THE EFFECT OF DRUGS IN FEED OR WATER ON THE RECOVERABILITY OF PATHOGENIC ORGANISMS FROM DISEASED POULTRY

By

CHARLES W. TITKEMEYER

A THESIS

Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

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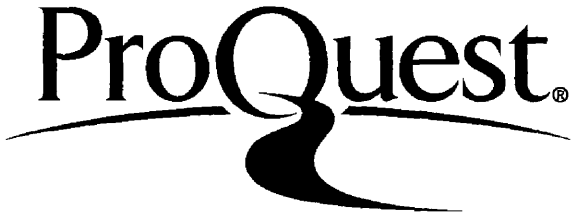

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ABSTRACT

The possibility that coccidiostats, growth-promoting additives, and therapeutic drugs in the feed or water might prevent the isolation of the pathogenic organism in diseased poultry has arisen. A study was made to determine the effects of six different drugs in feed or water on the recoverability of S. gallinarum from chickens given the organism either intraperitoneally or orally.

Representing the sulfas, sulfaquinoxaline at levels of 0.05 and 0.0175 per cent in the feed or 0.025 per cent in water and sulfamethazine at 0.1 per cent in the water reduced death losses but did not significantly prevent isolation of the causative organism. Growth-promoting drugs tested included chlortetracycline at 18 Gm. and 108 Gm. per ton of feed and penicillin at 4 Gm. per ton of feed. They neither reduced losses nor prevented recovery of the organism and, in some respects, seemed to increase the incidence of recovery of the organism. The broad spectrum antibiotics, chlortetracycline at 200 Gm. per ton of feed and 400 mg. per gallon of water and neomycin at 500 Gm. per ton of feed had little or no effect on death losses or recovery of the organism. A combination of chlortetracycline at 100 Gm. and neomycin at 500 Gm. per ton of feed resulted in greater death losses and higher incidences of recovery than no medication at all.

Furazolidone at 100 Gm. per ton of feed prevented death losses and almost completely prevented recovery of the organism.

Results of oral administration of the organism were the same as results of intraperitoneal administration, but the oral results were not as uniform in that not all chickens became infected. Time of administration of the drugs whether 48 hours before, at time of, or 48 hours after inoculation did not alter their effect on the recoverability of the organism.

Conclusions drawn were that sulfaquinoxaline, sulfamethazine, penicillin, chlortetracycline, and neomycin did not prevent recovery of S. gallinarum from chickens given the organism either orally or intraperitoneally, but that furazolidone prevented recovery of the organism in most cases.

ACKNOWLEDGMENTS

The author is grateful to Dr. Henrik J. Stafseth, Head, Department of Microbiology and Public Health, for his assistance in planning a course of study, his guidance in following it, and for the inspiration that his life work has created. The author is indebted to Dr. Samuel C. Schmittle for the splendid assistance he has given in planning and conducting the research project. He has been an inspiring and pleasant man with whom to work and associate.

Many thanks are due to Dr. M. Lois Calhoun, Head, Department of Anatomy, for her encouragement and for her fine cooperation in arranging work schedules that this project might be finished. Thanks also to Dr. J. Fred Smithcors, Department of Anatomy, for sharing a portion of the work load.

A word of appreciation is due Dr. Ralph D. Barner, Department of Pathology, for counsel and guidance.

The writer also wishes to thank Dr. Ralph C. Belding and Miss Mary Mayer of the Poultry Diagnostic Laboratory for advice and technical assistance.

It is with a deep and sincere appreciation that the author expresses his gratitude to the American Cyanamid Company for the financial assistance that made this project

possible. The generosity of this company not only with their finances but with their facilities, drugs, and technical advice has added immeasurably to this work. Dr. William Patton, a representative of this company, deserves a special note of thanks for his interest, untiring efforts, and helpful suggestions.

Thanks also to Merck and Company for furnishing a generous supply of sulfaquinoxaline and to Hess and Clark for furazolidone used in this project.

VITA

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candidate for the degree of

Doctor of Philosophy

Final Examination: 10:00 a.m., May 16, 1956, Room 101, Giltner Hall

Dissertation: The Effect of Drugs in Feed or Water on the Recoverability of Pathogenic Organisms from Diseased Poultry

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INTRODUCTION

The Isolation Problem

In any positive diagnosis of a disease, the demonstration of the causative organism is of great importance. If the organism cannot be isolated, a positive diagnosis is difficult to make. Diagnosticians have frequently experienced difficulty in isolating causative organisms and have speculated about it. One factor suspected as being a possible cause has been the use of coccidiostats, growth-promoting additives,and therapeutic drugs in the feed or water of the individual in question. Belding (1956), Henderson (1956), and Schmittle (1956) have at times suspected that antibiotics have decreased their chances of recovering the organism but they offer no proof of this assumption. Peckham (1956) has had some failures in isolation but blames them on faulty technique rather than on the presence of drugs in the feed. The only isolation difficulty Delaplane (1956) has experienced has been in isolating the fowl typhoid organism from birds on treatment with furazolidone. Smith (1955b) was able to recover Salmonella gallinarum in the feces of chickens treated with oxytetracycline, sulfamerazine, chloramphenicol and chlortetracycline but was unable to recover it consistently in chickens treated with furazolidone. Alberts (1950) reported

that experimental studies with the use of pyrimidine sulfonamides in chickens exposed to S. gallinarum gave clear evidence that the sulfonamides inhibited the number of isolations of the organism in birds receiving feed or water containing the drug at therapeutic levels, 0.5 and 0.2 per cent, respectively.

From the experiences of these poultry pathologists, there seems to be reasonable doubt concerning the effects of drugs in preventing recoverability of the organism in diseased chickens. This problem was undertaken in an attempt to determine the effects of drugs commonly used in poultry practice on the isolation of the causative organism of fowl typhoid.

REVIEW OF LITERATURE

The Sulfonamides

Because of their versatility, the sulfonamides have been used extensively in the control of poultry diseases. Sulfaquinoxaline has established itself as an effective agent against all of the known species of poultry coccidia of economic importance. Sulfamethazine has found wide usage in the control of many bacterial infections of chickens. Other members of the sulfa family have proved valuable in various disease conditions and have been used both prophylactically and therapeutically.

Use in control of fowl typhoid. Practically all of the sulfonamides have been used at one time or another in the prevention and treatment of fowl typhoid. Hammond (1945) found sodium sulfathiazole to be of definite value and phthalysulfathiazole and irradiation by sterile lamps of possible value in the control of fowl typhoid. He concluded that it was possible to eradicate fowl typhoid from a laying flock by the use of sodium sulfathiazole in the drinking water and removing reactors to the S. gallinarum rapid whole blood test. Moore (1946) tested five sulfonamides to determine their value in protecting birds artificially inoculated with S. gallinarum.

Sulfamerazine and two specially prepared sulfonamide compounds were found to be highly effective in reducing the mortality which ranged from 0.0 to 16.7 per cent. Sulfathalidine and sulfasuxidine were less effective with a mortality rate which varied from 33.3 to 83.3 per cent.

Holtman and Fisher (1946) reduced the losses in S. gallinarum infected day-old chicks from 80 per cent in the controls to four per cent in chickens given 0.1 per cent sulfathiazole in the drinking water for a period of one week. There was a recurrence of the infection within five days of the cessation of treatment which indicated that not all the organisms were killed.

Boney (1951) conducted a study of sulfaquinoxaline and sulfamethazine in fowl typhoid among breeder turkeys and found that sulfamethazine administered at 0.1 per cent in the drinking water did not control losses in field flocks. When administered intraperitoneally, it reduced the mortality temporarily, but, in order to maintain the prophylactic effect, it was necessary to repeat the injections every four or five days. This indicated that the organism was not completely destroyed. The average mortality was decreased from 12.5 per cent in the controls to 6.25 per cent in birds treated with sulfamethazine. Sulfaquinoxaline effectively reduced the mortality in two field outbreaks of fowl typhoid when given in the drinking water at the rate of 0.04 per cent for two days and then continued at levels of 0.025 per cent or 0.02 per cent.

Alberts (1950) found that a 0.4 per cent sulfamerazine-mash mixture or 0.2 per cent sodium sulfamerazine in the drinking water was effective in preventing losses caused by S. gallinarum in chicks during a seven day course of treatment and for two days after treatment was stopped. The disease recurred and losses were often severe unless an additional course of treatment was given. He also attempted to determine whether S. gallinarum remained in the tissues of exposed chickens that had continuous access to sodium sulfamerazine in the water or mash. Three days after oral exposure to 7.5×10^7 organisms, 250 chicks were separated into three groups. One group (Group 1) of 100 chicks received mash containing 0.4 per cent sulfamerazine which was kept before the birds for 21 days. A similar group (Group 2) of 100 birds had access to drinking water containing 0.2 per cent sodium sulfamerazine. The remaining 50 exposed chicks served as untreated controls. In Group 1, 85 birds were killed during four-day intervals while still on treatment. S. gallinarum was isolated from the hearts and livers of 48 (56.4 per cent) chickens. At necropsy, macroscopic lesions suggestive of fowl typhoid were observed in 24 (28.2 per cent) of the chickens. In Group 2, 90 birds were killed during four-day intervals while still on treatment. S. gallinarum was isolated from the livers and hearts of 37 (41.1 per cent) chicks. At necropsy, macroscopic lesions suggestive of fowl typhoid were observed in 17 (18.9 per cent) of the chickens.

