AN ANTIBIOTIC APPROACH TO THE PROBLEM OF PULLORUM DISEASE

By

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INTRODUCTION

Serious outbreaks of pullorum disease still occur among young chicks despite all protective measures. It is in such instances that a reliable antibiotic agent would be of immeasurable value.

The use of antibiotics in the control of pullorum disease can be considered from several important aspects. First of all, the drug should lend itself to oral administration (in feed or water), since it is obvious that methods involving the injection of a therapeutic agent are inefficient when applied to practical situations. Secondly, the antibiotic must maintain its potency under a variety of conditions and should possess a demonstrated affinity for the infecting organism. Finally, and most important, the antibiotic agent should eliminate the organism from the body of the host that survives infection. Drugs which merely suppress the symptoms without eliminating the infection only serve to perpetuate the cycle of transmission. The propagation of the carrier state is ultimately far more serious than the losses that might be sustained from non-treated flocks.

The present problem was undertaken with the above considerations in mind.

REVIEW OF LITERATURE

I. General

The causative agent of pullorum disease was first isolated by Rettger in 1900 (98). He described the condition as a fatal septicemia in young chickens. In 1914 Rettger, Kirkpatrick and Jones (100) established the cyclical transmission of this disease and emphasized the importance of infected ovaries in producing the carrier state among recovered birds. In 1915 Rettger et al (99) announced the practical utilization of a macroscopic tube agglutination test for the detection of infected birds.

Hinshaw, Upp and Moore (52) definitely proved that incubator transmission of pullorum disease was a serious control problem.

Runnells, Coon, Farley and Thorp (109) in 1927 introduced the rapid agglutination test as a diagnostic aid. This test was soon followed by a simplified modification devised by Bunyea, Hall and Dorset (16).

In 1931 Schaffer, MacDonald, Hall and Bunyea (111) and Coburn and Stafseth (23) proposed a rapid test employing whole blood with a stained antigen.

Insko (57) in 1941 stressed the value of formaldehyde as a fumigant, but warned against the use of this method as a substitute for adequate sanitation.

In 1941 Youmie (131) reported the existence of serologic variants in naturally infected flocks. The problem of antigenic variants was further investigated by Edwards and Bruner (36) who found that variants were stabilized with larger amounts of the XII_2 factor.

The effect of diet upon the eventual course of pullorum infection has been sporadically investigated throughout the years, but has never yielded any conclusive results. Rettger (100) indicated that sour milk feeding had a beneficial influence on the growth of chicks and lessened mortality from all causes. However, he doubted the value of sour milk as a therapeutic measure for pullorum disease. Roberts, Card and Severans (103) maintained that environmental factors influence resistance and susceptibility to infection in a variety of diseases. They demonstrated that one type of feed may cause a greater mortality than another, both among chicks inoculated with Salmonella pullorum and among non-inoculated birds. In one experiment 80.5 percent of the inoculated chicks fed a chick mash survived, while only 19.7 percent survived among chicks fed a laying mash. In experiments upon non-inoculated chicks, 98.1 percent of those fed a chick mash lived, while a survival rate of 71.8 percent was attained in those fed a laying mash. The two feeds differed in proportions of nutritive and of fibrous material.

A recent and more intensive study on the effect of

diet in pullorum infection has been conducted by Mann (78, 79, 80). He maintained that pullorum disease could not be sufficiently controlled by blood testing, when feeds of 20 percent protein content were fed to young chicks as the sole diet. Furthermore, Mann presented a new concept of the infective process as encountered in the usual cases of pullorum disease. Salmonella pullorum was regarded as a potential pathogen which must exist in a symbiotic relationship with other organisms in order to produce a pathogenic complex. Organisms of the "welchii type" are considered as the principal symbionts and a chick diet for the initial suppression of such symbionts is proposed.

During recent years the modification of intestinal flora by diet and antibiotics has occupied the attention of many investigators. The relationship of the intestinal flora to specific disease processes is still a problem of intensive research. Hull and Rettger (55) in 1917 stated that lactose, milk and mixed grains are specific articles of the diet which exert an influence on intestinal bacteria. They found that feeding of lactose to white rats for a three-day period, brought about a complete transformation of the intestinal flora. They also found that the feeding of a high carbohydrate diet to typhoid patients tended to reduce the putrefying types of bacteria and encouraged the emergence of "acidophilic" forms.

Johansson, Sarles and Shapiro (59) studied the effect of various carbohydrates in a biotin-deficient ration upon intestinal bacteria of chickens. They found that dextrin

stimulated the development of large numbers of coliform bacteria. Lactose-containing diets also encouraged a fecal coliform flora, but lactic acid bacteria proliferated more extensively in the intestines of birds fed such a diet. The effect of feeding sucrose as the principal carbohydrate produced a marked depression on fecal coliforms. Birds on a dextrin diet appeared to have the greatest numbers of microorganisms at all levels of the intestinal tract.

Romoser, Shorb, Combs and Pelczar (107) reported on the effect of diet composition and antibiotics on cecal bacteria and growth of chicks. Aerobacter aerogenes appeared to increase in the ceca of chicks fed penicillin at a level of 150 ppm, and increased further when a diet containing both penicillin and lactose was given. The addition of lactose and procaine penicillin to the diet produced a greater response in chicks than when penicillin alone was used.

Groschke and Evans (47) found that streptomycin and aureomycin exerted a definite stimulating effect on growth of young chicks. Streptomycin brought about a marked decrease in the coliform count of chick feces. It is suggested that the growth effect produced by antibiotics is mediated in some way through a changed intestinal microflora.

Anderson, Cunningham and Slinger (3) studied the effect of diets containing 17, 20, 23, and 26 percent protein on the cecal bacterial flora of chicks. These diets were

employed with and without antibiotics. An increase in protein content decreased the count of anaerobic and microaerophilic types, while aerobic forms remained constant. Penicillin lowered the pH. Both aerobic and anaerobic groups of organisms were influenced (increased count) by penicillin up to a protein content of 23 percent, followed by a definite decrease with 26 percent. Aciduric forms predominated over proteolytic types. Penicillin enhanced the coliform groups and depressed the enterococcal types. The use of aureomycin decreased the counts of both aciduric and proteolytic organisms with proteolytic forms being more inhibited. Coliform counts were also increased with this antibiotic, but the significance was reduced at a 23 percent protein level followed by a slight decrease with 26 percent.

The research being conducted on antibiotic feed supplements is voluminous and at the present time there is no proved explanation for the interaction between antibiotics and the microflora of normal animals.

Recently Jukes (60) attempted to summarize the various viewpoints regarding the growth-promoting effects of antibiotic feed supplements. According to this summary two theories are currently popular. One theory assumes that antibiotics have a direct vitamin effect on the animal, while the other holds that antibiotics act indirectly through an effect on the microorganisms in the intestine. The second theory is more widely accepted at the present time and is based on several assumptions: (1) toxin-pro-

ducing bacteria are eliminated, (2) competing microorganisms are eliminated, (3) vitamin-producing microorganisms are favored and finally (4) beneficial changes occur in the chemistry of the microorganisms. The second theory is supported by the observation that growth-stimulating effects of antibiotics are most pronounced when the animals are kept under unsanitary conditions and are suffering from subacute intestinal diseases.

The effect of antibiotics on protein requirements of chicks and other animals is still under investigation -- in some cases antibiotics have lowered the protein requirements of the animal. Some investigators believe that the response to an antibiotic is partly dependent on the type of feed ration employed. The composition of the microflora present in the intestinal tract also constitutes one of the many variable factors affecting the response to a particular antibiotic.

There is found among the many breeds of domestic fowl, a variability in resistance and susceptibility to disease. There not only exists a breed difference, but also a variation in genetic makeup within a particular breed.

Stafseth and Weisner (121) reported a breed difference in susceptibility to a variety of diseases. During an eight-year period of observation they found that White Leghorns were more susceptible to a wide range of diseases, while such breeds as Columbian Rocks, Dominiques and Black Minorcas were more resistant.

In a study of selection for resistance to fowl typhoid, Lambert (67, 68, 69) found that the results from five generations of selection showed a marked increase in resistance among the selected birds. The observed mortalities in the selected stock from the first to the fifth generation were as follows: 39.8, 29.3, 15.4, 15.0 and 9.4 percent. In the unselected birds (controls) the respective mortalities were 89.6, 93.2, 86.2, 86.4 and 85.0 percent.

Lambert also demonstrated a breed difference in susceptibility to artificial infection with the organism producing fowl typhoid. The Rhode Island Red and White Wyandotte chicks showed the greatest rate of mortality as well as total mortality, while the white Plymouth Rock chicks exhibited the slowest rate and the least total mortality. The White Leghorn was found to be the most resistant parent breed.

In experiments designed to ascertain breed susceptibility to infection with S. pullorum, Hutt and Scholes (56) noted that White Leghorns were always more resistant to artificial infection with S. pullorum. Leghorn chicks not artificially infected, but exposed to the disease by contact were much more resistant than Rhode Island Reds and Barred Rocks exposed in the same manner. Hutt and Scholes concluded that resistance to S. pullorum is a characteristic of the Leghorn breed.

DeVolt, Quigley and Byerly (30) concluded from their work, that strains of relatively pullorum-resistant chickens

can be developed by artificial selection and strains of relatively resistant chickens develop by natural selection in the presence of natural infection.

Roberts and Card (102) in a ten-year genetic study of resistance to pullorum disease found that heredity is an important factor. Evidence for hereditary factors consisted of the following: (a) selection produced more resistant strains, (b) selected stock was consistent in maintaining resistance through successive generations, (c) resistance is dominant to immunity, and (d) blood differences occur in resistant birds such as increase of erythrocytes and decrease in neutrophiles.

In a further study Severans, Roberts and Card (118) reported the following significant facts: (a) a decrease in the number of lymphocytes (by X-ray) produced a decrease in resistance to pullorum disease (b) the spleens of resistant chicks were larger than those of susceptible chicks, (c) a removal of the spleen from resistant chicks produced a decrease in the number of lymphocytes with a consequent reduction in resistance, (d) the lymphocyte level possessed by chicks, at the time the infecting organism reaches the blood stream, determines the degree of resistance, and (e) the temperatures of resistant chicks were slightly higher than those of susceptible chicks.

Bell (5) investigated the physiological factors associated with genetic resistance to fowl typhoid. His findings reveal that the ability of polymorphonuclear

leucocytes to digest phagocytosed bacteria appears to be a major factor in genetic resistance to Salmonella gallinarum. Differences in body temperature also play an important supporting role.

More recently Lerner and Taylor (70) obtained evidence for genetic differences in resistance to a respiratory disease among chickens, tentatively diagnosed as atypical infectious coryza.

II. Sulfonamides

The use of chemotherapautic agents in the control of poultry diseases has mainly been limited to the sulfonamides.

Delaplane (29) in 1941 successfully treated infectious coryza with sulfathiazole. He emphasized the fact that sulfathiazole was bacteriostatic rather than bactericidal.

Prior to the work of Delaplane, toxicity studies were conducted on some of the sulfa compounds (81, 101). Richardson found that the daily administration of 0.5 to 2.0 g of sulfanilamide per kg to mixed breeds of chickens produced an intense cyanosis within four to five days. This was attributed to the conversion of hemoglobin into methemoglobin by sulfanilamide.

Severans, Roberts and Card (117) tested sulfonamides with respect to their efficacy in reducing mortality from pullorum disease. Sulfadiazine and sulfamerazine were found to be the most effective. Sulfasuxidine, phthalylsulfathiazole and sulfanilamide were the least effective,

while sulfathiazole and sulfaquanidine were intermediate. Female birds (treated with sulfadiazine and sulfamerazine at one day of age) reacted negatively to pullorum agglutination tests at nine months of age.

Mullen (86) fed sulfamerazine to poults from pullorum-infected flocks at a concentration of 0.5 percent in starting mash. He recorded beneficial results in mortality reduction, but warned against use of the drug for periods longer than five days. After five days the treated mash had an adverse effect.

MacNamee (77) reported good results with sulfamerazine administered in the drinking water at a concentration of 0.5 percent. Medication after the fifth day offered no beneficial results. Sulfamerazine was also used prophylactically.

Bottorff and Kiser (13) evaluated sulfadiazine, sulfamethazine and sulfamerazine against pullorum disease in day-old chicks. Experimental trials revealed that sulfadiazine, sulfamethazine and sulfamerazine were equally effective in curtailing mortality due to pullorum disease. In the treated groups the minimum reduction in mortality was 30 percent and the maximum 56 percent. There was no significant weight gain at twenty-one days in the treated groups and no evidence of toxic effects was noted. About 90 percent of the surviving chicks reacted to the rapid whole blood test.

Beneficial effects were obtained by Holtman and Fisher (53) with sulfonamides in the treatment of fowl typhoid.

Failure of sulfamerazine therapy to control pullorum outbreaks in chicks has been reported by Petersen (89).

Considerable protection was afforded by a 0.2 percent concentration of sodium sulfamerazine to young chicks infected orally and by atomizing S. pullorum in the brooders (2).

Roberts, Card and Alberts (104) stated that administration of sulfonamides in the drinking water did not produce quite as good results as when given in dry feed. They urged treatment with sulfonamides immediately after exposure.

Adverse effects upon egg production following sulfa treatment has been noted by Bankowski (4).

Nine sulfonamides were evaluated by Pomeroy, Fenstermacher and Roepke (92). Sulfadiazine, sulfamerazine, sulfapyrazine, sulfaquinoxaline and sulfamethazine were the most effective against pullorum disease in chicks. Sulfasuxidine, sulfathalidine and combinations of both drugs were ineffective. Birds that survived the infection with drugs continued to react positively to the rapid whole blood agglutination test at five months of age.

Roberts, Eisenstark, and Alberts (105) demonstrated that S. pullorum could be adapted to grow in the presence of sodium sulfamerazine. The adapted pullorum strains were found to be more virulent for chicks than the non-adapted strains.

Cole (24) and Dickinson and Stoddard (31) could not

eliminate pullorum infection from yearling hens by prolonged feeding with sulfa drugs.

Swales (126) contended that the use of sulfonamides to reduce mortality from pullorum disease cannot be justified in Canada. He stated that birds with suppressed infection remain carriers.

Chang and Stafseth (21) found that normal chicken serum was bacteriostatic for S. pullorum and greatly enhanced the antibacterial activity of sulfadiazine.

Schweinburg and Rutenberg (114) justify the use of sulfonamide mixtures only when in vitro sensitivity tests demonstrate that the components of the mixture either possess an additive or potentiating effect. They suggested that additive effects may function in the majority of coccal infections, but not for infections due to gram-negative organisms.

In a review of therapeutics, Biester and Schwarte (7) indicated that such compounds as chinosol, metaphen, sulfuric acid, hydrochloric acid, mercuric chloride, resorcin, potassium permanganate, sulfocarbolates and hypochlorite solutions have been found to be without any beneficial effect in treating pullorum infection.

The sulfa compounds were the first to exhibit promising results in reducing mortality among young chicks.

III. Aureomycin - Chloromycetin - Streptomycin

Although the newer antibiotics have been used in the

field of veterinary medicine, their application in controlling poultry diseases has been rather limited. The use of newer antibiotics in reducing pullorum infection has been limited almost exclusively to streptomycin. The number of experiments reported with this antibiotic is limited.

Aureomycin was discovered by Duggar (32) during a program of intensive investigation to detect antibiotic-producing organisms.

Collins, Paine, Wells and Finland (25) evaluated the use of aureomycin against Salmonella typhosa, various severe salmonella infections and a colon bacillus bacteremia. The clinical and bacteriologic findings suggested that aureomycin had some beneficial effects, but the results in general were not striking.

Brainerd, Lennette, Meiklejohn, Bruyn and Clark (14) stated that aureomycin appears to exert a greater or lesser suppressive effect on the infecting organism without producing its complete destruction. Beneficial effects from aureomycin appeared to be limited or absent in typhoid, Salmonella, and Shigella infections. Aureomycin produced beneficial results in the rickettsial diseases and in brucella infections.

The sensitivity of five enteric strains of gram-negative bacilli to aureomycin was determined by Alexander, Leidy and Redman (1). They found that all of the strains were completely inhibited by less than 25 micrograms of

aureomycin per ml of broth medium.

In a series of *in vitro* experiments, Jackson, Gocke, Collins and Finland (58) showed that aureomycin inhibited 91 percent of 35 strains of *Salmonella typhosa* within a range of 12.5-25 micrograms of aureomycin per ml of medium. Fifty-five *Salmonella* strains (except *S. typhosa*) including *S. pullorum* were sensitive to aureomycin within a range of 25-50 micrograms per ml of broth medium. Aureomycin was fifth in order of diminishing activity for *S. typhosa* and other salmonellae among seven antibiotics tested. There did not seem to be any definite correlation between the sensitivity of any given strain to one antibiotic and its sensitivity to any of the others -- *S. pullorum* appeared to be an exception in that it was more sensitive than the other salmonellae to all of the antibiotics tested.

Knight, Sanchez, Sanchez, Shultz and McDermott (66) reported that the results of aureomycin therapy in typhoid fever were not uniform and frequently negligible.

The use of aureomycin in veterinary medicine is now receiving wider application. Peterson and Hymas (90) found that aureomycin produces beneficial results in the treatment of blue comb disease in chickens.

Burkhart (17) in a review of aureomycin therapy in veterinary medicine stated that this antibiotic has been effective in treating a large number of animal diseases. *In vitro* tests upon a variety of animal pathogens has revealed that all were inhibited by one microgram per ml of aureo-

mycin with the exception of Salmonella gallinarum. All were sensitive to low concentrations of aureomycin with the exception of Pseudomonas aeruginosa. Thus far there is little evidence of development of drug resistant strains with aureomycin. It was found to be effective in treating chicks infected with Pasteurella multocida. The survival rate of treated chicks was 50 to 80 percent higher than that of untreated controls.

