SYMPTOMS AND LESIONS IN DOGS AND RATS FOLLOWING PARENTERAL ADMINISTRATION OF FURACIN (R)

by

KADE-GOWDA THIMMAIAH

B. Sc. (Vet), Bombay University, Bombay, India, 1953.

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Physiology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1962

TABLE OF CONTENTS

INTRODUCTION

Investigations concerning the cellular changes which may occur after the administration of nitrofuran compounds is not a new one. Dodd (10) began his experiments in 1946 when he demonstrated the actions of nitrofuran compounds *in vitro* and *in vivo*. Krantz (23) employed mice to find out the pharmacodynamic actions of nitrofurazone in 1945 and reported little change in the hepatic cells. Featherstone, *et al.* (12) reported that the degeneration of seminiferous tubules occurred in rats after feeding with nitrofuran compounds. Nissim (33) utilized mice to find out the cellular alterations which might occur due to increased dosage of nitrofurazone and reported that the tissue alterations increased with increased dosages. Friedgood (14) and Wildermuth (56) employed nitrofuran compounds on an experimental basis to elicit the growth suppressive action on testicular carcinoma and seminoma. They reported that the growth suppressive action on these cancerous growths was observed.

Hammond (17) conducted an experiment to determine the action of nitrofurazone as a coccidiostat against *Eimeria bovis* in calves. When higher doses were administered, death followed, and cellular degeneration of liver and kidney was observed. Cooper, *et al.* (8) and Francis (13) conducted experiments separately to evaluate the efficiency or the side effects of nitrofurazone which produced atrophy of the testes and of the seminiferous tubules. Wildermuth in his personal communication to Robertson (42, 43) reported that in a human case, which was treated by employing nitrofurazone, he did not get the spermatogenesis to a normal condition even a year after treatment.

In human medicine the nitrofuran compounds, especially Furadantin (R), was used orally, as well as, intravenously against genitourinary infections and systemic diseases. Furadantin (R) was employed on a controlled basis which

produced the growth arrest of spermatozoa. Dodd (10) in 1946, when he tried to find out LD_{50} in rats and mice, administered nitrofurazone starting from low to a very high dose, orally as well as parenterally and observed cellular changes in the vital organs like liver and kidney. The cellular changes were more usual in animals which received nitrofurazone by the parenteral route. The cellular changes were almost complete loss of the normal architecture with a swelling of all the cells and a very marked cytoplasmic degeneration with considerable nuclear degeneration. The kidney revealed a marked degeneration of the tubular epithelium, particularly in the loops of Henle and the central portions of the tubules contained a precipitated protein material.

Purpose of study. Dodd in 1946 (10) showed that Furacin (R) was active against a number of gram positive and gram negative organisms, Treponema pallidium and Trypanosoma equiperdum. Evans (11) and Robertson (43) demonstrated that other trypanosomes were also susceptible to nitrofurazone. They used nitrofurazone for the treatment of Sleeping Sickness and obtained very significant results by oral administration. The experimental design was employed to elicit whether oral administration might produce results not only in trypansomiasis, but also in some other systemic diseases and the results were very encouraging. If nitrofurazone is employed by parenteral route, the percentage concentration in the plasma or blood or other body fluids may increase as it is obtained in case of nitrofurantoin (15). If nitrofurazone is to be employed by parenteral routes, the therapeutic and toxic dosages should be evaluated.

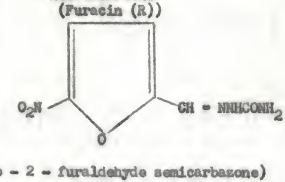
The experiments herein reported, were designed and conducted to investigate the cellular changes which might occur at different dosage levels of nitrofurazone by parenteral administration in rats and dogs.

LITERATURE REVIEW

Nitrofuran compounds (nitrated furans) are synthetic chemical agents which like sulfonamides and other chemical drugs are used to cure, prevent or alleviate diseases in animals and man. It is one of the chemical agents or drugs, which is in an equal position with several other chemical drugs and antibiotics to therapeutically treat diseases like genitourinary infections, coccidiosis, enteritis and trypanosomiasis (Sleeping Sickness).

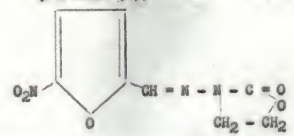
History. Active investigations of the furan compounds began in 1940 (37). Furfural is an agricultural by-product from corncobs and oat hulls. The addition of a nitro group in the five positions of the furan ring confers a high degree of antibacterial activity to the structure. The addition of various side chains, such as semicarbazone, amino-oxazolidone, or aminohydantoin, structures, controls to a major degree the physiological or pharmacological action of the nitrofurans. Many compounds were prepared, but very few were evaluated for their actions, absorption, and exertion. Today, three are in great use in veterinary medicine as well as in human medicine. The structural formulas for these nitrofurans are presented below:

1. Nitrofurazone (Furacin (R))



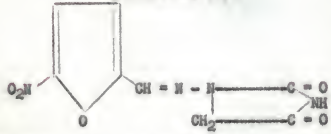
(5 - Nitro - 2 - furaldehyde semicarbazone)

2. Furazolidone (Furoxone (R))



(N - (5 - Nitro - 2 - furfurylidene) - 3 - amino - 2 - oxazolidone)

3. Nitrofurantoin (Furadantin (R))



(N - (5 - Nitro - 2 - furfurylidene) - 1 - aminohydantoin)

Furacin (R), Furoxone (R), and Furadantin (R) are registered trade names of Eaton Laboratories.

The primary factor for the wide spread use of the nitrofurans has been their remarkable effectiveness as chemotherapeutic agents against a large number of microbial organisms (10). The characteristics of the nitrofurans are responsible for their acceptance for different types of diseases. They are as follows:

1. A wide spectrum of activity against both gram positive and gram negative bacteria and in some instances against protozoa and fungi (10).
2. Bacterial action with little resistance on continued application (47).
3. In the recommended concentrations the nitrofurans have low toxicity to host tissues (15, 55). They are not protoplasmic poisons, rather they act by interrupting the enzymatic metabolic processes of the microbial

cell, preventing cell multiplication. Available evidence suggests that nitrofurans act by interfering with a key step of the carbohydrate cycle by which cells obtain their energy, hence, literally starving the invader (23). 4. The nitrofurans do not inhibit phagocytosis or retard healing. As such, the nitrofurans are used as preventive, curative or suppressive agents and in some cases as growth stimulating agents when the efficiency of feed utilization is of critical importance (1, 2, 24, 44).

Properties of nitrofuran compounds. Nitrofurazone is a lemon-bright yellow, odorless, crystalline powder which decomposes between 227° and 241° depending upon the speed of heating. One gram dissolves in about five liters of water. It is less soluble in ether, but dissolves in alcohol 1:740 and in propylene glycol 1:350 (23). The compound possesses bacteriostatic and bactericidal properties in solution dilutions of 1:2000,000 to 1:3000,000 and is inhibitory to bacterial growth and bactericidal at 1:50,000 to 1:75,000 dilutions in vitro. Nitrofurazone for soluble dressing is a 0.2 percent concentration in water soluble ointment like base. It is utilized for topical application in the prophylaxis and treatment of superficial mixed infections common to contaminated wounds, burns, ulcerations, and skin diseases. The soluble dressing has been used as an adjuvant in surgery for skin grafting and treatment of osteomyelitis. Organisms resistant to the sulfonamides, penicillin, and streptomycin may be susceptible to nitrofurazone.

Nitrofurazone solution is a light yellow, somewhat viscous liquid containing 0.2 percent nitrofurazone in polyethylene glycol solvent. This solution was employed in Experiment 1. Nitrofurazone is prepared in tablet form with dextrose as excipient. They are available in 50 mg, 100 mg, and 200 mg for oral administration.

Furazolidone is a yellow crystalline powder which is mixed in dry food to feed animals and birds to control, prevent, or cure many infections.

Nitrofurantoin is a yellow bitter powder with a slight odor and is prepared in tablet form for oral administration and 0.6 percent solution in polyethylene glycol in 10 c.c. vials which is to be diluted with glucose or saline for parenteral use (15).

Methods of analysis. Methods for analysis of nitrofurans are based primarily either on their physical characteristics or their antimicrobial activity. Most physical methods are based on ultraviolet light absorbing characteristics of the nitrofurans. The use of a narrow band spectrophotometer permits measuring the ultraviolet absorbing characteristics of a nitrofuran solution. It furnishes a widely employed method of laboratory analysis, identification and establishment of purity. This procedure is the basis for one of the assay methods for nitrofurans in feeds.

A modified procedure by Buzard (6) has been published in which nitrofuran is converted to a bright red phenylhydrazone permitting the use of less expensive wide band electric photometer. Special modifications of analytical methods have been utilized and are available for blood, serum, tissue, and other body fluids. Recently a chromatographic procedure for Furadantin (R) in urine has been developed.

The spectrophotometric methods have not been applied to bile. Bacterial assays have proven useful to confirm the spectrophotometric analysis by a biological assay for the active nitrofuran compounds. E. coli and K. pyogenes have been used for either broth dilution or cup-plate assay techniques using a series of graded reference nitrofuran standards. It was suggested that the use of both spectrophotometric and microbiological techniques may be better methods as a means of avoiding errors not readily apparent with either

technique alone.

Absorption, distribution and elimination of nitrofuran compounds. Busard in 1960 (7) reported that nitrofuran compounds are absorbed to a maximum extent in the small intestine, less in the colon and least absorption in the stomach and cecum. The experiments were conducted on rats by removing the digestive system part by part. Nitrofurantoin was utilized by oral administration via a stomach tube.

