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TOXICITY OF HALOGENATED OXYQUINOLINES IN DOGS. A CLINICAL STUDY

III. INTOXICATION EXPERIMENTS*

By

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LANNEK, BIRGITTA and PAUL LINDBERG: *Toxicity of halogenated oxyquinolines in dogs. A clinical study. III. Intoxication experiments.* Acta vet. scand. 1974, *15*, 398—418. — Oxyquinoline drugs, which are normally well tolerated by dogs, will cause disease when a dog's resistance is occasionally lowered. In a series of experiments in dogs we tried to reproduce the disease pattern of spontaneous cases of poisoning. Most of these experiments, using even high single or repeated oral doses, failed. It was observed, more or less by chance, that the intestinal absorption of ^{125}I-labelled vioform was greatly increased when the dog was not fasted. The consumption of fat, but not of protein or carbohydrates, was found to be the responsible factor. When the oxyquinoline drug was given in a fat emulsion, approx. ⅓ of the dogs fell ill. When fouled fish was also added, the dose necessary to produce disease was lowered to the range used in vioform therapy. We believe that phenolic substances, which may be produced from bacterial degradation of proteins in intestinal disorders, compete with oxyquinolines in metabolic and elimination processes.

v i o f o r m; o x y q u i n o l i n e s; d o g s; c o n v u l s i o n s; h e a r t i n j u r y; l i v e r i n j u r y; p o i s o n i n g; d i a r r h o e a; p h e n o l i c s u b s t a n c e s.

Observations on an acute clinical condition in dogs, suspected to be caused by the ingestion of halogenated oxyquinolines, have been published (*Hangartner* 1965, *Schantz & Wikström* 1965, *Müller* 1967, *Püschner & Fankhauser* 1969, *Lannek* 1973, 1974). Until recently, these preparations were used frequently in gastrointestinal disorders in this species, as in man. It was concluded that the ingestion of halogenated oxyquinolines by dogs is nor-

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mally tolerated without any apparent disturbances of hearth. Sporadically, however, and often following the administration of a single and small dose, a serious or even lethal disease would occur. It could thus be expected that trials aiming to reproduce the intoxication would meet with considerable difficulties and, at best, only rarely be successful. In the present study we have tried to produce in advance a condition hypothetically expected to render the experimental animals more sensitive to the deleterious effects of the preparations.

ANIMALS

Mongrel dogs and beagle dogs of both sexes were used. Most of them were young dogs, under 4 years of age. Except when a specially composed diet was used in the experiments, the dogs were fed a commercial well-balanced food ("Doggy" Vårgårda, "Spurt" Ferrosan, or "Pia" KF). Parasites were checked by regular deworming. The dogs were vaccinated against distemper and viral hepatitis (HCC). Only dogs considered to be in a good clinical health were used. Twenty-four separate experiments, including about 80 individual dogs, were performed (untreated controls not included). Dogs which did not fall ill during the experiments and dogs which became slightly ill and recovered were sometimes used for a second or third time in the toxicity experiments. Check-ups were always made to assure that the dog had a normal clinical appearance and normal laboratory findings before a new experiment started. In this way the dogs were used 184 times in all (untreated controls not included). Necropsies were performed at the Department of Pathology, and a detailed account of the post-mortem pictures will be presented (*Lannek & Jönsson* 1974).

Laboratory investigations (*Lannek* 1974) were made before the experiment started and then at varying intervals.

EXPERIMENTS AND RESULTS

Short-term experiments were made, in which 5-chloro-7-iodo-8-hydroxyquinoline (vioform) as Enterovioform® tablets (Ciba, Basle, Switzerland) was administered as a single dose, or as repeated daily doses for a week at the most. This series comprised 4 experiments in 30 dogs. The single doses varied from 80 to 1000 mg of vioform per kg body weight and

day, and the maximum sequential dose amounted to 7000 mg per kg. One dog (3/64) was given 125 mg per kg on each of 2 consecutive days and fell ill. Frequent twitches in the mimic musculature and in the muscles of shoulders and thighs were apparent on the day following the first administration. On the next day there was profuse salivation, and large amounts of saliva were seen in the cage. No further treatment was given and the dog recovered. Laboratory findings were normal. All other short-term experiments were negative.

A long-time experiment was performed in which 3 dogs were dosed daily for 5 months, 1 dog for 3½ months, 3 dogs for 2½ months, 10 dogs for 2 months, and 2 dogs for 1 month. All of them were given 100 mg of vioform per kg body weight. Altogether 12 dogs died with symptoms of distemper, and the diagnosis was confirmed at necropsy. No symptoms indicating oxyquinoline intoxication were observed.

Diarrhoea was produced in 2 dogs by administration of castor oil, 25 ml and 50 ml per day, respectively, and phenolphtalein, 130 mg per day, for 10 days. Diarrhoea occurred after 8 days of treatment and continued on the next 2 days, when vioform, 80 mg per kg, was given as a single dose. No other symptoms were observed. In another experiment (2 dogs) diarrhoea was produced by giving phenolphtalein, 650 mg per day, for 9 days. The dogs were deprived of water for the last 2 days in order to make them dehydrated, and a single dose of vioform, 80 mg per kg, was then given. No specific symptoms were noted.

A rauwolfia drug (Serpasil® Ciba) was used in 1 experiment to produce diarrhoea. The dose of Serpasil corresponded to 0.06—0.14 mg per kg body weight at each treatment. Seven dogs were used altogether. The treatment was varied according to the dogs' condition. In 2 cases, Serpasil was given for only 2 days. In some other experiments in this series, Serpasil was given daily for 4—5 days or every other day for a week. All dogs developed severe diarrhoea, marked apathy, and inappetence, but no CNS disturbances, during the Serpasil treatment. Vioform was given as a single tablet, when the dogs had diarrhoea, the dose varying between 80 and 150 mg per kg body weight. One dog (1/60) showed fibrillary muscle twitches and ataxia on the day it had received vioform. It was free from symptoms on the following day. Another dog (2/59) developed

severe convulsions after 4—5 hrs., marked aggressiveness, and a behaviour indicating blindness. These symptoms persisted until it was killed on the next day. A third dog (4/65) was found dead after 4 hrs. Laboratory findings indicating dehydration, such as a rise of packed blood cells, blood haemoglobin, total serum protein, and urea nitrogen, were noticed as a consequence of the rauwolfia treatment and before oxyquinoline had been given. The ECG showed slight bradycardia.

