One of several integrated studies of physiological fluoride effects, this series of tests explored the toxic effects in animals as a basis for studies in man. The tests demonstrated that an appreciable amount of fluoride is necessary to produce deleterious effects.

Acute and Subacute Toxicity Studies of Sodium Fluoride in Animals

By NICHOLAS C. LEONE, M.D., ERVING F. GEEVER, M.D., and NEIL C. MORAN, M.D.

CURRENT knowledge of the dosage of fluoride required to produce toxic effects in man is derived principally from suicidal or accidental poisonings (*1–5*). Since the exact doses ingested and absorbed are unknown, interpretation of the relationship between clinicopathological findings and dosage is difficult. The lack of reliable methods for determining minute blood fluoride levels adds to the difficulty of evaluation.

With the more widespread use of fluorides in industry, in agriculture, and in the home, there is need for additional evaluation of acute fluoride effects. Whereas some information is available concerning chronic toxicity, there is less concerning the acute toxic effects in man and animals.

The present study was undertaken to obtain data on the acute and subacute physiological and pathological effects of intravenously and orally administered sodium fluoride in animals. It was also undertaken to determine the safe limits of intravenous administration as an aid to the study of fluoride excretion in man.

The authors are all with the National Institutes of Health, Public Health Service. Dr. Leone is chief of Medical Investigations and Dr. Geever is pathology consultant with the National Institute of Dental Research. Dr. Moran is head of the Pharmacodynamics Section of the Laboratory of Chemical Pharmacology, National Heart Institute.

Technical assistance was given by William M. Butler, Jr., M.S., and James S. Watts, B.S., of the Laboratory of Chemical Pharmacology, and Patricia B. Geiser, M.S., Public Health Service Nurse Officer with the National Institute of Dental Research.

Methods

Two types of animals, dogs and mice, were used in the experiments.

The 27 dogs tested were unanesthetized mongrels of both sexes, weighing from 7 to 10 kilograms. For convenience the dogs were separated into five groups as follows:

Group 1. Five dogs were used for the determination of the acute lethal dose by continuous infusion to the point of death, using a calibrated infusion pump.

Group 2. Nine animals were given sodium fluoride in selected fractions of the acute lethal dose (with the same technique and infusion rate as in group 1) and observed for varying periods.

Group 3. Two dogs were given daily intravenous injections of sodium fluoride for 23 days.

Table 1. Summary of fluoride effects in dogs

Dog group	Dose of fluoride [1] mg./kg.	Rate of intravenous infusion [1] mg./minute	Average body weight (kg.)	Number of dogs	Number of spontaneous deaths	Survival time
Group 1	[2] 36	5.4	8.9	4	4	59–64 minutes.[3]
	31	1.1		1	1	219 minutes.[3]
Group 2	25	5.4	9.3	3	3	1, 18, and 31 hours.
	20	5.4		2	1	36 hours.
						7 days (S).
Group 2	15	5.4		4	0	36 hours (S).
						7 days (S).
						16 days (S, 2 dogs).
Group 3	5	[4] 1	7.9	2	0	23 days (S).

[1] Doses expressed as fluoride ion. [2] Mean of 4 dogs. [3] Death occurred during continuous infusion. [4] Single intravenous injection given daily for 23 days. S=Sacrificed.

Group 4. Four animals were given sodium fluoride in single doses by mouth.

Group 5. Seven untreated dogs were sacrificed by exsanguination to provide normal control material for the pathological anatomy studies.

Under local anesthesia, the femoral artery and vein of the dogs in groups 1 and 2 were cannulated for recording arterial pressure and for intravenous infusion. Blood pressure was recorded with an Anderson glass capsule manometer (*6*). Respiratory rates and electrocardiograms were recorded by routine methods. In all groups, pupil size and reactivity, tendon reflexes, emesis, defecation, urination, and other significant behavioral and neurological changes in the animals were also observed. In groups 1, 2, and 4, blood was taken before and after administration of sodium fluoride for the determination of serum calcium.

The term "acute lethal dose," as used throughout, refers to that dose at which death occurred as the result of continuous intravenous infusion (group 1). The term "subacute lethal dose" arbitrarily applies to that dose of fluoride causing death one or more hours following administration (group 2).