Chloromycetin was discovered by Burkholder (37) in a vast screening program for newer antibiotics. It possesses a wide range of activity against bacteria, rickettsiae and certain large viruses. Scheidy (113) reported that this antibiotic is of value in veterinary medicine, especially in the treatment of urinary tract infections, diarrheas and systemic bacterial infections in dogs.

Eastman, Schlingman, Manning and Eads (35) cited the value of chloromycetin in the treatment of both large and small domestic animals. Such diseases as hemorrhagic septicemia, conjunctivitis, keratitis, infectious diarrheas and otitis externa have responded very well to chloromycetin therapy.

Pharmacological and pathological studies of chloromycetin in animals were conducted by Gruhzit, Fisken, Reutner and Martino (48). They found that chloromycetin was relatively non-toxic to animals and possessed no cumulative toxic effect on oral dosage of 100 to 200 gm of chloromycetin per kg per day over a four month period. Oral administration of chloromycetin to dogs produced measurable

blood levels for eight hours.

The use of chloromycetin in the treatment of poultry diseases has not been reported to any appreciable extent.

Chloromycetin has been widely used for the symptomatic treatment of typhoid fever (11, 66). Rettger (100) in 1914 called attention to the similarity between the chronic typhoid carrier and the chronic carrier of pullorum disease among infected hens. It has therefore been of interest to follow the success of chloromycetin therapy in typhoid fever with a view toward possible application in pullorum disease.

Knight, Sanchez, Sanchez, Shultz and McDermott (66), along with Gabinus (42) and Cook and Marmion (28), noted dramatic responses to chloromycetin therapy in typhoid fever.

Stryker (125) reported the failure of chloromycetin to benefit a chronic typhoid carrier. Smadel, Woodward and Bailey (119) emphasized the fact that relapses occurred with chloromycetin therapy and proposed an eight day period of treatment in acutely ill patients.

More recently Boger, Schimmel and Matteucci (11) stated that, as the number of chloromycetin-treated cases of typhoid fever increases, it becomes apparent that, although patients respond dramatically to treatment, they are not always bacteriologically controlled. Relapses occur with moderate frequency. Bacteremia has persisted during chloromycetin treatment in a number of cases and it is now

acknowledged that chloromycetin is not effective in treating the carrier state. Chloromycetin is considered bacteriostatic and this probably accounts for failure to eliminate the carrier condition. *In vitro* studies with *S. typhosa* reveal that a concentration of 1000 micrograms per ml of chloromycetin fails to exert a bactericidal effect.

Jackson, Gocke, Collins and Finland (58) found that on a weight basis chloromycetin was third and streptomycin sixth in activity against *S. typhosa* and other salmonellae including *S. pullorum* (seven antibiotics were evaluated).

Alexander, Leidy and Redman (1) compared the *in vitro* action of streptomycin, chloromycetin and aureomycin against eight gram-negative organisms, including five enteric strains. Chloromycetin in a concentration of 10 micrograms per cubic milliliter of broth medium exerted a more rapid bactericidal action against *Hemophilus pertussis* and *Hemophilus parapertussis* than did aureomycin. Aureomycin and chloromycetin were equal in their speed of lethal action against *Hemophilus influenzae* and *S. typhosa*. Stasis of growth with chloromycetin persisted through twenty-four hours for all organisms tested except *Psuedomonas aeruginosa*.

Of the newer antibiotics, streptomycin has received wider application as a therapuetic agent in the control of pullorum disease. Streptomycin was discovered by Waksman (112) and is active against a large number of gram-positive and gram-negative organisms. It possesses little to no activity against fungi, viruses, yeasts, protozoa and

rickettsiae.

Benson (6) reported that streptomycin was of value in checking pullorum disease in baby chicks, but did not eliminate the carrier condition..

Gwatkin (50) conducted a series of experiments to determine the effect of streptomycin on pullorum infection. He found that streptomycin in the drinking water protected baby chicks artificially infected at two days of age. Streptomycin was not effective in curtailing pullorum infection after symptoms had appeared following artificial infection.

Chang and Stafseth (21) noted that streptomycin exhibited bactericidal action against S. pullorum in a concentration of 31 micrograms of streptomycin per ml of tryptose broth medium. The antibacterial action of streptomycin was not influenced by the serum of normal or infected chickens. They also found active blood levels in streptomycin-treated birds at the end of three hours following intramuscular injection.

Kirkpatrick, Moses and Baldini (65) noted beneficial results with streptomycin feed supplements in the treatment of infectious enteritis of quail.

Hughes and Farmer (54) recorded the order of descending resistance to streptomycin of a number of animal pathogens. The streptococci head the list with the Salmonella, Erysipelothrix rhusiopathiae, Erysipelothrix monocytogenes, Pasteurella and Escherichia coli following in order.

Seven strains of S. typhosa and 57 strains of Salmonella covering fifteen species (all isolated from carriers) were tested for in vitro sensitivity to streptomycin. All strains were inhibited by concentrations ranging from 0.004 to 0.064 micrograms of streptomycin per ml of broth medium (41).

Santivanez (110) stated that S. pullorum resisted the action of streptomycin (6.25, 12.5, 25, 50, 100 and 200 units) at zero and four hours, but at twelve hours it was inhibited by 50 units of streptomycin per ml of broth medium, and at 20 hours by 12.5 units of streptomycin per ml. Marked turbidity appeared after 24 hours of incubation.

Salmonella strains representing sixty different types were tested for streptomycin sensitivity by Seligmann and Wassermann (116). Most of the strains yielded in a range of from four to eight units. Mouse experiments utilizing per os infection with Salmonella typhi-murium, Salmonella interitidis et al. coupled with oral or oral and subcutaneous treatment resulted in the suppression of the normal fecal flora along with the pathogens. After termination of treatment the fecal flora and salmonellae reappeared.

IV. Penicillin

Penicillin was discovered by Fleming (40), although in the past few years this has been disputed by Brunel (15). The dramatic action of penicillin against diseases caused by gram-positive pathogens has obscured its value as an

effective agent in suppressing gram-negative organisms (with the possible exception of Neisseria gonorrhoeae).

Bigger and Daly (9) in 1946 reported unusual success in the treatment of typhoid carriers with penicillin and sulfathiazole.

Comerford and Kay (27) encouraged employment of the method used by Bigger in the treatment of typhoid carriers. They were able to duplicate the successful results of Bigger with penicillin and sulfathiazole.

McSweeney (82) modified the regimen of Bigger as far as dosage was concerned and also obtained good results in the treatment of typhoid fever with penicillin.

Parsons (88) claimed that penicillin therapy in typhoid did not produce very striking results, but admitted that the regimen recommended by McSweeney was not followed closely.

Bigger and Daly (8) again reported in 1949 the successful treatment of chronic typhoid carriers with penicillin and sulfathiazole.

With the discovery of chloromycetin and its pronounced effect against S. typhosa, the work of Bigger and McSweeney was obscured until 1951 when Boger, Schimmel and Matteucci (11) found that when penicillin was maintained at effective plasma levels, the symptoms of typhoid disappear dramatically and the carrier condition was eliminated. They stated that sulfathiazole has no appreciable effect on S. typhosa and that penicillin by itself is very effective in suppressing

this organism. They blamed the early failures of penicillin therapy on inadequate blood levels. A minimum concentration of 10 units per ml of plasma is recommended together with an agent such as Benemid to delay renal excretion and maintain proper blood levels of penicillin.

There are many reports in the literature concerning the *in vitro* action of penicillin on *S. typhosa* and other salmonellae.

Evans (38) in 1946 tested 66 different strains of *S. typhosa* against various concentrations of penicillin. He found that all strains were completely inhibited by 25 units of penicillin per ml of broth medium. Sixty-three strains were completely inhibited by 20 units of penicillin per ml and 15 units of penicillin per ml of broth medium was sufficient to inhibit completely the growth of 50 strains. A concentration of 2.5 units of penicillin per ml retarded growth of 28 strains. Mice inoculated intraperitoneally with virulent typhoid appeared to be benefited by penicillin therapy.

Pratt and Dufrenoy (96) stated that penicillin affects aerobic gram-positive and gram-negative organisms through the same chemical systems. The concentrations of penicillin required to inhibit gram-negative organisms is, however, much larger. They also revealed that trace amounts of cobalt added to agar markedly reduced the amount of penicillin needed to inhibit an organism. Trace amounts of cobalt are especially effective against gram-negative organisms that

are ordinarily resistant to penicillin. Cobalt also appeared to exert the same beneficial effect in vivo (93, 94, 124).

Stewart (122) found that S. typhosa, Salmonella paratyphi B and strains of Proteus vulgaris were inhibited by penicillin in concentrations of 8-20 units per ml.

Thomas and Hayes (128) tested 18 strains of S. typhosa and found that the growth of 15 were inhibited by 5 units of penicillin per ml of broth medium or less. During a 24-hour period of incubation at 37° C there was a 35 percent loss of penicillin potency.

Miller, Wilmer and Verwey (83) noted that when S. typhosa was used as the infecting organism in mice, there were marked differences in the amount of penicillin required for therapy depending upon the number and frequency of the doses used.

Spicer and Blitz (120) pointed out the fact that a few viable organisms always remain in cultures containing penicillin, but their multiplication was inhibited, which suggested that individual members of the bacterial population react differently to antibiotic influence.

Eagle, Fleischman and Musselman (33) studied the bactericidal action of penicillin in vivo and the participation of the host during therapy. They found that surviving organisms damaged by effective penicillin levels did not resume multiplication for a number of hours and were susceptible to the defense mechanisms of the host in the

absence of penicillin.

Eagle (34) in a further study of penicillin concluded that its bactericidal action was more rapid in vivo than in vitro. The bactericidal action in the infected animal is the sum of the direct effect of penicillin itself, plus the bactericidal action of the host mechanisms on infecting organisms, which are acted upon by the drug and thus made susceptible.

George and Pandalai (43) reported that penicillin-resistant organisms became penicillin sensitive when grown in the presence of other organisms or their metabolic products.

Thomas and Levine (127) found that S. pullorum was inhibited by 10 units of penicillin per ml in beef extract broth.

Jackson, Gocke, Collins and Finland (58) found that S. pullorum was inhibited by 3.8 micrograms of penicillin per ml of broth medium.

The oral use of penicillin has many decided advantages, but the dosage must be increased above that employed in parenteral administration. Keefer (63) contended that oral doses, 3 to 5 times the parenteral dose, give results comparable to those given parenterally.

Ross, Burke and McLendon (108) noted that adequate blood levels of penicillin could be attained by oral administration when the drug was protected against inactivation by gastric acidity.

Robinson, Hirsh and Dowling (106) also reported that oral administration of penicillin in dosages five times the customary intramuscular dosage produced results comparable to those obtained with parenteral penicillin.

Collins, Seeler and Finland (26) found that Caronamide in sufficient dosage enhanced and prolonged penicillin blood levels (penicillin was administered orally).

During recent years many substances have been used to maintain and prolong effective blood levels of penicillin. A few of the better known enhancing substances are Caronamide, vitamin K and Borrelidin (49, 91, 95, 124, 130).

V. Antibiotic Substances from Higher Plants

Antibiotic substances from higher plants have had limited application in the treatment of human and animal diseases.

Kahn (61) in 1933 noted that banana powder fed to infants produced a conversion of the intestinal flora from an almost completely gram-negative to an almost completely gram-positive state.

Bogert (12) in a review of the dietary uses of the banana indicated that bananas have been of benefit in alleviating many intestinal disturbances including diarrheas of typhoid fever.

Osborn (87) in 1943 reported on the antibacterial properties of higher plants.

Lucas and Lewis (76) in 1944 found that there was a

similarity of antibacterial actions throughout a given plant genus. They noted wide differences in the potency of active principles with genera and species.

Little and Grubaugh (73) in 1946 noted that some crude plant juices were much more active against animal pathogens than against those causing diseases of plants. Inhibition also appeared to be more pronounced against the gram-negative test strains than against gram-positive organisms. The juices of beans, corn, cabbage, tomatoes and cauliflower were tested.

In 1944, Cavallito, Buck, Suter (19) and Cavallito and Bailey (20) announced the isolation of an antibacterial substance from garlic cloves. The substance was found to be active against both gram-positive and gram-negative bacteria and was given the name of Allicin.

Rao, Rao, Natarajan and Venkataraman (97) reported in 1946 that Mycobacterium tuberculosis was inhibited by garlic extract.

In 1947 Stoll and Seebeck (123) isolated and defined the properties of alliin which is characteristic of certain kinds of garlic. They found that when alliin was acted upon by the enzyme alliinase, the antibacterial substance allicin was produced.

In 1941 Block and Tarnowski (10) reported favorable results from feeding bananas to dysentery patients.

More recently Scott, McKay, Schaffer and Fontaine (115) found several antibiotic substances in the banana with therapeutic possibilities.

The use of rutin, a flavonol glucoside, in the treatment of capillary fragility (46) has led some workers to investigate wider application of this drug in a variety of diseases.

Levitan (71, 72) claimed that rutin contributed to the stability of the tissue ground substance and that spread of infection and malignant growth is partly determined by the degree of permeability of ground substance.

Clark and MacKay (22) stated that judging by their experimental results it is unlikely that rutin is a vitamin-like compound. They further claimed that rutin does not exert any specific or therapeutic effect, but may elicit a non-specific stress or "alarm reaction" which might explain some of the observed, non-specific physiological phenomena.

Gottshall, Jennings, Weller, Redemann, Lucas and Sell (45) reported the presence of antibacterial substances in seed plants which are effective against Mycobacterium tuberculosis.

There are a number of higher plants which yield extracts exhibiting antibacterial properties. Hydrastis canadensis produced extracts effective in vitro against Micrococcus pyogenes var. aureus and Mycobacterium tuberculosis (44, 61, 87). Trigonella Foenum-graecum possesses mild antibacterial activity. Hypericum perforatum is highly active against gram-positive organisms, including Mycobacterium tuberculosis (74, 75).

MATERIALS AND METHODS

In Vitro Experiments

A. Sensitivity determinations

The tube dilution method was used in ascertaining the sensitivity of S. pullorum #89817 to aureomycin hydrochloride*, chloromycetin*, streptomycin (calcium chloride complex) and penicillin G* (oral, buffered, potassium salt). Veterinary preparations of streptomycin and chloromycetin were used for in vivo experiments.

The usual method of two-fold dilutions was modified to some extent and a stepwise increment of five was employed in the higher concentrations and a two-fold dilution in the lower ranges. Each series of concentrations extended from 35 micrograms to 0.625 micrograms per ml of broth medium. The units of penicillin per ml ranged from 100 to 5 units.

Stock solutions of aureomycin and chloromycetin** were prepared by dissolving appropriate amounts in distilled water in a concentration of 250 mg per 100 ml of solution.

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*The above antibiotics were kindly supplied through the courtesy of the following agencies: Aureomycin - Lederle Laboratories, Pearl River, N.Y.; Chloromycetin - Parke-Davis, Detroit, Mich.; Penicillin - The Upjohn Co., Kalamazoo, Mich.

**Sensitivity determinations with chloromycetin were later repeated, when a purified batch became available for this study.

A solution of streptomycin was made by adding an appropriate quantity of distilled water to the dry powder contained in a sterile vial. This was further diluted with distilled water to give a solution containing 250 mg of streptomycin base per 100 ml. A penicillin stock solution was prepared by dissolving an oral, buffered tablet in a given quantity of distilled water.

All the stock solutions were Seitz filtered to insure sterility and immediately used for the various tests.

In order to prepare desired concentrations of a particular antibiotic, varying amounts were taken from each stock solution and further diluted with sterile, distilled water. This was so calculated that one ml of the diluted stock solution when added to 9 ml of seeded broth gave the indicated concentrations.

Penassay broth (Difco) was employed for the determination of sensitivity to aureomycin, penicillin and chloromycetin. Mycin broth was used for streptomycin.

A flask containing 200 ml of broth was inoculated with one ml of a 24-hour broth culture of S. pullorum #89817. Nine ml of this seeded broth was then dispensed to each tube. The addition of one ml of antibiotic solution to each tube brought the total volume to 10 ml.

Each test series as well as each concentration of antibiotic was repeated at least in triplicate. Controls consisted of placing one ml of each concentration into 9 ml of unseeded broth. A second control consisted of only one

tube containing 10 ml of seeded broth without the addition of any antibiotic material.

The tubes were incubated for 24 hours at 37° C. and examined for visible turbidity. The lowest concentration of antibiotic preventing growth in 24 hours was taken as the endpoint reading.

The sensitivity of S. pullorum #89817 to garlic extract could not be determined by the tube-dilution method, since garlic extract imparts a cloudiness to broth media. An agar plate method was devised for testing sensitivity.

A stock solution of garlic extract was prepared by blending 20 g of dehydrated garlic with 400 ml of distilled water. This produced a stock solution containing 200 garlic units per ml. (The garlic unit is defined as the amount of active principle contained in one ml of a solution prepared by blending 0.1 g of dehydrated garlic with 400 ml of distilled water.) After blending, the mixture was centrifuged and the clear supernatant liquid Seitz filtered to insure sterility. Various amounts of sterile garlic solution and Penassay seed agar were uniformly mixed to give a range of garlic units from one to fifty per ml of garlic-agar mixture. A 24-hour culture of S. pullorum #89817 was used for streaking each plate. The plates were incubated at 37° C. and read within 48 hours. The lowest concentration of garlic units completely inhibiting growth of the test organism was regarded as the endpoint reading. Control plates contained seeded agar without garlic.