Paul, (36, 38) and Busard (6, 7) worked out the distribution of nitrofuran compounds in the various body fluids and tissues and the elimination from the body. Several nitrofuran compounds were employed by different routes of administration in different forms. The experimental animals utilized were rats, dogs, and other laboratory animals. These compounds were prepared in the form of suspension by using 0.7 percent carboxymethyl cellulose and water. Blood was withdrawn by cardiac puncture under ether anesthesia at the indicated time. The doses administered to these laboratory animals represent the range from a human therapeutic dose (8 - 10 mg/kg) to an acute toxic dose in laboratory animals. Typical toxic effects including convulsions and occasional deaths were observed at 500 mg/kg level. It is of interest to note that no detectable plasma concentration was found four hours after administering a dose of 10 mg/kg (human therapeutic dose of any of these compounds).

Nitrofuran compounds are distributed in all the body fluids in varying concentrations. In certain body fluids the concentration of nitrofuran compounds are in the concentration which is quite sufficient to produce bacteriostatic action. This work was very extensive and exhaustive, and the results which they obtained are given in Table 1.

Table 1. Plasma levels, recovery in urine and recovery in feces of selected nitrofurans given orally in rats.

Compound	Solubility in water mg/L	Dose mg/kg	Time after dose in hours	Blood level mg/L	Urine Dose mg/kg	Urine Amount percent of dose	Feces Dose mg/kg	Feces Amount percent of dose
Nitrofurazone	210	100	4	4.5	200	4.6	200	0.5
Nitrofurantoin	130	100	4	2.6	25	52	25	2.0
Furazolidone	40	100	4	2.5	600	Trace	600	13.0

Urinary excretion varies with the compound from little or none up to 75 percent of the administered dose. This is in line with the work reported by Paul (38). Some nitrofurans resisted degradation and appeared in the feces, but fecal excretion never accounted for a large portion of the compound administered. The nitrofuran compounds were destroyed in the digestive tract, when they are mixed with food materials or fecal suspension. Nitrofuran compounds are distributed in the bile, milk, cerebrospinal fluid and others. Nitrofurazone is distributed in cerebrospinal fluid to the extent that it will be able to destroy trypanosomes in Sleeping Sickness (42). Nitrofurantoin is distributed in the bile to the extent that it is capable of acting against bacteria there. After nitrofuran compounds are absorbed they are concentrated in the plasma. Certain percentages which are present in the plasma will be bound to plasma proteins and the protein binding percentage differs with different compounds. Plasma protein binding percentage was observed and evaluated (36).

Plasma Protein Binding of Various Nitrofurans

Compound	Average Plasma Binding Percent
Nitrofurazone	34
Furazolidone	30
Nitrofurantoin	53

Destruction of the nitrofurans. Some of the nitrofurans are converted in whole or in part by cellular action to biologically inactive structures which retain identical or similar spectral absorption characteristics (37). Nitrofurans are rapidly metabolized or broken down by all living tissues with the exception of blood (36). The reduction of the nitro group is thought to be an early step in the degradation of nitrofuran compounds. The end products vary from one nitrofuran compound to another. Paul (36) demonstrated that it was not cumulative in the cellular tissues. The absence of tissue concentrations of the nitrofurans utilized at therapeutic doses indicate the probability of degradation in the cellular tissues.

Several tissues when incubated with nitrofurazone and furazolidone apparently degrade the two nitrofurans at a somewhat similar rate. This could be interpreted to mean that the nitrofurans are destroyed by an enzyme common to most tissues or that they are susceptible to the action of several enzymes. The rate of breakdown indicates that furazolidone destruction is at the rate of at least 300 microgram/gm of tissue per hour (or 136 mg/lb of tissue per hour). The nitrofurans do not appear to be readily destroyed when incubated with mammalian blood, but they are readily destroyed when incubated with fecal suspension (36).

Action upon bacteria. It is believed that the nitrofuran compounds act upon bacteria by entering the dehydrogenase system. Nitrofuran compounds inhibit a step in bacterial carbohydrate catbolism and make them to die by starvation.

Pharmacodynamic action of nitrofuran compounds. Krantz et al. (23) conducted blood pressure studies under ether anesthesia and pentobarbital-sodium anesthesia. Saturated solutions of nitrofurazone in propylene glycol produced slight, transient rise in carotid blood pressure. Injections were calculated

on the basis of 0.5 c.c./kg. No changes in blood pressure were observed that could not be produced by the injection of solvent alone. Aqueous suspensions, 0.5 and 1 percent in 6 percent acacia were injected on the basis of 0.5 to 1 c.c./kg. These injections produced depressed responses upon repeated injections. However, as these changes were produced by similar substances like talc which are insoluble particles, the depressed action was considered due to such substances. No significant changes in rate or amplitude of respiration were recorded that could be attributed to the vascular effects of the injection of insoluble particles.

Electrocardiogram leads I, II, and III were taken under anesthesia and shortly after the injections of 0.5 percent suspensions of nitrofurazone. There appeared to be no significant difference in the regularity or form of the tracings. Nitrofurazone was agitated with Locke-Ringer's solution until saturated. This solution was perfused through the frog's heart. The experiments demonstrated no change in the heart rate, regularity or amplitude upon perfusion with the saturated solution of the furan derivative.

The defibrinated blood was mixed with Locke-Ringer's solution and perfused through the aorta. Electrocardiographic tracings were produced and recorded. At intervals, 5 c.c. volumes of saturated solution of nitrofurazone in Locke-Ringer's solution were introduced into the perfusion fluid with no changes in rate, regularity or amplitude of the heart beat.

Experiments and application. The first and elaborate experiment which was conducted by Dodd in 1944 (9) was published in 21 languages from 32 countries (37). Dodd utilized several furan compounds to elicit their pharmacological actions against pathogenic bacteria. He employed the following test organisms: (1) Staphylococcus aureus, (2) Streptococcus hemolyticus, (3) Diplococcus pneumoniae, (4) Eberthella typhi, (5) Escherichia coli, and (6) Pseudomonas pyocyanea.

Dodd demonstrated that out of twenty-five, twenty-four showed bacteriostatic activity in varying degrees, but Pseudomonas pyocyana showed resistant activity against these compounds. He showed that these compounds are active against both gram positive and gram negative organisms. Experiments utilizing subcultures and plate counts showed nitrofuran to be bacteriostatic in lower concentration and bactericidal in higher concentrations in 24 hours duration.

During this period of 1944 (37) when World War II was in progress, vigorous research was completed to ascertain better drugs to treat chronic refractory wounds which were very resistant to sulfonamides and antibiotics. The nitrofurazone was employed with very good results, as healing was speeded. Later, it was utilized in treatment of cervicitis, vaginitis, and urethritis and for sterilizing the surgical area prior to operations.

Dodd in 1946 (10) conducted an experiment by employing nitrofurazone, only. Using mice and rats as experimental animals, he studied: (1) the action of this compound in vitro and in vivo by varying the concentration of solutions; (2) the LD_{50} dose by oral and subcutaneous administration; (3) the toxic symptoms which might occur; and (4) the organs affected relative to the histopathology of vital organs.

The data in Table 2 indicate that nitrofurazone has a bacteriostatic action against a number of gram positive and gram negative organisms in rather low concentration. In experiments on mice and rats in vivo, Dodd employed: (1) S. aureus; (2) S. hemolyticus; (3) Salmonella; (4) Treponema pallidium; and (5) Trypanosoma equiperdum.

The organisms and protozoa became sensitive to this compound and 60 to 70 percent of animals survived by applying various dosages either by oral or subcutaneous administration of a single dose of 150 to 200 mgm/kg of body weight.

Application in human medicine. Friedgood (15) conducted treatment and bacteriological studies and in vitro tests in 26 cases. Furadantin was utilized

Table 2. In vitro study of bacteriostatic and bactericidal action of nitrofurasone on the following organisms and concentrations in broth was utilized.

Organisms used	Maximum bacteriostatic concentration in 24 hours	Maximum concentration producing complete inhibition for 4 days
1. S. aureus	1 - 100,000	1 - 80,000
2. S. hemolyticus	1 - 100,000	1 - 10,000
3. S. viridans	1 - 5,000	1 - 5,000*
4. S. faecalis	1 - 40,000	1 - 5,000
5. S. anhemolyticus	1 - 40,000	1 - 10,000
6. D. pneumoniae	1 - 40,000	1 - 5,000*
7. N. gonorrhoeae	1 - 100,000	1 - 100,000
8. N. intracellularis	1 - 100,000	1 - 40,000
9. Ps. pyocyanea	1 - 5,000*	1 - 5,000*
10. E. coli	1 - 100,000	1 - 80,000
11. S. schottmuelleri	1 - 100,000	1 - 100,000
12. S. paratyphi	1 - 100,000	1 - 100,000
13. E. typhosa	1 - 200,000	1 - 100,000
14. S. dysenteriae	1 - 200,000	1 - 100,000
15. Proteus vulgaris	1 - 20,000	1 - 10,000
16. Cl. welchii	1 - 20,000	1 - 5,000
17. Cl. tetani	1 - 20,000	1 - 5,000
18. Cl. novyi	1 - 200,000	1 - 40,000
19. tuberculosis (var-hominis-607)	1 - 200,000 (48 hours)	1 - 5,000
20. tuberculosis (Gary)	1 - 500,000 (30 days)	1 - 40,000 (30 days)

*Maximum solubility in broth.

to treat 26 patients with severe bacterial infections, including peritonitis with overwhelming bacteremia, acute pyelonephritis, cystitis, endocarditis, cholecystitis with cholangitis and others. All of the cases had some previous treatment with other antibiotic, and 17 cases had unsuccessful previous intensive therapy. The bacteria which were isolated were as follows: (1) Proteus sp; (2) Escherichia coli; (3) Pseudomonas sp; (4) Aerobacter aerogens; (5) Micrococci; (6) Enterococci, (7) Streptococcus hemolyticus, and (8) Streptococcus faecalis.

The bacteria were sensitive to the in vitro test. Most of the infections were caused by Proteus sp. showing in vitro resistance to most or all other antibiotics, but sensitive to nitrofurantoin. The 0.6 percent solution of nitrofurantoin in polyethylene glycol was diluted with about 20 volumes of 5 percent glucose and administered by intravenous drip. In three of the patients concomitant antibacterial therapy was employed; chloramphenicol parenterally in two and nitrofurazone topically in one.