Holtman and Fisher (1947) determined that relatively large infectious doses of S. gallinarum could be administered to young chicks without development of extensive fowl typhoid disease if sulfonamide therapy was begun prior to expiration of the incubation period of the disease. They experimentally infected ten 30-day old chicks and put them on 0.5 per cent sulfamerazine medication for 10 days. One chick was sacrificed each day following infection until all had been examined.

The results of culture on MacConkey and Salmonella-Shigella agar plates, with subsequent morphological and biochemical tests, indicated that the fowl typhoid organisms were present in the intestine only during the first 24 hours after treatment was started. Tests made on other organs, such as the ceca, gizzard, heart and liver were negative. After the sixth day of the test, two positive isolations were made from the gall bladder indicating that this organ may serve as a point of localization from which it may spread to produce a generalized infection when the resistance of the bird is lowered.

Hall, MacDonald and Legenhausen (1949) found that sulfamerazine when fed in 0.5 to 1 per cent concentration in mash caused a marked drop in mortality due to fowl typhoid for about a week following treatment after which mortality gradually climbed again. They stated that apparently this drug suppresses mortality during and shortly after the period of administration but that the organism survives the treatment and again multiplies after discontinuance of the drug.

Cox and Craig (1952) ran an experiment on the effects of various sulfonamides on fowl typhoid and found that of all treated birds which survived, the S. gallinarum organism was recovered from 11.5 per cent, while 95 per cent of the treated birds which died yielded the typhoid organism. In the untreated controls, the organism was recovered from 93.9 per cent of the birds when autopsy was performed.

Antibiotics Used for Growth Promotion

It is a well established fact that the addition of low level amounts of antibiotics to the ration enhances the growth rate of poultry. This is particularly true of poultry reared in houses used in preceding years. Porter (1953) found that in several hundred tests reported, antibiotics increased the growth rate of chickens by about 15 per cent and that of turkeys up to 30 per cent. Numerous theories have been advanced concerning the reason for this accelerated growth. Although not all authorities agree on the mode of action, most workers are agreed that antibiotics exert their effect through their action on the bacterial flora in the gut. Any reduction in the number of bacteria found here could be reflected in the recoverability of the pathogenic organism. Thus a problem of this type would be incomplete without consideration of growth-promoting additives.

Porter also reported that chlortetracycline, penicillin, and oxytetracycline have given the most consistent results and that there is probably little choice between these

antibiotics for pigs or chicks; with turkeys, however, penicillin promoted the best response. Mixtures of two or more of these antibiotics were no better than the single substance. In the light of his experiences with growth-promoting antibiotics, penicillin and chlortetracycline were chosen as the growth-promoting drugs to be used in this problem.

Glantz and Gordeuk (1955) conducted tests using various antibiotics as therapeutic agents for fowl typhoid. They found that chlortetracycline at a level of 1 Gm. per gallon of water was not effective. Chlortetracycline mixed with mash at the rate of 1 Gm. per pound reduced losses to 25 per cent and no relapse occurred when treatment was discontinued. The mortality was not reduced significantly when the 0.5 Gm. per pound level was used. These workers did not attempt to recover the organism from treated birds and gave no data on recoverability of the organism. Smith (1955b) reported that penicillin treatment for fowl typhoid was valueless.

Broad Spectrum Antibiotics

In recent years the broad spectrum antibiotics have assumed a place of great importance in the treatment of infectious diseases. As they are effective against both Gram positive and Gram negative organisms, they are especially useful when the identity of the causitive organism is unknown. Neomycin, a broad spectrum antibiotic, has found wide usage in the practice of veterinary medicine. Haas (1955) reported that neomycin hydrocortisone and neomycin-kaolin-pectin combinations

were particularly useful in veterinary practice. Drury (1952), Braeutigam (1953), Hinze (1952), Bunn and Scheidy (1952), Tucker and Johnson (1953), Konde and Monroe (1955), Christian, Harris, and Barr (1954), and Vigue (1954) reported neomycin sulfate to be an effective therapeutic agent for mastitis caused by streptococcus, staphylococcus, pseudomonas, proteus, and coliform organisms.

Braeutigam (1954), Davidson (1952), and Schultz (1955) reported good results with neomycin or neomycin sulfate in ophthalmic applications and in topical application for various dermatoses. Brown and Schirmer (1954) have used kaopectate with neomycin in the treatment of gastro-enteritis and have found that this combination gives consistently better results than products heretofore available. Dickson (1954) and McCarty (1953) have obtained excellent results in treating salmonellosis with neomycin sulfate. Davidson (1954) has successfully used a combination of hydrocortisone and neomycin in the treatment of chronic otitis externa in dogs. Bicek (1954) has used neomycin sulfate extensively as an adjunct in veterinary surgical procedures and has found it exceptionally effective in controlling those infections which so often complicate surgery conducted in the field.

Although neomycin has not found wide usage in the treatment of poultry diseases, it has given good results in "in vitro" trials against salmonella organisms. As it is one of the more widely used broad spectrum antibiotics, it warrants

usage in this problem. Other broad spectrum antibiotics have been used for fowl typhoid. Glantz and Gordeuk (1955) found that chloramphenicol when administered orally at the rate of 200 mg. per bird per day or 1 to 2 Gm. per pound of mash gave excellent protection when started on the day of infection. When treatment was delayed until the onset of symptoms, these rates of administration were not as effective. A relapse occurred when the chloramphenicol mash was discontinued. Chloramphenicol at the rate of 0.5 Gm. per pound of mash reduced mortality 30 to 40 per cent while birds were under treatment and no relapses occurred when treatment was discontinued. They did not run tests for recoverability of the organism. They found that polymyxin B alone was of little value. When combined with an equal amount of chloramphenicol either 0.25 Gm. or 0.5 Gm. per pound of mash, the combination lowered the mortality rate to 20-30 per cent and no relapse occurred when the antibiotics were discontinued.

Furazolidone, a Drug that is Particularly Effective Against S. gallinarum

In the more recent articles concerning the treatment and control of salmonellosis, furazolidone has been receiving great acclaim. Wilson (1955) ran a series of experiments using various methods of inoculation and several different organisms to determine the efficacy of furazolidone in salmonellosis. In his first experiment, he infected 150 day-old chicks with S. gallinarum by introducing a suspension of the

organisms directly into the crop. Twenty-four hours later, 75 of the chicks were given 0.02 per cent furazolidone in mash. This treatment was continued for five days. Losses in the treated pen during the first 21 days amounted to four chicks (5.3 per cent) compared with 68 (90.6 per cent) in the untreated controls. However, 20 days after cessation of treatment, a serious outbreak occurred resulting in the deaths of nine chicks during the next four days. Sporadic deaths occurred for the next two weeks until a total of 20 (26.7 per cent) of the treated birds died. The surviving birds were tested by the rapid whole blood technique six weeks later. Six were positive for fowl typhoid. S. gallinarum was isolated from four of these birds and was also isolated from six birds which had given negative reactions to the test. This experiment indicated that 0.02 per cent furazolidone in mash for a period of five days greatly decreased death losses but did not completely eliminate the organism.

In a second experiment, Wilson inoculated the chickens by the spray method. Treatment with 0.02 per cent furazolidone was begun 24 and 72 hours after infection. Mortality was completely prevented during the period of treatment, while it was almost 100 per cent in the controls. As in the previous experiment, a breakdown occurred several days after treatment ceased resulting in deaths of 31.9 per cent of the birds receiving furazolidone 24 hours after inoculation and 12.8 per cent of the birds receiving the drug 72 hours after

inoculation. These results confirmed that 0.02 per cent furazolidone is not bactericidal.

In a third experiment, using furazolidone against S. typhimurium, very few death losses occurred in either controls or infected birds. However, the organism was isolated from four of 30 chicks receiving 0.04 per cent furazolidone, 11 of 32 receiving 0.02 per cent furazolidone, and 18 of 29 in the control pen.