A second method for determining sensitivity was developed and differs somewhat from the above procedure. Two ml of a 24-hour culture of S. pullorum #89817 was added to 200 ml of penassay seed agar. Various concentrations of garlic standard were placed in a series of Petri dishes and seeded agar added in amounts necessary for the various desired concentrations. The lowest concentration of garlic completely inhibiting the growth of the test organism for 72 hours was regarded as the endpoint.

The following table illustrates the method of obtaining the range of concentrations used in this study.

TABLE 1

PREPARATION OF GARLIC DILUTIONS

Ml of standard garlic solution	Garlic units	Ml of seeded agar	Concentration per ml of mixture in units
0.1	20	19.9	1
0.2	40	19.8	2
0.3	60	19.7	3
0.4	80	19.6	4
0.5	100	19.5	5
1.0	200	19.0	10
2.0	400	18.0	20
3.0	600	17.0	30
4.0	800	16.0	40
5.0	1000	15.0	50

B. Antibiotic stability in the presence of feed constituents

Six feed samples of varying composition were utilized for stability tests. Aureomycin was incorporated at a level of 1 mg per g of feed. Chloromycetin was added to prepared feed samples in concentrations of 1 mg per g of feed, but two forms of this antibiotic were employed. One form consisted of chloromycetin plus binder (1 mg containing 0.5 mg of pure antibiotic). The other form, which was obtained at a later date, was a purified preparation (1 mg = 1 mg of pure natibiotic). The latter preparation was evaluated in a commercial feed sample. Streptomycin base was incorporated at a concentration of 0.25 mg per g of feed, while penicillin levels consisted of 1000 units per g of feed. Garlic constituted 15 percent of each feed sample. Feed samples containing varying amounts of ingredients were prepared as shown in Table 2.

Aureomycin and streptomycin were also combined with regular commercial feed samples. A veterinary streptomycin mixture was used here instead of the calcium chloride complex. This mixture was an oral preparation which contained 0.36 g of streptomycin base per g of mixture (this mixture was later evaluated qualitatively).

The sample feed mixtures containing antibiotic material were stored for a one-month period and then tested for antibiotic activity. The method used was in general the same for all samples.

Five g of feed-mixture were suspended in a dilution

TABLE 2

PREPARATION OF SAMPLE FEED MIXTURES*

Ingredient	Feed mixture number					
	1	2	3	4	5	6
Corn	280	92	0	0	0	0
Dextrin	0	0	0	0	222	72
Soybean meal	92	280	150	300	150	300
Sucrose	0	0	222	72	0	0
Alfalfa meal	20	20	20	20	20	20
Limestone	4	4	4	4	4	4
Bone meal	2	2	2	2	2	2
Salt	2	2	2	2	2	2
Percent protein	15	30	15	30	15	30

*Total weight of each sample = 400 g

blank containing 50 ml of distilled water (for aureomycin, streptomycin and chloromycetin samples). Ten g of feed-garlic mixture were suspended in 50 ml of distilled water, while penicillin-feed samples were added at the rate of one gram in 100 ml. The penicillin solution produced after settling of feed particles was further diluted to give a theoretical concentration of one unit per ml of solution. All dilution blanks were vigorously shaken in order to produce a complete solution of the particular antibiotic. After shaking, the suspensions were allowed to settle and the clear liquid portions decanted into a Petri dish. Standard

paper discs (740-E, 12.7 mm diameter) were immersed in the solution and placed on seeded agar plates which were prepared in the following manner: 18 ml of penassay base agar was poured into Petri plates and allowed to solidify. A flask containing 200 ml of penassay seed agar was then inoculated with 2 ml of a 24-hour broth culture of S. pullorum #711 and vigorously rotated to insure a uniform distribution of the organism. Three ml of the seeded agar was then overlayered onto the solidified base and the Petri dish gently rotated back and forth to distribute the seed agar uniformly over the entire surface. Micrococcus pyogenes var. aureus #9144 was used as the test organism in penicillin potency determinations.

The zones of inhibition observed were then evaluated for actual antibiotic concentration by referring to standard reference curves (Figs. 2 - 10). Theoretically the antibiotic concentration per ml of feed-water suspension was known from the amount of feed sampled and the level of antibiotic concentration originally added. However, any inactivation by feed constituents or any strong adsorption of the antibiotic to feed, would result in the formation of inhibition zones that were smaller than those produced by theoretical concentrations. Controls consisted of feed samples without antibiotics.

Standard reference curves were prepared by utilizing a modified version of the paper disc plate method for streptomycin assay (129). Essentially the plates were

prepared like those described for stability testing. Originally M. pyogenes var. aureus #9144 was employed as the test organism for preparing penicillin reference curves and stability tests. This organism was later replaced by S. pullorum #89817 which revealed moderate to high sensitivity in the presence of appropriate amounts of penicillin.

The reference curves employed in this study consisted usually of four to five points -- each point being the average of triplicate or quadruplicate trials. Sterile discs were immersed in different concentrations of antibiotic solution and placed on seeded plates. The zones of inhibition were measured after 18 hours of incubation at 37° C. S. pullorum consistently showed poor growth on mycin agar which was replaced by penassay seed agar (in streptomycin determinations).

In reading streptomycin plates it was found that this antibiotic produced elliptical zones of inhibition with a consistent long axis. The long axis was therefore used in measuring zones of inhibition.

Antibiotic stability tests were conducted at intervals of one month and twelve months (penicillin tested at one and six month intervals).

C. Effect of penicillin on S. pullorum

The following numbered strains of S. pullorum were obtained from the Michigan State College Poultry Pathology Laboratory: 89817, 1304-BS, 1273-1, 1300-B1, 711 and 5.

Each strain was tested by the previously described seeded-plate method. Concentrations of 50 and 100 units per ml of penicillin were used.

The action of penicillin on S. pullorum was determined by subculturing into plain penassay broth from various tubes containing different concentrations of penicillin. These transfers were carried out at 24, 48 and 72 hours. Some surviving organisms were identified by the usual sugar reactions in order to ascertain any damage that may have occurred through penicillin contact. Agar plates were streaked in addition to subculturing in broth.

D. The enhancement of penicillin action by cobalt against S. pullorum

A series of seeded plates were prepared with S. pullorum #89817 as the test organism. An aqueous solution of cobalt was prepared by dissolving cobalt chloride in a concentration of 0.2 mg per ml of solution. Three ml of this solution was carefully added to each seeded plate. The plates were then incubated for 30 minutes at 37° C. After incubation the cobalt solution was carefully decanted from each plate. Sterile paper discs were immersed in a stock solution of penicillin (containing 100 units per ml) and immediately placed on each plate. Controls consisted of seeded plates without addition of cobalt. All plates were then incubated for 24 hours at 37° C and read.

In order to measure the degree of enhancement by cobalt,

a standard reference curve was made with seeded plates containing S. pullorum. Six plates were used for each concentration of penicillin. The range of concentrations extended from 50 units per ml of solution to 400 units.

Toxicity tests on cobalt were performed by the paper disc method. The discs were immersed in a solution of cobalt and placed on plates seeded with S. pullorum. The formation of a zone of inhibition would indicate an inhibiting effect by the selected concentration of cobalt.

In Vivo Experiments

A. Feed

Regular commercial starter feed was used in all experiments with the exception of the first and third series. Feeds that did not contain any antibiotic supplements were chosen.

In the first series, birds were given feeds containing 15 and 30 percent protein. In the same series another group was supplied with a diet of oats for 72 hours prior to feeding with 15 and 30 percent protein. In the third series of experiments one of the feeds contained sucrose as the principal carbohydrate.

These special feeds were composed of the following ingredients:

Ingredient	Percent protein 15	30	Sucrose-15% protein
Sucrose	0	0	60.3
Corn	73.4	35.4	0
Soybean meal	17.0	55.0	30.0
Alfalfa	5.0	5.0	5.0
Bone meal	3.0	3.0	3.0
Calcium carbonate	0.5	0.5	0.5
Salt	0.5	0.5	0.5
BY feed	0.3	0.3	0.3
Viadex	0.1	0.1	0.1
APF	0.1	0.1	0.1
Magnesium sulfate	0.05	0.05	0.05
Choline chloride	0	0	0.1
Niacin	0.05	0.05	0.05

All above figures given in percent.

B. General arrangement of each experimental series

Each series consisted of six experimental groups and two control groups. One control was composed of birds which were artificially infected, but did not receive any therapeutic treatment. The second control consisted of birds that were not infected and did not receive any antibiotic therapy.

A total of 25 birds were included in each experimental group. Four different breeds of chicks were used in the course of this study.

C. Preparation of the inoculum

Large nutrient agar slants (20 mm tubes) were inoculated with a 24-hour culture of S. pullorum #89817 and incubated for 24 hours. To each slant 5 ml of sterile physiological

saline was added and the organisms gently removed with a wire loop. The bacterial suspensions were pooled in sterile flasks. For the first 5 experimental series the suspension was adjusted to tube number 4 of the McFarland nephelometer (1,200,000,000 organisms per ml). Beginning with the sixth series the number of organisms per ml of suspension was determined by plating methods. The average number of organisms was found to be approximately 1,350,000,000 per ml of saline suspension.

The inoculum was administered orally to each chick in a volume of 0.5 ml.

D. Method of antibiotic therapy

In general most of the antibiotic agents were administered as a prophylactic measure. The period of prophylactic feeding started when the birds were one day of age and continued for 72 hours in the first two series of experiments. This period was reduced to 48 hours in succeeding series and finally down to 24 hours.

When the time of prophylactic feeding was terminated, the birds were inoculated with the infecting organism and continued on a therapeutic diet for the duration of the experiment.

All the antibiotic agents were incorporated into feed at the various indicated levels. In several groups of the first series, dietary constituents were evaluated for their ability to check the course of infection.

E. Preparation of an antigen-mash

The following S. pullorum strains were used in preparing a mixed antigen: 1300-B1, 1304-BS, 1273-1, 5, 711 and 89817. All strains were obtained from the Michigan State College Poultry Pathology Laboratory.

Antigen preparations were added to the feed and removed after the birds were inoculated with the infecting organism.

Large concentrations of the various pullorum strains were prepared by adding 2 ml of a 24-hour broth culture of each strain to a flask containing 500 ml of nutrient broth. The number of organisms per ml of broth was determined by plating methods.

Several procedures were used for attenuating the organisms. After a 24-hour period of incubation at 37° C in either nutrient or tryptose broth, the organisms were killed by adding three volumes of absolute alcohol and the mixture allowed to stand for one hour. The ethanol-broth mixture was then poured over a given quantity of feed and mixed thoroughly. The alcohol was evaporated by a stream of air at room temperature. After evaporation the feed on which the organisms were adsorbed was thoroughly stirred and added to the bulk quantity of feed.

Other methods for antigen production involved heat attenuation and adsorbing of the organism on charcoal. The procedures used for charcoal adsorption and heat attenuation were as follows:

1. Heat attenuation - 512 ml of antigen broth was heated to a temperature of 90-95° C and held at this temperature range for 30 minutes. The broth was then cooled with frequent stirring and incorporated into 10 kg of feed by thorough mixing.

2. Charcoal adsorption - 512 ml of antigen broth was heated and cooled in the same manner as described above. Two hundred g of activated carbon (Norit A) was gradually added to the cooled broth with continuous stirring. The resulting suspension was thoroughly mixed with two kg of feed and dried. The dry product was further mixed with the remainder of feed to give a total of 10 kg.

F. Method of cobalt administration to chicks

Chicks were allowed to drink water which contained cobalt chloride at a concentration of 0.2 mg per ml of solution. Cobalt-water was given for 24 hours prior to the time of artificial infection. During this period the chicks were kept on plain starter feed without antibiotic. Thirty minutes after infection each chick was again given 0.5 ml of cobalt water (0.2 mg per ml) orally. Cobalt water and plain feed were then removed from the troughs and feed containing penicillin at a level of 2290 units per g and plain water were substituted.

The penicillin used in the last series of experiments was an oral preparation which assayed 1560 units per mg. Calcium carbonate had to be added as a buffer for this particular preparation.

G. Recovery of the infecting organism

The following method was used for isolating S. pullorum from dead chicks: Portions of the heart, lung, liver and intestine were placed in tetrathionate enrichment broth and incubated for 18 hours at 37° C. From here material was streaked on bismuth sulfite agar and incubated from 24 to 48 hours. Typical colonies were then transferred to Kligler's iron agar slants. If a characteristic reaction for S. pullorum appeared, transfers were made to Sims medium, sucrose and mannitol broth. The use of bismuth sulfite agar as a choice medium for the isolation of S. pullorum has been confirmed by other investigators (18).

A large number of birds from these experiments was kept for carrier studies. Most of the birds were sacrificed at twelve weeks of age (aureomycin birds sacrificed at 9 months of age) and carefully examined for the presence of S. pullorum. Portions of the heart, liver, spleen, lung, testes or ovaries and intestinal bifrication from each bird were pooled in tetrathionate broth. After enrichment, transfers were made to bismuth sulfite agar, Kligler's medium, etc.

In examining a large number of birds it was imperative not to carry over infecting organisms from one bird to another by way of instruments and hands. Therefore, after each dissection all instruments were immersed in a germicidal detergent for three to five minutes (hands were also washed in detergent). After this period of time, the instruments

were removed and the excess fluid allowed to drain. This procedure did not appear to interfere with the isolation of S. pullorum. Previously, all instruments were dried with sterile gauze pads to remove residual detergent solution. However, the usual number of isolations was obtained with either method. In order to ascertain the efficacy of this method of instrument and hand sterilization, swabs were taken from the instruments and hands and placed in tetrathionate enrichment. From here, material was transferred to the various differential media.

The virulence of the infecting organism was at first maintained by chick passage, but it was later found that virulence could be maintained in stock cultures (nutrient agar slants) by transferring the organism every two weeks. S. pullorum #89817 is a standard strain, but does not produce gas at 37° C.

RESULTS AND DISCUSSION

The Sensitivity of S. pullorum to Aureomycin, Chloromycetin and Streptomycin

There are many factors which affect the response of a given bacterial population to a selected concentration of an antibiotic. This is true for both *in vitro* and *in vivo* studies.

In performing the tube-dilution assay, certain conditions must be standardized so that results can be duplicated within allowable limits. A number of factors in this procedure can be regulated, while others are almost impossible to control.

Reasonable accuracy can be attained by this method in replicate trials, if the following factors are kept in mind: (1) the test organism must be sensitive to the antibiotic, (2) the organism must be of the same strain and age in every assay, (3) incubation temperatures should be the same in every trial. Generally, the duration of incubation is shorter when the temperature is increased. Endpoint readings are sharper at certain incubation temperatures and tend to shift either into a higher or lower concentration with a change in the time of incubation. Variable endpoint readings may be due to the development of

bacterial resistance or the gradual destruction of the antibiotic, (4) for certain antibiotics the number of organisms initially present must be constant in every test and finally (5) the composition of the test medium should not be varied.

However, a given sample of antibiotic may be assayed under identical conditions and still exhibit a variable potency in successive tests. A partial explanation for this phenomenon may be due to the fact that any given bacterial population is not perfectly homogeneous in regard to the minimal concentration of antibiotic necessary to inhibit or kill each individual member. If penicillin is used as an example, (other antibiotics could be included) only those cells that are in an active metabolic phase would be susceptible to minimal inhibiting concentrations of the antibiotic. As the more sensitive members of the bacterial population are killed, there is a release of cellular constituents which may serve as essential nutrients for those members that are in a phase of decreasing metabolic activity. These inactive cells become metabolically active and are subject to minimal inhibiting concentrations of the antibiotic. Thus there may appear waves of growth within a definite period of incubation time and a consequent shift in the endpoint reading.

The <u>in vivo</u> activity of antibiotic agents may not necessarily parallel the <u>in vitro</u> activity. Certain con-

stituents of normal body fluids may inactivate all or a portion of the antibiotic or else enhance its antibacterial properties. Eagle, Fleischman and Musselman (33) found that organisms damaged by penicillin contact *in vivo*, did not recover as rapidly as those contacted *in vitro*. They also noted that penicillin-damaged organisms were susceptible to the defense mechanisms of the host in the absence of any demonstrable penicillin levels for a certain length of time.

Variations in sensitivity tests have been reported by Jackson, Gocke, Collins and Finland (58). They used a number of salmonella species including *S. typhosa* and *S. pullorum*. They reported a four-fold variation with streptomycin. A two-fold difference was found in tests with chloromycetin at different times. Results with aureomycin varied as much as eight-fold from one test to another. The authors conclude that the results are valid only for the conditions under which the tests were performed. They obtained the following endpoint readings for *S. pullorum*: streptomycin, 25 micrograms per ml.; aureomycin, 12.5 micrograms; and chloromycetin, 1.6 micrograms per ml (all readings in 48 hours).

In general most investigators have reported that many species of *Salmonella* are susceptible to low concentrations of streptomycin as determined by *in vitro* methods (41, 1, 116).

The *in vivo* activity of aureomycin against various *Salmonella* species has not been very pronounced. This is

especially true for infections due to S. typhosa (14, 25, 66).

The in vivo activity of chloromycetin against S. typhosa is much superior to that of aureomycin (66).

The in vitro sensitivity of S. pullorum #89817 to chloromycetin (impure and purified forms), aureomycin and streptomycin has been recorded in Tables 3 - 6. It will be noted that there occurs a shift in the endpoint readings (more marked with streptomycin), but this recorded difference is within allowable limits.