N e c r o p s y showed focal myocardial necrosis and hydropic liver cell degeneration in 1 of the dogs (2/59) and myocardial degeneration and congestion of liver, lungs, brain and kidneys in the one (4/65) that was found dead. As more than 5 hrs. elapsed between death and necropsy an evaluation of the CNS lesions could not be made (*Lannek & Jönsson* 1974).

Four dogs did not develop any further symptoms after the vioform treatment and recovered completely in a few days.

Eight dogs which had s p o n t a n e o u s d i a r r h o e a were treated experimentally with vioform tablets. Two had suffered from diarrhoea for about 2 months. They had a heavy Toxocara infection and were repeatedly treated with Piperazine and Diphenthan-70+Toluen (Vermithana Vet.®, Ferrosan). Six dogs developed diarrhoea in association with testing of different commercial feeds. They had been sick for about a month. Vioform was given daily for 3 consecutive days and then at intervals of 1—2 days for about a week, 100 mg per kg body weight on each occasion.

One dog (Sl.), belonging to the last-mentioned group (beagle, male, 21 months old), showed aggressiveness on day 4, soon followed by convulsions, apathy, ataxia, and excessive salivation. There was some improvement in its condition on day 5. Vioform treatment was repeated on days 6 and 8, the last time with radioactive vioform suspension. From day 6 and onwards, the symptoms were aggravated, and the dog died on day 10 in severe convulsions. From day 5 and onwards, ECG showed small but significant ST depressions (Fig. 1). Other laboratory findings were normal.

P o s t - m o r t e m e x a m i n a t i o n of the heart, liver, kidneys, and lungs was performed. Focal myocardial necrosis and congested lungs were the only findings. No other dogs developed symptoms indicating oxyquinoline poisoning.

Three dogs (A, B, C) were used in an experiment to study

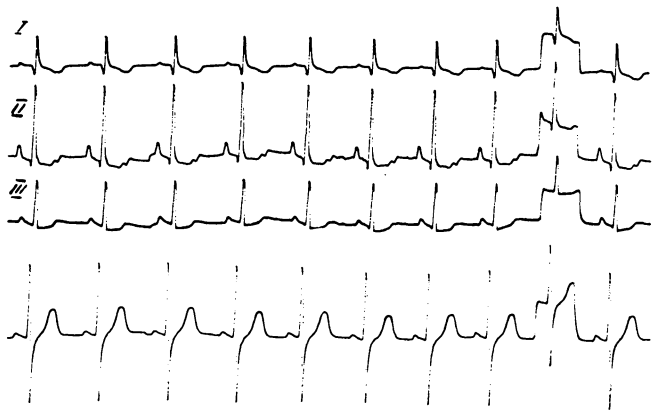

Figure 1. ECG from dog Sl. showing small but significant ST depressions in leads II and III.

the absorption and elimination of vioform. For this purpose, ^{125}I-labelled vioform was used synthesized from 5-chloro-8-hydroxyquinoline (*Haskins et al.* 1950). Dog A had spontaneous diarrhoea but was otherwise in good condition. About 3 to 4 hrs. after treatment with 100 mg of vioform per kg body weight, he was hyperactive and very excited, and had to be sedated. Thereafter he was quiet, but the symptoms reappeared after some hours. The dog was difficult to handle and was slightly aggressive. Laboratory examinations showed no abnormalities. On the next day he was free from symptoms. The other dogs showed no symptoms.

Because diarrhoea may be complicated with acidosis, it was thought to be of interest to investigate a possible effect of acidosis on the tolerance to vioform. Three dogs were treated with repeated doses of acetazolamid (Diamox®, Lederle), 5 mg, 10 mg, and 20 mg per kg body weight, respectively, until the serum bicarbonate in 2 of them, which received the high doses, had fallen from about 25 mmol/l to 16 mmol/l. One dose of vioform was given to each of the 3 dogs, 50 mg, 75 mg, and 100 mg per kg body weight, respectively. No symptoms of disease were observed.

Trials to promote the absorption of vioform by stimulating the gastric secretion with a histamin analogue (Histalog®, Lilly) 2—5 mg per kg in 8 dogs, or a 5 % solution of alcohol, 20 ml per kg, in 4 dogs, gave negative results. Vioform,

100 mg per kg, was given 45 min. and 20 min., respectively, after the stimulation of gastric secretion.

In order to obtain a more regular absorption, a vioform suspension was prepared by crushing vioform tablets in 75 ml of water, to which 5 drops of Tween 80® and 10 mg of sodium lauryl sulphate were added. The suspension was given through a stomach tube.

Vioform suspension, 100 mg of vioform per kg body weight, was given daily to 8 starved dogs for 3 days. The suspension was also used to 12 newborn puppies, less than 12 hrs. old, 6 of which had not been allowed to nurse before the first dose. They were treated daily for 4 days. No disease occurred.

In spontaneous cases of oxyquinoline poisoning, liver injuries are often seen. The liver-cell damage ranges from cloudy swelling to hydropic degeneration with depositions of lipids (*Lannek* 1973, *Lannek & Jönsson*). It was therefore decided to provoke liver injury by administration of carbon tetrachloride (*Reichard* 1959) before giving vioform. Five dogs were thus given CCl_4, 1 ml per kg body weight, orally. The development of liver injury was followed by determination of serum-aspartate-aminotransferase, SASAT, serum-alanine-aminotransferase, SALAT (*Sigma Technical Bulletin* 1967), serum-ornithin-carbamyl-transferase, SOCT (*Reichard*), bromsulphtalein (BSP) retention at 0, 24, 48, and 72 hrs. (Table 1) and morphological examination of liver-biopsy specimens (*Lannek* 1968) at 0 and 48 hrs. CCl_4 was given at 0 hrs. and vioform, 100 mg per kg body weight, at 48 hrs.

Table 1. Parameters of liver condition, extreme values, in CCl_4 experiment. Five dogs are represented throughout.