To compare the relative effects of furazolidone and sulfaquinoxaline against S. pullorum, Wilson (1955) inoculated chicks and placed 50 of them on 0.04 per cent furazolidone and 49 of them on 0.05 per cent sulfaquinoxaline 72 hours after infection. None of the chicks receiving furazolidone died and only 2 chicks were positive for S. pullorum when the livers were cultured. In the control group receiving no medication, 45 of 48 birds died of pullorum disease and the surviving three showed extensive lung lesions when destroyed at the end of the experiment. Ten of the 49 chickens receiving sulfaquinoxaline died from pullorum disease during treatment and five died 48 hours after treatment had stopped. There was gross evidence of the disease in every one of the 31 survivors from 30 of which S. pullorum was isolated by direct culture from the liver.

In a companion experiment, Wilson placed 49 healthy day-old chicks in a brooder near chickens infected with pullorum. On the sixth day when losses began, treatment with 0.04 per cent furazolidone was instigated and was continued for 10 days.

There were 28 survivors, three of which were undersized. Of these survivors, S. pullorum was isolated from 10 (35.7 per cent). This test indicated that treatment after the infection is well under way is helpful but not as effective as treatment begun earlier. Against S. thompson, furazolidone was not nearly as effective as against the other Salmonellae used.

Harbourne and Sellers (1955) conducted trials using furazolidone on field outbreaks of fowl typhoid. They found that this treatment caused a marked reduction in mortality but did not prevent isolation of the organism from some of the surviving birds. They felt that this "carrier state" did not necessarily indicate that the drug was ineffective but reflected the difficulty, under field conditions of ensuring that all birds received the drug in adequate amounts.

Smith (1955b) has worked extensively with furazolidone as a treatment for fowl typhoid. From an experiment comparing the recoverability of S. gallinarum in the feces of chickens on various antibiotic agents, he reported the following results:

Agent	Per cent of chicks discharging organisms in feces
Furazolidone	0.0
Oxytetracycline	78.5
Sulfamerazine	88.9
Chloramphenicol	92.3
Chlortetracycline	70.8
Dihydrostreptomycin	84.2
Penicillin	100.0
Untreated	82.6

He concluded that furazolidone was greatly superior to the other drugs tested and that this superiority was associated with the fact that furazolidone was bactericidal for S. gallinarum in low concentrations whereas the other agents were only bacteriostatic. Penicillin treatment was useless. The occurrence of fecal excreters of S. gallinarum was very uncommon among chickens that had been treated with furazolidone. A concentration of 0.04 per cent was necessary to achieve this when furazolidone was given in the mash for 10 days. Fecal excreters were found more frequently when the concentration and the period of administration were decreased. Most of the chickens treated with other agents became fecal excreters. Chickens treated with furazolidone early in the course of the experimental disease developed very little immunity as evidenced by the absence of agglutinins to S. gallinarum in their blood and their susceptibility to reinfection. Apart from a slight depression in the growth rate, the continuous feeding of healthy chicks and young chickens on mash containing 0.02 or 0.04 per cent furazolidone for three weeks was not accompanied by any signs of toxicity.

In the treatment of S. typhimurium infection in turkey poults and chicks with furazolidone, Smith (1955c) found a somewhat different picture. The results indicated that furazolidone was of considerable value in the control of natural outbreaks of S. typhimurium infection in poultry although a high proportion of the treated chicks and poults remained carriers of the infection. In spite of the carrier state,

they appeared clinically healthy at the end of the treatment period and grew normally thereafter. In poults, S. typhimurium was isolated in the feces or organs of 64 to 91 per cent of the treated groups compared with 100 per cent in the untreated. In chickens the organism was isolated in from 24 to 50 per cent of the survivors when treatment was started three days after infection. When furazolidone was administered three days before infection, a very low percentage died but 71 per cent were carriers. Thus the proportion of treated survivors that were carriers was disappointingly high as compared with results using S. gallinarum or S. pullorum. Smith explained that a high proportion of treated survivors were carriers of S. typhimurium because this organism was able to colonize in the alimentary contents of chickens whereas any S. gallinarum found in the contents originated from an infected focus in the tissues. He also pointed out that furazolidone taken per os is distributed almost entirely in the cephalic third of the alimentary tract. In tests that he ran, he found high concentrations in the crop and gizzard contents, lower concentrations in the duodenal contents, and none in the ileum, ceca, or rectum. The ability of S. typhimurium to colonize in the alimentary contents plus the fact that very little furazolidone could be demonstrated in the lower bowel of the chicken provided a reasonable explanation for the large numbers of carriers that arose after treatment.

Gordon and Tucker (1955) found furazolidone to be significantly effective against chronic carriers of S. pullorum.

When furazolidone at both 0.02 per cent and 0.04 per cent was fed continuously for 10 days in the rations of 13 birds positive to the rapid whole blood test in the field, 11 were subsequently found to be free from S. pullorum infection. All of the untreated controls showed generalized S. pullorum infection. Two treated birds failed to respond to the drug, maintained a high titre, and continued to shed organisms in the feces.

Libby and Schaible (1955) tested the effects of furazolidone at low levels as a growth-promoting factor. They reported that the relative insolubility of furazolidone reduced absorption by the chicken and thereby diminished the toxicity of the drug. This insoluble phase acted as a reserve supply and became available continuously throughout the intestinal tract to replenish the effective concentration. Furazolidone at low levels in the feed consistently improved the early growth rate of chickens especially under heavily contaminated environments.

Grumbles, Wills, and Boney (1954) reported that furazolidone fed in the feed at levels of 0.011 per cent and 0.0055 per cent was found to be effective in preventing mortality associated with fowl typhoid infection in turkeys. No evidence of toxicity or unpalatability was encountered. When birds were put on non-medicated feed, an outbreak recurred. Treatment was again instigated for ten days with no further losses. The surviving birds were moved to a clean range with

uncontaminated equipment and given non-medicated feed. No further losses occurred. No conclusions were drawn as to whether the reinfection occurred from fecal excretors or from the contaminated environment.

From this literature review on the use of furazolidone in salmonelloses, data both proving and disproving the recoverability of the organism from treated poultry were presented. All workers agreed that furazolidone was highly effective in reducing mortality but not all felt that it was completely bactericidal.

MATERIALS AND METHODS

Chickens

The chickens used in all trials except one were Hyline cockerels, a hybrid variety of the Leghorn breed. They were purchased from Gulliver's Hatchery of Eaton Rapids, Michigan as day-old chicks and were kept under observation for one week before being infected during which time all weak birds were destroyed. When Gulliver's Hatchery was unable to supply birds on the exact date needed on one occasion, a group was purchased from Klager's Hatchery of Bridgewater, Michigan.

Housing

The chickens were housed in a well-ventilated animal room in which no diseased chickens had been kept. Temperature was regulated by controlling the heat of the entire room rather than by units in the individual pens. All inoculated chickens were kept in a 20-unit battery (Figure I). Non-inoculated controls were kept in a separate battery in the same room but several feet distant. Since the organism was never recovered from non-inoculated controls, this arrangement proved adequate.

Figure I. Battery Used for Inoculated Chicks

Feed

The feed used was a non-medicated chick starter mash purchased from the Valley City Milling Company of Portland, Michigan. The composition of the feed was:

Ingredients	Total Lbs.
Ground Yellow Corn	455.00
Pulverized Oats	100.00
Delsterol	.50
4% Soybean Oil Meal	220.00
Red Fish Meal	5.00
Meat Scraps	50.00
Middlings	100.00
17% Alfalfa Meal	25.00
Poultry Mineral A	15.00
Ground Fermentation Solubles	20.00
Condensed Fish Solubles	10.00
Vitamin A Premix	1.25
Salt	2.50
Manganese Sulphate	.13
B-12 Supplement	.67
Choline Chloride	.50
	1,005.55

Drugs

Drugs chosen as representative therapeutic agents for this problem were sulfaquinoxaline and sulfamethazine representing the sulfas; chlortetracycline and penicillin as growth-promoting drugs; neomycin, chlortetracycline, and combinations of the two as broad spectrum antibiotics; and furazolidone as a drug showing specificity for S. gallinarum.

Inoculum

S. gallinarum, the etiological agent for fowl typhoid, was the organism chosen for this study. It was chosen because of the frequency of occurrence of salmonelloses (pullorum disease, typhoid and paratyphoid) in poultry. This particular species, S. gallinarum, was considered more suitable for an experimental problem than other salmonella species because of its adaptability. Its pathogenicity can be controlled by serial passage to the extent that death losses can be governed to suit the problem. By proper control of the virulence of the organism, an optimal number of deaths will occur and a suitable number of affected birds will survive the experiment to show the effects of the drugs on isolation of the organism. In preliminary trials, S. pullorum proved to be less suitable because it primarily affects very young birds and its acuteness often caused the death of all the birds before the drug had a chance to mask its recovery. Any of the paratyphoid organisms could have been used but as there are over 150 types, the problem of positive identification makes work with these organisms more tedious than with either S. gallinarum or S. pullorum.