The Sensitivity of S. pullorum to Penicillin

As was mentioned previously, the unusual success of treating infections caused by gram-positive organisms with penicillin, had obscured the possible use of this antibiotic against gram-negative organisms. Previously, organisms which required concentrations above one or two units per ml for inhibition were thought of as being rather resistant to penicillin (in view of the fact that a large number of streptococci were inhibited by concentrations as low as 0.06 units per ml). The term units, by which penicillin concentrations are designated, can be rather deceiving when sensitivity concentrations are considered. If a certain batch of penicillin has been assayed at 1560 units per mg, it would mean that an organism inhibited by 10 units per ml is actually subjected to approximately 6 micrograms per ml on a weight basis. When units are evaluated in micrograms, the term assumes new meaning as far as sensitivity deter-

TABLE 3

THE SENSITIVITY OF SALMONELLA PULLORUM TO AUREOMYCIN

Micrograms of aureomycin per ml of broth	Positive control	Negative control	Seeded broth plus aureomycin		
			1	2	3
Experiment 1					
35	+	-	-	-	-
30	+	-	-	-	-
25	+	-	-	-	-
20	+	-	-	-	-
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	-	-	-
2.5	+	-	-	-	-
1.25	+	-	-	-	-
0.625	+	-	+	+	+
Experiment 2					
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	-	-	-
2.5	+	-	-	-	-
1.25	+	-	+	+	+
0.625	+	-	+	+	+
Experiment 3					
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	-	-	-
2.5	+	-	-	-	-
1.25	+	-	-	-	-
0.625	+	-	+	+	+

Positive control - seeded broth without aureomycin
Negative control - unseeded broth plus aureomycin
+ = presence of growth; - = complete absence of growth

TABLE 4

THE SENSITIVITY OF SALMONELLA PULLORUM TO CHLOROMYCETIN (IMPURE)

Micrograms of chloromycetin per ml of broth	Positive control	Negative control	Seeded broth plus chloromycetin		
			1	2	3
Experiment 1					
35	+	-	-	-	-
30	+	-	-	-	-
25	+	-	-	-	-
20	+	-	-	-	-
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	-	-	-
2.5	+	-	+	+	+
1.25	+	-	+	+	+
0.625	+	-	+	+	+
Experiment 2					
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	-	-	-
2.5	+	-	+	+	+
1.25	+	-	+	+	+
0.625	+	-	+	+	+
Experiment 3					
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	-	-	-
2.5	+	-	+	+	+
1.25	+	-	+	+	+
0.625	+	-	+	+	+

Positive control - seeded broth without chloromycetin
Negative control - unseeded broth plus chloromycetin
+ = presence of growth; - = complete absence of growth

TABLE 5

THE SENSITIVITY OF SALMONELLA PULLORUM TO CHLOROMYCETIN (PURE)

Micrograms of chloromycetin per ml of broth	Positive control	Negative control	Seeded broth plus chloromycetin		
			1	2	3
Experiment 1					
25	+	-	-	-	-
12.5	+	-	-	-	-
6.25	+	-	-	-	-
3.125	+	-	-	-	-
1.56	+	-	-	-	-
0.78	+	-	+	+	+
0.39	+	-	+	+	+
0.195	+	-	+	+	+
0.0975	+	-	+	+	+
0.04875	+	-	+	+	+
Experiment 2					
25	+	-	-	-	-
12.5	+	-	-	-	-
6.25	+	-	-	-	-
3.125	+	-	-	-	-
1.56	+	-	-	-	-
0.78	+	-	+	+	+
0.39	+	-	+	+	+
0.195	+	-	+	+	+
0.0975	+	-	+	+	+
0.04875	+	-	+	+	+

Positive control - seeded broth without chloromycetin
Negative control - unseeded broth plus chloromycetin
+ = presence of growth; - = complete absence of growth

TABLE 6

THE SENSITIVITY OF *SALMONELLA PULLORUM* TO STREPTOMYCIN

Micrograms of streptomycin per ml of broth	Positive control	Negative control	Seeded broth plus streptomycin		
			1	2	3
Experiment 1					
35	+	-	-	-	-
30	+	-	-	-	-
25	+	-	-	-	-
20	+	-	-	-	-
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	+	+	+
2.5	+	-	+	+	+
1.25	+	-	+	+	+
0.625	+	-	+	+	+
Experiment 2					
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	-	-	-
2.5	+	-	+	+	+
1.25	+	-	+	+	+
0.625	+	-	+	+	+
Experiment 3					
15	+	-	-	-	-
10	+	-	-	-	-
5	+	-	+	+	+
2.5	+	-	+	+	+
1.25	+	-	+	+	+
0.625	+	-	+	+	+

Positive control - seeded broth without streptomycin
Negative control - unseeded broth plus streptomycin
+ = presence of growth; - = complete absence of growth

minations are concerned.

Pratt and Dufrenoy (96) stated that penicillin affects aerobic gram-positive and gram-negative organisms by blocking the catabolism of mononucleotides. However, the minimal concentration of penicillin required to produce this effect is far greater for gram-negative organisms than for gram-positive.

Reports in the literature concerning the use of penicillin against gram-negative organisms are rather numerous (8, 9, 11, 27, 38, 83, 127). Stewart (122) stated that "When applied to gram-negative bacteria, the term sensitivity to penicillin must be interpreted with reservations." In his study of penicillin action on gram-negative organisms, the range of penicillin sensitivity extended from 8-128 units of penicillin per ml of broth medium.

A shifting endpoint was obtained when S. pullorum #89817 became subjected to varying concentrations of penicillin (tube dilutions). However, the endpoint reading (in 24 hours) was constant for a given experiment, but tended to shift in repeated determinations. This was observed despite every effort to duplicate each step in successive trial assays. This may be explainable on the basis that bacterial populations exhibit post lytic growth responses when in contact with penicillin. It is reasonable to assume that various members of the bacterial population possessed different sensitivities to inhibiting concentrations. The phenomenon of bacterial lysis and

release of essential nutrients has already been discussed.

It will be noted that in high concentrations of penicillin (90-100 units per ml) the endpoint is constant. The fact should be mentioned that endpoint readings were made in 24 hours and that the true endpoint at the end of 72 hours was an entirely different value (determined by subculture technique rather than visible turbidity). This determination will be discussed shortly.

The sensitivity of S. pullorum to penicillin has been reported sporadically in the literature, but rarely as a specific evaluation against S. pullorum. This organism has served as one of the many species in a bacterial spectrum.

With the reported success of penicillin in the treatment of typhoid infections (9, 11, 27), it was decided to evaluate penicillin against the test strain of S. pullorum used in this study as well as a number of other pullorum strains. The results presented in Table 7 were obtained on plates seeded with different pullorum strains (obtained from the Michigan State College Poultry Pathology Laboratory).

The results obtained in successive tube dilution tests are given in Tables 8 and 9.

The minimal inhibitory concentration of penicillin varied in some of these sensitivities tests, but once the endpoint reading was attained (in 24 hours), it remained constant thereafter. However, it was also noted that tubes showing a definite presence of growth did not increase in visible turbidity after 24 hours, and as a matter of fact

TABLE 7

THE PLATE SENSITIVITY OF SIX *SALMONELLA PULLORUM* STRAINS TO PENICILLIN

Strain number	Plates minus penicillin	Units per ml					
		3.125	6.25	12.5	25	50	100
1304-BS	+	+	+	+	+	20.0	25.5
1273-1	+	+	+	+	+	17.0	24.3
1300-B1	+	+	+	+	+	17.8	23.0
711	+	+	+	+	+	17.3	22.8
5	+	+	+	+	+	17.0	23.3
89817	+	+	+	+	+	19.8	25.0

+ = complete growth of test organism
zone diameter measured in mm

in 48 hours all tubes exhibited a decreasing amount of turbidity. At 72 hours and 96 hours all the tubes became clear and a slight sediment was visible at the bottom of each tube. This was observed in every test. A comparable phenomenon was not noted with aureomycin, chloromycetin or streptomycin. With these antibiotics the endpoint reading (in a given experiment) would shift after 24 hours to the next highest dilution. A similar action was seen on plates seeded with *S. pullorum*. Penicillin produced a zone of inhibition that was very sharp and distinct. The borders of such zones retained their sharpness for weeks without any encroachment of the test organism. In plate assays with aureomycin, chloromycetin and streptomycin, the zones of inhibition would begin to disappear after 48 hours and

TABLE 8

THE PENICILLIN SENSITIVITY OF A STOCK CULTURE OF SALMONELLA PULLORUM # 89817

No. of trials	Positive control	Negative control	Units per ml									
Experiment 1			100		50		25		12.5		6.25	
1	+	-	-		+		+		+		+	
2	+	-	-		+		+		+		+	
3	+	-	-		-		+		+		+	
4	+	-	-		-		+		+		+	
Experiment 2			60		55		50		45		40	35
1	+	-	-		-		-		-		-	-
2	+	-	-		-		-		-		-	-
3	+	-	-		-		-		-		-	-
4	+	-	-		-		-		-		-	-
Experiment 3			55	60	65	70	75	80	85	90	95	100
1	+	-	-	-	-	-	-	-	-	-	-	-
2	+	-	-	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	-	-	-
4	+	-	-	-	-	-	-	-	-	-	-	-
Experiment 4			50	45	40	35	30	25	20			
1	+	-	-	-	-	-	-	-	-			
2	+	-	-	-	-	-	-	-	-			
3	+	-	-	-	-	-	-	-	-			
4	+	-	-	-	-	-	-	-	-			
Experiment 5			20		15		10		5			
1	+	-	+		+		+		+			
2	+	-	+		+		+		+			
3	+	-	+		+		+		+			
4	+	-	+		+		+		+			

Positive control - seeded broth without penicillin
Negative control - unseeded broth plus penicillin
+ = presence of growth; - = complete absence of growth

TABLE 9

THE PENICILLIN SENSITIVITY OF A RECENT ISOLATE OF *SALMONELLA PULLORUM* #89817

Units per ml	Positive control	Negative control	Trials 1	2	3
Experiment 1					
100	+	-	-	-	-
95	+	-	+	+	+
90	+	-	+	+	+
85	+	-	+	+	+
80	+	-	+	+	+
75	+	-	+	+	+
70	+	-	+	+	+
65	+	-	+	+	+
60	+	-	+	+	+
55	+	-	+	+	+
50	+	-	+	+	+
45	+	-	+	+	+
40	+	-	+	+	+
35	+	-	+	+	+
30	+	-	+	+	+
25	+	-	+	+	+
20	+	-	+	+	+
15	+	-	+	+	+
10	+	-	+	+	+
5	+	-	+	+	+
Experiment 2					
100	+	-	-	-	-
95	+	-	+	+	+
90	+	-	+	+	+

Positive control - seeded broth without penicillin
Negative control - unseeded broth plus penicillin
+ = presence of growth; - = complete absence of growth

within a few days only a slight haze of inhibition was visible.

In the penicillin-tube assay which employed a ten-day isolate of S. pullorum, the gradual clearing of visible turbidity occurred. At the end of 96 hours all tubes were clear and it was decided to subculture each tube into plain broth. The results produced by subculturing are recorded in Table 10.

The results of subculturing indicated that penicillin exerted a bactericidal effect within 96 hours. However, a further detailed study was needed for determining more accurately the length of time required for a bactericidal effect to become evident. A tube dilution series was prepared which extended from 5 units of penicillin per ml of broth medium to 35 units. Subcultures were made in penassay broth. The results from this study are recorded in Table 11.

It is evident that the use of a broth medium is superior to that of a solid medium for subculturing techniques. Some organisms which survived penicillin contact continued to proliferate when subcultured in broth, but did not grow when streaked on agar plates.

The bactericidal action of penicillin against S. pullorum could have a partial explanation in the theory of post-lytic growth responses. As described previously, members of any bacterial population will vary in response to a minimal inhibiting concentration of penicillin.

TABLE 10

THE SURVIVAL OF SALMONELLA PULLORUM AFTER 96 HOURS OF PENICILLIN CONTACT AS DETERMINED BY SUBCULTURE TECHNIQUE

Units per ml of subcultured tube	Positive control	Negative control	Trials 1	2	3
100	+	-	-	-	-
95	+	-	-	-	-
90	+	-	-	-	-
85	+	-	-	-	-
80	+	-	-	-	-
75	+	-	-	-	-
70	+	-	-	-	-
65	+	-	-	-	-
60	+	-	-	-	-
55	+	-	-	-	-
50	+	-	-	-	-
45	+	-	-	-	-
40	+	-	-	-	-
35	+	-	-	-	-
30	+	-	-	-	-
25	+	-	-	-	-
20	+	-	-	-	-
15	+	-	-	-	-
10	+	-	-	-	+
5	+	-	+	+	+

Positive control - seeded broth without penicillin
Negative control - unseeded broth plus penicillin
+ = presence of growth; - = complete absence of growth

TABLE 11

THE GROWTH OF SALMONELLA PULLORUM IN THE PRESENCE OF PENICILLIN FOR SUBCULTURE STUDIES

24 hours of incubation									
Trials	Positive control	Negative control	Units per ml						
			35	30	25	20	15	10	5
1	+	-	+	+	+	+	+	+	+
2	+	-	+	+	+	+	+	+	+
3	+	-	+	+	+	+	+	+	+

Units per ml	24-hour reading			48-hour reading			72-hour reading		
	1	2	3	1	2	3	1	2	3
Subcultures in broth after 24 hours of incubation									
35	+	+	-	+	+	-	+	+	-
30	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+
Subcultures in broth after 48 hours of incubation									
35	-	-	-	-	-	-	-	-	-
30	+	-	-	+	-	-	+	-	-
25	+	-	+	+	-	+	+	-	+
20	+	-	+	+	-	+	+	-	+
15	+	+	-	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+
Subcultures in broth after 72 hours of incubation									
35	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-
25	-	-	+	-	-	+	-	-	+
20	-	-	-	+	-	-	+	-	-
15	-	-	-	-	-	-	-	-	-
10	-	+	+	-	+	+	-	+	+
5	+	+	+	+	+	+	+	+	+

TABLE 11 CONTINUED

Units per ml	Subcultures on solid media								
	24-hour incub.			48-hour incub.			72-hour incub.		
	1	2	3	1	2	3	1	2	3
35	-	-	-	-	-	-	-	-	-
30	+	-	+	-	-	-	-	-	-
25	+	+	+	-	-	-	-	-	-
20	+	+	-	-	-	-	-	-	-
15	+	+	+	+	-	-	-	-	-
10	+	+	+	+	+	-	-	-	-
5	+	+	+	+	+	+	+	+	-

Positive control - seeded broth without penicillin
Negative control - unseeded broth plus penicillin
+ = presence of growth; - = complete absence of growth

Those members that are killed first, release essential nutrients upon lysis of the cells. Penicillin is known to exert its lethal action against those cells that are in an active metabolic state. Those members of the bacterial population that are less active are stimulated to greater metabolic activity when essential nutrients are made available through lysis of more susceptible members. The increased metabolic activity of formerly inactive cells, renders these cells susceptible to a minimal inhibiting concentration of penicillin. Thus the killing action of penicillin is exerted through a series of cycles, in which non-susceptible cells are continually made susceptible until a point is reached where all cells are eventually killed. This phenomenon may not only explain shifting endpoint readings, but also the rather slow bactericidal action observed in this study. A beginning bactericidal action was noted after 48 hours of penicillin contact (for minimal inhibiting concentrations of penicillin). Apparently some organisms were able to survive 72 hours of penicillin contact. In 25 units per ml of penicillin and in 20 units, one out of three tubes still showed growth. These organisms either developed resistance or the antibiotic underwent deterioration.

Thomas and Hayes (128) found that during a 24-hour period of incubation at 37° C there was a 35 percent loss of penicillin potency. This is interesting when minimal inhibiting concentrations of penicillin are considered.

In the present study 20 units of penicillin per ml of broth medium may be considered as the bactericidal end-point for S. pullorum #89817 at the end of 72 hours, but this concentration may have a lower value (decrease in potency at 37° C). When viewed in this respect the bactericidal concentration of penicillin is probably lower than 20 units per ml of broth medium.

It was of interest to determine whether S. pullorum had been damaged by penicillin contact, so as to influence the biochemical reactions characteristic for this organism. The organisms were removed from several different concentrations of penicillin in which they still exhibitied a growth response and placed in the following differential media: Kligler's iron agar, sucrose and mannitol broth and Sims medium. The following reactions were obtained:

TABLE 12

THE BIOCHEMICAL CHARACTERIZATION OF SALMONELLA PULLORUM AFTER CONTACT WITH SEVERAL VARYING CONCENTRATIONS OF PENICILLIN

Unitage derivation of selected transfer	Kligler's		Individual sugars		Gas	Sims	
	Butt	Slant	Mannitol	Sucrose		H_2S	Motility
48-hour							
Pos. control	A	AK	A	AK	-	+	-
5 units	A	AK	A	AK	-	+	-
20 units	A	AK	A	AK	-	+	-
30 units	A	AK	A	AK	-	+	-
72-hour							
10 units	A	AK	A	AK	-	+	-
20 units	A	AK	A	AK	-	+	-
25 units	A	AK	A	AK	-	+	-

A = acid reaction; AK = alkaline reaction

Apparently, penicillin contact does not alter the biochemical activity of S. pullorum. The recorded reactions on differential media are typical for the strain used in this study. Microscopic examination of these organisms did not reveal any gross morphological changes. Occasionally, the organism appeared more elongated than usual.