A sterile, pyrogen-free solution of sodium fluoride (10 mg. of F ion per ml.) was used for intravenous administration; sodium fluoride powder was used for oral administration. All doses are expressed in terms of the fluoride ion.

Necropsy was performed as soon after death as possible, in most instances immediately. Animals that did not die as a direct result of administered fluorides were sacrificed by exsanguination following sodium pentobarbital anesthesia. Necropsy was not performed on the animals that received fluoride by mouth. Tissues were fixed in 10 percent formalin for microscopic examination, and routine methods of tissue preparation, paraffin embedding, and hematoxylin and eosin stains were employed. Special fat stains using oil red O-hemalum and

Table 2. Emetic, cardiotoxic, and lethal doses of sodium fluoride during continuous intravenous infusion of group 1 dogs

Experiment dog No.	Dose [1] at which—			Terminal ECG event
	Emesis occurred	Arrhythmias appeared	Death occurred	
1	19.8	29.4	36.0	Asystole.
2	20.8	30.7	36.6	Asystole.
3	20.5	33.6	36.8	VF.
4	17.6	28.7	34.6	VF.
Mean	19.7	30.6	36.0	

[1] Doses expressed as milligrams of fluoride ion per kilogram. All animals infused at a rate of 5.4 mg. per minute.

VF denotes ventricular fibrillation.

Nile blue were made on renal tissue from 7 controls and 11 fluoride-treated dogs from groups 1, 2 and 3.

For mice the oral and intravenous lethal doses (LD_{50}) were determined by using fasted, male, white mice of uniform weight (10 grams). Each dose level was evaluated in groups of 10 or more mice. The LD_{50} was calculated by the method of Litchfield and Wilcoxon (7). The arbitrary end point was 24 hours after administration.

Physiological Effects in Dogs

Group 1

Four dogs were given sodium fluoride by continuous infusion at a rate of 5.4 mg. of fluoride ion per minute to death. The mean acute lethal dose was 36.0±0.5 mg./kg. (tables 1 and 2). An additional animal was infused at a rate of 1.1 mg. per minute to a lethal dose of 31 mg./kg. but is not included in the calculation of the mean acute lethal dose because of the difference in infusion rate.

No significant change in arterial blood pressure occurred until an average dose of 15 mg./kg. was reached. Beyond this point there was a moderate decrease in pulse pressure. From 20 to 30 mg./kg. there was a moderate fall in systolic and diastolic pressure, after which a rapid, progressive fall of blood pressure occurred (fig. 1).

Little change in mean heart rate was noted at low doses; however, after 20 mg./kg. had been given, there was a progressive decline in rate to zero at death (fig. 1).

Consistent electrocardiographic changes did

Figure 1. Respiratory and cardiovascular effects of sodium fluoride administration to group 1 dogs.