Two strains of S. gallinarum were employed. For intraperitoneal inoculation, a lyophilized culture procured from

the University of Illinois was used. It was reconstituted in nutrient broth and serially passed through birds until a desired virulence was reached. Subsequent groups were inoculated with organisms recovered from the hearts and livers of infected chickens used in the preceding run. For oral inoculation, a culture obtained from the Lederle Laboratories was used. It was more virulent than the Illinois strain if given intraperitoneally, but if administered orally caused about the same number of deaths as was caused by intraperitoneal inoculation of the Illinois strain. The difference in the virulence compensated for the difference in the route of administration.

Maintenance of proper virulence proved to be the most difficult part of the problem. An optimal mortality rate of 25 per cent could not be maintained uniformly because of the many variables affecting the death rate. As this problem dealt primarily with the recoverability of the organism and not with the therapeutic properties of the drug, death of the birds was not necessarily essential for proof of infection.

Administration of the Organism

Intraperitoneal inoculation was accomplished with a 1 ml. tuberculin syringe and a 22 gauge needle. All oral administration was by the use of a 10 ml. syringe and a three-inch 16 gauge needle that had been curved and blunted. The inoculum was placed slowly in the crop thereby preventing over-flow into the esophagus and by reverse peristalsis into the trachea.

In all runs except one, the administration of the organism was in nutrient broth. One-half ml. of a 24 hour culture was given either intraperitoneally or orally. Turbidity tests were not run to determine the number of organisms given.

Smith (1955a) reported that the number of organisms given above a certain level was not important. He ran a trial testing the number of organisms necessary to cause infection in day-old chicks given S. gallinarum orally. He caused 100 per cent infectivity when chickens were given 50 million, 5 million, 0.5 million, or 0.05 million organisms. When 0.005 million organisms were given, only three per cent became infected. The number of organisms in one-half ml. used in this problem was far more than necessary to cause infection. With oral administration any excess was passed in the feces during the first 24 hour period after inoculation. Smith found that at three hours and six hours after oral administration, large numbers of the organism could be recovered in the feces but that at 24 hours, the feces became negative and remained so until the bacteremic phase of the disease. This bacteremic phase usually occurred on the third day after inoculation. As this problem is concerned with the recoverability of the organism from the diseased bird in the bacteremic phase as is done in diagnostic laboratories, the excess inoculum passed in the feces had no significant effect on the results.

Administration of the Drugs

Administration of the drugs was per os either through the feed or water. The correct amount of drug after being

hand mixed in a quart of feed was poured into an operating mixer (Figure II) simultaneously with 50 pounds of non-medicated feed. Running the mixer for three 10 minute intervals plus hand mixing between intervals ensured the absence of pockets of unmixed feed. Adequate grounding prevented charged particles from adhering to the sides of the mixer.

The sulfamethazine and one level of soluble sulfaquinoxaline were administered in the drinking water according to the manufacturers' directions.

Figure II. Machine Used for Mixing Drugs in Feed

PROCEDURE

The experimental test for each drug followed the same procedure. Fifty week-old chickens were divided into five groups of 10 birds each. On the first day of the test, one group received medicated feed. Forty eight hours later, a second group was placed on medicated feed. At this time, all birds except the non-inoculated controls received the organism either intraperitoneally or orally. Forty-eight hours later, a group of 10 of the inoculated chicks was put on medication. Thus, for each experimental run, one group of 10 birds received medication 48 hours before inoculation, one group at the time of inoculation, and one group 48 hours after inoculation. The fourth and fifth groups served as controls either inoculated or non-inoculated.

On the third day after inoculation and on alternate days thereafter, one bird from each group was sacrificed until all 10 were used. Portions of the liver, heart, and spleen were removed and placed in 50 ml. of selenite F* broth. Selenite F is an enrichment medium for the isolation of typhoid, other salmonellae, and some types of dysentery bacilli. After an 18 to 24 hour incubation period at 37° C. a loop of this material

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*Selenite F Enrichment, Baltimore Biological Laboratory, Baltimore, Maryland.

was streaked on a plate of MacConkey agar.* This agar is used for the isolation of Shigella and Salmonella. The MacConkey plates were incubated 24 hours at 37° C. and the organism identified by accepted carbohydrate tests.

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*MacConkey agar, Difco, Detroit, Michigan.

RESULTS AND DISCUSSION

Intraperitoneal Inoculation

Drugs chosen for use in this project were tested first against organisms which were injected intraperitoneally. This is an unnatural method of producing fowl typhoid but it has certain advantages over oral administration. With intraperitoneal inoculation, birds will go through a bacteremic phase. They may recover from the disease completely and may rid themselves of all organisms after a period of time but they will have an initial infection. With oral inoculation, 100 per cent infectivity cannot uniformly be attained.

Onset of the disease was very rapid following this method of administration. By the third day, infected birds became depressed, refused food, and sought segregation in a corner away from the other birds. They became very droopy and soon became moribund. Death usually occurred from the second to the fifth day after inoculation.

Principal pathological changes included enlargement of the liver and spleen (Figure III), mottling of the liver, and a catarrhal inflammation of the digestive tract.

Experiment 1--Sulfas and their effects. The first trial consisted of a study of the effects of sulfaquinoxaline and

Figure III. Enlarged liver and spleen typical of fowl typhoid on the left compared to the normal on the right. The spleens are indicated by arrows.

sulfamethazine on the recovery of S. gallinarum from the hearts, livers and spleens of chickens intraperitoneally inoculated with one-half ml. of a 24 hour culture of nutrient broth. Fourteen groups of 10 chickens each were placed on trial. These included three groups for each of the four levels of drugs plus two groups as controls. Sulfaquinoxaline* was used at two levels in the feed, 453 Gm. per ton (0.05 per cent) and 160 Gm. per ton (0.0175 per cent). In one group, it was given in the water at the rate of four Gm. of soluble sulfaquinoxaline (.025 per cent) per gallon of water. Sulfamethazine (Sulmet**) was given in the water according to the manufacturer's directions, (0.1 per cent). For each drug level, one group of 10 birds received medication 48 hours before inoculation, one at the time of inoculation, and one 48 hours after inoculation.

Death losses in this particular experiment were not heavy. Only 10 birds (7.7 per cent) died. Of these, two birds which were to be given sulfamethazine 48 hours after inoculation died on the second day before having access to the medicated water. Two other birds, one from this same group and one from the 0.05 per cent sulfaquinoxaline (48 hours after) died on the third day after having access to medication less than 24 hours. All deaths except three occurred by the end of the third day. There were no deaths among the

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*Sulfaquinoxaline, Merck and Company, Inc., Rahway, N. J.

**Sulmet, Lederle Laboratories, Pearl River, New York.

birds receiving sulfaquinoxaline in water and none among those receiving either 0.05 per cent sulfaquinoxaline or sulfamethazine 48 hours before or at the time of inoculation. Only one infected control died. Table 1 shows a complete breakdown of the losses as they occurred.

TABLE 1

DEATH LOSSES IN CHICKS RECEIVING VARIOUS LEVELS OF SULFA

	Days after Inoculation					
	1	2	3	4	5	Totals
0.05% Sulfaquinoxaline in feed						
48 hours prior	0	0	0	0	0	0
Simultaneous	0	0	0	0	0	0
48 hours after	0	0	1	0	1	2
0.0175% Sulfaquinoxaline in feed						
48 hours prior	0	0	0	1	0	1
Simultaneous	0	0	2	0	0	2
48 hours after	0	0	0	0	0	0
0.025% Sulfaquinoxaline in water						
48 hours prior	0	0	0	0	0	0
Simultaneous	0	0	0	0	0	0
48 hours after	0	0	0	0	0	0
0.1% Sulfamethazine in water						
48 hours prior	0	0	0	0	0	0
Simultaneous	0	0	0	0	0	0
48 hours after	0	2	1	0	1	4
Controls						
Inoculated	0	0	1	0	0	1
Non-inoculated	0	0	0	0	0	0
Daily Losses	0	2	5	1	2	10

Table 2 shows the results of cultures of portions of the hearts, livers, and spleens of the inoculated birds. Isolation was accomplished by placing portions of these organs in 50 ml. of selenite F broth and incubating for 18 hours. A loop of this broth was streaked on MacConkey agar and incubated.

As indicated in Table 2, the sulfas had little effect in preventing recovery of the organism. During the first seven days after inoculation, the period during which birds most likely would be brought to a diagnostic laboratory during a field outbreak, S. gallinarum was recovered in considerable numbers from all inoculated birds. After the seventh day, a few hardy individuals were free of the organism. As time elapsed, the organism was recovered from fewer birds. Since natural recovery may occur with any disease, a certain amount of these negatives should be attributed to this. Of the 30 birds in each group on sulfa, 22 (73 per cent) birds receiving 0.05 per cent sulfaquinoxaline were positive, 25 (83 per cent) receiving either sulfaquinoxaline or sulfamethazine in water were positive as were 27 birds (90 per cent) receiving 0.0175 per cent sulfaquinoxaline in feed. All 10 inoculated controls were positive. Time of medication, whether before, at time of, or after inoculation, had no effect on recovery of the organism.