	Hours			
	0	24	48	72
SASAT[1]	28 — 36	136 — 185	210 — 3000	180 — 1940
SALAT[1]	20 — 34	310 — 1100	3400 — 5600	4200 — 6200
SOCT[2]	1.2 — 4.2	8.4 — 21.6	72.0 — 222.0	173.0 — 356.0
BSP[3]	0.2 — 0.5	1.3 — 2.2	2.2 — 4.4	1.3 — 2.5

[1]: Sigma-Frankel units.
[2]: Reichard units.
[3]: mg per 100 ml.

The biopsy specimens were normal at 0 hrs., and showed centrolobular degeneration and/or necrosis and fatty degeneration of the liver cells at 48 hrs.* The consistency of the organ, as observed at the puncturing for biopsies, changed from firm-elastic at 0 hrs. to soft-brittle at 48 hrs. All dogs developed a marked bleeding tendency which could already be observed at 24 hrs. Otherwise no symptoms of disease occurred. They were killed at 96 hrs., and the livers were examined postmortem. They showed the same changes as those seen in biopsy specimens.

The possible influence of varying feedstuffs was investigated in a series of experiments. Three groups of dogs, 5 in each group, were starved for 24 hrs. and then given gruel (Semper, saccharose-free, for human use) or whole milk or cheese ad lib. for 4 days. Vioform, 100 mg per kg as tablets, were given daily during the same period. No disease was observed.

During investigations using ^{125}I-labelled vioform (*Lannek & Lindberg* 1974) it was observed that in dogs which had eaten shortly before the administration the activity in the blood was markedly higher than in dogs which had been fasted. It was thus concluded that the ingestion of feedstuffs had a promoting effect on the absorption of vioform. It was found in further experiments that this effect was produced by fat, whereas carbohydrate and protein had a weak, if any, effect. For this reason it was important to ascertain the influence of feeding fat on the tolerance to vioform (*Lannek & Lindberg* 1972 a). Eleven dogs were fasted for 24 hrs. and then fed a 20 % soya-oil emulsion (Intralipid®, Vitrum) by stomach tube. The amount of oil was 40 ml (80 cal.) per kg. Three dogs vomited after the administration and were withdrawn from the experiment. Thereafter, 10 to 20 ml of oil (20—40 cal.) per kg were given to 9 dogs. Vioform suspension was given at the same time.

In preliminary experiments, a vioform dose of 100 mg per kg or lower gave high blood levels when given with fat, but no clinical toxicity was evident (*Lannek & Lindberg* 1972 a). The doses of vioform were therefore increased. Single doses were used. The dogs were kept under continuous observation for at least 14 hrs. after the administration, and then at intervals.

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* Kindly prepared and examined by Dr. L. Jönsson, the Department of Pathology, Royal Veterinary College, Stockholm.

Typical reactions were observed in 7 dogs out of 17. In 3 dogs (Sk. and Ma., 300 mg per kg, Fi., 500 mg per kg) fibrillary muscle twitches were seen after 4—6 hrs. Other symptoms were apathy interrupted by hyperactivity and excitation, and profuse salivation. Convulsions appeared after about 8 hrs. (Fig. 2). One

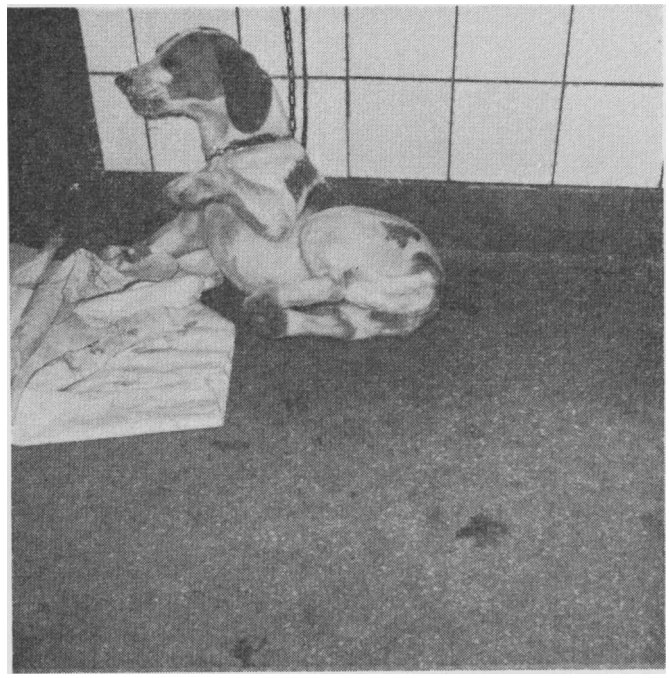

Figure 2. Convulsions in dog Sk. 8 hrs. after soya-oil emulsion and vioform suspension (300 mg per kg) given to the dog by stomach tube.

dog (Ma.) recovered after 1 day and another (Fi.) after 4 days. The third dog (Sk.) died in violent convulsions after 26 hrs. Sk. developed changes indicating severe liver and kidney injury (Table 2). ECG showed advanced ST-T depression (Fig. 3). Necropsy revealed congested liver and brain. Unfortunately, histological sections of the myocardium could not be evaluated because of post-mortem changes. In Fi. there was some increase of serum enzymes after about 12 hrs. and of BSP retention (1.8 mg per 100 ml on day 8). ECG showed marked ST depression in leads II and III, first noted after about 8 hrs. and then persisting for the next 4 days. The laboratory examinations in dog

Ma. are inconclusive owing to haemolysis. The ECG tracings could not be evaluated because of artefacts (excitation and hyperactivity).

Table 2. Laboratory findings in experimental dog Sk. The dog was given vioform suspension (300 mg per kg) and fat emulsion at 08.15 on day 1 (cf. p. 8).

	Day 1		Day 2
	08.00	20.00	08.00
SASAT[1]	47	620	216
SALAT[1]	38	290	400
SOCT[2]	1.6	—	100
Urea N[3]	8	25	50
Serum Ca[4]	9.1	10.3	8.9
Serum P[4]	5.6	12.2	23.6
Serum K[5]	5.0	—	10.2
PCV[6]	33	50	66

[1]: see Table 1.
[2]: see Table 1.
[3]: mg per 100 ml blood.
[4]: mg per 100 ml serum.
[5]: meq. per 1 serum.
[6]: ml per 100 ml.