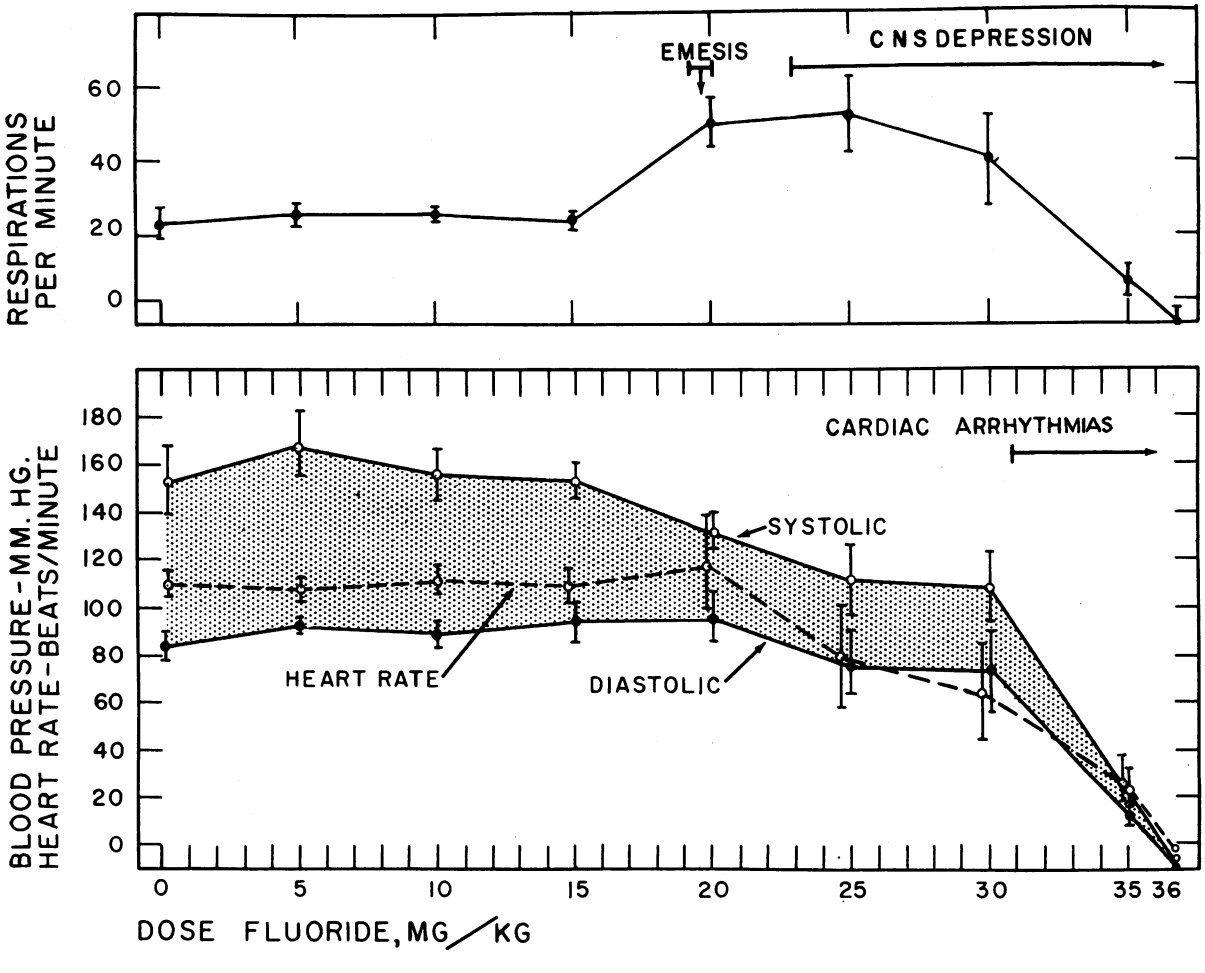
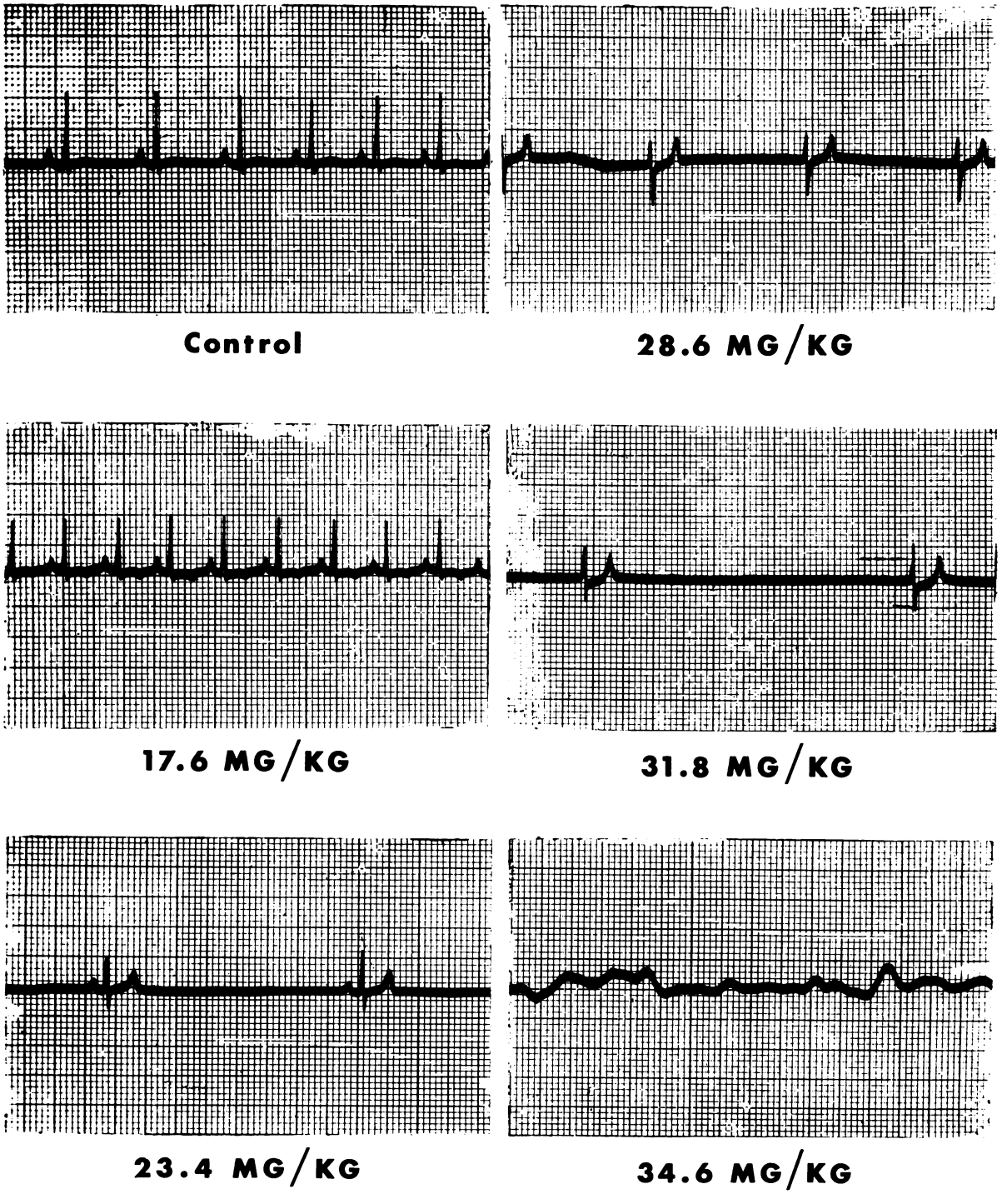