All of the cases demonstrated prompt clinical improvement. Blood and urine cultures became sterile in all but four patients. Extraneous complications caused the death of two patients some days after therapy had been discontinued following cure of the infection.

Other workers (21, 25, 27, 46) conducted the in vitro test to compare the action of nitrofuran compounds with sulfathiazole and antibiotics, which showed that when the nitrofurans were employed for therapeutic purposes, they gave good results even though the cases were refractory to sulfonamides and antibiotics. Experiments were conducted in the animal field employing in vitro and in vivo tests utilizing the sulfonamides and antibiotics in comparison with nitrofuran compounds. Nitrofurazone and Furazolidone gave good results (31, 45).

Nitrofurazone was used in vivo against Trypanosoma equiperdium and was found to be sensitive to the action of nitrofurazone (10). Nitrofurazone was tested in search to find a better drug in curing refractory cases of Sleeping Sickness. Packchanian (35) tried in vivo experiments on mice against Trypanosoma gambiense and Trypanosoma rhodesiense which showed that it was having a curative effect. Packchanian used 150 to 450 mgm/kg body weight and observed the result of cure from 22 to 100 percent. Survival was increased as the dose was increased. Nitrofurazone has also been shown to have a suppressive effect on Trypanosoma cruzi infection in mice.

Nitrofurazone was also tested with good results against Trypanosoma gambiense and Trypanosoma rhodesiense in guinea pigs by Evans (11). Latter nitrofurazone was employed by Evans (11) in a limited number of patients who were having a refractory type of the disease which was not responding to other common trypanosomacidic drugs. He was able to cure some patients and in certain patients the disease was suppressed. He did not record any untowards effects due to nitrofurazone in African negroes. Evans noted that nitrofurazone was effective against trypanosomes in the blood as well as in cerebrospinal fluid. The usual trypanosomacides were effective on trypanosomes in the peripheral blood and some were effective in peripheral blood as well as cerebrospinal fluid, but they were highly toxic with usual dosage for extended periods of time.

Robertson (42) used nitrofurazone to cure Sleeping Sickness which was caused by Trypanosoma gambiense. He noted that the African negroes were susceptible to the hemolytic action of nitrofurazone. Robertson (43) showed that nitrofurazone might be used with caution as in the case of other hemolytic agents. He mentioned that: (1) hemoglobin or hematocrit values should be tested often during nitrofurazone treatment if the person had the hemolytic trait; (2) the

drug should be given in small doses (eg. 0.5 gram daily) and increased according to the reaction; (3) it was advised that the hematocrit value should not be allowed to fall below 25 percent or the hemoglobin to below 8.0 gram per 100 ml; if the hemoglobin or hematocrit value goes down the drug should be stopped immediately; (4) it is also reasonable that the fluid intake also is to be increased after the nitrofurazone is stopped to promote excretion. As the mass of younger insensitive cells are built up during the recovery phase, longer and longer courses of nitrofurazone are tolerated. It was stated that it is advisable to limit the length of an individual course to seven days with an interval of at least a week between each course. The hemolytic action of nitrofurazone and nitrofurantoin in ordinarily harmless doses has been shown to be associated with a unique intrinsic erthrocyte abnormality which occurs similarly with primaquine and other related compounds. The hemolysis is due to erythrocyte glucose-6-phosphate dehydrogenase deficiency trait (3).

The incidence of hemolysis among various tribes as reported by Knight which was quoted by Robertson (42)(43) showed an incidence of about 15 percent among Bantu in the Tororo area of Uganda, 10 percent in American Negroes. Friedgood (15) noted only 2 percent in American Negroes. The coastal area and highland areas of Africa had only 1.7 to 1.9 percent. There was a very low incidence among Caucasians, less than 1 percent in Chinese and Jews.

Nitrofurazone alone and with combination of trypanosomacidic drugs has given good results in treating refractory cases of Sleeping Sickness in Africa. The hemolysis and anemia is assumed to be self limiting in the use of nitrofurazone just as in other cases of hemolytic drugs like primaquine. Recovery from hemolytic reaction takes place inspite of continued administration of the drug. It is considered reasonable to use nitrofurazone in an attempt to cure refractory cases of Sleeping Sickness even if they have the hemolytic trait

especially when no alternative treatment is available. (42, 43)

Nitrofurazone has been reported (14, 56) to have decreased effect upon the growth of Sarcoma. In a comparative study utilizing nitrofurazone and two nitrofuran derivatives, furadroxyl and furadantin, it was found that the nitrofurazone decreased testicular cells formation in rats and carcinoma of the human testes. He observed marked degeneration of the tumor cells, increased fibrosis and decreased cellularity of the tumor in four cases of human seminoma.

Application in large animal diseases. Vigue and coworkers (54) studied the bacteria in the bovine genital tract with a recovery of 27 different bacterial organisms. All of the bacteria were sensitive to the in vitro test. Five hundred and thirty cattle were treated with vaginal inserts containing nitrofurazone. There was qualitative evidence that the total number of organisms were reduced following treatment. The average number of services per conception was 1.38 in the treated and 2.85 in the untreated cows. The average number of days for calving was 385 in the treated and 447 in the untreated animals.

Nitrofurazone with urea (Furea) in the form of tablets (9 to 12 grams) was introduced into the uterus to treat endometritis and other untoward sequelae. Following the insertion of "Furea intrauterine" into the uterus in case of retained placenta of the bovine produced rapid cessation of malodor, discharge and other signs of infection (22). (Furea contains 1.45 percent micro-pulverized nitrofurazone (N.F. Furacin (R)) and 98.55 percent powdered crystalline urea). The action of nitrofurazone differs quantitatively from that of other antibiotics and the sulfonamides. The pathogenic organisms which have become resistant to the latter two classes of drugs remain susceptible to Furacin (R). The drug is harmless topically in therapeutic concentrations to mamalian tissues

and does not inhibit phagocytosis, delay in healing, or epithelization of wounds. It remains antibacterial in the presence of pus, serum or organic detritus and is effective against most gram positive and gram negative pathogenic organisms.

Furacin (R) was introduced into the mammary gland of a cow to treat mastitis by Mire (28). With encouraging results Mire (29) experimented and treated with the colloboration of *in vitro* test for bacteria which may occur in mastitis. Their (Eaton Laboratory) *in vitro* test revealed that the following bacteria were sensitive to Furacin (R).

Organisms	Minimal 24 hour bacteriostatic concentrations of Furacin in broth
Streptococcus agalactiae	1: 20,000
Streptococcus uberis	1:100,000
Streptococcus aureus hemolyticus	1: 20,000
Coliform bacteria	1: 40,000

The *in vitro* experiments results indicated that Furacin (R) was effective in high dilutions against many organisms of mastitis. Ten thousand cases were treated in a period of three years with good results obtained in 78 percent of the cases with acute clinical mastitis and in 90 percent of dry cows with a history of mastitis in their previous lactation period.

Shumard (49) utilized different coccidin-oocysts to elicit the value of Furacin (R) in controlling coccidiosis in lambs. He observed that the disease was alleviated and the mortality and morbidity was reduced. It was also noted that the infected medicated lambs gained more weight than the control.

Guthrie, *et al.* (18) applied nitrofurazone and furasolidine against salmonella choleraesuis enteritis in swine and found they were effective in controlling the disease.

Application in small animal diseases. Mosier and Coles in 1958 (31) conducted in vitro and clinical tests on several organisms which were isolated from the infected urinary system of dogs and cats. The various strains of bacteria isolated were tested for sensitivity to Furadantin (R) without any complete resistance to the drug. However, in one case, the sensitivity test indicated that the organisms were only slightly susceptible to Furadantin (R) after 4 days of treatment. Furadantin (R) was given orally at a dosage ranging between 4 and 10 mg per pound of body weight per day for 5 to 15 days. These doses were divided into three or four equal portions and given with meals to reduce nausea or emesis. Mosier and Coles reported that the Furadantin (R) was effective in producing rapid clinical improvement in 29 of 32 patients. However, sterilization of urine was achieved in less than half of the cases. Urine became sterilized in 40.6 percent and symptomatic cure was obtained in 90.6 percent of the cases.

Pollock (39) isolated (1) E. coli, (2) Proteus vulgaris, and (3) Proteus mirabilis in a canine which was suffering from prostatic abscess. These organisms were tested in vitro for their sensitivity with antibiotics and nitrofurantoin, and found that they were susceptible to nitrofurantoin. They were resistant to the tetracycline group, erythromycin, penicillin, streptomycin and bacitracin, only moderately sensitive to polymyxin, but highly sensitive to nitrofurantoin and chloramphenical.

Application in poultry and turkeys. Harwood (18) utilized nitrofurazone for poultry coccidiosis, which was effective for the treatment of acute epidemics caused by Eimeria tenella and E. necatrix. The nitrofurans have been used extensively in the prophylaxis of avian coccidiosis. This treatment was profitable and of value for prevention of losses from infections with E. tenella and E. necatrix. When the nitrofurazone was fed to chickens in the

absence of coccidia, the feed conversion was improved significantly.

Bierer, 1961 (4) employed feed containing one pound per ton of a nitrofurazone furazolidone preparation which has been effective against Eimeria tenella and Eimeria necatrix in poultry. Nitrofurazone furazolidone preparation reduced morbidity and mortality in poultry of pullorum disease when used as a feed additive for the control of coccidiosis. Short (48) and Shumard (49) were of the same opinion in controlling the coccidiosis in poultry and further observed that the drug did not interfere with the development of immunity to the coccidia nor did it produce any loss of feed conversion efficiency in uninfected birds.

Stephenson (52) fed furazolidone to breeding hens at the level of 50 gm furazolidone per ton of feed which resulted in five to twenty percent improvement in egg production, fertility and hatchability.

Schaible (44) utilizing low level of furazolidone indicated that the addition resulted in an improved rate of growth in chickens and the feed efficiency was also improved in a majority of cases.