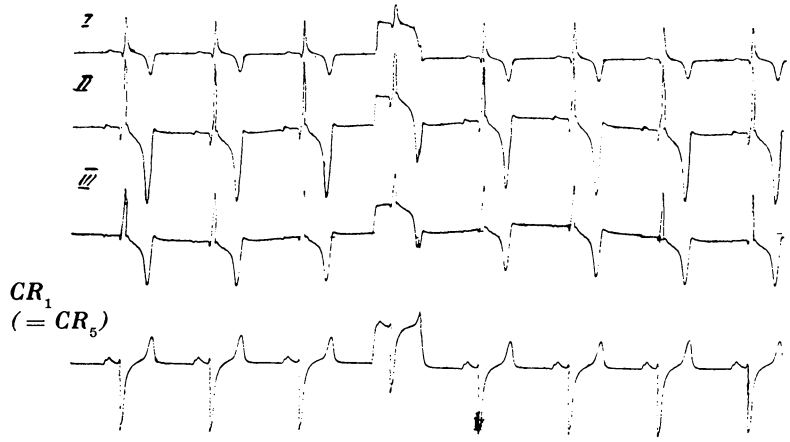

Figure 3. Marked depression of ST-T in dog Sk. about 24 hrs. after the administration of vioform (300 mg per kg) and fat emulsions (cf. p. 8).

Two dogs (Un., 50 mg per kg, and Mo., 500 mg per kg) showed marked listlessness, beginning 6—8 hrs. after the administration of vioform. They took little or no notice of the surroundings and did not seem to observe the approach of human beings, whom they would welcome normally. They showed general stolidity and would nearly fall asleep in a sitting posture. It should be mentioned that the dogs were observed by one of the authors (B.L.), who knew the dogs individually and their special behaviour. The dogs were normal on the next day. One dog (Al., 500 mg per kg) became hyperactive after 6—7 hrs. It jumped and barked or howled almost incessantly. If it calmed down for a while, the attacks were provoked again by a sudden noise. The dog was normal on the next day. One dog (St., 300 mg per kg) developed aggressiveness, beginning after 4—6 hrs. It seemed to have hallucinations as expressed by sudden and furious attacks on imaginary objects and bristling and snapping in the air. The condition lasted for about 4 hrs. In Un., Mo., Al., and St. no laboratory or ECG abnormalities were noticed.

It was accepted as a working hypothesis that, as oxyquinoline drugs are normally given to dogs with intestinal disorders, the elimination of the oxyquinoline via the liver may have to compete with aromatic products which arise in excess from bacterial degradation of protein (*Lannek & Lindberg* 1972 b). Such products are phenolic substances which, like the halogenated oxyquinolines, are coupled to glucuronic and sulphuric acids for elimination in bile and urine. Fresh herring in air-tight jars was exposed to 40°C for 48 hrs. This treatment will produce an intensive and extremely offensive odour. The fish has a soft consistency but still retains its shape. The fouled fish was eaten by some dogs, but to others it had to be mixed with tasty meat products to be accepted. All dogs which were used for vioform administration had eaten at least half a herring per kg body weight per day. No other feed was given during the experiment. Altogether 25 dogs were used. Herring was given to 4 dogs for only 1 day, but to 10 dogs for 3 days and to 11 for 4 days. On the last day and 2—3 hrs. after the dogs had eaten their herring ration, a fat emulsion (Intralipid®, Vitrum) and vioform suspension were given. The vioform doses were 50 mg per kg body weight in 13 dogs, 100 mg per kg in 11 dogs, and 300 mg per kg in 1 dog. The amount of fat emulsion was 10 ml per kg. Symptoms indicating oxyquinoline poisoning were noted in 10

dogs, 5 with 50 mg, 4 with 100 mg, and 1 with 300 mg per kg. One dog (Mal., 50 mg per kg) showed hyperactivity after 2—3 hrs. The symptoms subsided and no significant symptoms were seen for the next 2 days. On the third day this dog developed aggressiveness and attacked the keepers. This occurred especially when the latter tried to capture or fasten the dog in some way. At intervals the dog was deeply depressed (Fig. 4). Other symp-

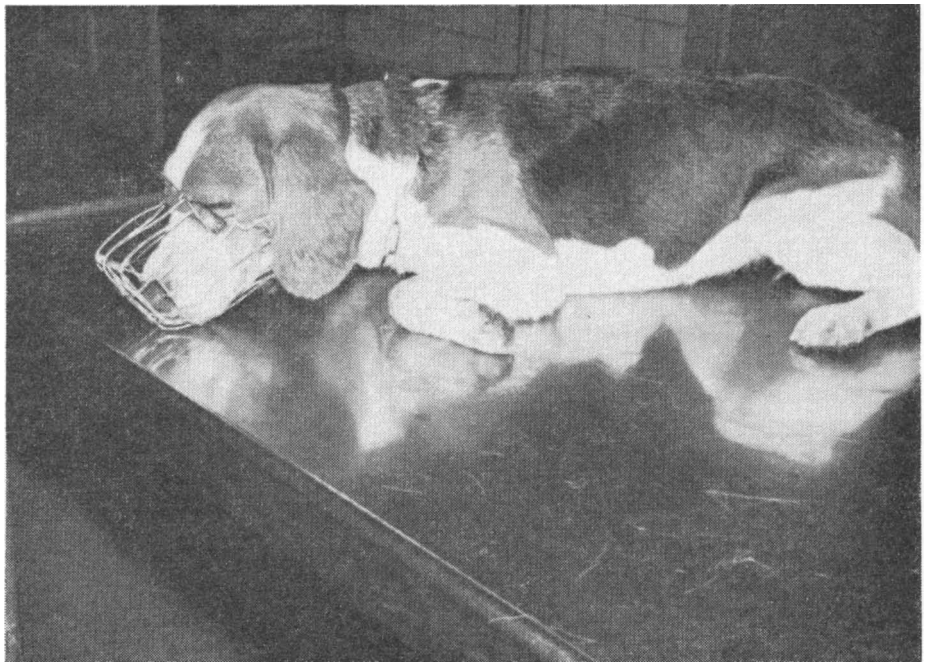

Figure 4. Dog Mal. showing apathy on day 4 after vioform suspension (50 mg per kg). This dog had been fed fouled herring for 4 days before receiving the soya-oil and vioform.

toms were ataxia, profuse salivation, and convulsions. By day 4 the symptoms had become aggravated and periods of apathy to near coma intervened. The dog was moribund on day 4. There was a moderate serum-enzyme elevation on day 4 (SASAT 135, SALAT 130, and SOCT 13.2 units). ECG showed myocardial injury, namely depressed ST segments in multiple leads.