Figure 2. Electrocardiograph tracings (standard limb lead II) show the progressive effects of increasing doses of sodium fluoride on the heart of an unanesthetized dog. The rate of intravenous infusion was 5.4 milligrams of fluoride ion per minute in a 9.2 kilogram dog.



not appear until a mean dose of 30.6 mg./kg. was reached, when there was conversion in every dog to atrioventricular nodal or ventricular rhythm. The terminal cardiac event was either ventricular fibrillation or asystole (table 2). Occasional changes in the amplitude and direction of the T waves and S–T segments were noted before loss of sinus rhythm but were not consistent. Durations of PR, QRS, and QT intervals did not change until abnormal rhythms occurred. Figure 2 illustrates the electrocardiographic tracings of a representative experiment.

On necropsy the hearts were usually found in systolic contracture. When arrested in diastole, slight mechanical stimulation of the heart induced contracture.

Average respiratory rates did not change appreciably until a dose of about 20 mg./kg. was reached, when an increase occurred. From 30 mg./kg. to the lethal dose, a progressive depression of rate was observed (fig. 1) as also was amplitude. In most of the animals there were frequent short periods of tachypnea, often occurring during the phase of central nervous system depression.

Depression of the central nervous system progressing to coma appeared in all group 1 animals at doses of from 23 to 31 mg./kg. Pupil size and reflex response to light were unaffected until moderately severe central depression developed. At that stage dilation and hypoactive reflexes were noted. Terminally, maximal dilation and areflexia of the pupils occurred. The corneal reflex persisted until the period immediately prior to death. Depression of tendon reflexes paralled the central depression. Convulsions were not observed in the animals of this group in which the infusion rate was fast. However, in the one case with a slower infusion rate, a stage of neuromuscular hyperexcitability was prominent in the dose range of from 22 to 25 mg./kg. Characteristic of this phase were hyperactive tendon reflexes with clonus, tonic convulsions, and muscle fasciculations. This stage merged into the comatose state described in other animals.

Emesis and defecation were consistent effects with a mean emetic dose of 19.7±0.8 mg./kg. (table 2). Defecation usually occurred shortly before or immediately after emesis.

Group 2

The dogs in group 2 were infused at the rate of 5.4 mg. per minute. The infusions were stopped at arbitrarily chosen fractions of the acute lethal dose of group 1 (15, 20, 25 mg./kg.), and the animals were observed until death or sacrifice.