Pomeroy (40) studied blue comb disease of turkeys by employing antibiotics and nitrofurans. He noticed that the furazolidone alone or furazolidone and antibiotics combined reduced the mortality rate of turkeys more than the antibiotic alone. Schmittle (45) reported that the furazolidone proved effective in a high percentage of sinusitis of turkeys and was superior to streptomycin sulfate and dihydrostreptomycin sulfate.

Nitrofurans side effects. Nitrofuran compounds produced degeneration of the seminiferous tubules and arrested the growth of spermatozoa. Featherstone (12) employed rats to trace the effects. Francis and Cooper (13, 8) utilized chickens and found glandular atropy but no other effects were noticed. However, Francis (13) cautioned not to feed nitrofuran compounds to breeding stock.

Nissim (33) noticed the arrest of spermatocytes at the primary spermatocyte stage and unassociated with any abnormality of the interstitial cells. Featherstone, et al. (12) employed rats and observed that the testes were atrophied to 1/3 of the original size when they were fed nitrofurazone for 30 days. The size of the testes returned to the original size in 30 to 42 days. Friedgood (15) observed the degeneration of seminiferous tubules in humans after they had received treatment with nitrofuran compounds. He also noticed that the cells of seminiferous tubules returned to their original condition within a month or two depending upon the duration of treatment with nitrofuran compounds. In his personal communication to Robertson (42, 43) Wildermuch reported that by employing nitrofurazone in a human case he observed that the spermatogenesis did not become normal a year after treatment. Robertson (43) found that nitrofurazone caused a serious polyneuropathy resembling beriberi and burning feet syndrome when nitrofurazone was used for a long time to cure Sleeping Sickness in man. Parenteral administration of thiamine, riboflavine, nicotinamide, pyridoxine, and calcium pantothenate did not prevent symptoms and signs of polyneuropathy though they were mild. The other minor side effects were nausea, vomiting, skin rash, malaise, headache, and gastralgia. (15)

Toxicity. Dodd (10) has tried to evaluate LD_{50} in rats by oral and subcutaneous routes. He administered nitrofurazone orally starting from 100 to 700 mgm/kg for different groups for a single dose. Nitrofurazone was injected subcutaneously into mice and rats in doses up to 3 grams per kilogram of body weight. This dose failed to produce a sufficient number of deaths but did produce toxic symptoms. The most likely explanation for this failure of such large doses to cause death is again the poor solubility of the compound which probably permits only a very slow absorption from the site of injection. The

solubility of the compound precludes the determination of an LD_{50} by intravenous administration without the use of solvents. However, 0.5 c.c. to 1.0 c.c. of 10 percent solution was administered by this route to mice and rats, the same type of toxic symptoms were produced. The symptoms noticed were: (1) hyperirritability; (2) tremors; (3) weakness; (4) convulsions; (5) death occured after 2 to 48 hours; (6) death was assumed to be due to respiratory failure. This was in accordance with the experiment which was conducted by Krantz (23). Guthrie et al. (16) observed that the acute toxic dose of nitrofurazone for swine was approximately 300 mg per kilogram of body weight. Symptoms were inappetite, lethargy, and locomotor ataxia, followed by an almost complete motor paralysis and death. Chronic toxicity studies indicated that hogs tolerated a level of 0.09 percent in the feed for eight weeks.

Brion and Fontaine (5) employed 0.05 to 0.2 gram of nitrofurazone per kg of feed of three to six weeks old chickens and observed the toxic symptoms. The symptoms were anemia, asthenia, and anorexia, causing the death of a number of subjects within a few hours to several days. Some of them showed diarrhea and limping. The sick birds were unable to walk and developed skeletal deformities which were similar to clinical symptoms to an intense rickets. In cases of rapid death, the macroscopic lesions were very characteristic of a hemorrhagic syndrome. The mucosae were pale and there were petechial hemorrhages on the heart and the intestinal mucosa and hemorrhages in the marrow of long bones. There was a pericardial transudate, marked duodenal congestion, myocarditis, hepatic or renal degeneration and slight icterus. X-rays confirmed the generalized osteoporosis, and the almost complete disappearance of calcified tissue in the epiphyses. Nelson (32) observed that in females similar doses of nitrofurazone had no significant effect on the estrus cycles, ovulation, conception, or implantation; but they interrupt gestation by direct action on

the fetus.

Histopathology. Dodd (10) fed mice and rats single doses of 150, 200 and 300 mgm/kg. The significant finding which occurred, was a slight cloudy swelling of the liver in some of the animals. Similar studies were made on mice and rats fed 100 to 150 mgm/kg every eight hours for four to six days. The result of examination was the same as above. These animals failed to show any pathological condition when they were fed 5 mgm/20 gm of body weight along with their feed per day for four to six days.

The subcutaneous injection of 3 gm/kg of the compound, although it did not cause death of mice and rats, did produce definite lesions in the liver and kidney. The sections of liver showed an almost complete loss of the normal architecture with a swelling of all the cells and a very marked cytoplasmic degeneration. There was considerable nuclear degeneration. The sections of the kidney revealed a marked degeneration of the tubular epithelium, particularly in the loops of Henle and the central portions of the tubules contain a precipitated protein material.

Krantz and Evans (23) failed to show any definite physiological or histological evidence as to the cause of the toxic symptoms produced by the oral administration of nitrofurazone. However, massive subcutaneous doses did produce a severe toxic hepatitis and an extensive degeneration of the tubular epithelium of the kidney. Guthrie, et al. (16) reported using nitrofurazone and furazolide separately in swine that the most conspicuous lesion was an intense congestion of the meninges and petechial hemorrhages in the thymus. The pathologic histology consisted of degeneration of cortex and cord neurons, with congestion and degeneration of the cells in the convoluted tubules of the kidney. Chronic toxicity studies did not reveal any cellular changes. This was in close agreement with Krantz (23).

Cooper (8) employed nitrofurazone in poultry and observed a striking change with a great number of tubules showing atrophy while the remainder showed hypertrophy in testes. In sections showing complete atrophy there was a total disappearance of cells, the tubules were reduced in size and the lumen was filled with eosinophilic material. Brion and Fontaine (5) observed the rarefaction of myeloid tissue and few stem cells in their nitrofurazone toxicity study on chickens.

MATERIALS AND METHODS

The experiments were conducted by employing rats in one group and dogs in another group. Furacin (R) was suspended in water to use intraperitoneally for rats and a solution of 0.1 percent in polyethylene glycol was used for dogs intravenously.

Experiment 1

A total of 21 dogs were employed in this study. The dogs were of common breeds, ranging in age from five months to five years and weighing between 7 to 34 pounds. There were 11 males and 10 females. All the dogs were kept under observation for a period of 24 hours preceding the experiments to record their general state of health as registered by temperature, rate of pulse, and respiration, condition of the visible mucous membrane, nature of appetite, character of secretion and excretions. Only those animals which were apparently healthy were used for experimental study.

Furacin (R) which was in solution form, had 2 mgm Furacin (R) per c.c. of solution in polyethylene glycol. This was used on a scheduled dose basis by one injection per day intravenously for five successive days with three dogs per group. The scheduled dosage form with the particulars of concentration of solution employed for each group of dogs presented in Table 3.

Table 3. Scheduled dosage of nitrofurazone on the basis of five pounds body weight in dogs for a period of five days.

Group number	No. of animals in each group	Dose	Concentration of solution
1	3	½ mg	1 mg/c.c.
2	3	1 mg	1 mg/c.c.
3	3	3 mg	1 mg/c.c.
4	3	5 mg	1.3 mg/c.c.
5	3	10 mg	1.3 mg/c.c.
6	3	15 mg	1.5 mg/c.c.
7	3	Control with normal saline 3 c.c. for every 5 lbs. of body weight.	

The animals were fed and watered daily. All the animals were anesthetized and sacrificed with Equi-Thesin (R).* The following specimens were taken for histopathological sectioning; liver, kidney, heart, testes or the ovary, and duodenum. The gross lesions were also noted and mentioned in the following tables, from Table 4 through 10.

Results. All the three dogs of Group 1, during their treatment and after treatment were in good health. Appetite, temperature and other activities were normal. Necropsy showed no gross changes of the tissues except a slight enlarged spleen in one dog (No. 2). Number 3 dog was a spayed female, the ovary was not available.

*Equi-Thesin (R): Manufactured by Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri. Each 500 c.c. contains: Chloral hydrate 328 gr., pentobarbital 75 gr., Magnesium sulfate 164 gr. in aqueous solution of propylene glycol and alcohol. (Veterinary product).

Table 4. Group 1: Effect of Furacin (R) in dogs utilizing 0.5 mgm/5-lbs of body weight per day.

Animal: No.	Sex	Approx. age	Weight in lbs pre	Weight in lbs post	Total Furacin (R) injected (1) per day (2) for 5 days	Symptoms observed	Lesions observed	Histopathology
1	female	1½	21	21	(1) 2.1 mgm (2)10.5 mgm	normal	normal	normal
2	male	2½	24	24	(1) 2.4 mgm (2)12.0 mgm	normal	normal	normal
3	female	1½	30	30	(1) 3.0 mgm (2)15.0 mgm	normal	normal	normal

Table 5. Group 2: Effect of Furacin (R) in dogs utilizing one mgm/5-lbs. of body weight per day.

Animal No.	Sex	Approx. age	Weight in lbs pre	Weight in lbs post	Total Furacin (R) injected (1) per day (2) for 5 days	Symptoms observed	Lesions observed	Histopathology
4	male	2½	22	21	(1) 4.4 mgm (2)22.0 mgm	normal	normal	normal
5	male	4	31	32½	(1) 6.2 mgm (2)31.0 mgm	normal	normal	normal
6	male	2½	38	39	(1) 7.6 mgm (2)38.0 mgm	normal	normal	normal

Table 6. Group 3: Effect of Furacin (R) in dogs utilizing 3 mgm/5-lbs of body weight per day.