One dog (Un., 50 mg per kg) was listless after 2—3 hrs. This progressed to unconsciousness on the next day, and the dog was killed. Un. had already been used once, 2 weeks before, in this series of experiments. He had then received 300 mg of vioform

per kg. Listlessness had been observed after 3—4 hrs. and lasted for about 12 hrs. Serum enzymes had risen significantly, being SASAT 670, SALAT 480, and SOCT 66 units after 24 hrs.; the values were normal after a week (38, 40, and 3.6 units, respectively). Some laboratory findings representing the conditions a few hours before he was killed showed i.a. severe dehydration and liver injury (PCV 66 %, Hb 24 g per 100 ml, urea N 58 mg per 100 ml, SASAT 2320, SALAT 1480, and SCOT 148 units). ECG showed marked tachycardia (300/min.).

A third dog (St., 100 mg per kg) showed convulsions after 9 hrs. which was followed by listlessness. On the next day she had repeated convulsions and was apparently blind. She was killed on the same day. Laboratory findings on the day she was killed showed a slight rise of serum enzymes. ECG revealed advanced myocardial injury with deeply depressed ST segments and high peaked T waves (Fig. 5).

A fourth dog (Al.) was used on 3 different occasions in fouled fish-oil experiments (intervals 14 days and 7 months). He showed symptoms each time. On 2 occasions (50 mg and 100 mg per kg) he had convulsions after 6—7 hrs.; on the third occasion (50 mg per kg) he showed hyperactivity and nervousness. The symptoms subsided each time within a day and the dog survived. In the last experiment, when the dog had been given 50 mg per kg, there was a moderate rise of serum enzymes (SASAT 600, SALAT 880, SOCT 94 units), and ECG showed depressed ST-T in multiple

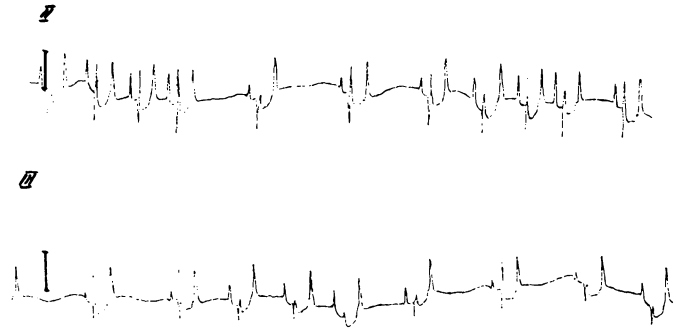

Figure 5. ECG from dog St. showing deeply depressed ST segments and high peaked T waves in leads II and III. The dog was fed fouled herring 4 days before treatment with soya-oil and vioform (100 mg per kg).

leads. In the first 2 experiments laboratory findings were normal. ECG could not be recorded because of the dog's hyperactivity.

Two dogs (Fim. and Pe., 100 mg and 50 mg per kg) developed hyperactivity after 2—3 hrs. It was very marked in Fim. which nearly strangled herself in the leash. She was treated with phenobarbital, and the symptoms had disappeared when she woke up the next day. Pe. showed only nervousness for a couple of hours. One dog (Mo., 100 mg per kg) became listless to apathic after a few hours but had recovered on the next day. Laboratory findings were normal in Fim., Pe., and Mo. ECG showed ST depressions in Pe. and Mo. ECG could not be recorded in Fim.

Necropsy. In 3 dogs (Mal., Un., St.) necropsy was performed immediately after death. In 1 of them (Mal.) retrograde aortic perfusion of fixation medium was applied when the dog was in a moribund state; severe myocardial damage and nerve-cell degeneration were found in all the dogs, liver-cell degeneration as well in 2 (Mal. and Un.) (*Lannek & Jönsson* 1974).

Intravenous administration of 8-hydroxyquinoline

Oxyquinoline was dissolved in 2.5 % citric acid to give a 2.0 % solution. It was slowly administered intravenously (about 5 min.) at a dose rate of 5 mg (2 dogs), 20 mg (2 dogs), 30 mg (2 dogs), 40 mg (4 dogs), and 50 mg (3 dogs) per kg body weight. Three control dogs were given the citric acid solution, 2.0 ml per kg, without oxyquinoline in a corresponding way.

All dogs which received more than 5 mg of oxyquinoline solution per kg body weight (nos. 11/65, 4/60, 10/65, Fu., Lu., 100/65, 9/65, 11/65, 3/60, 4/60, and 5/64) showed principally the same reactions. General clonic convulsions commenced before the injection was terminated. Tonic muscular contractions with maximally extended legs and neck (opisthotonus) then began and lasted for several minutes. These were followed by clonic convulsions in the jaws, excessive salivation, coughing, and vomiting. When the dogs were able to stand on their feet after some 10—20 min., they staggered and sometimes moved in circles. They had an anxious look. Two dogs were markedly aggressive. The dogs behaved normally after 2—3 hrs. In 3 cases, nos. 3/60, 4/60 (20 mg per kg), and no. 5/64 (40 mg per kg), the symptoms and reactions were less apparent. The dogs recovered more

rapidly and seemed to be normal again after ½ hr. No abnormal changes were observed at laboratory or electrocardiographic examinations, except in 1 dog (no. 5/64). In this case depressed ST segments in leads I, II, III, and CR_3 ($= CR_{6u}$) were noted within only ½ hr. after the injection and were even more advanced on the next 2 days.

