poisoning with brain lesions (Pienaar & Basson, unpublished observations, 1969).

From the results in our experiments and the fact that the diamidines accumulate in the tissues, it is quite evident that therapy with these drugs should never be exceeded or repeated during the course of a disease such as babesiosis. According to Boyt *et al.* (1968) even repetition at intervals of several months was found to be poisonous in certain instances where dogs were injected prophylactically with Berenil. Until proved otherwise in domestic animals, it must be assumed that the danger of accumulation, especially in the brain tissue, does exist. In South Africa the administration of multiple doses of the diamidines is further facilitated by the free access that laymen have to these babecides. Frequently they therefore treat animals without consulting a veterinarian, and this is a fact that is not always brought out when the help of a veterinarian is eventually sought.

When the first dose of a babecide given at the recommended rate is ineffective in clearing up an infection, a second administration of the same or chemically related compound could hardly be expected to be effective. According to Bigalke (Veterinary Research Institute, Onderstepoort, personal communication, 1970), practical experience also indicates that whenever diamidines are ineffective, quinoronium sulphate is usually also ineffective and therapy with completely different drugs (e.g. the dyes) should, therefore, be resorted to immediately.

The inability to produce brain lesions with quinoronium sulphate, even at 15 daily repetitions at four times the therapeutic dose, was not unexpected as it is not chemically a diamidine. In addition, the apparent lack of even any other acute or chronic ill-effects, suggests the desirability of possible critical re-evaluation of this drug as a babecide, especially in the dog.

SUMMARY

Multiple therapeutic and higher dosages of Berenil, Phenamidine and quinoronium sulphate were administered to 44 apparently healthy dogs and four normal cattle in order to determine the toxic effects of these drugs. The diamidines caused severe nervous symptoms such as imbalance, rolling movements, extensor rigidity, opisthotonus, nystagmus and terminal paralysis in dogs. Marked haemorrhagic and malacic lesions in these animals were demonstrated mainly in the cerebellum, midbrain and thalamus. The cattle showed only mild nervous symptoms but none of the brain lesions seen in the dogs were noticed. Degenerative lesions, mainly of a fatty nature, occurred in the liver, kidneys, myocardium and skeletal muscles of some animals. These lesions were more prominent in the cattle. Two repeated daily doses of 20 mg/kg of Phenamidine caused brain lesions in one dog after a latent period of 2 days. The results of the experiments clearly indicated that the therapeutic dosages of diamidines should never be exceeded or repeated in the course of treatment of a disease. None of the animals which were given quinoronium sulphate succumbed.

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Addendum

Since the completion of this study Losos & Crockett, 1969, (*Vet. Rec.* 85, 196) have reported the

occurrence of extensive haemorrhagic malacia in the brains of dogs receiving single doses of 15 to 60 mg/kg of Berenil.

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