Animal No.	Sex	Appr. age	Weight in lbs pre	Weight in lbs post	Total Furacin (R) injected (1) per day (2) for 5 days	Symptoms observed	Lesions observed	Histopathology
7	female	1	21	22½	(1)12.6 mgm	Appeared to be weak following the second injection	normal	normal
					(2)63.0 mgm			
8	male	5	23	23½	(1)13.8 mgm	Appeared to be weak following the second injection	Slightly enlarged liver. The intestine was full of worms.	Slight edematous condition in the cells of the liver. Spots of hemosidirosis were observed in the kidney section.
					(2)69.0 mgm			
9	male	2½	26	25½	(1)15.6 mgm	Appeared to be weak following the second injection	No lesions were observed	normal
					(2)78.0 mgm			

Table 7. Group 4: Effect of Furacin (R) in dogs utilizing 5 mgm/5-lbs of body weight per day.

Animal No.	Sex	Approx. age	Weight in lbs pre	Weight in lbs post	Total Furacin (R) injected (1) per day (2) for 5 days	Symptoms observed	Lesions observed	Histopathology
10	female	4½	14	15	(1) 14 mgm (2) 70 mgm	The animal was dull following the second injection. Appetite was lost.	Slightly enlarged liver.	Edematous condition in the liver cells. No other abnormalities were observed in any other specimens.
11	female	2½	17	16	(1) 17 mgm (2) 85 mgm	The animal was dull following the third injection. Appetite was lessened.	No abnormalities	normal
12	male	4½	28	29½	(1) 28 mgm (2)140 mgm	Became dull on the second day and constipation developed	normal	normal

Table 8. Group 5: Effect of Furacin (R) in dogs utilizing 10 mgm/5-lbs of body weight per day.

Animal No.	Sex	Approx. age	Weight in lbs pre	Weight in lbs post	Total Furacin (R) injected (1) per day (2) for 5 days	Symptoms observed	Lesions observed	Histopathology
13	female	1	20	18½	(1) 40 mgm (2)200 mgm	Became dull following the first injection. Appetite was lost. The diarrhea developed.	Slightly enlarged liver	Edematous cells in the liver and kidney were observed
14	female	3½	20	18½	(1) 40 mgm (2)200 mgm	Same symptoms were observed and the constipation developed.	normal	normal
15	male	3	22	19¼	(1) 44 mgm (2)220 mgm	Same symptoms as they were in case number 13 and diarrhea was developed	normal	normal

Table 9. Group 6: Effect of Furacin (R) in dogs utilizing 15 mgm/5-lbs of body weight per day.

Animal No.	Sex	Appr. age	Weight in lbs pre	Weight in lbs post	Total Furacin (R) injected (1) per day (2) for 5 days	Symptoms observed	Lesions observed	Histopathology
16	female	2	32	33.5	(1) 96 mgm	Became dull on the second day. Improved appetite for a day. Developed diarrhea on the third day and vomiting on the fourth day.	Slightly enlarged liver; petechial hemorrhages were noticed on the liver and kidney.	Degeneration of the cells of the liver and vacuolations were noticed. Edema of the tubular cells of the kidney were observed.
					(2)480 mgm			
17	male	2½	34	35.5	(1)102 mgm	Same symptoms were observed as in case number 16.	normal	Slight degeneration of the cells of the seminiferous tubules was observed.
					(2)510 mgm			
18	female	2	25	25.5	(1) 75 mgm	Became dull on the second day. Left off-eating. Developed constipation.	Hyperemia of the liver and kidney were noticed; with petechial hemorrhages.	Hemorrhagic spots in the liver and kidney were noticed.
					(2)375 mgm			

Table 10. Group 7: Effect of saline in dogs utilising approximately 3 c.c./5-lbs of body weight per day.

Animal No.	Sex	Appr. age	Weight in lbs pre	Weight in lbs post	Total saline injected (1) per day (2) for 5 days	Symptoms observed	Lesions observed	Histopathology
19	male	1½	29	29.5	(1) 20 c.c. (2)100 c.c.	normal	normal	normal
20	female	6 mos.	10	10	(1) 10 c.c. (2) 50 c.c.	normal	normal	normal
21	male	5 mos.	7	7	(1) 8 c.c. (2) 40 c.c.	normal	normal	normal

Group No. 2 appeared to be normal except dog No. 5 showed 103.4°F temperature on the fourth day and continued to the last day. The visceral organs on necropsy appeared to be hyperemic in all three dogs. Group No. 3 dogs appeared to be somewhat weak. Whenever these animals were lifted from the cages they evidenced some sort of pain in the abdominal cavity by producing low cry. Lesions on necropsy were slightly enlarged spleen, engorged and enlarged liver with petechial hemorrhages, congested kidney and engorged blood vessels in all the visceral organs and peritonium. Dog No. 3 had small white patches over the intestine. Group No. 4, all the three were not eating well after the third day. They appeared to be weak and were producing the same type of low cry whenever they were handled. Necropsy lesions were the same as Group No. 3 with higher intensity. Group No. 5 had the same type of symptoms as Group No. 4, but they were somewhat severe in these animals. Dog No. 15 showed the temperature of 103.8°F after the second day of treatment to the last day. Dog No. 16 was constipated from the third day onward. Necropsy lesions were more severe than in the fourth group. Group No. 6 were all of the same age. They were very strong. Quiescence of the animals was noted. Following the first injection, two of them developed diarrhea on the second day which was of bad odor. The fecal material was mixed with bubbles of gas. The dogs' appetites improved for a day or two and they drank considerable water. After the third day they lost their appetite and were very weak, but the movement of the animals was normal. There was lacrimation. Temperature was normal, urine was tinged to yellow. Dog No. 18 had developed constipation and refused food from the third day. Necropsy revealed the same lesions with more severe intensity in nature. Group No. 7 was normal to the last day and necropsy revealed no untoward effects.

Discussion. Krantz (23) quoted Gilman and Pickens reported that several furan derivatives would elicit local anesthetic action and Koch and Cahan reported that after a study of the five membered ring, furan, on several species

concluded, that while nitrofuran compounds produced some anesthetic and analgesic properties, its inherent toxicity eliminated it from consideration in therapeutics. In this author's experience in working with dogs an agreement with the quotation of Krantz (23) was reached in connection with analgesic and anaesthetic properties which were shown by dogs after the administration of Furacin (R) intravenously. This was especially true when higher doses were administered.

Some of the dogs exhibited a great deal of strength and resisted handling. It was found that these animals had to be handled with the utmost care to inject the Furacin (R) solution into the vascular system. Afterwards the dogs were very quiet and various ones had to be awakened to be returned to the cages. They appeared dull and inactive after the first injection.

According to the histopathological results obtained and mentioned in the above tables number 4 to 10 inclusive, there was an indication of hepatitis. This change occurred in Group 3 through Group 6. This change was noticed in only 1/3 of the animals in each group, and it was assumed that the slight evidence of hepatitis and hemosiderosis in dog number 8, group 3 might be due to parasitic infection. In the other three groups these changes in the liver were due to the toxicity caused by Furacin (R). These results in Experiment 1 and 2 were in full agreement with the results obtained by Dodd (10) and Krantz (23) who reported that furacin did not cause uniform toxicity in rats. The author assumed that this type of random toxicity might be due to individual susceptibility rather than the general toxicity of the Furacin (R). In acute toxicity studies in swine some changes were observed in the nerve fibres and neurons (16). Further investigation of the nervous system and the enzyme system might reveal the cause of toxicity and death in which no other lesions or cellular changes were observed except the slight change in the liver and kidney referred to by the author and the previous workers.

Summary and conclusions. The experiments were conducted by employing 21 dogs in seven groups and there were three dogs in each group. The dogs were examined and kept under observation for a period of twenty-four hours. The apparently healthy dogs were utilized for the experiment. The Furacin (R) which was employed was in the solution form in the concentration as to adjust the quantity of the solution and to lessen the viscosity of Furacin (R) solution. The dose and concentration of the Furacin (R) were ½, 1, 3, 5, 10, and 15 mgm/5 lb. body weight and 1, 1.3 and 1.5 mgm/c.c. of prepared solution respectively. The Furacin (R) was injected for all the groups of dogs in the varied dose level and the seventh group was treated with saline which was approximately fixed as 3 c.c./5 lbs. of body weight. The injections were made for five days and the symptoms and temperature were recorded daily. The animals were sacrificed on the sixth day and the lesions noted. The specimens from liver, kidney, heart, testes or the ovary and duodenum were collected for histopathological study. Histopathological study revealed the origin of hepatitis in 1/3 of the animals in each group from Group 4 through 6 and slight cellular changes were observed in the kidney of the same dogs which showed hepatitis in Group 5 and 6. The cellular changes in the seminiferous tubules were observed in Group 6. As there were no lesions observed in Group 1, 2, and 3 it was concluded by the author that the highest dose among the 3 groups i.e. 3 mg. per 5 pounds of body weight, might be used for a short duration of treatment and the next higher dose of one mg. per five pounds of body weight might be used for longer duration of treatment. The histopathological examination in these two above recommended dosages treated animals was in agreement with Waisbern (55) who employed Furadantin (R) for human treatment on therapeutical dose.

Experiment 2

A total number of 43 rats were employed for the nitrofurazone intraperitoneal injection experiment. They were all virgin Spraque Dawley female rats, and their weight ranged from 240 to 270 grams. These were divided into nine groups, consisting of five rats in eight groups and three rats in the ninth group which was used as a control group. Each group was kept in a separate cage and fed and watered daily.

The Furacin (R) was in tablet form containing 50 mgm Furacin (R) with dextrose as excipient. These tablets were ground and suspended in water. Irrespective of the quantity each dose was suspended in 2 c.c. of water and this 2 c.c. suspension was injected intraperitoneally to rats. The scheduled dosage form and the particulars of concentrations of Furacin (R) used in different groups of rats presented in Table 11.

Table 11. The scheduled dose of Furacin (R) for each rat per day.