Three dogs which received 25 mg./kg. of fluoride died within 1 to 31 hours after the end of the infusion. One of the two dogs given 20 mg./kg. died at 36 hours, while the other survived until sacrificed at 7 days. None of the four animals administered 15 mg./kg. died as a result of the fluoride. An approximate subacute lethal dose 50 (LD_{50}) of 20 mg./kg., therefore, might be assigned to fluoride when given by this method of administration. Table 1 summarizes the results in this group.

Up to the point where the infusions were stopped in the group 2 animals, the cardiovascular and respiratory effects were similar to those of group 1 (fig. 1). There were no consistent electrocardiographic changes in these animals although recordings were not obtained in the period immediately preceding death in those animals which succumbed to the fluoride.

Depression of the central nervous system began before completion of the infusion in all three dogs given 25 mg./kg. and deepened progressively until death. Similar depression was observed in the animal that died as a result of 20 mg./kg. of fluoride. All of the other group 2 animals passed through a short stage of reduced activity, but no severe depression was observed.

Emesis occurred in all of the animals that received 20 or 25 mg./kg. Two of the four dogs receiving 15 mg./kg. of fluoride vomited about 30 minutes after cessation of the infusion.

Group 3

Group 3 consisted of two dogs given fluoride by daily intravenous injections of 5 mg./kg. for 23 days. Blood pressure and respiratory rates were not recorded. The animals remained in good condition with no evidence of toxic effects or weight loss. Electrocardiographs remained normal. The results are summarized in table 1.

Group 4

The four animals in group 4 were each given a single dose of sodium fluoride by mouth in powder form. One received the compound mixed in food and the others in gelatin capsules. Doses of 38, 81, 260, and 3,100 mg./kg. of fluoride were given. The main effects observed were vomiting and frequent defecation. In one case (3,100 mg./kg.) the vomitus was grossly bloody and the animal was mildly stuporous for a short period. In every dog in this group there was apparently complete recovery in 18 to 24 hours.

Physiological Effects in Mice

The oral LD_{50} with standard error was 46.0± 1.6 mg./kg. as compared with an intravenous LD_{50} of 23.0±0.9 mg./kg. (table 3). Mice dying within 2 hours after injection showed successively cyanosis, dilatation of ear vessels, depression of respiration, tremors, clonic convulsions, paralysis of the hind legs, loss of righting reflex, depression, respiratory arrest, and death. Those with longer survival periods (2 to 24 hours) went through similar, but less severe, stages, progressing to a long terminal depression.

Serum Calcium and Blood Clotting

The effects of fluoride on serum calcium were determined on 11 dogs (table 4). Whole blood was drawn for serum calcium determinations prior to infusion and again immediately before death in the group 1 animals. In the group 2 animals, blood was drawn prior to and at the end of infusion. Blood was drawn from 1 animal in group 4 approximately 1 hour after administration. There was a slight drop in serum calcium in 9 of 11 animals. This finding has been reported by others (*3*, *8*, *9*).

Although no clotting-time determinations were done, soft, friable, poorly contracted clots were observed in blood samples drawn after fluoride administration.

Pathological Anatomy of Dogs

The only gross pathological changes noted in dogs were generalized hyperemia in the animals that died after fluoride administration, whether acute or delayed and, in addition, focal hemorrhages in those dogs that died from 18 to 36 hours after fluoride administration.

Table 3. Intravenous and oral lethal dose determinations of sodium fluoride in mice

Dose of fluoride [1] mg./kg.	Ratio of deaths to mice used	Percent deaths
Intravenous [2]		
18	0/10	0
21	3/10	30
24	7/10	70
27	9/10	90
36	10/10	100
48	10/10	100
Oral [3]		
24	0/10	0
36	1/10	10
42	1/10	10
48	3/10	30
54	4/10	40
60	10/10	100
72	10/10	100
84	9/10	90

[1] Expressed as fluoride ion.
[2] Intravenous LD_{50}=23.0±0.9 mg./kg.
[3] Oral LD_{50}=46.0±1.6 mg./kg.