8-hydroxyquinoline was dissolved in dimethyl sulfoxide (DMSO) to give a 12—15 % solution. It was administered slowly intravenously. The dose of 1 mg per kg (1 dog) produced no symptoms. Ten mg per kg (1 dog, 14/65) was followed by immediate anxiety and whining. The reactions disappeared soon after the injection had been completed. In a third dog (13/65) the planned dose was 50 mg per kg, but after a volume corresponding to about 20 mg per kg had been injected, the dog showed such violent reactions that the injection had to be stopped. The symptoms were anxiety, temporary unconsciousness, vomiting, and staggering gait. These were soon followed by tonic muscular contraction (extension) of legs and neck, lasting for about 15 min. The dog recovered and had a normal appearance after a few hours. Serum-enzyme values are set out in Table 3. In dog 13/65 ECG showed an arborization block and in dog 14/65 depressed T waves from shortly after the experiment and for the next 2 days.

Table 3. Serum enzymes in dogs given DMSO or vioform (OQ) dissolved in DMSO intravenously (cf. above).

		DMSO (dog As. control)	DMSO + OQ 10 mg/kg (dog 14/65)	DMSO + OQ 20 mg/kg (dog 13/65)
SASAT[1]	before	16	24	15
	3.5 hrs.	18	47	75
	24 hrs.	12	45	38
SALAT[1]	before	24	28	17
	3.5 hrs.	20	31	74
	24 hrs.	17	41	83
SOCT[2]	before	—	3.0	3.6
	3.5 hrs.	2.4	7.4	10.2
	24 hrs.	2.6	—	1.8

[1]: see Table 1.
[2]: see Table 1.

Two control dogs which were given DMSO alone (0.1 ml per kg) showed no symptoms.

DISCUSSION

The halogenated 8-hydroxyquinolines have long been used as antiseptics. They are of special value in treating intestinal amoebiasis, as dusting powders for wounds, eczemas, etc., and as vaginal inserts in Trichomonas vaginitis. For amoebiasis, the drug is given orally as tablets, and such preparations have also been used rather uncritically in diarrhoeic conditions of unspecific aetiology. Little is known about the fate of the substance after oral administration, but most of an ingested dose is supposed to pass through the intestinal tract without being absorbed (*Goodman & Gilman* 1971). However, systemic absorption does occur, and a varying part of the substance is excreted in the urine as conjugates of glucuronic and sulphuric acid (*Palm* 1932, *David et al.* 1944, *Knight & Miller* 1949, *Haskins & Luttermoser* 1953, *Ritter & Jermann* 1966, *Berggren & Hansson* 1968). Among the 7 possible isomerides of monohydroxyquinolines it is only 8-hydroxyquinoline, also known as 8-quinolinol or oxine, which possesses antibacterial and antifungal activity. Much work has been conducted to find out how oxine excerts its effect on the microorganisms. *Albert et al.* (1947) showed that oxine is the only monohydroxyquinoline that besides its antibacterial properties is capable of chelating. This means that the activity of this type of drug is determined by its ability to combine with polyvalent metals and to form complexes which are devoid of the characteristic properties of metal ions. *Albert* (1968) has also shown that these substances function by disorganising trace-metal mechanisms which are essential for bacterial growth. The combination of the drug and some metal(s) takes place on the bacterial surface.

Tablets containing halogenated 8-hydroxyquinolines were available on the Swedish market without a doctor's or veterinarian's prescription until June 1972. They seem to have been widely used for treating gastrointestinal disorders in man as well as in dogs. Untoward side-effects occur rather seldom, but 100 cases of spontaneous intoxications in dogs have been diagnosed at the medical clinic of the Veterinary College in the period 1963—1969 (*Lannek* 1973, 1974). The object of the present work was to reproduce the intoxication in experimental dogs.

The first experiments of this kind were made in 1964, when our experience of the spontaneous disease was still fairly limited. We did not know, for example, that listlessness and/or hyperactivity, which may be apparent only for a few hours, is the mildest manifestation of the disease. Recording of this symptom requires more or less continuous observation. Therefore the possibility cannot be excluded that some slight reactions that might have been present in the early experiments were overlooked or neglected. Taking this possibility into consideration, it is still obvious that the mere administration of an oxyquinoline drug to unprepared dogs, even in single doses up to 1000 mg per kg body weight, is usually well tolerated. Only 1 of the 30 short-term experiments was recorded as successful, namely in a dog which had received 125 mg per kg for 2 consecutive days. This again supports the clinical experience (*Lannek* 1973) that poisoning or tolerance is largely independent of the dose ingested, at least in the dose range of 10—1000 mg per kg body weight.

From the growing clinical experience it further emerged that the tolerance of an individual dog must vary within wide limits. Histories of spontaneous cases commonly contained the information that the dog had been given tablets of halogenated oxyquinolines earlier without developing disease. This fact was 1 reason for performing the long-term experiment. It was assumed that if a group of dogs is given vioform tablets daily for a long time, there is the likelihood that, sporadically, a dog in a low phase of tolerance would be treated. A weakness of the assumption is obviously that it leaves out factors responsible for the lowered resistance, such as dietary ones.

In the long-term experiment described here, no symptoms of disease were seen. It is noteworthy, however, that a number of dogs fell ill with distemper while they were under treatment with Enterovioform. They had been vaccinated according to a scheme which normally provides adequate protection. As they were kept at the clinic, it can be assumed that the experimental dogs were frequently exposed to the distemper virus. The question arises whether the daily administration of oxyquinoline may have lowered the resistance to the virus infection. At present, we know of no evidence of such a relationship. That oxyquinoline therapy may precipitate a bacterial infection has been demonstrated by *Mentzing & Ringertz* (1968) who found that travellers to the Mediterranean area who regularly used oxyquinolines prophy-

lactically showed higher incidence of Salmonella infections than those who did not take the drug.

Available reports on experimental vioform-tolerance studies in dogs are either very short or unpublished and lack data which would permit a closer comparison with the present study. *Tateishi et al.* (1971) apparently produced acute poisoning in dogs, running with convulsions, as well as a chronic picture resembling subacute myelo-optic neuropathy (S.M.O.N.) in man. In their experiments, 17 out of 20 dogs fell ill after treatment with clioquinol (vioform). In the chronic cases they used a dosage of 60 to 144 mg per kg body weight and day. Attempts to replicate their results in the laboratories of Ciba-Geigy were unsuccessful (*Cohn & Harun* 1972). *Griffith* (1969) noticed increased SALAT levels in dogs given broxyquinoline (5,7-di-bromo-8-hydroxyquinoline) for 26 weeks, and liver lesions were observed at necropsy. Symptoms such as listlessness, ataxia, and paresis appeared in dogs which were given the highest dose, 400 mg per kg daily. Definite conclusions as to the true nature of these observations were not drawn, however.