Group number	Dosage	Concentration of Furacin (R)
1	1 mg/day	0.1%
2	3 mg/day	0.3%
3	5 mg/day	0.5%
4	10 mg/day	1.0%
5	15 mg/day	1.5%
6	25 mg/day	2.5%
7	50 mg/day	5.0%
8	75 mg/day	7.5%
9	2 c.c. saline per day for each rat	

The rats were injected intraperitoneally for five successive days at the interval of 24 hours between each injection. The bottles were shaken properly before nitrofurasone was used for each injection to secure uniform distribution of the drug. Every day they were watched for the symptoms and deaths. The symptoms and the time of death were recorded.

Results. All the rats of six groups starting from one up to sixth group except one in sixth group and the ninth control group were alive. These were anesthetized by ether and sacrificed 24 hours following the fifth injection of Furacin (R). The macroscopic lesions were noted: In Group No. 8 one died on the same day following the injection after 8 hours period and one died from the seventh group after about 12 to 14 hours. They symptoms were shown after about four to eight hours respectively. After the 2nd day injection, one in each of the seventh and eighth groups died after about eight and 10 hours respectively. The symptoms were observed after about four to six hours. The third day one died in Group No. 8 after an eight hour interval from the time of injection on that day. The fifth day two from Group No. 8, one from Group No. 7 and one from Group No. 6 died, after about eight to twelve hours interval after the injection on that day.

Symptoms were of the same type in the rats that died. After they received injections, they remained quiet for one or two hours. Afterwards they were well off though they looked somewhat dull. If the cage was tapped by finger or by some metal rod they appeared to be very much irritated. In such condition some especially the one which died on that day ran about for a few seconds and fell down with convulsive movements. Some used to get the symptoms of tetanic convulsions whenever they were irritated. These symptoms varied according to the variation of the dose. They were highest in the eighth group, medium in the seventh group and low in the sixth group. Whenever they were in

convulsive movements they would lay down on their back or sides by stretching their legs. Sometimes, these convulsive movements were observed even without any disturbance. They overcame these convulsions and afterwards some recovered slowly. Some appeared to be very weak without any movement and with half closed eyes. If they were disturbed, they would move in a wobbling manner. They would die without any observable symptom in their occupied spot. Lesions in dead animals were of the similar type in all the rats.

Organs searched for lesions and lesions observed.

1. Heart was hard, congested and with petechial hemorrhagic spots.
2. Lungs were necrosed at the tip and the rest of the part congested, hyperemic with petechial hemorrhages.
3. Pleura was congested.
4. Liver was necrosed at the tips from 10 to 15% and the rest of the part was highly congested including petechial hemorrhages. It was usually dark red in color with gross swelling and somewhat firm in its consistency.
5. Digestive tract: blood vessels were congested, full of gas, fluid, and stained with yellow color.
6. Peritoneum: was in a state of congestion with engorged blood vessels. There were patches of petechial hemorrhage. The tissue at the site of injection necrosed or very highly congested with blood, and dark red in color.
7. Kidney: 1/3 to 1/2 the part necrosed, dark red in color, the rest was highly congested with petechial hemorrhages.
8. Adrenal glands: were very much congested.
9. Peritoneal and thoracic cavities had blood tinged exudate.
10. The horns of the uterus and ovaries were congested with blood vessels.

11. Pancreas was congested.

12. The mesentric glands were swollen and red in color.

13. The spleen was very firm in consistency, dark red in color, and swollen.

14. The unabsorbed Furacin (R) flakes were present in the abdominal cavity and they were greenish-yellow in color.

In one case where the drug was administered intramuscularly by accident it was there, like a caseous dark, green-yellow colored substance.

Lesions noted in each group after they were sacrificed.

Control Group No. 9: No lesions were observed.

Group No. 8: They were treated with 75 mgm of Furacin (R) which was suspended in water. One died on the day of injection after it had all the symptoms which were mentioned above. It died after about eight hours of injection. Death occurred approximately eight hours following injection. Necropsy was conducted and the lesions were noted. All the visceral organs, peritoneum, heart, and lungs were congested with blood. Heart was hard like a stone mass. The unabsorbed Furacin (R) flakes were observed. There was blood tinged discharge from all the openings including eyes. The other one died on the second day after about eight hours of injection. On the third day one died after about eight hours interval from the time of injection. Two rats died on the fifth day after 8 to 12 hours of lapse of time. All the rats which died showed the similar symptoms prior to death, and same type of lesions were observed.

Group No. 7: These rats received 50 mgm per animal intraperitoneally. The first one died following the first day's treatment after approximately 12 to 14 hours. One on the second day after about 10 hours, and another on the fifth day after about 12 hours. All of the rats showed the same symptoms and similar lesions on necropsy. The remaining two were sacrificed on the sixth day.

Heart was hard, congested and had petechial hemorrhages. Five percent of the lungs were necrosed. Liver was enlarged, dark in color, congested, firm in consistency, and had petechial hemorrhages. Kidney was swollen and had petechial hemorrhages. Spleen was hard and swollen, dark in color, and the blood vessels of the digestive system, peritoneum, mesentery and other visceral organs were congested. The place of injection was highly congested. The remaining two rats sacrificed under the effect of ether anesthesia.

Group No. 6: These rats were injected with 25 mgm of Furacin (R) daily per animal for five days. One rat died on the fifth day after about 12 hours; following injections the rat showed the similar signs before and after the death. The remaining were sacrificed on the sixth day. The lesions which were mentioned in the seventh group were similar for these rats too, but the intensity was somewhat less and all the organs, which were mentioned for the seventh group were affected.

Group No. 5: They received 15 mgm of Furacin (R) per rat. They were very much alerted with fear whenever they were irritated. Necropsy showed that the vital organs were inflammed and no other significant lesions were observed.

Group No. 4: They were treated with 10 mgm of Furacin (R) per rat, and no significant symptoms or the lesions were noted except a hyperemic condition at the site of injection.

Group Nos. 3, 2, and 1: They received 5, 3 and 1 mgm of Furacin (R) per rat in each group respectively. Their appearance was normal and no lesions were observed. All the groups except Group 9 remained in a very quiet state from 20 to 30 minutes to 1 to 2 hours. This sign appeared to be an analgesic to milder form of anesthetic condition. These rats were weighed before and after the experiment and the reduction of five to twenty grams of body weight was recorded. The effect of Furacin (R) in different groups of rats in varying dosage presented in Tables 12 to 20 inclusive.

Table 12. Group 1: Effect of Furacin (R) on Rats.

Animal No.	Weight in grams: Pre-Expt'l.	Weight in grams: Post-Expt'l.	Reduction in weight	Daily dose of Furacin (R) injected in mgs	Total Furacin (R) injected in mgs for 5 days	Death after injections in hours	Percentage of deaths
1	248	240	8	1	5	---	---
2	242	228	14	1	5	---	---
3	236	222	14	1	5	---	---
4	267	250	17	1	5	---	---
6	262	244	18	1	5	---	---

Table 13. Group 2: Effect of Furacin (R) on rats.

Animal No.	Weight in grams: Pre-Expt'l.	Weight in grams: Post-Expt'l.	Reduction in weight	Daily dose of Furacin (R) injected in mgm	Total Furacin (R) injected in mgm for 5 days	Death after injections in hours	Percentage of deaths
1	268	254	14	3	15	- - -	- - -
2	258	252	6	3	15	- - -	- - -
3	278	266	12	3	15	- - -	- - -
5	246	232	14	3	15	- - -	- - -
6	264	246	18	3	15	- - -	- - -

Table 14. Group 3: Effect of Furacin (R) on rats.

Animal No.	Weight in grams: Pre-Expt'l.	Weight in grams: Post-Expt'l.	Reduction in weight	Daily dose of Furacin (R) injected in mgs	Total Furacin (R) injected in mgs for 5 days	Death after injections in hours	Percentage of deaths
1	261	246	15	5	25	- - -	- - -
3	269	260	9	5	25	- - -	- - -
4	253	242	11	5	25	- - -	- - -
5	268	253	15	5	25	- - -	- - -
6	255	242	13	5	25	- - -	- - -

Table 15. Group 4: Effect of Furacin (R) on rats.

Animal No.	Weight in grams		Reduction in weight	Daily dose of Furacin (R) injected in mgs	Total Furacin (R) injected in mgs for 5 days	Death after injections in hours	Percentage of deaths
	Pre-Expt'l.	Post-Expt'l.					
1	271	251	20	10	50	- - -	- - -
2	241	224	17	10	50	- - -	- - -
3	286	275	11	10	50	- - -	- - -
4	259	246	13	10	50	- - -	- - -
6	287	269	18	10	50	- - -	- - -

Table 16. Group 5: Effect of Furacin (R) on rats.

Animal No.	Weight in grams: Pre-Expt'l.	Weight in grams: Post-Expt'l.	Reduction in weight	Daily dose of Furacin (R) injected in mgm	Total Furacin (R) injected in mgm for 5 days	Death after injections in hours	Percentage of deaths
1	265	244	21	15	75	---	---
2	282	272	10	15	75	---	---
3	247	234	13	15	75	---	---
5	278	254	24	15	75	---	---
6	279	260	19	15	75	---	---

Table 17. Group 6: Effect of Furacin (R) on rats.

Animal No.	Weight in grams: Pre-Expt'l.	Post-Expt'l.	Reduction in weight	Daily dose of Furacin (R) injected in mgs	Total Furacin (R) injected in mgs for 5 days	Death after injections in hours	Percentage of deaths
1	270	248	22	25	125	12*	20
2	262	248	14	25	125	- - -	
3	245	230	15	25	125	- - -	
4	244	232	12	25	125	- - -	
5	271	248	23	25	125	- - -	

*After 5th injection

Table 18. Group 7: Effect of Furacin (R) on rats.

Animal No.	Weight in grams: Pre-Expt'l.	Weight in grams: Post-Expt'l.	Reduction in weight	Daily dose of Furacin (R) injected in mgm	Total Furacin (R) injected in mgm for 5 days	Death after injections in hours	Percentage of deaths
2	276	258	18	50	250	- - -	
3	246	229	17	50	250	- - -	
4	255	244	11	50	50	10*	60
5	266	246	20	50	100	12**	
6	257	241	16	50	250	10***	

*After 1st injection
**After 2nd injection
***After 5th injection

Table 19. Group 8: Effect of Furacin (R) on rats.