Table 4. Effects of fluoride on serum calcium in 11 dogs

Group	Number of dogs	Dose of fluoride [1] mg./kg.	Serum calcium mg./100 cc.	
			Prior to fluoride administration	Immediately after fluoride administration
1	3	[2] 36	8. 94 6. 51 8. 00	7. 23 7. 75 6. 70
2	3	[2] 25	11. 15 8. 50 8. 60	9. 65 6. 50 7. 20
2	2	[2] 20	10. 30 9. 70	10. 30 9. 40
2	2	[2] 15	9. 20 9. 40	8. 60 8. 20
4	1	[3] 3, 100	8. 50	7. 90

[1] Expressed as fluoride ion.
[2] Intravenous administration.
[3] Oral administration.

Microscopically, all animals that died after fluoride administration and one surviving until sacrificed at 36 hours showed generalized hyperemia and acute focal hemorrhages. All other animals showed some focal hyperemia and focal hemorrhages, but the conditions were no more severe than those in the control group.

Although special attention was given to brain, heart, liver, kidneys, lungs, gastrointestinal tract, and bone marrow, no other histological evidence of cellular injury could be found in any animal. Differences in fat concentration were found, but variations were as great among the controls as among the animals exposed to fluoride. The fat appeared to be liberated from the cytoplasm of the tubular epithelium, particularly along the cortico-medullary junction.

Incidental findings, common to the treated and the control dogs, included roundworm and tapeworm infestation of the intestinal tract and microscopic acute and chronic nonspecific inflammation of the lungs, kidneys, liver, and, less often, of the myocardium.

Discussion

On the basis of the present study several points stand out in regard to the acute toxicity of sodium fluoride in dogs.

First, the doses of intravenously administered fluoride required to produce acute toxic effects in animals are high, and there is no evidence of cumulative effects on daily administration of sublethal doses for a period of 3 weeks.

Second, the more uniform effects with a constant rate of intravenous infusion of sodium fluoride are in contrast to the more erratic results and difficulty in producing toxic effects with oral administration.

Third, the physiological effects and pathological changes seen in dogs given toxic doses of fluoride resemble those reported in human fluoride poisoning (*1–4*).

The greater uniformity of results seen in groups 1 and 2 on intravenous administration is most evident in the relatively precise doses at which the various physiological effects occur. In contrast, the erratic results observed when fluoride is given to dogs by mouth are largely due to the loss of undetermined amounts of the administered dose through vomiting.

Diarrhea and bloody stools were observed in some animals in which death was delayed from 1 to 36 hours. Similar findings have been reported in human cases (*1*, *3*, *4*).

The vasodepressor action of fluoride seen in this study has been demonstrated by Greenwood and associates (*10*). It probably is due to a combined depression of the vasomotor centers of the brain and of the vascular smooth muscle. The cardiac arrhythmias, resembling those resulting from high serum potassium, are probably caused by enhanced sensitivity of the myocardium to potassium, secondary to decreased serum calcium concentration. The terminal systolic contracture is of particular interest in view of Loewi's recent observations of a positive inotropic effect of sodium fluoride on the depressed frog heart (*11*). He suggests this action is caused by the formation of a complex of fluoride, a cellular constituent and calcium, and that this complex restores the cellular membrane excitability to normal. However, no conclusive evidence is available to suggest a comparable action in mammalian hearts.

The central nervous system and neuromuscular effects of sodium fluoride may be related to two actions: one, a reduction of ionizable calcium through a fluoride binding effect, resulting in a state of neuromuscular hyperexcitability, that is hypocalcemic tetany, and, second, a depression of the central nervous system as a possible result of enzyme inhibition (*12*).

Routine pathological studies did not demonstrate a specific mechanism of death in experimental fluoride exposure, but at the levels used in this study, and with the short interval to death in many instances, direct toxic cellular effects cannot be ruled out despite the lack of histological evidence of injury. The gross and microscopic pathological examination showed only generalized congestion and acute focal hemorrhages. Similar changes in man and animals have been described for deaths due to fluoride poisoning (*1*, *4*, *13*, *14*,). These changes are interpreted as resulting from injury to vascular endothelium.

Fatty changes have been described in the

tubular epithelium of rat kidneys following prolonged administration of sodium fluoride (*15*). In our dogs, shift of renal fat was noted, but no consistent pattern could be found on comparison with the normal controls.