TABLE 2

EFFECT OF SULFAS ON RECOVERY OF
S. GALLINARUM INOCULATED INTRAPERITONEALLY

	Days after Inoculation									
	3	5	7	9	11	13	15	17	19	21
0.05% Sulfaquinoxaline in feed										
48 hours prior	≠	≠	≠	-	≠	≠	≠	≠	-	-
Simultaneous	≠	≠	≠	≠	-	≠	≠	-	-	-
48 hours after	*≠	*	≠	≠	≠	≠	≠	-	≠	...
0.0175% Sulfaquinoxaline in feed										
48 hours prior	≠	*≠	≠	≠	≠	≠	≠	≠	≠	...
Simultaneous	**	≠	≠	≠	≠	≠	≠	≠	-	...
48 hours after	≠	≠	≠	≠	≠	-	≠	≠	≠	-
0.025% Sulfaquinoxaline in water										
48 hours prior	≠	≠	≠	≠	≠	-	≠	≠	-	≠
Simultaneous	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
48 hours after	≠	≠	≠	≠	≠	≠	≠	-	-	≠
0.1% Sulfamethazine in water										
48 hours prior	≠	≠	≠	≠	≠	-	≠	-	-	-
Simultaneous	*≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
48 hours after	**	*≠	≠	≠	-	≠	≠	...	...	...
Controls										
Inoculated	*	≠	≠	≠	≠	≠	≠	≠	≠	≠
Non-inoculated	-	-	-	-	-	-	-	-	-	-

≠ organism recovered
* dead chick, organism recovered
- organism not recovered
... no chick available due to death loss

Experiment 2--Effects of growth-promoting additives. Experiment 2 was conducted in the same manner as experiment 1 with the objective of determining the effects of growth-promoting additives on the recovery of S. gallinarum. Chlortetracycline and penicillin were chosen as representative drugs in this category. They were given at levels recommended by the Poultry Department of Michigan State University. These levels were those employed for growth promotion and were below the therapeutic levels recommended by the manufacturers if the drugs were to be used in the control of the disease. Due to the wide usage of feeds containing antibiotics, this experiment was considered important from the standpoint of recovery of the organism although no therapeutic values were expected at these levels.

Chlortetracycline used in the feed was Aurofac 2A*. It was given at two levels, five pounds (18 Gm. active ingredient) per ton and thirty pounds (108 Gm. active ingredient) per ton. Penicillin used was Pro-Pen "2:3"** at the rate of two pounds (4 Gm. active ingredient) per ton. The chickens were divided into pens of 10 birds each with one group for each drug receiving medication 48 hours before inoculation, one group at the time of inoculation, and one group 48 hours after inoculation.

Death losses were rather high in this experiment. Thirty-three of the 100 inoculated birds died. The fact that the

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* Aurofac, Lederle Laboratories, Pearl River, New York.

** Pro-Pen "2:3", Merck and Company, Inc., Rahway, New York.

medication was at a growth-promoting rather than a therapeutic level may have caused this. As in the previous experiment, most of the losses occurred from the second to the fifth day. Twenty-two deaths occurred by the third day including six on the higher level of chlortetracycline, 10 on the lower level, five on penicillin and only one which received no treatment. Death losses began to taper off on the fifth day and ceased entirely after the seventh.

TABLE 3

DEATH LOSSES IN CHICKS RECEIVING GROWTH-PROMOTING DRUGS

	Days after Inoculation								Totals
	1	2	3	4	5	6	7	8	
Chlortetracycline 108 Gm/ton									
48 hours prior	0	2	1	0	1	1	0	0	5
Simultaneous	0	2	0	0	0	0	0	0	2
48 hours after	1	0	0	0	1	0	0	0	2
Chlortetracycline 18 Gm/ton									
48 hours prior	0	3	0	1	0	0	0	0	4
Simultaneous	0	1	2	1	0	1	0	0	5
48 hours after	0	4	0	0	1	0	0	0	5
Penicillin 4 Gm/ton									
48 hours prior	0	1	1	0	1	0	0	0	3
Simultaneous	1	0	1	0	0	0	0	0	2
48 hours after	0	1	0	1	0	0	1	0	3
Controls									
Inoculated	0	1	0	1	0	0	0	0	2
Non-Inoculated	0	0	0	0	0	0	0	0	0
Daily Losses	2	15	5	4	4	2	1	0	33

Overall death losses included nine of 30 birds (30 per cent) receiving the higher level of chlortetracycline, 14 (46.7 per cent) receiving the lower level, eight (26.7 per cent) receiving penicillin and only two (20 per cent) receiving no medication at all. Although no attempt was made in this problem to evaluate these drugs from the standpoint of prevention of death losses, it appeared that at growth-promoting levels, chlortetracycline and penicillin were worse than no treatment.

Table 4 shows the results of attempts at recovering the organism. The drugs at the levels used had no effect whatsoever in preventing recovery. At the lower level of chlortetracycline, the organism was recovered from all 30 chickens. It was recovered from 29 of 30 chickens (97 per cent) at the higher level of chlortetracycline, 28 of 30 (92.3 per cent) receiving penicillin and seven of 10 birds (70 per cent) receiving no medication at all. These results lead one to suspect that low levels of antibiotics in feed may enhance the growth of bacteria rather than retard it. Pratt and Dufrenoy (1953) have found that antibiotics definitely stimulate the growth of organisms at very low levels of concentration. In the standard biologic assay of penicillin, they "seeded" nutrient agar with a thin layer of penicillin-sensitive bacteria and then placed small glass cylinders containing penicillin on the seeded agar surface. After suitable incubation there was seen around each cylinder or reservoir of penicillin solution a circular clear zone (zone of inhibition) in which

TABLE 4

EFFECT OF ANTIBIOTICS AT GROWTH PROMOTION LEVELS ON RECOVERY OF S. GALLINARUM INOCULATED INTRAPERITONEALLY

	Days after Inoculation									
	3	5	7	9	11	13	15	17	19	21
Chlortetracycline 108 Gm/ton of feed										
48 hours prior	***	*	*	/	...	/	/	/	...	/
Simultaneous	**	...	/	/	/	/	/	/	/	/
48 hours after	*	*	/	/	/	/	/	/	/	-
Chlortetracycline 18 Gm/ton of feed										
48 hours prior	***	*	...	/	/	/	/	/	...	/
Simultaneous	***	*	*	/	...	/	/	/	...	/
48 hours after	****	*	...	/	...	/	/	/	...	/
Penicillin 4 Gm/ton of feed										
48 hours prior	**	*	...	/	/	/	/	/	/	/
Simultaneous	**	...	/	/	/	/	-	/	-	/
48 hours after	*	*	*	/	/	/	/	/	/	/
Controls										
Inoculated	*	*	/	/	-	/	/	/	-	-
Non-inoculated	-	-	-	-	-	-	-	-	-	-

/ organism recovered
* dead chick, organism recovered
- organism not recovered
... no chick available due to death loss

multiplication of the bacteria had been blocked by the antibiotic. The diameter of the inhibition zone was, within limits, a function of the concentration of the penicillin in the solution under test. Outside the clear area, no inhibition occurred. However, between the zone of inhibition and zone of no inhibition, there was a narrow ring where growth had been enhanced and where the bacterial population was much more dense than in the uninhibited portion of the assay plate. Diagrammatically, the plates appeared as in Figure IV.

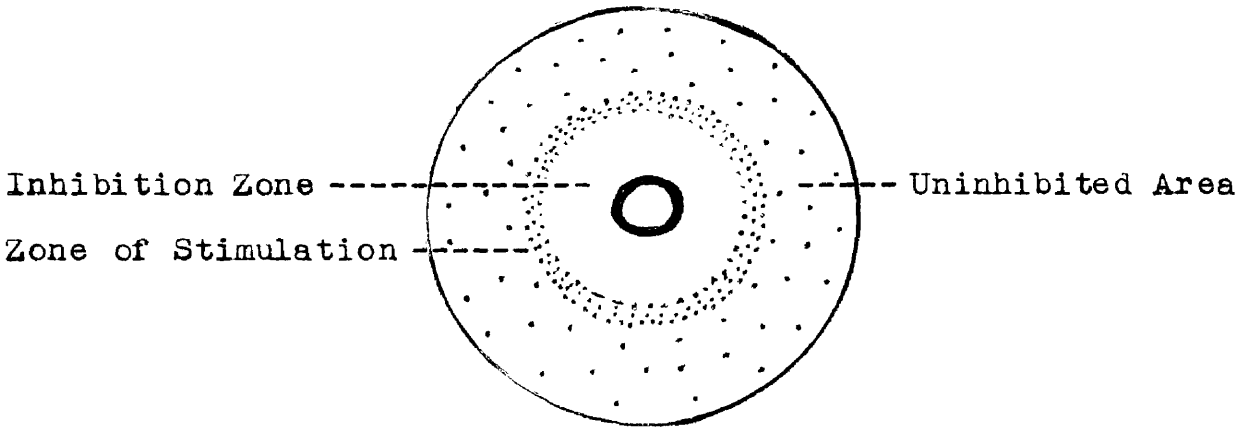

Figure IV. Diagrammatic Representation of a Penicillin Assay Plate

Just what is the level of stimulation for the various antibiotics is beyond the scope of this problem. However, due to the lowered intake of food by a sick chicken, the amount of antibiotic consumed would be lowered and could well fall into the correct concentration for either stimulation or no inhibition.