Animal No.	Weight in grams: Pre-Expt'l.	Weight in grams: Post-Expt'l.	Reduction in weight	Daily dose of Furacin (R) injected in mgm	Total Furacin (R) injected in mgm for 5 days	Death after injections in hours	Percentage of deaths
1	250	239	11	75	150	8**	
2	254	250	4	75	75	8*	
3	268	255	13	75	225	8***	100
5	260	246	14	75	375	8****	
6	265	241	24	75	375	8****	

*After 1st injection
**After 2nd injection
***After 3rd injection
****After 5th injection

Table 20. Group 9: Effect of Saline on rats.

Animal No.	Weight in grams: Pre-Expt'l.	Post-Expt'l.	Reduction in weight	Daily dose of Saline injected in c.c.	Total Saline injected in c.c. for 5 days:	Death after injections in hours	Percentage of deaths
3	248	240	8	2	10	---	---
5	251	245	6	2	10	---	---
5(a)	273	268	5	2	10	---	---

Discussion. Krantz (23) worked with rats to elicit the LD_{50}. He administered nitrofurazone to rats, starting from 10 mgm/100 gm body weight to 100 mgm/100 gm body weight. The number of deaths after 48 hours was observed. Animals of the group which died showed premonitory symptoms of hyperirritability, hyperreflexia, tremors, weakness, convulsive seizures and respiratory arrest appeared to be the ultimate cause of death.

Krantz (23) employed 20 mgm/100 gm body weight observed that 20 percent of the rats died, 30 mgm/100 gm body weight gave the same result as above; 40 mgm/100 gm killed 32 percent of the rats, 50 mgm/100 gm 42 percent of the rats, 60 mgm/100 gm killed 35 percent; 70 mgm/100 gm killed 40 percent, 80 mgm/100 gm killed 80 percent and 100 mgm/100 gm killed 100 percent of the rats. Krantz (23) conducted the same type of experiment on mice weighing approximately 15 gm. He obtained more or less the same results. Dodd (10) administered nitrofurazone by oral subcutaneous and intravenous routes, and obtained the same result. Death of rats started from 2 to 48 hours after administration of the drug and the majority died within 2 to 12 hours.

Dodd and Krantz (10, 23) agreed and observed major cellular changes in the liver and kidney, and the minor cellular changes in spleen, bladder, lymph glands, digestive tract and others. These figures of deaths, and those obtained by previous workers. The histopathological study was not prepared. Though nitrofurazone was injected daily for five days in the same variation of dosage, instead of only one dose, the death rates did not vary with previous work in the work of Dodd (10) and Krantz (23). It was assumed that though nitrofurazone was injected daily for five days the absorption did not take place as it was not dissolved in peritonial fluid and it was in line with the work of Dodd (10) who employed nitrofurazone by subcutaneous route, without using specific solvent. The analgesic and low anesthetic effect of Furacin (R) which was mentioned

in Experiment 1 in dogs was observed in rats also. These effects varied from 20 minutes to two hours after the injection of Furacin (R) which was due to variation in dosage of Furacin (R) and after this effect of analgesic and low anesthetic was over they remained active for the rest of the day.

Summary and conclusions. The experiments were conducted on rats by using Furacin (R) in suspension form in water, starting from 1 mgm to 75 mgm per rat which had the body weight of 240 to 270 grams. The toxic symptoms were produced at the dosage level of 25, 50 and 75 mgm per rat. The development of toxic symptoms varied according to the variation in dosage of Furacin (R). They were observed after four to six hours duration following the injection of Furacin (R). Death occurred approximately eight hours following the first injection to 110 hours after the first injection. Each group was receiving the same treatment with Furacin (R) daily with a 24 hour interval between each injection. One rat in each Group No. 7 and 8 died with the effect of one dose (50 and 75 mgm). One rat from each Group No. 7 and 8 died after they received two doses. One rat from Group No. 8 died after it had three injections of Furacin (R). Two rats from the Group No. 8, one from Group No. 7, and one from Group No. 6 died after the fifth injection. Similar symptoms and lesions were observed in all of the rats that died.

The remaining 34 rats were sacrificed 24 hours following the last injection of Furacin (R). The first two groups and control group did not reveal any observable lesions except hyperemic area at the site of injection. In the remaining three groups lesions were observed and these lesions varied according to the quantity of the drug injected. The death rates were the same as in the previous work of Krantz (23) and Dodd (10) where they employed only one single dose orally and subcutaneously; instead of five successive intraperitoneal injections employed in this experiment by the author with the interval of 24

hours for each injection. The author was of the opinion that nitrofurazone had a wide margin of safety and if Furacin (R) is employed by parenteral route, it should be prepared in the proper solvent which might be specific to Furacin (R).

ACKNOWLEDGMENTS

Grateful acknowledgment to Dr. G. K. L. Underbjerg, Head of the Department and professor of Physiology, who gave valuable counsel, encouragement and guidance during this experimental study.

Grateful acknowledgment to Dr. A. H. Swanson, instructor in Physiology for the ready help at all times during the course of these studies.

Thanks are due to Dr. J. E. Mosier, Head of the Department of Surgery and Medicine for the kind supply of Furacin (R) and necessary suggestions. The author is grateful to Dr. Christenson, instructor in Materia Medica and Therapeutics, for his valuable suggestions. Thanks are due to Dr. M. J. Twiehaus, Head of the Department of Pathology, for the facilities afforded for the preparation of histopathologic specimens.

The author is obliged for the kind help extended by Robert Snyder, Loren Ray, Mrs. Audyne Self, and Miss Kaye Smith. The author is grateful to Mrs. Bonnie Pearson for executing the typing of the manuscript in a very up-to-date manner.

BIBLIOGRAPHY

1. Belloff, G. B., J. Buzard and H. D. B. Roberts.
The effect of furazolidone consumption on drug content of chicken tissues and eggs, color of shell, yolk and whole egge homogenate. Poult. Sci. 37:223-227. 1958.

2. Berg, L. R., C. M. Hamilton and G. E. Bearse.
Nitrofurazone and nicarbazin as growth stimulant and coccidiostatic agents for young chicks. Poult. Sci. 35:1394-1396. 1956.

3. Beutler, E.
The hemolytic effect of primaquine and related compounds. A review. Blood 14:103-109. 1959.

4. Bierer, B. B.
Nitrofuran coccidiostat aids in control of pullorum disease under stress condition. Jour. Vet. Med. Assn. 139:258-259. 1961.

5. Brion, A. J. and M.Fontaine.
Nitrofurazone: A hemorrhagic and rachitic like syndrome in chickens due to nitrofural medicated feed. Poult. Sci. 37:1071-1074. 1958.

6. Buzard, J. A., D. M. Vrablic and M. F. Paul.
Colorimetric determination of nitrofurazone, nitrofurantoin and furazolidone in plasma. Antibiot. and Chemother. 6:702-707. 1956.

7. Buzard, J. A., J. D. Conklin, E. O. Keefe and M. F. Paul.
Studies on the absorption, distribution and elimination of nitrofurantoin in the rat. Jour. Pharmacol. and Expt. Therap. 130-131, 38-43. 1960.

8. Cooper, D. M. and G. Skuleki.
The effect of continuous feeding of furazolidone on the growth of gonads in single comb White Leghorn cockerels. Vet. Rec. 67:541. 1955.

9. Dodd, M. C. and D. B. Stillman.
The in vitro bacteriostatic action of some simple furan derivatives. Jour. Pharmacol. and Expt. Therap. 82:11-18. 1944.

10. Dodd, M. C.
The chemotherapeutic properties of 5-nitro-2-furaldehyde-semicarbazone (Furacin) Jour. Pharmacol. and Expt. Therap. 86:311-323. 1946.

11. Evans, F., K. Niemegeers and A. Packchanian.
Chemotherapy of Trypanosoma gambiense and Trypanosoma rhodesiense infections in guinea pigs with nitrofurazone. Amer. Trop. Med. and Hyg. 6:658-664. 1957.

12. Featherstone, R. M., W. O. Nelson, F. Welden, E. Marberger, and A. Bocabella.
Pyruvate oxidation in testicular tissues during furadroxyl-induced spermatogenic arrest. Endocrinology 56:727-736. 1955.

13. Francis, D. D. and C. S. Shaffner.
An investigation of the morphological changes in young chickens and reproductive performance of adult chickens fed furazolidone or nitrofurazone. Poult. Sci. 34:1195. 1955.

14. Friedgood, C. E., A. L. Danza and A. Bocca-bella.
The effect of nitrofurans on the normal testes and on testicular tumors (seminoma) Cancer Res. 12:262. 1952.

15. Friedgood, C. E.
Intravenous nitrofurantoin (Furadantin) in the treatment of systemic infections caused by antibiotic resistant bacteria. New York State. Jour. Med. 58:2044-2048. 1958.

16. Guthrie, J. E. and J. Briggs.
Tolerance and specific therapeutic value of nitrofurans in swine. 1st Nat'l. Symp. on Nitrofurans in Agri. Michigan State University, East Lansing, Michigan p. 96-103. 1956.

17. Hammond, D. M.
Results of experiments with nitrofurazone and sulfomethazine for controlling coccidiosis in calves. Cornell Vet. 50:351-362. 1960.

18. Harwood, P. D. and D. I. Stuns.
Nitrofurazone in the medication of Avian Coccidiosis. Jour. Parasitol. 35:175-182. 1949.

19. Hasen, H. B. and T. D. Moore.
Nitrofurantoin (Furadantin) A study in vitro and in vivo in one hundred cases of urinary infection. Jour. Amer. Med. Assn. 155: 1470. 1954.