The Enhancement of Penicillin Action Against S. pullorum by Proper Concentrations of Cobalt

In 1947 Pratt, Dufrenoy and Strait (93, 94, 124) reported that trace amounts of cobalt enhanced the effectiveness of penicillin. They found that trace amounts of cobalt added to agar plates increased the effectiveness of dilute penicillin solutions in producing inhibition zones. The test organism used was Micrococcus pyogenes var. aureus. A four-fold to eight-fold enhancement of penicillin action was recorded. The degree of enhancement depended on the test organism used and the minimal inhibiting concentration of penicillin that could be detected by their method of testing. A cobalt concentration of one mg per liter of nutrient agar was found to be the most optimal.

They also observed that the time required to produce discernible zones of inhibition was much shorter than on plates containing no cobalt. They postulated that trace amounts of cobalt lowered the required minimal inhibiting concentration of penicillin.

These investigators found further evidence for the

enhancement of penicillin action by cobalt. Test plates seeded with Escherichia coli and incubated for 16 hours at 37° C produced readable zones of inhibition with 10 units of penicillin per ml of aqueous solution. The zones measured 12 mm in diameter without the addition of cobalt. However, when the test agar contained cobalt chloride in a concentration of one mg per liter of nutrient agar, a zone of inhibition measuring 15 mm in diameter was produced by only one unit of penicillin per ml of aqueous solution. This was not the result of an additive effect of two antibiotic agents. A solution containing only cobalt chloride did not have any inhibitory effect on the test organism.

The cobalt effect on penicillin was also noted in serial dilution experiments. Tubes containing cobalt required only half the usual minimal inhibiting concentration of penicillin.

The cobalt effect on penicillin was also noticed with such test organisms as Proteus vulgaris and Bacillus subtilis.

It was also found that a short period of incubation with cobalt solution prior to penicillin contact produced the most striking results. This period of incubation varied with the test organism employed.

The in vivo effect of cobalt on penicillin was then determined. Mice were inoculated with S. typhosa and then given 256 micrograms of cobalt chloride. After a short in vivo incubation period, 2000 units of crystalline sodium benzyl penicillin was injected. This combination exerted a

protective effect equal to 4000 units of penicillin. Concentrations as low as 64 micrograms of cobalt chloride produced some enhancement of penicillin effectiveness. Cobalt in these concentrations was non-toxic and conferred no protection on the animals.

The results of cobalt action on penicillin reported by Pratt and Dufrenoy (124) were extremely interesting and the possibility of extending their findings to such an organism as S. pullorum seemed plausible.

The first task was to find a suitable concentration of cobalt that would be effective against S. pullorum. The recommended concentration of one mg per liter of solution was not effective. A much higher concentration of cobalt was found to give very striking results. The concentration used in this study was 0.2 mg of cobalt chloride per ml of distilled water. This concentration was not toxic to S. pullorum, although a concentration of 0.4 mg per ml appeared to have some slight toxic effect. Plates seeded with S. pullorum and incubated with cobalt solution for 30 minutes produced zones of inhibition that were three to ten mm larger than control plates containing no cobalt. This is illustrated in Figure 1.

The cobalt enhancement of penicillin action against S. pullorum was then evaluated by means of a penicillin reference curve for this particular test organism (Figure 11). It was found that cobalt produced approximately a four-fold degree of enhancement. In other words, 100 units per ml of

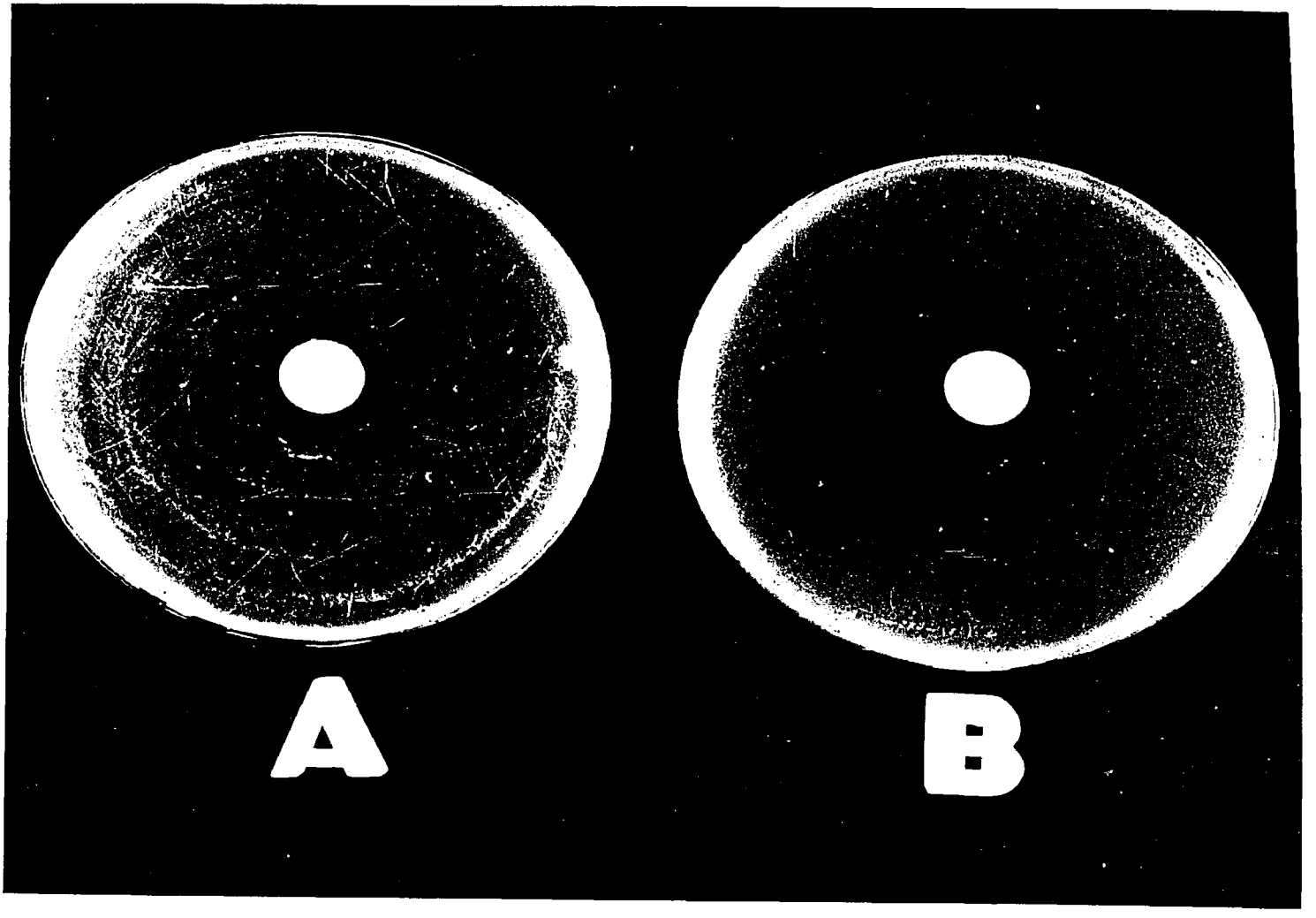


Figure 1. The enhancement of penicillin action against Salmonella pullorum by cobalt

Both plates were seeded with Salmonella pullorum #89817 and subjected to equal concentrations of penicillin (100 u/ml). The zone of inhibition on plate A measured 22 mm, while that of plate B measured 32 mm. Organisms on plate B were incubated for 30 minutes in the presence of cobalt (0.2 mg/ml) before penicillin contact. Organisms on plate A received no cobalt treatment.

penicillin plus cobalt was equivalent to 400 units per ml of penicillin without cobalt.

The concentration of cobalt used in this study may not be the optimal, since there was not enough time to investigate a series of concentrations below 0.2 mg. The information obtained here was later used for in vivo experiments which will be discussed shortly.

Pratt, Dufrenoy and Strait (93) proposed some theories regarding the mechanism of cobalt action. They stated that "the effect of cations may ultimately be associated with the formation of complexes with -SH containing groups or with some other essential component of an energy-providing oxidation-reduction system. The degree of inhibition effects exhibited by the various cations may be related to the degree of binding of the cations in the complex. Thus cadmium and silver, which form stable complexes, are highly toxic, whereas cobalt, which forms loose complexes with -SH groups is much less toxic."

The authors also postulated a general theory regarding the role of cobalt. They stated that penicillin exerts its greatest antibiotic effect on susceptible organisms when they are in a phase of logarithmic increase. Penicillin exerts its optimal effect on bacteria which are thriving in an enviornment favorable for their growth. Conditions which favor the multiplication of bacteria also increase the rate of penicillin action. Suitable concentrations of cobalt stimulate the metabolism or growth of

the organisms and ultimately render them more susceptible to penicillin action.

The results of cobalt action on S. pullorum are recorded in Table 13.

Marked in vitro enhancing effects can be duplicated if three ml of cobalt solution is used in a concentration of 0.2 mg per ml.

The Sensitivity of S. pullorum to Garlic Extract

Alicin, the active principle of garlic cloves, has been found to exhibit a rather wide antibacterial spectrum. Its action in vitro against S. pullorum #89817 was very pronounced. The zones of inhibition produced on seeded plates were very sharp and clear. There was a marked similarity between these zones and those produced by penicillin.

A streak-plate technique and a seeded agar method were used in testing S. pullorum sensitivity (Tables14 and 15).

It appears that both methods can be used for the determination of garlic sensitivity.

The Stability of Aureomycin, Chloromycetin, Streptomycin, Penicillin and Garlic in the Presence of Various Feed Mixtures (Tables 16 and 17)

The results obtained from stability determinations indicate that all the antibiotics tested were relatively

TABLE 13

THE IN VITRO ENHANCEMENT OF PENICILLIN ACTION BY APPROPRIATE CONCENTRATIONS OF COBALT

Trials	Toxicity control 2 ml cobalt (0.2 mg/ml) minus penicillin	Penicillin 100 u/ml	
		2 ml cobalt (0.2 mg/ml) 30 min. incub.	minus cobalt
1	+	26	23
2	+	26	22.5
3	+	24.5	22
4	+	24	21.5
5	+	27.5	23.5
6	+	29	21.5
7	+	26	23
8	+	28	22.5
9	+	28	22
10	+	26.5	21
11	+	25	21
12	+	26	21.5
13	+	25.5	24
Average diameter		26.3	22.2

Trials	Penicillin 100 u/ml			
	1 ml cobalt (0.2 mg/ml) 30 min. incub.	2 ml cobalt (0.2 mg/ml) 30 min. incub.	2 ml cobalt (0.2 mg/ml) 120 min. incub.	minus cobalt
1	24.5	30	24	23
2	28.5	30	25	22.5
3	26.5	32	26.5	23.5
4	26	29	25	22
5	27	29	27	21.5
6	26.5	27.5	25.5	22.5
7	24.5	27.5	25.5	20.5
8	26	28.5	--*	20.5
9	--	28	--	22
10	--	29	--	22.5
Average diameter	26.2	29	25.5	22

TABLE 13 CONTINUED

Trials	Penicillin 100 u/ml				
	30 min. incub.				minus cobalt
	cobalt (0.2 mg/ml)		cobalt (0.4 mg/ml)		
	2 ml	3 ml	2 ml	3 ml	
1	25	31	29	zone	22.5
2	26	28.5	28	open	21
3	24	28.5	29.5	"	22
4	25	27	31	"	22
5	26	28.5	--*	"	23
6	23.5	27.5	--	"	23
7	24	30	--	"	21.5
8	24	31.5	--	"	24
Average diameter	24.7	29.1	29.4		22.4

Trials	Penicillin 100 u/ml		
	1 ml cobalt	30 min. incub.	minus cobalt
	0.2 mg/ml	0.002 mg/ml	
1	26	23	24
2	26.5	23	24
3	27	24.5	23.5
4	27	23	22.5
5	--*	--	24
Average diameter	26.6	23.4	23.6

Trials	Toxicity control 3 ml cobalt (0.2 mg/ml) minus penicillin 30 min. incub.	Penicillin 100 u/ml	
		3 ml cobalt (0.2 mg/ml) 30 min. incub.	minus cobalt
1	+	31.5	23.5
2	+	29	23
3	+	27	22.5
4	+	29.5	23
5	+	30	22.5
6	+	29.5	23
7	+	30	22.5
8	+	26.5	22.5
Average diameter		29.1	22.8

+ = complete growth of organism
zone diameter measured in mm
*Replicates not carried out beyond last reading

TABLE 14

A STREAK-PLATE METHOD FOR DETERMINING THE GARLIC SENSITIVITY OF SALMONELLA PULLORUM

Units per ml of mixture	Trials (48-hour read.) 1	2	Agar without garlic
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
10	+	-	+
20	-	-	+
30	-	-	+
40	-	-	+
50	-	-	+

+ = presence of growth; - = complete absence of growth

TABLE 15

A SEEDED-AGAR METHOD FOR DETERMINING THE GARLIC SENSITIVITY OF SALMONELLA PULLORUM

Units per ml of mixture	Trials (72-hour read.) 1	2	Agar without garlic
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
10	+	+	+
20	+	+	+
30	-	-	+

+ = presence of growth; - = complete absence of growth

TABLE 16

THE STABILITY OF SELECTED ANTIBIOTICS IN FEED SAMPLES AFTER A ONE-MONTH PERIOD OF STORAGE

Antibiotic	Feed mixture	Trials 1	2	3	4	Ave. zone diameter
Aureo-mycin	15% protein	23	22	21	22	22
	30% protein	23.5	22	22	22	22.4
	Sucrose + 15% protein	23	22	22	22	22.3
	Sucrose + 30% protein	22.5	22	20	20.5	21.3
	Dextrin + 15% protein	23.5	23.5	23.5	23	23.4
	Dextrin + 30% protein	24	24	23	23	23.5
	Commercial	20	20.5	21	21	20.6
Chloro-mycetin	15% protein	21	20	19	19	19.6
	30% protein	18.5	18	18.5	17.5	18.1
	Sucrose + 15% protein	23	21	20.5	20.5	21.2
	Sucrose + 30% protein	20	20	20.5	18.5	19.8
	Dextrin + 15% protein	20.5	20.5	21.5	21.5	21
	Dextrin + 30% protein	20	18	17	18.5	18.4
	Commercial	25	24	25	25.5	24.9
Strepto-mycin	15% protein	15	15	15	15.5	15.1
	30% protein	15.5	15	15.5	16	15.5
	Sucrose + 15% protein	15	15	16	16	15.5
	Sucrose + 30% protein	16	16	16	16	16

TABLE 16 CONTINUED

Antibiotic	Feed mixture	Trials 1	2	3	4	Ave. zone diameter
Strepto-mycin	Dextrin + 15% protein	16	15	15	15	15.2
	Dextrin + 30% protein	16	15	15	15.5	15.4
Penicillin	15% protein	22	22	21	22	21.8
	30% protein	26	27	25.5	24	25.5
	Sucrose + 15% protein	25	26	25	24.5	25.1
	Sucrose + 30% protein	23.5	24	24	25	24.1
	Dextrin + 15% protein	23	24	25	24	24
	Dextrin + 30% protein	25	24.5	24.5	25.5	24.9
Garlic	15% protein	21.5	22.5	22	20.5	21.6
	30% protein	23	23	23.5	22.5	23
	Sucrose + 15% protein	22	20.5	22.5	21	21.5
	Sucrose + 30% protein	22	22	23.5	23	22.6
	Dextrin + 15% protein	24.5	26	26	27	25.9
	Dextrin + 30% protein	24	24	24	23	23.8

Zone diameter measured in mm

Control (feed minus antibiotic) - no antibiotic activity detected in all tested feed samples

The zones of inhibition produced by theoretical levels of antibiotic in tested feed samples (from reference curve data)

Aureomycin - 25.0 mm Penicillin - 25.8 mm

Chloromycetin - sample mixture 20.3 mm; commercial sample 25.7 mm

Streptomycin - 15.3 mm Garlic - 22.0 mm

TABLE 17

SUMMARY OF ANTIBIOTIC STABILITY AFTER A ONE-YEAR PERIOD

Antibiotic - feed mixture	Ave. zone diameter (mm)	Zone produced by original concentration (from ref. curve data)
Aureo-15% protein	21.8	24.5
Aureo-30% protein	21.2	"
Aureo-15% protein-sucrose	18.8	"
Aureo-30% protein-sucrose	19.0	"
Aureo-15% protein-dextrin	20.8	"
Aureo-30% protein-dextrin	21.7	"
Aureo-commercial sample	20.6	"
CM-15% protein	20.5	19.5
CM-30% protein	18.7	"
CM-15% protein-sucrose	19.2	"
CM-30% protein-sucrose	20.5	"
CM-15% protein-dextrin	21.0	"
CM-30% protein-dextrin	18.7	"
CM-commercial sample	23.6	25.8
SM-15% protein	15.3	15.8
SM-30% protein	15.2	"
SM-15% protein-sucrose	15.5	"
SM-30% protein-sucrose	no zones	"
SM-15% protein-dextrin	15.8	"
SM-30% protein-dextrin	no zones	"
Garlic-15% protein	20.5	22.0
Garlic-30% protein	20.0	"
Garlic-15% protein-sucrose	20.8	"
Garlic-30% protein-sucrose	19.8	"
Garlic-15% protein-dextrin	21.5	"
Garlic-30% protein-dextrin	22.0	"

Aureo = aureomycin
CM = chloromycetin
SM = streptomycin

stable over a one-year period. Aureomycin appeared to undergo a slight to moderate decrease in potency at the end of four weeks, but this was probably due to an initial adsorption to feed constituents rather than outright deterioration. This conclusion is based on the fact that similar zones of inhibition were produced at the end of twelve months (identical amounts of feed-mixture were tested at one and twelve months). The slight variations in zone diameter are negligible when the errors inherent in plate-assay procedures are considered.