Smith (1955b) found that even at higher levels of antibiotics, although less deaths occurred, the percentage of

chickens excreting organisms in their feces was often higher than in the controls. For proof of this, he offered the following results:

Agent	Per cent of chicks discharging organisms in feces
Furazolidone 25 mg./kg. once daily	0
Oxytetracycline 50 mg./kg. once daily	78.5
Sulfamerazine 150 mg./kg. twice daily	88.9
Chloramphenicol 150 mg./kg. twice daily	92.3
Chlortetracycline 50 mg./kg. once daily	70.8
Dihydrostreptomycin 25 mg./kg. twice daily	84.2
Penicillin 150 mg./kg. once daily	100.0
Untreated	82.6

His experiment indicated that with the exception of furazolidone, the drugs tested gave poorer results than no treatment at all. In four of the six cases, a higher percentage of fecal excretors was found than in the controls. The overall percentage of the drugs (exclusive of furazolidone) was 85.8 per cent of fecal excretors compared with only 82.6 per cent among the untreated birds.

Experiment 3--Broad spectrum antibiotics at therapeutic levels and furazolidone. This experiment was a combination test to show the effect upon the recovery of the organism of both broad spectrum antibiotics at therapeutic levels and furazolidone. The broad spectrum antibiotics used were chlortetracycline at 200 Gm. per ton of feed and at 400 mg. per gallon of water, neomycin at 500 Gm. per ton of feed, and a combination of the two drugs with chlortetracycline at 100 Gm. and neomycin at 500 Gm. per ton. The levels of neomycin were considerably higher than would be economically feasible.

However, this high level was used to determine whether it had any effect rather than to establish a therapeutic level. Furazolidone was given at the level recommended by the manufacturer, two pounds of NF 180* (50 Gm. of furazolidone per pound) per ton of feed.

Table 5 shows the death losses. A total of 47 deaths (29.4 per cent) occurred in 160 chickens. None died among those receiving furazolidone or among the non-inoculated controls. Again death losses occurred mostly from the second to the fifth day. Deaths included seven birds (23.3 per cent) receiving 200 Gm. of chlortetracycline per ton of feed; 16 (53.3 per cent) receiving 400 mg. of chlortetracycline per gallon of water; eight (26.7 per cent) receiving neomycin; 13 (43.3 per cent) receiving a combination of two drugs as compared to three of 10 (30 per cent) non-medicated controls. The high death losses in the combination of the two drugs could have been due to the antagonistic action of the antibiotics when used together.

Scheidy (1952) grouped antibiotics as follows:

> "group 1 includes penicillin, streptomycin, bacitracin, neomycin, and polymixin. When used together, they will possibly help each other or be synergistic in their action. In group 2 are chlortetracycline, chloramphenicol, and oxytetracycline. When they are combined or given in combination, they have an additive effect. When antibiotics of group 1 and group 2 are added in the animal at the same time, they are antagonistic. In other words, for good therapy, one perhaps should not mix the members of group 1 with those of group 2 in the treatment of certain infections."

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*NF 180, Hess and Clark, Ashland, Ohio.

TABLE 5

DEATH LOSSES IN INTRAPERITONEALLY INOCULATED CHICKS RECEIVING FURAZOLIDONE OR BROAD SPECTRUM ANTIBIOTICS

	Days after Inoculation								
	1	2	3	4	5	6	7	8	Totals
Furazolidone 100 Gm/ton									
48 hours prior	0	0	0	0	0	0	0	0	0
Simultaneous	0	0	0	0	0	0	0	0	0
48 hours after	0	0	0	0	0	0	0	0	0
Chlortetracycline 400 mg/gal H_2O									
48 hours prior	0	2	1	1	1	0	0	0	5
Simultaneous	0	1	4	0	0	0	0	0	5
48 hours after	0	0	1	0	1	3	0	1	6
Chlortetracycline 200 Gm/ton									
48 hours prior	0	0	1	1	0	1	0	0	3
Simultaneous	0	0	1	0	1	0	0	0	2
48 hours after	0	0	1	1	0	0	0	0	2
Neomycin 500 Gm/ton									
48 hours prior	0	0	1	0	1	0	0	0	2
Simultaneous	0	0	1	1	0	0	0	0	2
48 hours after	0	1	1	0	1	1	0	0	4
Neomycin and Chlortetracycline									
48 hours prior	0	0	3	1	0	0	0	0	4
Simultaneous	0	0	1	0	1	0	0	0	2
48 hours after	0	4	2	0	1	0	0	0	7
Controls									
Inoculated	0	0	1	1	0	1	0	0	3
Non-inoculated	0	0	0	0	0	0	0	0	0
Daily Losses	0	8	19	6	7	6	0	1	47

Results of attempts at recovering the organism shown in Table 6 bear out the picture presented by the death losses. Furazolidone almost completely negated the organism. When one considers that the organism was injected intraperitoneally where it rapidly came in contact with the blood stream, this almost complete inhibition of the organism was remarkable.

With chlortetracycline in feed, S. gallinarum was recovered in 24 of 30 chickens (80 per cent). With chlortetracycline in water, neomycin alone, or neomycin in combination with chlortetracycline, the recovery was from 27 of 30 chickens (90 per cent). It was recovered from all of the infected controls.

The results indicate that furazolidone had a definite effect in preventing the recovery of the organism even if given intraperitoneally. Chlortetracycline and neomycin, either alone or in combination, did not prevent isolation by the usual cultural procedures.

Oral Inoculation

Natural infection of fowl typhoid occurs normally by ingestion of contaminated material by a susceptible bird. Any experiment of this nature would be incomplete without data determining the inhibitory effect of the drugs when infection is produced by oral administration. Certain problems are encountered with this method that are not met with when the organism is given intraperitoneally. A healthy chicken raised under ideal conditions has considerable

TABLE 6

EFFECT OF FURAZOLIDONE AND BROAD SPECTRUM ANTIBIOTICS ON RECOVERY OF S. GALLINARUM INOCULATED INTRAPERITONEALLY

	Days after Inoculation									
	3	5	7	9	11	13	15	17	19	21
Furazolidone 100 Gm/ton										
48 hours prior	≠	-	-	-	-	-	-	-	-	-
Simultaneous	-	-	≠	-	-	-	-	-	-	-
48 hours after	-	≠	-	-	-	-	-	-	-	-
Chlortetracycline 200 Gm/ton										
48 hours prior	*	*	*	≠	≠	≠	≠	-	-	-
Simultaneous	*	*	≠	-	≠	≠	≠	-	≠	≠
48 hours after	*	*	≠	≠	≠	-	≠	≠	≠	≠
Chlortetracycline 400 mg/gal										
48 hours prior	***	**	...	≠	...	≠	-	≠	...	≠
Simultaneous	*****	...	...	≠	...	≠	≠	≠	...	≠
48 hours after	*	*	***	*	...	≠	-	≠	...	-
Neomycin 500 Gm/ton										
48 hours prior	*	*	≠	≠	≠	≠	≠	-	≠	-
Simultaneous	*≠	*	≠	≠	≠	≠	-	≠	≠	≠
48 hours after	**	*	*≠	≠	≠	≠	≠	...	≠	...
Chlortetracycline 100 Gm/ton and Neomycin 500 Gm/ton										
48 hours prior	***	*	≠	≠	≠	≠	-	...	-	...
Simultaneous	*	*	≠	≠	≠	≠	≠	≠	≠	≠
48 hours after	******	*	≠	-	...	...	...	...	≠	...
Controls										
Inoculated	*	*	*	≠	≠	≠	≠	≠	≠	≠
Non-inoculated	-	-	-	-	-	-	-	-	-	-

≠ organism recovered
* dead chick, organism recovered
- organism not recovered
... no chick available due to death loss

resistance to infection. With oral administration, results are not uniform in that some of the birds resist infection. This resistance is influenced by such factors as breed difference, resistant strains within breeds, age of bird, environmental conditions and the pH of the digestive tract at the time of administration of the organism.