20. Horton-Smith, C. and P. L. Long.
Nitrofurazone in the treatment of coccidia. Brit. Vet. Jour. 108: 47-57. 1952.

21. Jewets, E., J. Hopper and D. R. Smith.
Nitrofurantoin in chronic urinary tract infection. Amer. Med. Assn. Arch. Int. Med. 100:549-557. 1957.

22. Jones, S. V., G. B. Belloff, H. D. B. Roberts.
Chemical use of a new nitrofuran dosage form in the retained placenta syndrome in cattle. Vet. Med. 51:413-415. 1956.

23. Krantz, J. C., Jr. and W. E. Evans, Jr.
A contribution to the pharamacology of 5-nitro-2-furaldehyde-semicorbazone. Jour. Pharmacol. and Expt. Therap. 85:324-331. 1945.

24. Libby, D. A. and P. J. Schaible.
The effect of furazolidone in poultry feeds upon early growth of chickens. Poult. Sci. 34:1207. 1955.

25. Lyman-Stewart, B. and H. J. Rowe.
Nitrofurantoin in treatment of urinary tract infections. Jour. Amer. Med. Assn. 160:1221. 1956.

26. Meyer-Jones, L.
Nitrofuran derivatives, veterinary pharmacology and therapeutics. Iowa State University Press. Iowa. 2nd Ed. p. 466-468. 1959.

27. Mintzer, S., E. R. Madison, D. H. Shlaes and O. Felsenfeld.
Treatment of urinary tract infections with a new antibacterial nitrofuran. Antibiot. and Chemother. 3:151-157. 1953.

28. Mires, M. H. and R. H. Chadwick.
A preliminary report on the use of 5-nitro-2-furaldehyde-semicarbazone in the inflammatory therapy of bovine mastitis. Vet. News 10:3-4. 1947.

29. Mires, M. H.
Nitrofurazone: A practical antibacterial agent for bovine mastitis. Jour. Amer. Vet. Med. Assn. 117:49-51. 1950.

30. Mosier, J. E.
A preliminary report on the use of nitrofurantoin in treatment of Canine Tracheo Bronchitis. Vet. Med. 50:605-606. 1955.

31. Mosier, J. E. and H. E. Coles.
Nitrofuran compounds, urinary tract infection of small animals clinical observation and treatment. Vet. Med. 53:649-654. 1958.

32. Nelson, D. O. and Steinberger.
Effect of nitrofuran compounds on the testes of the rat. Fed. Proc. 12:103. 1953.

33. Nissim, J. A.
Increased pituitary gonadotrophin activity after degeneration of seminiferous tubules produced by nitrofurazone. Lancet 272:304-305. 1957.

34. Olson, Norman O.
The use of streptomycin and dihydrostreptomycin for infectious sinusitis in turkeys. Jour. Amer. Vet. Med. Assn. 118:174-175. 1951.

35. Packchanian, A.
Chemotherapy of African Sleeping Sickness: Chemotherapy of experimental Trypanosoma gambiense infection in mice (Mus-musculus) with nitrofurazone. Amer. Jour. Trop. Med. and Hyg. 4:705-711. 1955.

36. Paul, M. F., H. E. Paul, R. C. Bender, F. Kopko, C. M. Harrington, V. R. Ellis and J. A. Busard.
Studies on the distribution and excretion of certain nitrofurans. Antibiot. and Chemother. 10:287-302. 1960.

37. Paul, H. E.
Research background on the nitrofurans. 1st Nat'l. Symposium on Nitrofurans in Agri. Michigan State University, East Lansing, Michigan. p. 6-10. 1956.

38. Paul, M. F., R. C. Bender, and E. G. Nohle.
Renal Excretion of nitrofurantoin (Furadantin). Amer. Jour. Physiol. 197:580-584. 1959.

39. Pollock, S.
Prostatic abscess in the dog. Amer. Vet. Med. Assn. 129:274. 1956.

40. Pomery, B. S.
Use of furazolidone and antibiotics in Blue Comb disease of turkeys. 1st Nat'l. Symposium of Nitrofurans in Agri. Michigan State University, East Lansing, Michigan. p. 75-79. 1956.

41. Richards, D. A., E. Rice, E. H. Kass and M. Finland.
Nitrofurantoin: Chemical and laboratory studies in urinary tract infections. Amer. Med. Assn. Arch. Int. Med. 96:437-450. 1955.

42. Robertson, D. H. H.
Nitrofurazone induced hemolytic anemia in a refractory case of Trypanosoma rhodesiense Sleeping Sickness: The hemolytic trait and self-limiting hemolytic anemia. Ann. Trop. Med. Parasitol. 55:49-63. 1961.

43. _____.
The hemolytic of primaquine and nitrofurazone in cases of sleeping sickness with the hemolytic trait. Ann. Trop. Med. Parasitol. 55: 278-86. 1961.

44. Schaible, P. J.
Effect of low levels of furazolidone in poultry feed. 1st Nat'l. Symposium on nitrofurans in Agri. Michigan State University. East Lansing. Michigan. p. 51-55. 1956.

45. Schmittle, S. C. and William B. Putnam.
Experimental treatment of infectious sinusitis in turkey with furazolidone. Poult. Sci. 35:1169. 1956.

46. Schneierson, S. S.
Bacterial sensitivity to nitrofurantoin. Antibiot. and Chemother. 6: 212-214. 1956.

47. _____.
Bacterial susceptibility to nitrofurantoin as compared to 1953 to 1955. Antibiot. and Chemother. 10:747-752. 1960.

48. Short, C. E.
Research on feed medication in chickens. 1st Nat'l. Symposium on nitrofurans in Agri. Michigan State University. East Lansing, Michigan. p. 24-35. 1956.

49. Shumard, R. F.
The activity of soluble furacin (Nitrofurazone) against the Coccidian: Eimeria-necatrix. 1st Nat'l. Symposium on Nitrofurans in Agri. Michigan State University. East Lansing, Michigan. p. 36-42. 1956.

50. _____.
Experimentally induced ovine Coccidiosis use of water soluble Nitrofurazone as a therapeutic agent. Vet. Med. 54:477-479. 1959.

51. Smith, H. H.
The treatment of Salmonella pullorum infection in chicks with furazolidone, sulfamerazine and chloramphenicol. Vet. Rec. 66:493-95. 1954.

52. Stephenson, E. L.
The effect of furazolidone on growth and reproduction of chickens. 1st Nat'l. Symposium on Nitrofurans in Agri. Michigan State University. East Lansing, Michigan. p. 56. 1956.

53. Stewart, B. L. and H. J. Howe.
Nitrofurantoin (Furadantin) in treatment of urinary tract infections. Jour. Amer. Med. Assn. 160:1221. 1956.

54. Vigue, R. F., D. H. Fitzgerald and R. F. Costrucci.
Management of bovine genital infections. Intravaginal therapy. Jour. Amer. Vet. Med. Assn. 134:308-311. 1959.

55. Waisbren, B. A. and W. Crowley.
Nitrofurantoin, Clinical and laboratory evaluation. Amer. Assn. Arch. Int. Med. 92:653-661. 1955.

56. Wildermuth, O.
Testicular Cancer. Management of metastases with report of a new chemotherapeutic agent. Radiology 65:599-662. 1955.

SYMPTOMS AND LESIONS IN DOGS AND RATS FOLLOWING PARENTERAL ADMINISTRATION OF FURACIN (R)

by

KADE-GOWDA THIMMAIAH

B. Sc. (Vet), Bombay University, Bombay, India, 1953.

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Physiology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1962

In this study an attempt was made to evaluate the probable therapeutic dose of Furacin (R) which would not cause the toxicity or the cellular changes in the tissues of the animal and to study the probable changes in the tissues which might occur in higher doses of Furacin (R). Totally 21 dogs and 43 rats were employed for the experiments. There were seven groups of dogs and each group had three dogs. Six groups were treated with Furacin (R) in varied and according to scheduled doses of ½, 1, 3, 5, 10 and 15 mgm per five lbs of body weight. The remaining one group was employed as control and treated with saline. The dose of saline was fixed approximately at 3 c.c./5 lbs of body weight. Furacin (R) was in the solution form for dogs which was in the concentration of 2 mgm/c.c. in polyethylene glycol. It was diluted with water to make the solution to the concentration of 1, 1.3, and 1.5 mgm per c.c. of solution. Furacin (R) was diluted to lessen the viscosity of the Furacin (R) and adjusted to lessen the quantity of Furacin (R) for intravenous injection. The Furacin (R) was injected intravenously into dogs for five successive days. During these days the symptoms were noted. They were sacrificed on the sixth day by using Equi-Thesin (R). The lesions were recorded and specimens from the liver, kidney, heart, testes or the ovary and duodenum were collected and preserved in buffered formalin. The sections were stained by hemotoxylion-eosin stain. The signs of hepatitis were observed in 1/3 of the animals in Group 4 through 6 and slight changes in the kidneys were observed in Group 5 and 6 in the same animals which had hepatitis. The male dog in Group 6 showed cellular changes in the seminiferous tubules of the testes.

There were nine groups of rats and each group up to eight and inclusive had five rats and in the ninth group there were three. These three were employed as controls by giving 2 c.c. saline for each rat intraperitoneally. The Furacin (R) which was employed for rats was in tablet form with dextrose.

Each tablet had 50 mgm of Furacin (R). These tablets were ground and suspended in water. The dose of Furacin (R) varied from 1 to 75 mgm per rat. Each dose was suspended in 2 c.c. of water irrespective of quantity. The Furacin (R) was injected intraperitoneally according to the scheduled dose of 1, 3, 5, 10, 15, 25, 50 and 75 mgm per rat for five days. All the five rats from group number 8, two from group number 7 and one from group number 6 died at different intervals of time. The remaining 34 rats were sacrificed by the application of ether. The symptoms of hyperirritability and convulsions were observed in the higher doses of 25, 50 and 75 mgm per rat. The necrosis and inflammatory changes were noticed in liver kidney and lungs of the dead animals. Hyperemic and petechial hemorrhages were observed in liver, kidney, heart and lungs of the rats which were of group numbers 5, 6 and 7 inclusive, after they were posted.