A number of in vivo experiments covered a period of two to three weeks and it was necessary to know whether a particular antibiotic would remain stable for that length of time. It was also of interest to know whether these antibiotics would retain their potency over a protracted period of time under practical storage conditions.

At the end of one year, streptomycin exhibited evidence of deterioration in two of the sample mixtures.

Penicillin mixtures produced zones of inhibition that were almost identical at one and six month intervals. This is probably due to the fact that oral penicillin preparations are usually well buffered to withstand gastric acidity.

Qualitative determinations were made on veterinary preparations of chloromycetin and streptomycin. These antibiotic mixtures produced ill-defined zones of inhibition that could not be evaluated with accuracy.

An In Vivo Evaluation of 15 Experimental Series

(Tables 18 - 46)

A. General considerations

A total of four series failed to produce valid results (Series 2, 5, 10 and 14). These failures were evidenced by very low or non-existent mortality in all experimental groups. In this study, the only criterion for successful, artificial infection was a demonstrable difference in mortality among non-treated, infected controls and treated infected groups. However, this method of evaluation may be somewhat severe, in view of the fact that chicks infected with S. pullorum may exhibit symptoms of the disease without succumbing to the infection (these birds are dangerous because they may be carriers). The infected controls of the present experimental series (invalid series) exhibited such pullorum symptoms as pasted vents, drowsiness, ruffled feathers, extreme thirst and respiratory difficulties. However, within a few days all symptoms disappeared and to all outward appearances the chicks appeared healthy. There is a good possibility that S. pullorum could have been recovered, if cloacal swabs had been taken at this time. Several factors may have contributed to low or no mortality among the non-treated, infected controls.

One factor may be that a critical number of infecting organisms are required to produce drastic results (in certain

cases). In series 14, the usual method of preparing an inoculum, resulted in a bacterial count of 900,000,000 organisms per ml of saline suspension (the usual average being 1,350,000,000 organisms per ml of suspension). On the other hand, in series 10, the bacterial count was 1,600,000,000 organisms per ml of saline suspension.

Another factor to consider is the present day practice of careful genetic selection for resistant birds. Genetic variations among different breeds and within a particular breed only serve to complicate the results obtained in studies of this nature. It is known, for instance, that the White Leghorn breed is rather resistant to pullorum infection (30, 56).

The virulence of the infecting organism is also of prime importance. This factor cannot be predicted with certainty from one experiment to the next. Certain procedures such as chick passage are of benefit, but even here the method can be open to question. It was found in this study that the virulence of the infecting organism could be maintained for a long period of time in stock cultures, if transfers were made every two weeks.

The degree of pullorum infection is also dependent upon the age of the chick at the time of exposure. In this particular experiment a 72-hour period of prophylactic feeding was used in the first two experimental series. This was later changed to 48 hours and then to 24 hours. It is a known fact that chicks rapidly develop resistance

to pullorum infection after the first day of life. Artificial exposure to infection beyond the first day will not produce as many fatalities as infection at one day of age. This may have been a factor in causing low mortality rates among some non-treated, infected controls.

Chicks which died of pullorum infection during the experimental periods were necropsied and examined for any gross lesions. Usually consolidation of the lungs and nodulation of the heart appeared in 12 to 14 days after infection. Chicks dying of overwhelming infection during the first two or three days generally did not exhibit any unusual lesions. An attempt was made to isolate the infecting organism from each chick that died, but this was not always possible in every experimental group and therefore only a representative number were sampled in such instances.

It should be stressed that <u>in vivo</u> experiments are usually subject to many variables. Some of these variables are known, while others remain undetected. A large number of experiments are therefore necessary in order to determine the validity of all results obtained. In this particular study, a number of experiments have been repeated in successive trials. However, this is not sufficient evidence for making definite conclusions about the efficacy of any particular antibiotic therapy (or other treatment). The experiments in this study have merely provided a basis for further work. The results indicate a trend or direction

that should be followed either with additions or modifications of present procedures.

B. Specific considerations

1. The value of feed modifications with respect to protein and carbohydrate content. Mann (78, 79, 80) considers S. pullorum as only a potential pathogen and that its existence is dependent on a symbiotic relationship with certain gram-positive organisms. He therefore claims that a diet which suppresses gram-positive organisms will check the eventual course of pullorum infection. Mann proposes that chicks be fed an oat diet for 72 hours in order to establish an acid condition in the intestinal tract. This will provide an environment unfavorable to gram-positive organisms of the "welchii type", which are regarded as the principal symbionts for S. pullorum. After 72 hours, the oat diet was supplanted by feed containing 14 percent protein. Mann holds that feeds containing 20 percent protein favor the growth of gram-positive symbionts.

The work of Mann is interesting in view of our present knowledge concerning the effect of antibiotics on the normal intestinal microflora. However, the findings reported by Mann are vague in many places and the proof of symbiotic relationships are inconclusive. The inclusion of oats in the diet would appear to be rather unnecessary, since the intestinal tract of young chicks is normally in

an acid condition.

Nevertheless it was of interest to test the hypothesis of Mann in this study. His proposals were modified so that feeds containing two extremes of protein content were used. A 30 percent protein feed was employed in order to exaggerate the harmful effects of high protein diets on the course of pullorum infection (as believed by Mann).

The results of the first series of experiments indicate that oat feeding and a lowered protein content of feed do not reduce mortality. When aureomycin was added to these diets, there was a marked decrease in mortality.

Perhaps more experiments would be needed to confirm or refute the findings by Mann, but at present the indications do not seem to support the original hypothesis.

Johansson, Sarles and Shapiro (59) found that sucrose as the principal carbohydrate in chick feed, produced a marked depression on coliform organisms. Dextrin, on the other hand, stimulated the development of organisms at all levels of the intestinal tract. It was of interest to determine the effect of these carbohydrates on the eventual course of pullorum infection. There was no opportunity for evaluating dextrin, but sucrose was included in one experimental group. The results were not striking and the usual mortality rates from infection <u>per os</u> were obtained. The aureomycin group of this particular series (Series 3) had a 13.3 percent mortality, while for sucrose it was 24 percent. However, a combination of sucrose plus aureomycin resulted

in no mortality. There may be a synergistic effect between sucrose and aureomycin, but this cannot be stated with certainty from one group of experiments.

2. Aureomycin. This antibiotic appears to be effective in reducing mortality rates due to pullorum infection. The synergistic effect of this drug in combination with other antibiotics remains to be determined. Of special interest would be the use of aureomycin along with appropriate levels of penicillin.

3. Chloromycetin. This drug also seems to be of value in the treatment of pullorum infection. Its effectiveness in synergistic combinations remains to be ascertained. Chloromycetin was combined with aureomycin and also with penicillin, but the results were not conclusive as far as a synergistic phenomenon is concerned.

4. Penicillin. The use of oral penicillin in feed mixtures involves some important considerations. The amount of penicillin given by oral administration must be at least three to five times greater than a parenteral dose. This is due to several factors. First, absorption of oral penicillin in the intestinal tract is erratic. Secondly, organisms present in the intestinal tract may inactivate a large proportion of the original concentration by elaboration of the enzyme penicillinase. Thirdly, the gastric juices may also destroy penicillin to a certain extent, even

though oral preparations are highly buffered.[1] Therefore high concentrations of penicillin must be used to offset these various factors.

The effect of penicillin on the course of pullorum infection has been beneficial in a number of experimental groups (Series 3, 7, 9, 12, 15). The erratic nature of penicillin absorption at inadequate concentration levels is best illustrated by the results obtained in several experimental groups. In Series 3, 7 and 9, low mortality figures at 660 and 330 units per g of feed were recorded. Poor results were obtained in Series 13, which included penicillin groups having concentrations of 2000 and 2290 units per g of feed. Favorable results were observed in Series 12 and 15, at levels of 2936 and 5500 units of penicillin per g of feed. It is therefore extremely important to maintain an adequate dosage level. This level is probably well above 5000 units of penicillin per g of feed.

From in vitro results, the indications are that penicillin blood levels of 15 to 20 units per ml of plasma may be necessary at all times during the acute stage of infection. However, a study should be made on the actual blood levels of chicks being fed a penicillin mash. Perhaps the actual concentration of penicillin will have to be based on a series of blood level determinations plus a number of in vitro studies on various pullorum strains.

The in vitro studies indicated that appropriate concentrations of cobalt markedly reduced the minimal inhibiting

concentration of penicillin. Cobalt produced a four-fold enhancement of penicillin action. This phenomenon was also observed *in vivo*. Penicillin, when incorporated at a level of 2290 units per g of feed, did not reduce mortality figures to any appreciable extent, but with cobalt treatment there was a striking reduction in mortality. In Series 15 the non-treated, infected control had a mortality figure of 32 percent, while three cobalt groups had mortalities of 4, 12 and 0 percent respectively. The proper use of cobalt may greatly reduce the required amount of penicillin. Studies should also be made on the optimal concentration of cobalt necessary for maximum enhancement (*in vivo*) of penicillin action.

Sodium benzoate will prolong penicillin blood levels after oral ADMINISTRATION ~~injection~~. This is due to the fact that sodium benzoate inactivates the enzyme penicillinase which is produced by such intestinal organisms as *Escherichia coli*. Sodium benzoate was used in Series 7, 8 and 9. However, no conclusive results were obtained since low concentrations of penicillin were employed in these groups. *In vitro* studies should also be conducted with various concentrations of sodium benzoate. Cobalt along with sodium benzoate may prove to be valuable agents in reducing the minimal inhibiting concentration of penicillin.

There are indications that a marked increase in oxygen uptake occurs when certain bacterial species are placed in contact with appropriate concentrations of cobalt. It would

be extremely interesting to perform a series of manometric studies on the oxygen uptake of S. pullorum in the presence of cobalt.

It might also be mentioned that penicillin at higher concentrations did not appear to exert any toxic effect on chicks.

Cobalt without the addition of penicillin may enhance the virulence of S. pullorum. This may be true in view of the current belief that cobalt accelerates metabolic activity among certain microorganisms.

5. Streptomycin. The results obtained by using a veterinary streptomycin mixture were not very pronounced. Prophylactic feeding of this mixture did not seem to reduce the symptoms of pullorum infection. Birds given aureomycin, chloromycetin and penicillin (in adequate dosages) exhibited slight transient symptoms. The use of other streptomycin preparations may produce better results. However, it is a well known fact that a number of organisms will quickly develop resistance to rather high concentrations of streptomycin. Therefore, it would be important to conduct a series of in vitro tests on the development of resistance to streptomycin by S. pullorum. From in vitro determinations it would appear that streptomycin is mainly bacteriostatic in action against S. pullorum. These factors may preclude the further use of streptomycin in the control of pullorum infection.

6. Antibiotic substances from higher plants. Antibiotic substances from higher plants do not appear to be beneficial in the control of pullorum disease. Such substances as Trigonella Foenum-graecum actually exaggerated the symptoms of this disease. Birds that were given this preparation developed extreme pasting of the vent and pronounced respiratory symptoms. Mortality was very high in this group, 60 percent. The use of banana meal may have possibilities, but there is not sufficient evidence for any definite conclusions. A mortality of 34.5 percent was recorded for non-treated, infected controls in the third series of experiments. A mortality of 16 percent occurred among birds given banana meal (Maqueno variety) in the diet. For many years the beneficial results of banana feeding were thought to be due to the exceptional nutritional qualities of this plant. The recent discovery of several promising antibiotic fractions in the banana by Scott, McKay, Schaffer and Fontaine (115) has put new emphasis on the use of the banana for therapeutic puproses.

Garlic is perhaps the most promising of all the higher plants. The in vitro action of garlic against S. pullorum is very similar to that of penicillin. Zones of inhibition on seeded plates were very sharp and remained clearly defined for many weeks without any encroachment by the test organism. The effect of garlic appears to be bactericidal for S. pullorum. The use of garlic in several in vivo experiments did not produce very favorable results. However,

this may have been due to the fact that the concentrations employed were too high and actually exerted a toxic effect on the chicks. Experiments should be tried in which very low levels are used over a longer period of time. In the present study garlic constituted 15 and 6 percent of feed mixtures respectively. It would be of interest to use a concentration of one or two mg of garlic powder per g of feed.

7. The use of an antigen-mash. In this experiment oral antigen preparations were incorporated in the feed. The antigen was either adsorbed on plain feed or on a charcoal preparation. The results obtained in these experimental groups were poor, and it appeared that charcoal had a slight toxic effect on young chicks when given for a prolonged period of time.

Ingestion of a prepared antigen would undoubtedly have a decided advantage over conventional methods of immunization. An antigen-mash may have value in protecting young chicks from later infection.

What appeared to be a successful method of immunization against pullorum disease was reported by Morcos, Zaki and Zaki in 1946 (85). Their method of immunization consisted of testing mature chickens for the presence of pullorum infection by the agglutination technique. If these chickens proved to be absolutely negative, they were inoculated intramuscularly with a heat-killed antigen (10^9 organisms per ml). Each bird received 0.5 ml, one ml, and one ml at

weekly intervals. Two weeks after the last injection the birds were tested by the agglutination method. Each bird possessed a high titer as compared to non-vaccinated controls. The eggs of vaccinated birds were collected and kept for hatching. The same was done with non-vaccinated controls. Eighty chicks from vaccinated parents survived, while nineteen survived from the non-vaccinated controls (these chicks were not exposed to S. pullorum). When the chicks were two months old they were tested by the agglutination method. The chicks from vaccinated birds gave a titer of between 1:80 and 1:320, while the non-vaccinated chicks did not exceed a titer of 1:5.

The authors conclude that "agglutinins were transmitted from the vaccinated mother to the chick through the egg".

The Percentage of Carrier Birds Produced by Treatment with Aureomycin, Chloromycetin, Penicillin and Penicillin plus Cobalt (Table 47)

A total of 278 birds were retained for carrier studies. Most of the birds were killed at three months of age (aureomycin group nine months) and carefully examined for the presence of S. pullorum. The aureomycin group contained the smallest number of birds (thirteen).

The percentage of carriers recorded among the several different groups were as follows: aureomycin, 0 percent; chloromycetin -- first group, 0 percent, second group, 28

percent, and third group, 16 percent; penicillin -- 4500 units per g feed, 31.9 percent, 5500 units per g, 10.5 percent; penicillin (2290 units per g) plus cobalt, 6.6 percent and non-treated, infected controls, 16 percent.

The listed results cannot be taken at face value. Some important factors must be considered before any definite analysis can be made. First of all, a much larger group of birds from repeated experiments would have to be sampled. Secondly, birds given different antibiotics (or different dosage levels of the same antibiotic) should be isolated within their respective groups. This must be done in order to evaluate the efficacy of a particular antibiotic (or dosage level) in removing the infecting organism from the host. Recovered birds which no longer harbor the infecting organism (due to an effective antibiotic) may again become infected with the same organism if placed in contact with other birds that are still infected (due to an ineffective antibiotic).

Unfortunately, the facilities for extensive carrier studies were rather limited in this experiment and various groups had to be kept in the same housing area.

Finally, the optimal concentration of antibiotic necessary to eliminate carriers, may not have been attained in a number of experimental groups. This is probably very true of penicillin. It is believed that the effective concentration of penicillin required to eliminate carriers is well beyond the highest levels employed in this study (a

limited supply of penicillin prevented the use of higher concentration levels). The number of infecting organisms used for artificial infection may be far in excess of that likely to be encountered in natural infections. This possibility might decrease the required minimal, inhibiting concentration of antibiotic (as indicated in this study) for natural infections. However, artificial infections can never duplicate the conditions operating in nature and therefore all determinations must be based on laboratory situations.

SUMMARY

Aureomycin, chloromycetin and penicillin were effective in reducing fatalities among young chicks artificially infected with S. pullorum. The protective action of a veterinary preparation of streptomycin and that of garlic was not very pronounced.

In vitro studies indicate that S. pullorum #89817 is sensitive to low concentrations of aureomycin (2 micrograms of aureomycin per ml of broth medium) and chloromycetin (1.56 micrograms per ml of broth medium). S. pullorum #89817 also possesses a moderate sensitivity to penicillin (15 units per ml), garlic (20 to 30 units per ml) and streptomycin (6.6 micrograms per ml). Five pullorum strains, in addition to #89817, were also found to be sensitive to penicillin action.

Antibiotic substances from higher plants did not seem to exert any beneficial effect on the course of pullorum infection. In vitro doterminations indicate that garlic may have therapeutic possibilities. The concentrations employed in this study were too toxic for young chicks.

Appropriate concentrations of cobalt chloride markedly enhanced the action of penicillin against S. pullorum. This was true for both in vivo and in vitro tests. Cobalt produced approximately a four-fold enhancement of penicillin action.

An attempt to immunize young chicks against pullorum infection by use of an antigen-feed did not produce any significant results. However, the possibility of immunization by other methods still exists.

Aureomycin, chloromycetin, streptomycin and garlic were found to be relatively stable in various feed mixtures over a one-year period. Penicillin was also found to be stable when tested at the end of a six-month period.

Carrier birds were found among those groups treated with chloromycetin, penicillin and penicillin plus cobalt. No carriers were found among birds treated with aureomycin. The small number of birds in the aureomycin group, precludes any definite statements regarding the value of this antibiotic in the elimination of pullorum carriers.

A combination of cobalt plus penicillin produced the lowest carrier percentage among the larger groups of birds tested (6.6 percent).