Garren and Barber (1955) in their studies on endocrine and lymphatic gland changes in young chickens with fowl typhoid found considerable differences among breeds in their resistance to infection by the S. gallinarum organism. They orally inoculated chickens that were approximately six weeks of age with 1 ml. of a saline suspension of the organism. They used three different breeds--New Hampshire Reds, Rhode Island Reds, and White Leghorns. The mortality rate was 85 per cent in the New Hampshire Reds, 100 per cent in the Rhode Island Reds, and 0 per cent in the White Leghorns. With the New Hampshire Reds and the Rhode Island Reds, they found a marked decrease in feed and water consumption, a decrease in body weight, a heavy mortality, a pituitary hypertrophy, a tremendous adrenal enlargement, an involution of the bursa of Fabricius, and histological evidence of a decreased thyroid activity. The only pronounced effect following inoculation of young White Leghorns with fowl typhoid organisms was a decrease in bursa weight. The authors suggested the possibility that the changes noted could have resulted from the adverse physiological conditions induced by successful infection rather than by the chickens' attempt to resist infection.

From their experimentation, it is obvious that there is a great deal of variation in the susceptibility of the different breeds and that White Leghorns have a certain degree of natural immunity to oral infection with fowl typhoid.

Biester and Schwarte (1952) found that selection of resistant strains within breeds for resistance to fowl typhoid resulted in a decided decrease in the mortality of selected stocks. Smith (1955a) found that with oral administration, age of the birds had a pronounced effect upon susceptibility. He reported that one-day-old chicks were most susceptible and that a higher degree of resistance was exhibited by three-day-old birds. Little difference was noted between the resistance of 17 and 3 day-old birds but the results for 4, 9, and 14 week-old chickens showed a progressive increase of resistance with age as indicated by the longer survival times and lower death rates.

Environmental conditions have an effect upon susceptibility. Any stress reaction such as vaccination against fowl pox, chilling, or restriction of water or food intake makes a chicken more susceptible to oral infection.

The pH of the digestive tract at the time of administration of the organism has a definite bearing upon susceptibility. Horton-Smith and Long (1955) in attempting to produce blackhead in hen chicks tried fasting them in order to raise the pH. The pH of the gizzard contents of feeding chicks varied from 2.9 to 3.3 while that of chicks fasted for 18 hours rose to 6.3 to 7.0. When the pH of the gizzard contents was

raised to 7.3 to 7.6 by administering an alkali mixture to fasted chickens, patent infections were produced in most cases. When alkali was given to feeding chickens, the pH of the gizzard content rose to 6.2 to 6.5 and infections were produced in much the same proportion of cases as in fasted chickens not receiving alkali.

Smith (1955a) in producing infection with S. gallinarum routinely fasted the chickens for 18 hours and then gave them 0.1 ml. of an 18 hour broth culture which was suspended in 1 ml. of distilled water containing 0.3 grams of alkali. This neutralized the bactericidal action of the gastric juice in the gizzard and allowed a higher percentage of infectivity.

With oral administration, the disease took a chronic rather than an acute course. Onset of the disease was rather slow with the first deaths appearing about the sixth day after inoculation. The chickens became emaciated and anemic. Their food consumption was reduced and growth rate greatly curtailed. Principal pathological lesion was the proliferative cellular reaction in the heart. Large white nodular masses infiltrated the myocardium causing enlargements on the heart surface (Figure V). These masses changed the contour of the organ and caused a great increase in its size.

Experiment 4--Effects of furazolidone and broad spectrum antibiotics with oral administration of the organism. For this experiment, a slant culture of S. gallinarum, strain 605,

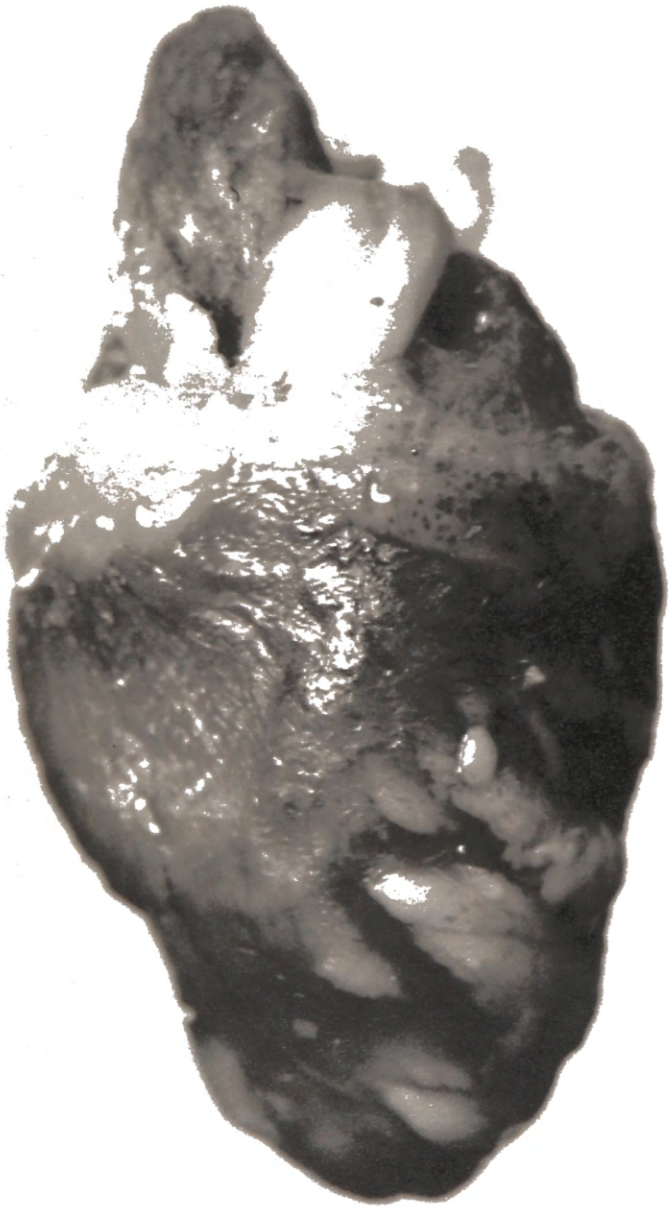

Figure V. Enlargements on the heart surface typical of fowl typhoid.

was provided by Lederle Laboratories. According to Patton (1956), one-half ml. of a 10^{-1} dilution of a 24-hour yeast extract broth culture administered intraperitoneally resulted in 100 per cent mortality in white mice. This organism was serially passed through chickens three times and then used for oral inoculation.

The same drugs and the same levels were used as in experiment 3. The only other difference was that chickens were sacrificed and cultured on alternate days beginning with the sixth day after inoculation rather than the third. This was done because symptoms did not appear until the fifth day.

Of the 130 chickens orally inoculated, 14 (10.8 per cent) died. Table 7 shows a breakdown of the death losses. There were no death losses in chickens receiving furazolidone and none in the inoculated controls. No particular reason can be given for this survival of the controls. However, in view of the fact that Garren and Barber (1955) could readily produce deaths in New Hampshire and Rhode Island Reds but not in White Leghorns and the fact that only 10.8 per cent of all 130 inoculated chickens died, it is not unreasonable for these 10 birds to survive. Four of 30 birds (13.3 per cent) receiving either chlortetracycline or neomycin died and six birds (20 per cent) receiving a combination of the two drugs died.

TABLE 7

DEATH LOSSES IN ORALLY INOCULATED CHICKS RECEIVING FURAZOLIDONE OR BROAD SPECTRUM ANTIBIOTICS

	Days after Inoculation				Totals
	6	8	10	12	
Furazolidone 100 Gm/ton					
48 hours prior	0	0	0	0	0
Simultaneous	0	0	0	0	0
48 hours after	0	0	0	0	0
Chlortetracycline 200 Gm/ton					
48 hours prior	0	0	0	1	1
Simultaneous	0	0	0	0	0
48 hours after	1	0	1	1	3
Neomycin 500 Gm/ton					
48 hours prior	0	0	1	0	1
Simultaneous	1	1	1	0	3
48 hours after	0	0	0	0	0
Neomycin and Chlortetracycline					
48 hours prior	0	1	1	0	2
Simultaneous	0	0	0	0	0
48 hours after	1	1	1	1	4
Controls					
Inoculated	0	0	0	0	0
Non-inoculated	0	0	0	0	0
Daily Losses	3	3	5	3	14

Results of attempts to recover the organism (Table 8) are not uniform. This concords with a report by Moore (1946) that oral inoculation did not give as uniform results as when the organism was given intraperitoneally or rectally. Many of the birds resisted infection. However, the overall picture is much the same as when the organism was given intraperitoneally. Again furazolidone completely inhibited the organism. The organism was not recovered from any of the 30 chickens receiving this drug. Further evidences of good health in this group of birds were rapid growth, high food intake, and absence of any pathological lesions. The organism was recovered from 15 birds (50 per cent) on chlortetracycline, 17 birds (56.7 per cent) on neomycin, six of 10 birds (60 per cent) inoculated but receiving no treatment and 20 birds (66.7 per cent) receiving a combination of neomycin and chlortetracycline. This combination of two drugs again appeared to exhibit antagonism and gave poorer results than no treatment. In comparing the results of birds on chlortetracycline, neomycin, or a combination of the two with birds receiving no medication, one would have to say that these drugs did not prevent recoverability of the organisms. The same cannot be said for furazolidone.