Reviewing the results of this study, it is interesting to compare the findings in animals with human cases of fluoride poisoning (*1–4*). Outstanding is the study of Lidbeck, Hill, and Beeman, who reported a mass accidental poisoning of 263 persons with 47 fatalities (*1*). They described respiratory and cardiovascular depression as a common finding in addition to a number of other manifestations also observed in the present study. An example is: "General collapse developed in most instances but at variable periods of time, apparently depending upon the concentration of the poison. This was characterized by pallor, weakness, absent or thready pulse, shallow, unlabored respiration, weak heart tones, wet cold skin, cyanosis, and equally dilated pupils. When this picture was pronounced, death almost invariably occurred." This description closely follows the findings in our animals receiving a fatal dose of fluoride.

In the present study and the clinical cases described by Lidbeck and associates, convulsions were not regularly observed. However, the carpopedal spasm and spasm of the extremities, described by these authors in a few patients who recovered or where death was delayed, resembled the neuromuscular hyperexcitability seen in one dog in the present study. These effects may be manifestations of hypocalcemic tetany. Generalized convulsions which have been described by others could be on the basis of cerebral anoxia or severe hypocalcemia (*2*).

On a practical basis, the data from this study may be of value in the evaluation and treatment of cases of acute fluoride poisoning in man. For instance, electrocardiographic demonstration of a cardiac arrhythmia in the absence of known heart disease would suggest that about three-quarters of the acute lethal dose had been absorbed and would imply poor prognosis. Central nervous system depression to a marked degree might also denote the absorption of more than half of a lethal dose.

The intravenous lethal dose of fluoride determined in the present study agrees well with those previously reported if differences in injection rates and in species are considered (*10, 16, 17*).

This information, establishing the relatively high dosage of fluoride required to produce acute toxic effects in animals, suggests that small intravenous doses may be used with safety for human studies. The absence of toxic effects in dogs given 5 mg./kg. of fluoride intravenously daily for 3 weeks also suggests a considerable margin of safety and an apparent absence of accumulative toxic effects in this period of time.

Summary

In this limited study, designed to evaluate the acute toxic effects of sodium fluoride in dogs and mice, it was demonstrated that:

1. The mean acute lethal dose of sodium fluoride in unanesthetized dogs infused to death by continuous intravenous infusion at the rate of 5.4 mg. of fluoride ion per minute was 36.0± 0.5 mg./kg. The principal effects were progressive depression of blood pressure, heart rate, and central nervous system with vomiting and defecation, all occurring with the administration of approximately 20 mg./kg. At a mean dose of 30.6 mg./kg. there was a depression of respiratory rate and a conversion to atrioventricular nodal or ventricular rhythm with terminal ventricular fibrillation or asystole.

2. In a group of dogs infused intravenously with selected fractions of the acute lethal dose, an approximate LD_{50} was estimated to be 20 mg./kg. The major effects observed in this group were vomiting, defecation, and central nervous system depression. In the fatal cases death occurred in 1 to 36 hours.

3. In a group of dogs given 5 mg./kg. intravenous injections daily for 23 consecutive days, no toxic effects were observed.

4. In dogs, single doses up to 3,100 mg./kg. of fluoride by mouth produced only vomiting, defecation, and transient moderate depression.

5. The intravenous LD_{50} in mice was 23.0± 0.9 mg./kg. and the oral LD_{50}, 46.0±1.6 mg./kg.

6. A slight drop in serum calcium followed the infusion of fluoride in most dogs in which serum calcium was determined.

7. The pathological findings in those animals which died directly as a result of sodium fluoride administration consisted of generalized hyperemia and acute focal hemorrhages.

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Inventory of Water Needs

At least one of every four large urban areas have reported they need additional water supplies to meet anticipated municipal and industrial growth, according to a nationwide inventory taken by the Water Supply and Water Pollution Control Program of the Public Health Service.

Of 1,532 community water facilities, 367 serving a population of nearly 20 million need additional water supplies. About one-fourth of the water supply facilities need additional distribution systems, partly because of the growing practice among municipalities to provide water service to suburban areas. More than half of the facilities need improvement or enlargement.

The inventory covered all facilities serving 10,000 people or more and a 40-percent sample of facilities serving between 5,000 and 10,000 people. It lists population served, source of supply, treatment provided, capacity, storage, and the improvements which local officials consider necessary to maintain satisfactory service. Additional details are available from the Water Supply and Water Pollution Control Program, Public Health Service, Washington 25, D. C.