Nearly all the dogs (98 out of 100) which were admitted to the clinic with the poisoning syndrome had been treated with oxyquinolines because of diarrhoea. It was therefore natural to test the hypothesis that the presence of diarrhoea would lower the resistance to the deleterious effetcs of the drugs. Diarrhoea is of course only 1 symptom of intestinal disorder. As mentioned earlier (*Lannek* 1973), the diarrhoeic condition in most of the dogs treated with oxyquinoline preparations reflected a mild disease or a temporary intestinal dysfunction.

The limited experiments made in dogs in which diarrhoea had been produced by laxatives (castor oil and phenolphtalein) were clearly negative. The results of the rauwolfia experiments are difficult to interpret because the drug in itself is so toxic. Reserpine, 0.05—0.1 mg per kg i.v., causes depression, contraction of the pupil, relaxation of the membrana nictitans, and slight stupor in dogs. Larger doses, 0.25—0.5 mg per kg, are followed by a condition with shivering, tremors, and diarrhoea, which may be fatal (*Garner* 1961). The dogs in the present work received repeated doses of 0.06—0.14 mg per kg i.v., and fell ill after a few hours with diarrhoea and apathy but no other CNS disturbances, and later on a poor general condition. It appears also from the medical literature that rauwolfia therapy in man is com-

plicated by numerous side-effects (*Goodman & Gilman*). Nevertheless, 3 dogs out of 7 used in the present experiments fell ill after the administration of vioform, showing symptoms indicating oxyquinoline poisoning. We are inclined to think that dogs influenced by reserpine may be less resistant to vioform than normally. Without further investigations no conclusions can be drawn as to which rauwolfia effect would be responsible.

Two dogs with spontaneous diarrhoea fell ill during the vioform therapy. One of them (Sl.) showed a typical syndrome and died, and there can be no doubt that the cause was the ingestion of vioform. The other one (A) was very excited, slightly aggressive, and difficult to handle. This dog recovered. However, the fact that only 2 out of 9 treated dogs fell ill hardly supports the hypothesis that the presence of a gastrointestinal disturbance makes a dog more liable to develop disease due to treatment with an oxyquinoline preparation.

The experiments in dogs made acidotic did not reveal any increased sensitivity. However, it should be remembered that these and other trials presented here have the character of pilot experiments. They were designed to form parts of a screening of several possible causal conditions and in no way to be elaborate and conclusive investigations.

It is somewhat surprising that the severe liver injury caused by carbon tetrachloride failed to lower the resistance to vioform under the experimental conditions. Table 1 shows that there was a marked rise of SALAT which is considered to be relatively liver-specific in dogs (cf. *Candlin* 1968) and of SOCT which is most liver-specific (*Reichard* 1959). The increase of BSP retention also shows that the elimination mechanism of BSP was inhibited. This indicates that lowered resistance to oxyquinoline is not, or not essentially, a consequence of decreased elimination of the drug. Either increased absorption of oxyquinoline from the intestine and/or an impaired metabolism of the substance seems to be more a probable cause of intoxication (*Lannek & Lindberg* 1974). The rapidly appearing symptoms during intravenous injection of oxyquinoline in citric acid or DMSO support the view that the acute disease is closely associated with the concentration of oxyquinoline in the blood. This was further supported in experiments where the blood concentration of ^{125}I-labelled vioform was studied after oral application in combination with fat (*Lannek & Lindberg* 1972 a, b, 1974).

In absorption studies using ^{125}I-labelled vioform it was found that dogs which had not been fasted showed higher activity in the blood than did fasted dogs. Experiments designed to find out which feed component might be responsible showed clearly that it was the fat (*Lannek & Lindberg* 1972a). Experiments in which oxyquinoline was administered orally together with a fat emulsion resulted in 7 positive events out of 17. There was an indication, however, that, when given with fat, only high doses of oxyquinoline produced disease. Thus, in the positive experiments the dose was 300 mg per kg or more in 6/7, whereas in the negative experiments the dose was 100 mg per kg or less in 9/10. In the spontaneous cases the median dose was 44 mg per kg (*Lannek* 1973). It was therefore hypothesized that phenolic substances produced by an abnormal intestinal bacterial activity, as in a diarrhoeic condition, might compete with oxyquinoline in the elimination. During bacterial digestion of protein various toxic substances (e.g. indole, skatole, phenol) are formed. These are partly excreted in the faeces and partly absorbed, and conjugated in the liver and kidneys with e.g. sulphuric or glucuronic acid. The conjugates are eliminated through the kidneys into the urine. Conjugation with sulphuric and, especially, with glucuronic acid is a most important detoxication process. Phenolic substances as well as 8-hydroxyquinoline are detoxicated and eliminated in this way (*Palm* 1932, *David et al.* 1944, *Knight & Miller* 1949, *Haskins & Luttermoser* 1953, *Ritter & Jermann* 1966, *Cantarow & Schepartz* 1967, *Albert* 1968, *Berggren & Hansson* 1968). The fouled-herring experiments (*Lannek & Lindberg* 1972b) in which 9 dogs out of 25 were positive with doses of 100 mg per kg body weight or less support the hypothesis that a competition of the liver's detoxicating capacity takes place. This may result in incomplete conjugation of absorbed oxyquinoline with intoxication as a consequence.

REFERENCES

Albert, A.: Selective Toxicity. 4th Ed., Methuen & Co. Ltd., London 1968.

Albert, A., S. D. Rubbo, R. J. Goldacre & B. G. Balfour: The influence of chemical constitution on antibacterial activity. Part III: A study of 8-hydroxyquinoline (oxine) and related compounds. Brit. J. exp. Path. 1947, *28*, 69—87.

Berggren, L. & O. Hansson: Absorption of intestinal antiseptics derived from 8-hydroxyquinolines. Clin. Pharmacol. Ther. 1968, *9*, 67—70.

Candlin, F. T.: The liver. *In* Canine Medicine. 1st Catcott Ed., Amer. vet. Publ., Inc., California 1968.

Cantarow, A. & B. Schepartz: Biochemistry. 4th Ed., W. B. Saunders Co., Philadelphia & London 1967.