TABLE 18

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 1)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
15% protein (infected control)	0	24	72	9	37.5
30% protein	0	25	72	6	24.0
Oats - followed by 15 % protein	0	25	72	7	28.0
Oats - followed by 30% protein	0	25	72	7	28.0
15% protein + aureomycin	1	29	72	1	3.4
30% protein + aureomycin	1	29	72	0	0
Oats - followed by 15% protein + aureomycin	1	29	72	3	10.3
Oats - followed by 30% protein + aureomycin	1	29	72	2	6.9

Breed of chicks: White Leghorn

TABLE 19

THE RECOVERY OF SALMONELLA PULLORUM FROM DEAD CHICKS (SERIES 1)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Oats-30% protein	+	A	AK	A	AK	+	-	-
Oats-30% protein	+	A	AK	A	AK	+	-	-
Oats-30% protein	+	A	A	AK	AK	N	N	N
Oats-30% protein	+	A	A	A	NR	N	N	N
Oats-15% protein	+	A	AK	A	AK	+	-	-
Oats-15% protein	+	A	AK	A	AK	+	-	-
Oats-15% protein	+	A	AK	A	AK	+	-	-
Oats-15% protein	+	A	AK	A	AK	+	-	-
30% protein	+	A	AK	A	AK	+	-	-
30% protein	+	A	AK	A	AK	+	-	-
30% protein	+	A	AK	A	AK	+	-	-
30% protein	+	A	AK	A	AK	+	-	-
15% protein	+	A	AK	A	AK	+	-	-
15% protein	+	A	AK	A	AK	+	-	-
15% protein	+	A	AK	A	AK	+	-	-
15% protein	+	A	AK	A	AK	+	-	-
15% protein	+	A	AK	A	AK	+	-	-
15% protein + aureomycin	-	N	N	N	N	N	N	N
Oats-15% protein + aureomycin	+	A	AK	A	AK	+	-	-

16 positive isolates

A - acid reaction NR - no reaction
AK - alkaline reaction N - reaction not carried further

TABLE 20

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH *SALMONELLA PULLORUM* (SERIES 2)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	30	0	0	0
Infected control	0	23	0	1	4.3
Penicillin	550 units	32	72	1	3.1
Sucrose	0	30	72	0	0
Sucrose + aureomycin	1	25	72	0	0
Aureomycin	1	25	72	1	4
Garlic	150	25	72	16	64.0
Banana	150	25	72	2	8

Breed of chicks: White Leghorn
Feed: Kellogg starter

TABLE 21

THE RECOVERY OF SALMONELLA PULLORUM FROM DEAD CHICKS (SERIES 2)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Aureomycin	+	A	AK	A	AK	+	-	-
Banana	+	A	AK	A	AK	+	-	-
Penicillin	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	A	N	N	N
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-

12 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further

TABLE 22

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 3)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	24	0	0	0
Infected control	0	29	0	10	34.5
Penicillin	660 units	25	24	1	4.0
Sucrose	0	24	24	6	25.0
Sucrose + aureomycin	1	25	24	0	0
Aureomycin	1	30	24	4	13.3
Garlic	60	27	24	6	22.2
Banana	150	25	24	4	16.0

Breed of chicks: White Leghorn
Feed: Kellogg starter

TABLE 23

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 3)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	AK	AK	A	AK	N	N	N
Aureomycin	+	AK	AK	AK	AK	N	N	N
Aureomycin	+	A	AK	A	AK	+	-	-
Aureomycin	+	A	AK	A	AK	+	-	-
Aureomycin	+	A	AK	A	AK	+	-	-
Banana	+	A	AK	A	AK	+	-	-
Banana	+	A	AK	A	AK	+	-	-
Banana	+	A	AK	A	AK	+	-	-
Penicillin	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Garlic	+	A	AK	A	AK	+	-	-
Sucrose	+	A	AK	A	AK	+	-	-
Sucrose	+	A	AK	A	AK	+	-	-
Sucrose	+	A	AK	A	AK	+	-	-
Sucrose	+	A	AK	A	AK	+	-	-
Sucrose	+	A	AK	A	AK	+	-	-

20 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further

TABLE 24

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 4)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	25	0	1	4.0
Infected control	0	24	0	7	29.2
Penicillin - 1	660 units	29	48	14	48.3
Penicillin - 2	990 units	25	48	11	44.0
Aureomycin - 1	1	24	48	4	16.7
Aureomycin - 2	1	25	48	1	4.0
Antigen mash (ethanol attenuated)	0	25	48	4	16.0
Antigen mash (heat attenuated)	0	25	48	6	24.0

Breed of chicks: Rhode Island Red
Feed: Kellogg starter

TABLE 25

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 4)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	HS	HS	N	N	N	N	N
Antigen mash (ethanol)	+	A	AK	A	AK	+	-	-
Antigen mash (ethanol)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Aureomycin - 1	+	HS	HS	N	N	N	N	N
Aureomycin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	A	N	N	N
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-

23 positive isolates

A - acid reaction HS - Excess H_2S
AK - alkaline reaction N - reaction not carried further

TABLE 26

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 5)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	18	0	1	5.6
Infected control	0	30	0	3	10.0
Penicillin - 1	660 units	24	48	8	33.3
Penicillin - 2	990 units	25	48	3	12.0
Aureomycin - 1	1	25	48	3	12.0
Aureomycin - 2	2	25	48	1	4.0
Antigen mash - 1 (ethanol attenuated)	0	24	48	5	20.8
Antigen mash - 2 (ethanol attenuated)	0	25	48	1	4.0

Breed of chicks: Rhode Island Red
Feed: Kellogg starter

TABLE 27

THE RECOVERY OF SALMONELLA PULLORUM FROM DEAD CHICKS (SERIES 5)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Non-infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Antigen mash - 1	NR	N	N	N	N	N	N	N
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Aureomycin - 1	NR	N	N	N	N	N	N	N
Aureomycin - 1	NR	N	N	N	N	N	N	N
Aureomycin - 1	+	A	AK	A	AK	+	-	-
Aureomycin - 2	NR	N	N	N	N	N	N	N
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	NR	N	N	N	N	N	N	N
Penicillin - 1	NR	N	N	N	N	N	N	N
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 2	NR	N	N	N	N	N	N	N
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-

16 positive isolates

A - acid reaction
Ak - alkaline reaction
N - reaction not carried further
NR - no reaction

TABLE 28

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH *SALMONELLA PULLORUM* (SERIES 6)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	25	0	1	4.0
Infected control	0	25	0	7	28.0
Penicillin - 1	660 units	24	48	9	37.5
Penicillin - 2	990 units	25	48	8	32.0
Aureomycin - 1	1	27	48	1	3.7
Aureomycin - 2	2	27	48	1	3.7
Antigen mash - 1 (heat attenuated)	0	25	48	6	24.0
Antigen mash - 2 (heat attenuated)	0	25	48	8	32.0

Breed of chicks: New Hampshire
Feed: Kellogg starter

TABLE 29

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 6)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	A	A	A	N	N	N
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Aureomycin - 1	+	A	AK	A	AK	+	-	-
Aureomycin - 2	+	A	AK	A	AK	+	-	-

TABLE 29 CONTINUED

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-

34 positive isolates

A - acid reaction
AK - Alkaline reaction
N - reaction not carried further

TABLE 30

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 7)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	30	0	2	6.7
Infected control	0	23	0	7	30.4
Rutin - 1	1	25	48	3	12.0
Rutin - 2	2	24	48	5	20.8
Antigen mash - 1 (heat attenuated)	0	25	48	6	24.0
Antigen mash - 2 (heat attenuated)	0	24	48	7	29.2
Sodium benzoate control	1	22	48	5	22.7
Penicillin + sodium benzoate	660 units	25	48	2	8.0

Breed of chicks: New Hampshire
Feed: Kellogg starter

TABLE 31

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 7)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Non-infected control	+	AK	AK	AK	AK	N	N	N
Non-infected control	+	AK	AK	AK	AK	N	N	N
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	A	A	AK	N	N	N
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Sodium benzoate control	+	AK	AK	AK	AK	N	N	N
Sodium benzoate control	+	A	AK	A	A K	+	-	-
Sodium benzoate control	+	A	AK	A	AK	+	-	-
Sodium benzoate control	+	A	AK	A	AK	+	-	-
Sodium benzoate control	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	HS	HS	N	N	N	N	+
Antigen mash - 1	NR	N	N	N	N	N	N	N
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-
Antigen mash - 1	+	A	AK	A	AK	+	-	-

TABLE 31 CONTINUED

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Rutin - 1	+	A	AK	A	AK	+	-	-
Rutin - 1	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	AK	AK	AK	AK	N	N	N
Rutin - 2	+	A	AK	A	AK	+	-	-

27 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further
HS - Excess H_2S
NR - no reaction

TABLE 32

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 8)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	32	0	0	0
Infected control	0	25	0	6	24.0
Rutin - 1	1	25	48	1	4.0
Rutin - 2	2	25	48	5	20.0
Antigen mash - 1 (heat attenuated) charcoal adsorbed	0	25	48	0	0
Antigen mash - 2 (heat attenuated)	0	24	48	6	25.0
Sodium benzoate control	1	25	48	2	8.0
Penicillin + sodium benzoate	660 units	24	48	6	25.0

Breed of chicks: Barred Rock x White Rock
Feed: Kellogg starter

TABLE 33

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 8)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Sodium benzoate control	+	A	AK	A	AK	+	-	-
Sodium benzoate control	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	A	N	N	N	N	N
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Antigen mash - 2	+	A	AK	A	AK	+	-	-
Penicillin + sodium benzoate	+	A	A	N	N	N	N	N
Penicillin + sod. benz.	+	A	A	N	N	N	N	N
Penicillin + sod. benz.	+	A	AK	A	AK	+	-	-
Penicillin + sod. benz.	+	A	AK	A	AK	+	-	-
Penicillin + sod. benz.	+	A	AK	A	AK	+	-	-
Penicillin + sod. benz.	+	A	AK	A	AK	+	-	-
Rutin - 1	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-

23 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further

TABLE 34

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 9)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	30	0	0	0
Infected control	0	25	0	5	20.0
Rutin - 1	0.25	24	48	2	8.3
Rutin - 2	0.5	24	48	7	29.2
Antigen mash (heat attenuated)	0	25	48	7	28.0
Penicillin + sodium benzoate	330 units	25	48	0	0
Chloromycetin	1	25	48	0	0
Streptomycin	1	24	48	2	8.3

Breed of chicks: Barred Rock x White Rock
Feed: Kellogg starter

TABLE 35

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 9)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt.	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	HS	HS	N	N	N	N	N
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Antigen mash (heat)	+	A	AK	A	AK	+	-	-
Rutin - 1	+	A	AK	A	AK	+	-	-
Rutin - 1	+	A	AK	A	AK	+	-	-
Rutin - 1	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Rutin - 2	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-

19 positive isolates

A - acid reaction
AK - alkaline reaction
HS - Excess H_2S
N - reaction not carried further

TABLE 36

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 10)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	30	0	0	0
Infected control	0	25	0	0	0
Chloromycetin	1	25	48	0	0
Streptomycin	1	25	48	0	0
Penicillin	660 units	23	48	0	0
Antigen mash (heat attenuated) charcoal adsorbed	0	20	48	4	20.0
Streptomycin + aureomycin	1	19	48	2	10.5

Breed of chicks: Barred Rock x White Rock
Feed: Kellogg starter

TABLE 37

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH *SALMONELLA PULLORUM* (SERIES 11)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	32	0	1	3.1
Infected control	0	25	0	8	32.0
Chloromycetin	1	22	24	3	13.7
Streptomycin	1	25	24	11	44.0
Aureomycin + streptomycin	1	24	24	2	8.3
Aureomycin + chloromycetin	1	25	24	3	12.0
Antigen mash (heat attenuated) charcoal adsorbed	0	25	24	12	48.0
Trigonella	60	25	24	15	60.0

Breed of chicks: Barred Rock x White Rock
Feed: Kellogg starter

TABLE 38

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 11)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Antigen mash	+	A	AK	A	AK	+	-	-
Antigen mash	+	A	AK	A	AK	+	-	-
Aureomycin + streptomycin	+	A	AK	A	AK	+	-	-
Chloromycetin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Trigonella	+	A	AK	A	AK	+	-	-

8 positive isolates

A - acid reaction
AK - alkaline reaction

TABLE 39

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 12)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control (143 g)*	0	43	0	0	0
Infected control (114 g)	0	25	0	6	24.0
Penicillin - 1 (143 g)	2936 units	23	0	1	4.3
Penicillin - 2 (137 g)	2936 units	23	0	2	8.7
Streptomycin (143 g)	2.75	25	24	8	32.0
Hydrastis (123 g)	10	14	24	2	14.3
Hypericum (130 g)	10	25	24	7	28.0
Chloromycetin (150 g)	1.5	25	24	0	0

*Average weight per chick
Breed of chicks: White Leghorn
Feed: Kellogg starter

TABLE 40

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 12)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	A K	+	-	-
Infected control	+	A	AK	A	A K	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	A K	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Hydrastis	+	A	AK	A	AK	+	-	-
Hydrastis	+	NR	NR	N	N	N	N	N
Penicillin	+	A	AK	A	AK	+	-	-
Hypericum	+	A	AK	A	AK	+	-	-
Hypericum	+	A	AK	A	A K	+	-	-
Hypericum	+	A	AK	A	A K	+	-	-
Hypericum	+	A	AK	A	AK	+	-	-
Hypericum	+	A	AK	A	AK	+	-	-
Penicillin + sod. benz.	+	A	AK	A	AK	+	-	-
Penicillin + sod. benz.	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-
Streptomycin	+	A	AK	A	AK	+	-	-

20 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further
NR - no reaction

TABLE 41

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH SALMONELLA PULLORUM (SERIES 13)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	35	0	1	2.8
Infected control	0	25	0	10	40.0
Penicillin - 1	2290 units	25	24	7	28.0
Penicillin - 2	2290 units	25	24	6	24.0
Penicillin - 3	3500 units	25	24	5	20.0
Penicillin - 4	2000 units	24	24	7	28.0
Chloromycetin	1.5	25	24	3	12.0
Chloromycetin + penicillin	1.5 + 1145 units	25	24	3	12.0

Breed of chicks: White Leghorn
Feed: Kellogg starter

TABLE 42

THE RECOVERY OF *SALMONELLA PULLORUM* FROM DEAD CHICKS (SERIES 13)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	AK	AK	N	N	N	N	N
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Chloromycetin	+	A	AK	A	AK	+	-	-
Chloromycetin	+	A	AK	A	AK	+	-	-
Chloromycetin	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-

TABLE 42 CONTINUED

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Penicillin - 3	+	A	AK	A	AK	+	-	-
Penicillin - 3	+	A	AK	A	AK	+	-	-
Penicillin - 3	+	NR	NR	N	N	N	N	N
Penicillin - 3	+	A	AK	A	AK	+	-	-
Penicillin - 4	+	A	AK	A	AK	+	-	-
Penicillin - 4	+	A	AK	A	AK	+	-	-
Penicillin - 4	+	A	AK	A	AK	+	-	-
Penicillin - 4	+	A	AK	A	AK	+	-	-
Penicillin - 4	+	A	AK	A	AK	+	-	-
Penicillin - 4	+	A	AK	A	AK	+	-	-
Penicillin - 4	+	A	AK	A	AK	+	-	-
Penicillin + chloromycetin	+	A	AK	A	AK	+	-	-
Penicillin + chloromycetin	+	A	AK	A	AK	+	-	-
Penicillin + chloromycetin	+	A	AK	A	AK	+	-	-

35 positive isolates

A - acid reaction
AK - alkaline reaction
NR - no reaction
N - reaction not carried further

TABLE 43

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH *SALMONELLA PULLORUM* (SERIES 14)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control	0	30	0	1	3.3
Infected control	0	25	0	1	4.0
Penicillin - 1	4500 units	24	24	1	4.2
Penicillin - 2	4500 units	25	24	1	4.0
Penicillin + cobalt (in feed)	2290 units	25	24	3	12.0
Chloromycetin - 1	2.2	25	24	0	0
Chloromycetin - 2	2.2	25	24	2	8.0
Cobalt control (2.5 mg/15 lb feed)	0	25	24	7	28.0

Breed of chicks: White Leghorn
Feed: Kellogg starter

TABLE 44

THE RECOVERY OF SALMONELLA PULLORUM FROM DEAD CHICKS (SERIES 14)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Non-infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Cobalt control	+	A	AK	A	AK	+	-	-
Cobalt control	+	A	AK	A	AK	+	-	-
Cobalt control	+	A	AK	A	AK	+	-	-
Cobalt control	+	A	AK	A	AK	+	-	-
Cobalt control	+	A	AK	A	AK	+	-	-
Chloromycetin - 2	+	A	AK	A	AK	+	-	-
Chloromycetin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 1	+	A	AK	A	AK	+	-	-
Penicillin + cobalt	+	AK	AK	N	N	N	N	N
Penicillin + cobalt	+	A	AK	A	AK	+	-	-
Penicillin + cobalt	+	A	AK	A	AK	+	-	-
Penicillin + cobalt	+	A	AK	A	AK	+	-	-

13 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further

TABLE 45

EVALUATION OF THE EFFECT OF FEEDING THERAPEUTIC AGENTS TO CHICKS INFECTED WITH *SALMONELLA PULLORUM* (SERIES 15)

Experimental group	Concentration of antibiotic agent mg/g feed	Number of birds	Hours of prophylactic feeding	Number of dead birds	Percent mortality in 21 days
Non-infected control (147 g)*	0	35	0	1	2.8
Infected control (124 g)	0	25	0	8	32.0
Penicillin - 1 (125 g)	5500 units	25	24	2	8.0
Penicillin - 2 (126 g)	5500 units	25	24	4	16.0
Penicillin + cobalt (0.2 mg/ml) - 1 (139 g)	2290 units	25	24	1	4.0
Penicillin + cobalt (0.2 mg/ml) - 2 (135 g)	2290 units	25	24	3	12.0
Penicillin + cobalt (0.2 mg/ml) - 3 (137 g)	2290 units	25	24	0	0
Chloromycetin (143 g)	1	25	24	1	4.0