Experiment 5--Effects of sulfas with oral administration of the organism. The results of this experiment were inconclusive. Due either to a lowered virulence or to fewer organisms in the inoculum, the organism failed to produce

TABLE 8

EFFECT OF FURAZOLIDONE AND BROAD SPECTRUM ANTIBIOTICS ON RECOVERY OF S. GALLINARUM GIVEN ORALLY

	Days after Inoculation									
	6	8	10	12	14	16	18	20	22	24
Furazolidone 100 Gm/ton										
48 hours prior	-	-	-	-	-	-	-	-	-	-
Simultaneous	-	-	-	-	-	-	-	-	-	-
48 hours after	-	-	-	-	-	-	-	-	-	-
Chlortetracycline 200 Gm/ton										
48 hours prior	≠	-	-	*	≠	-	≠	-	-	≠
Simultaneous	≠	-	≠	≠	-	≠	-	-	-	≠
48 hours after	*	≠	*	*	-	-	≠	-	-	-
Neomycin 500 Gm/ton										
48 hours prior	≠	≠	*	-	≠	≠	-	-	-	≠
Simultaneous	*	*	*	-	-	≠	≠	≠	-	-
48 hours after	≠	≠	-	-	-	-	≠	≠	-	≠
Chlortetracycline 100 Gm/ton and Neomycin 500 Gm/ton										
48 hours prior	≠	*	*	≠	-	-	≠	≠	-	≠
Simultaneous	≠	≠	-	-	-	≠	-	-	≠	≠
48 hours after	*	*	*	*	≠	≠	≠	≠	-	-
Controls										
Inoculated	≠	≠	-	≠	≠	-	≠	≠	-	-
Non-inoculated	-	-	-	-	-	-	-	-	-	-

≠ organism recovered
* dead chick, organism recovered
- organism not recovered

infection in many chickens. No death losses occurred. The organism used was the same strain as used in the previous experiment. It was injected intraperitoneally into 16 chicks, 15 of which died within six days. The organism could not be recovered from the sixteenth chick. A culture from the liver and spleen of one of the dead chickens was used as the source for the oral inoculum in this trial.

Recovery of the organism was not consistent. As can be seen in Table 9, isolation of S. gallinarum was accomplished from only one of the 10 inoculated controls, from six of the 30 birds (20 per cent) receiving sulfamethazine, nine (30 per cent) receiving 0.05 per cent sulfaquinoxaline, 11 (37 per cent) receiving 0.0175 per cent sulfaquinoxaline, and three (10 per cent) receiving sulfaquinoxaline in water.

The very low rate of infectivity especially in the inoculated controls invalidates the results. The most that can be said is that recovery of the organism was accomplished at least as often and usually more often in the medicated birds as in the non-medicated controls.

Experiment 6--Effect of selenite F broth as an inoculum. This experiment was conducted primarily to determine the effect of fasting on the percentage of birds becoming infected. As previously discussed, raising the pH of the gastro-intestinal tract either by addition of alkali or by fasting reportedly reduces its bacteriostatic action. In this trial, birds were fasted 18 hours before oral administration of the organism.

TABLE 9

EFFECTS OF SULFAS ON RECOVERABILITY
ON S. GALLINARUM GIVEN ORALLY

	Days after Oral Inoculation									
	6	8	10	12	14	16	18	20	22	24
0.1% Sulfamethazine in H_2O										
48 hours prior	/	-	-	-	-	-	-	-	-	-
Simultaneous	-	/	/	/	-	-	-	-	-	-
48 hours after	/	-	-	-	/	-	-	-	-	-
0.05% Sulfaquinoxaline in feed										
48 hours prior	-	-	/	/	/	/	-	-	-	-
Simultaneous	-	/	-	-	-	-	-	/	-	/
48 hours after	-	/	/	-	-	-	-	-	-	-
0.0175% Sulfaquinoxaline in feed										
48 hours prior	-	/	-	-	-	-	/	-	/	-
Simultaneous	-	/	/	/	-	-	-	-	-	-
48 hours after	/	/	/	-	-	-	/	-	/	-
0.025% Sulfaquinoxaline in H_2O										
48 hours prior	-	-	-	-	-	-	/	-	-	-
Simultaneous	-	-	-	/	-	-	-	-	-	-
48 hours after	-	-	-	/	-	-	-	-	-	-
Controls										
Inoculated	-	-	-	-	/	-	-	-	-	-
Non-inoculated	-	-	-	-	-	-	-	-	-	-

/ organism recovered
- organism not recovered

To augment further its infectivity, the organism was administered in selenite F broth rather than in nutrient broth. This proved to be a gross error. Ninety chickens were fasted 18 hours and then given 1 ml. of an 18-hour culture of selenite F enrichment broth. Immediately after inoculation, feed was placed before them. In spite of the 18-hour fasting period, they exhibited no interest in the feed and appeared listless and in a state of shock. Within two hours, death losses began. During the next 48 hours, 86 of the 90 chickens died. Death was attributed to poisoning from selenium, the inhibitory agent in the selenite enrichment broth. To rule out the possibility of the effects of fasting and of the organism, four healthy chickens were given 1 ml. of sterile selenite F broth. Three of them died within the next few days and the other one showed severe toxic symptoms. This experiment was a failure insofar as determination of effects of fasting, but it was instrumental in proving the toxicity of selenite as an oral inoculum.

SUMMARY AND CONCLUSIONS

Various levels of six different drugs were tested to determine their effects on preventing the recovery of S. gallinarum from inoculated chickens. White Leghorn chicks at one week of age were divided into groups of 10 and given the organism either orally or intraperitoneally. One group received the test drug in food or water 48 hours before inoculation, one at the time of inoculation, and one 48 hours after inoculation. A chicken from each group was sacrificed on the third day after inoculation if the organism was given intraperitoneally and on the sixth day after inoculation if given orally and on alternate days thereafter until all 10 chicks were used. Portions of the heart, liver, and spleen were incubated 18 hours at 37° C. in selenite F enrichment broth. A loop of this was streaked on a MacConkey agar plate and incubated 24 hours at the same temperature. Identification of the organism was by suitable carbohydrate tests.

Representing the sulfas, sulfaquinoxaline at levels of 0.05 and 0.0175 per cent in the feed or 0.025 per cent in water reduced death losses but did not significantly prevent isolation of the causitive organism. Sulfamethazine at the level of 0.1 per cent in the water gave similar results.

Growth-promoting drugs tested included chlortetracycline and penicillin. Chlortetracycline at levels of 18 Gm. and 108 Gm. per ton of feed and penicillin at 4 Gm. per ton of feed neither reduced losses nor prevented recovery of S. gallinarum. In some respects, they seemed to increase its incidence of recovery.

Broad spectrum antibiotics at therapeutic levels used were chlortetracycline, neomycin, and a combination of the two. Chlortetracycline at 200 Gm. per ton of feed or 400 mg. per gallon of water and neomycin at 500 Gm. per ton of feed had little or no effect on death losses or recovery of the organism. A combination of chlortetracycline at 100 Gm. and neomycin at 500 Gm. per ton of feed resulted in greater death losses and higher incidences of recovery than no medication at all.

Furazolidone at the level of 100 Gm. per ton of feed prevented death losses and almost completely prevented recovery of the organism.

Oral administration of the various drugs gave the same results as intraperitoneal administration but they were not as uniform in that not as many chickens became infected. Time of administration of the drugs whether 48 hours before, at the time of, or 48 hours after inoculation did not alter their effect on the recoverability of the organism.

Selenite F enrichment broth was used as the inoculum in one experiment and proved very toxic. One ml. of this broth administered orally caused the death of 86 of 90 chickens.

Conclusions:

1. Sulfaquinoxaline, sulfamethazine, penicillin, chlortetracycline, and neomycin at various levels did not prevent recovery of S. gallinarum from chickens given the organism either orally or intraperitoneally.

2. Furazolidone prevented recovery of the organism in most cases.

3. Time of administration of the drug as used in this problem had little or no effect on recoverability of the organism.

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