Cohn, H. D. & J. S. Harun: Letter to the Editor. J. Amer. med. Ass. 1972, *220,* 276.

David, N. A., N. M. Phatak & F. B. Zener: Iodochlorhydroxyquinoline and diiodohydroxyquinoline: animal toxicity and absorption in man. Amer. J. trop. Med. 1944, *24,* 29—33.

Garner, R. J.: Veterinary Toxicology. 2nd Ed., Baillière, Tindall and Cox, London 1961.

Goodman, L. S. & A. Gilman: The Pharmacological Basis of Therapeutics. 4th Ed., The Macmillan Company, New York 1971.

Griffith, R. W.: Intestopan, a 26-week oral toxicity study in dogs. Sandoz Ltd., Dept. Pharm. Tox., March 28, 1969 (unpublished).

Hangartner, P.: Troubles nerveux observés chez le chien après absorption d'Entéro-Vioforme Ciba. (Nervous symptoms observed in dogs after absorption of Enterovioform Ciba). Schweiz. Arch. Tierheilk. 1965, *107,* 43—47.

Haskins, W. T., G. W. Luttermoser & F. J. Brady: The physiological disposition of diodoquin, vioform and chiniofon in the rabbit as determined with radioiodine. Amer. J. trop. Med. 1950, *30,* 599—612.

Haskins, W. T. & G. W. Luttermoser: Urinary excretion of vioform and diodoquin in rabbits. J. Pharmacol. exp. Ther. 1953, *109,* 201—205.

Knight, A. A. & J. Miller: Comparative studies on the iodine absorption of Anayodin, Chiniofon, Diodoquin, and Vioform in man. Ann. intern. Med. 1949, *30,* 1180—1187.

Lannek, B.: Leverbiopsi hos hund. Ett diagnostiskt hjälpmedel. (Liver biopsy in dogs. A diagnostic aid). Svensk Vet.-Tidn. 1968, *21,* 591—594.

Lannek, B.: Toxicity of halogenated oxyquinolines in dogs. A clinical study. I. A survey of cases. Acta vet. scand. 1973, *14,* 723—744.

Lannek, B.: Toxicity of halogenated oxyquinolines in dogs. A clinical study. II. History, symptoms, laboratory findings, therapy, and follow-up. Acta vet. scand. 1974, *15,* 219—238.

Lannek, B. & L. Jönsson: Toxicity of halogenated oxyquinolines in dogs. A clinical study. V. Pathological findings. Acta vet. scand. 1974, *15.* In press.

Lannek, B. & P. Lindberg: Vioform poisoning in dogs. The absorption of vioform from the intestine. Svensk Vet.-Tidn. 1972 a, *11,* 438—441.

Lannek, B. & P. Lindberg: Toxicity of halogenated oxyquinolines to dogs. The effect of feeding fouled herring. Acta vet. scand. 1972 b, *13,* 441—442.

Lannek, B. & P. Lindberg: Toxicity of halogenated oxyquinolines in dogs. A clinical study. IV. Tissue distribution and elimination in urine. Acta vet. scand. 1974, *15,* 419—435.

Mentzing, L. O. & O. Ringertz: Salmonella infection in tourists. 2. Prophylaxis against Salmonellosis. Acta path. microbiol. scand. 1968, *74*, 405—413.

Müller, L. F.: Die Mexaformvergiftung des Hundes (Klinische Demonstration). (The mexaform poisoning in the dog. Clinical demonstration). Kleintier-Prax. 1967, *12*, 51—52.

Palm, A.: Untersuchungen in der Chinolinreihe. X. Mitteilung: Dijodverbindungen. (Investigations of the quinoline derivatives. X. Communication: Diiodo compounds). Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. 1932, *166*, 176—185.

Püschner, H. & R. Fankhauser: Neuropathologische Befunde bei experimenteller Vioform-Vergiftung der weissen Maus. (Neurological symptoms after experimental Vioform poisoning in the white mouse). Schweiz. Arch. Tierheilk. 1969, *111*, 371—379.

Reichard, H.: Ornithine Carbamyl Transferase in dog serum on intravenous injection of enzyme, choledochus ligation, and carbontetrachloride poisoning. J. Lab. clin. Med. 1959, *53*, 417—425.

Ritter, P. & M. Jermann: Vergleichende Untersuchungen über Resorption und Stoffwechsel einiger therapeutisch angewendeter 8-Hydroxychinoline. (Comparative study on the absorption and the metabolism of some 8-hydroxyquinolines for therapeutic use). Arzneimittel-Forsch. 1966, *16*, 1647—1652.

Sigma Technical Bulletin No. 505. The colorimetric determination of glutamic-oxalacetic and glutamic-pyruvic transaminases. 1967.

Schantz, B. & B. Wikström: Suspected poisoning with oxyquinoline preparations in dogs. Svensk Vet.-Tidn. 1965, *4*, 106—107.

Tateishi, J., S. Kuroda, A. Saito & S. Otsuki: Myelo-optic neuropathy induced by clioquinol in animals. Lancet 1971, *2*, 1263—1264.

SAMMANFATTNING

Toxiciteten av halogenerade oxikinoliner hos hund. En klinisk studie. III. Intoxikationsförsök.

Oxikinolinpreparat, vilka normalt tolereras väl av hundar, kan framkalla sjukdom när hundens resistens tillfälligt är minskad. Författarna har försökt framkalla sjukdomsmönstret vid spontan oxikinolinförgiftning i en försöksserie på hundar. Flertalet av dessa försök, även med enstaka eller upprepade höga doser givna per os, misslyckades. Av en tillfällighet observerades, att absorptionen från tarmen av ^{125}J-märkt vioform ökade, när hundarna hade ätit strax före givan. Intagandet av fett, men ej protein eller kolhydrat, visade sig vara den ansvariga faktorn. När oxikinolin gavs i en fettemulsion, insjuknade ungefär ⅓ av hundarna. Tillsattes rutten strömming, kunde den sjukdomsframkallande dosen sänkas till samma nivå som vid spontan vioformförgiftning. Författarna anser, att substanser av fenolkaraktär, vilka bildas genom nedbrytning av proteiner vid tarmåkommor, „tävlar" med oxikinolinerna vid nedbrytning och elimination.

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