*Average weight per chick
Breed of chicks: White Leghorn
Feed: Kellogg starter

TABLE 46

THE RECOVERY OF SALMONELLA PULLORUM FROM DEAD CHICKS (SERIES 15)

Experimental group	Growth on bismuth sulfite agar	Kliglers		Individual sugars		Sims		Gas
		Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	NR	NR	N	N	N	N	N
Infected control	+	A	AK	A	A K	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Infected control	+	A	AK	A	AK	+	-	-
Chloromycetin	+	NR	NR	N	N	N	N	N
Penicillin - 1	+	NR	NR	N	N	N	N	N
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin - 2	+	NR	NR	N	N	N	N	N
Penicillin - 2	+	A	AK	A	AK	+	-	-
Penicillin + cobalt - 1	+	A	AK	A	AK	+	-	-
Penicillin + cobalt - 2	+	A	AK	A	AK	+	-	-
Penicillin + cobalt - 2	+	A	AK	A	A K	+	-	-
Penicillin + cobalt - 2	+	A	AK	A	AK	+	-	-

15 positive isolates

A - acid reaction
AK - alkaline reaction
NR - no reaction
N - reaction not carried further

TABLE 47

THE ISOLATION OF *SALMONELLA PULLORUM* FROM SUSPECTED CARRIER BIRDS

Non-treated Infected Controls

Bird number	Tetrathionate enrichment of pooled organs	Growth on bismuth sulfite agar	Transfers to differential media
301	+	-	-
302	+	-	-
303	+	-	-
304	+	-	-
305	+	-	-
306	+	-	-
307	+	-	-
308	+	-	-
309	+	+	+
310	+	-	-
311	+	-	-
312	+	+	+
313	+	+	+
314	+	-	-
315	+	-	-
316	+	+	+
317	+	-	-
318	+	-	-
319	+	-	-
320	+	-	-
321	+	-	-
322	+	-	-
323	+	-	-
324	+	-	-
325	+	-	-
326	+	-	-
327	+	+	+
328	+	-	-
329	+	+	+
330	+	-	-
331	+	-	-
332	+	-	-
333	+	-	-
334	+	+	+
335	+	-	-

TABLE 47 CONTINUED

Bird number	Tetrathionate enrichment of pooled organs	Growth on bismuth sulfite agar	Transfers to differential media
336	+	-	-
337	+	-	-
338	+	+	+
339	+	+	+
340	+	-	-
341	+	-	-
342	+	-	-
343	+	-	-
344	+	-	-
345	+	-	-
346	+	-	-
347	+	+	+
348	+	-	-
349	+	-	-
350	+	-	-
351	+	-	-

Non-treated Infected Controls
Further Characterization (Biochemical) of Suspected Carriers

Bird number	Kliglers		Individual sugars		Sims		Gas
	Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
309	A	AK	A	AK	+	-	-
312	A	A	N	N	N	N	N
313	A	AK	A	AK	+	-	-
316	A	AK	A	AK	+	-	-
327	A	AK	A	AK	+	-	-
329	A	AK	A	AK	+	-	-
334	Excess H_2S	Excess H_2S	N	N	N	N	N
338	A	AK	A	AK	+	-	-
339	A	AK	A	AK	+	-	-
347	A	AK	A	AK	+	-	-

8 positive isolations
A - acid reaction
AK - alkaline reaction
N - reaction not carried further
NR - no reaction

TABLE 47 CONTINUED

Aureomycin (1 mg/g feed)

Bird number	Tetrathionate enrichment of pooled organs	Growth on bismuth sulfite agar	Transfers to differential media
3503	+	-	-
3504	+	-	-
3505	+	-	-
3509	+	-	-
3510	+	-	-
3511	+	-	-
3512	+	-	-
3513	+	-	-
3515	+	-	-
3516	+	-	-
3517	+	-	-
3521	+	-	-
3537	+	-	-

TABLE 47 CONTINUED

Chloromycetin - Group I (1.5 mg/g feed)

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
3802	+	+	A	AK	A	AK	+	-	-
3803	+	+	HS	HS	N	N	N	N	N
3805	+	+	A	AK	A	AK	+	-	-
3808	+	+	HS	HS	N	N	N	N	N
3810	+	+	A	AK	AK	AK	N	N	N
3811	+	+	HS	HS	N	N	N	N	N
3814	+	+	HS	HS	N	N	N	N	N
3815	+	+	NR	NR	N	N	N	N	N
3817	+	+	A	AK	AK	AK	N	N	N
3819	+	+	A	A	N	N	N	N	N
3822	+	+	HS	HS	N	N	N	N	N
3823	+	+	NR	NR	N	N	N	N	N
3827	+	+	A	A	N	N	N	N	N
3828	+	+	A	AK	A	AK	+	-	-
3831	+	+	HS	HS	N	N	N	N	N
3833	+	+	HS	HS	N	N	N	N	N
3834	+	+	A	A	N	N	N	N	N
3836	+	+	NR	NR	N	N	N	N	N

3 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further
HS - Excess H_2S
NR - no reaction

TABLE 47 CONTINUED

Chloromycetin - Group II (2.2 mg/g feed)

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
3020	+	+	A	A	N	N	N	N	N
3023	+	+	HS	HS	N	N	N	N	N
3024	+	+	A	A	N	N	N	N	N
3051	+	+	HS	HS	N	N	N	N	N
3053	+	+	NR	NR	N	N	N	N	N
3054	+	+	HS	HS	N	N	N	N	N
3057	+	+	A	AK	A	AK	+	-	-
3058	+	+	A	A	N	N	N	N	N
3059	+	+	A	AK	A	AK	+	-	+
3066	+	+	A	AK	A	AK	+	-	-
3068	+	+	NR	NR	N	N	N	N	N
3070	+	+	A	AK	AK	AK	N	N	N
3080	+	+	A	AK	A	AK	+	-	-
3084	+	+	NR	NR	N	N	N	N	N
3098	+	+	A	AK	A	AK	+	-	-
4827	+	+	HS	HS	N	N	N	N	N
4829	+	+	A	A	N	N	N	N	N
4830	+	+	HS	HS	N	N	N	N	N
4864	+	+	A	AK	A	AK	+	-	-
4865	+	+	A	AK	A	AK	+	-	-
4867	+	+	A	AK	A	AK	+	-	+
4870	+	+	NR	NR	N	N	N	N	N
4871	+	+	A	AK	A	AK	+	-	-
4873	+	+	A	A	A	A	N	N	N
4875	+	+	HS	HS	N	N	N	N	N

7 positive isolates

A - acid reaction HS - Excess H_2S
AK - alkaline reaction NR - no reaction
N - reaction not carried further

TABLE 47 CONTINUED

Chloromycetin - Group III (1.5 mg/g feed)

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
3305	+	+	NR	NR	N	N	N	N	N
3306	+	+	HS	HS	N	N	N	N	N
3314	+	+	A	AK	AK	AK	N	N	N
3315	+	+	NR	NR	N	N	N	N	N
3324	+	+	NR	NR	N	N	N	N	N
3332	+	+	NR	NR	N	N	N	N	N
3333	+	+	NR	NR	N	N	N	N	N
3334	+	+	HS	HS	N	N	N	N	N
3337	+	+	NR	NR	N	N	N	N	N
3362	+	+	A	AK	AK	AK	N	N	N
3369	+	+	A	AK	AK	AK	N	N	N
3373	+	+	HS	HS	N	N	N	N	N
3391	+	+	NR	NR	N	N	N	N	N
3853	+	+	NR	NR	N	N	N	N	N
3863	+	+	HS	HS	N	N	N	N	N
3881	+	+	HS	HS	N	N	N	N	N
3887	+	+	A	AK	AK	AK	N	N	N
3896	+	+	NR	NR	N	N	N	N	N
4826	+	+	HS	HS	N	N	N	N	N
4837	+	+	NR	NR	N	N	N	N	N
4841	+	+	NR	NR	N	N	N	N	N
4845	+	+	NR	NR	N	N	N	N	N
4848	+	+	NR	NR	N	N	N	N	N

No positive isolations

A - acid reaction HS - Excess H_2S

AK - alkaline reaction NR - no reaction

N - reaction not carried further

TABLE 47 CONTINUED

Penicillin (4500 u/g feed)

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
818	+	+	A	A	A	A	N	N	N
819	+	+	A	AK	A	AK	+	-	-
820	+	+	A	AK	A	AK	+	-	-
821	+	+	A	AK	A	AK	+	-	-
822	+	+	A	AK	A	AK	+	-	-
823	+	+	A	AK	A	AK	+	-	-
824	+	+	A	AK	A	AK	+	-	-
825	+	+	A	AK	A	AK	+	-	-
826	+	+	A	AK	A	AK	+	-	-
827	+	+	A	AK	A	AK	+	-	-
828	+	+	A	AK	A	AK	+	-	-
829	+	+	A	AK	A	AK	+	-	-
830	+	+	A	AK	A	AK	+	-	-
831	+	+	A	AK	A	AK	+	-	-
832	+	+	A	AK	A	AK	+	-	-
833	+	+	A	AK	A	AK	+	-	-
834	+	+	NR	NR	N	N	N	N	N
835	+	+	NR	NR	N	N	N	N	N
836	+	+	NR	NR	N	N	N	N	N
837	+	+	NR	NR	N	N	N	N	N
838	+	+	NR	NR	N	N	N	N	N
887	+	+	HS	HS	N	N	N	N	N
888	+	+	HS	HS	N	N	N	N	N
890	+	+	HS	HS	N	N	N	N	N
891	+	+	HS	HS	N	N	N	N	N
892	+	+	A	A	N	N	N	N	N

TABLE 47 CONTINUED

Penicillin (4500 u/g feed) Continued

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
866	+	+	NR	NR	N	N	N	N	N
867	+	+	A	A	N	N	N	N	N
868	+	+	NR	NR	N	N	N	N	N
869	+	+	A	A	N	N	N	N	N
870	+	+	NR	NR	N	N	N	N	N
871	+	+	NR	NR	N	N	N	N	N
872	+	+	NR	NR	N	N	N	N	N
873	+	+	NR	NR	N	N	N	N	N
874	+	+	NR	NR	N	N	N	N	N
875	+	+	HS	HS	N	N	N	N	N
876	+	+	A	A	N	N	N	N	N
877	+	+	HS	HS	N	N	N	N	N
878	+	+	NR	NR	N	N	N	N	N
879	+	+	HS	HS	N	N	N	N	N
880	+	+	NR	NR	N	N	N	N	N
881	+	+	NR	NR	N	N	N	N	N
882	+	+	HS	HS	N	N	N	N	N
883	+	+	HS	HS	N	N	N	N	N
884	+	+	HS	HS	N	N	N	N	N
885	+	+	HS	HS	N	N	N	N	N
886	+	+	NR	NR	N	N	N	N	N

15 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further
HS - Excess H_2S
NR - no reaction

TABLE 47 CONTINUED

Penicillin (5500 u/g feed)

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
3326	-	+	N	N	N	N	N	N	N
3328	-	+	N	N	N	N	N	N	N
3330	-	+	N	N	N	N	N	N	N
3338	+	+	A	AK	AK	AK	N	N	N
3339	-	+	N	N	N	N	N	N	N
3344	+	+	HS	HS	N	N	N	N	N
3355	+	+	A	AK	AK	AK	N	N	N
3358	-	+	N	N	N	N	N	N	N
3359	-	+	N	N	N	N	N	N	N
3377	+	+	HS	HS	N	N	N	N	N
3378	+	+	A	A	AK	AK	N	N	N
3379	+	+	A	AK	A	AK	+	-	-
3383	+	+	A	AK	AK	AK	N	N	N
3386	-	+	N	N	N	N	N	N	N
3387	-	+	N	N	N	N	N	N	N
3388	-	+	N	N	N	N	N	N	N
3389	+	+	A	AK	AK	AK	N	N	N
3393	-	+	N	N	N	N	N	N	N
3399	+	+	HS	HS	N	N	N	N	N
3400	+	+	A	AK	A	AK	+	-	-
3530	+	+	A	AK	A	AK	+	-	-
3574	-	+	N	N	N	N	N	N	N
3582	+	+	A	AK	A	AK	+	-	-
3598	+	+	HS	HS	N	N	N	N	N
3599	+	+	A	AK	AK	AK	N	N	N

TABLE 47 CONTINUED

Penicillin (5500 u/g feed) Continued

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	Gas
3600	-	+	N	N	N	N	N	N	N
3847	-	+	N	N	N	N	N	N	N
3856	-	+	N	N	N	N	N	N	N
3861	+	+	HS	HS	N	N	N	N	N
3864	+	+	HS	HS	N	N	N	N	N
3872	-	+	N	N	N	N	N	N	N
4833	-	+	N	N	N	N	N	N	N
4835	-	+	N	N	N	N	N	N	N
4836	-	+	N	N	N	N	N	N	N
4838	+	+	HS	HS	N	N	N	N	N
4840	-	+	N	N	N	N	N	N	N
4844	+	+	A	A	A	A	N	N	N
4849	-	+	N	N	N	N	N	N	N

4 positive isolates

A - acid reaction
AK - alkaline reaction
N - reaction not carried further
HS - Excess H_2S
NR - no reaction

TABLE 47 CONTINUED

Penicillin (2290 u/g feed) - Cobalt (0.2 mg/ml water)

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
3302	+	+	NR	NR	N	N	N	N	N
3303	-	+	N	N	N	N	N	N	N
3307	+	+	HS	HS	N	N	N	N	N
3308	-	+	N	N	N	N	N	N	N
3309	-	+	N	N	N	N	N	N	N
3312	+	+	HS	HS	N	N	N	N	N
3313	+	+	HS	HS	N	N	N	N	N
3317	-	+	N	N	N	N	N	N	N
3320	-	+	N	N	N	N	N	N	N
3322	-	+	N	N	N	N	N	N	N
3323	-	+	N	N	N	N	N	N	N
3325	+	+	HS	HS	N	N	N	N	N
3329	+	+	HS	HS	N	N	N	N	N
3331	+	+	A	A	A	A	N	N	N
3336	+	+	HS	HS	N	N	N	N	N
3341	-	+	N	N	N	N	N	N	N
3342	+	+	HS	HS	N	N	N	N	N
3351	+	+	A	A	A	AK	N	N	N
3352	-	+	N	N	N	N	N	N	N
3353	-	+	N	N	N	N	N	N	N
3365	+	+	HS	HS	N	N	N	N	N
3372	+	+	HS	HS	N	N	N	N	N
3379	+	+	A	AK	A	AK	+	-	-
3380	-	+	N	N	N	N	N	N	N
3381	+	+	HS	HS	N	N	N	N	N

TABLE 47 CONTINUED

Penicillin (2290 u/g feed) - Cobalt (0.2 mg/ml water) Continued

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
3382	-	+	N	N	N	N	N	N	N
3384	-	+	N	N	N	N	N	N	N
3385	-	+	N	N	N	N	N	N	N
3392	-	+	N	N	N	N	N	N	N
3394	+	+	HS	HS	N	N	N	N	N
3395	-	+	N	N	N	N	N	N	N
3400	+	+	A	AK	A	AK	+	-	-
3532	+	+	HS	HS	N	N	N	N	N
3530	+	+	A	AK	A	AK	+	-	-
3582	+	+	A	AK	A	AK	+	-	-
3849	+	+	A	AK	AK	AK	N	N	N
3852	-	+	N	N	N	N	N	N	N
3855	+	+	HS	HS	N	N	N	N	N
3857	-	+	N	N	N	N	N	N	N
3858	-	+	N	N	N	N	N	N	N
3859	+	+	A	AK	AK	AK	N	N	N
3862	+	+	HS	HS	N	N	N	N	N
3865	+	+	HS	HS	N	N	N	N	N
3866	+	+	A	AK	AK	AK	N	N	N
3868	-	+	N	N	N	N	N	N	N
3869	-	+	N	N	N	N	N	N	N
3873	+	+	A	AK	AK	AK	N	N	N
3876	-	+	N	N	N	N	N	N	N
3878	+	+	HS	HS	N	N	N	N	N
3879	+	+	HS	HS	N	N	N	N	N

TABLE 47 CONTINUED

Penicillin (2290 u/g feed) - Cobalt (0.2 mg/ml water) Continued

Bird number	Growth on bismuth sulfite agar	Tetrathionate enrichment of pooled organs	Kliglers		Individual sugars		Sims		Gas
			Butt	Slant	Mannitol	Sucrose	H_2S	Motility	
3882	+	+	HS	HS	N	N	N	N	N
3883	-	+	N	N	N	N	N	N	N
3884	-	+	N	N	N	N	N	N	N
3886	-	+	N	N	N	N	N	N	N
3888	+	+	A	AK	AK	AK	N	N	N
3891	-	+	N	N	N	N	N	N	N
3895	-	+	N	N	N	N	N	N	N
4842	-	+	N	N	N	N	N	N	N
4843	-	+	N	N	N	N	N	N	N
4846	-	+	N	N	N	N	N	N	N
4847	-	+	N	N	N	N	N	N	N

4 positive isolates

A - acid reaction HS - Excess H_2S

AK - alkaline reaction NR - no reaction

N - reaction not carried further

FIG. 2 AUREOMYCIN-REFERENCE CURVE FOR SALMONELLA PULLORUM (1 month)

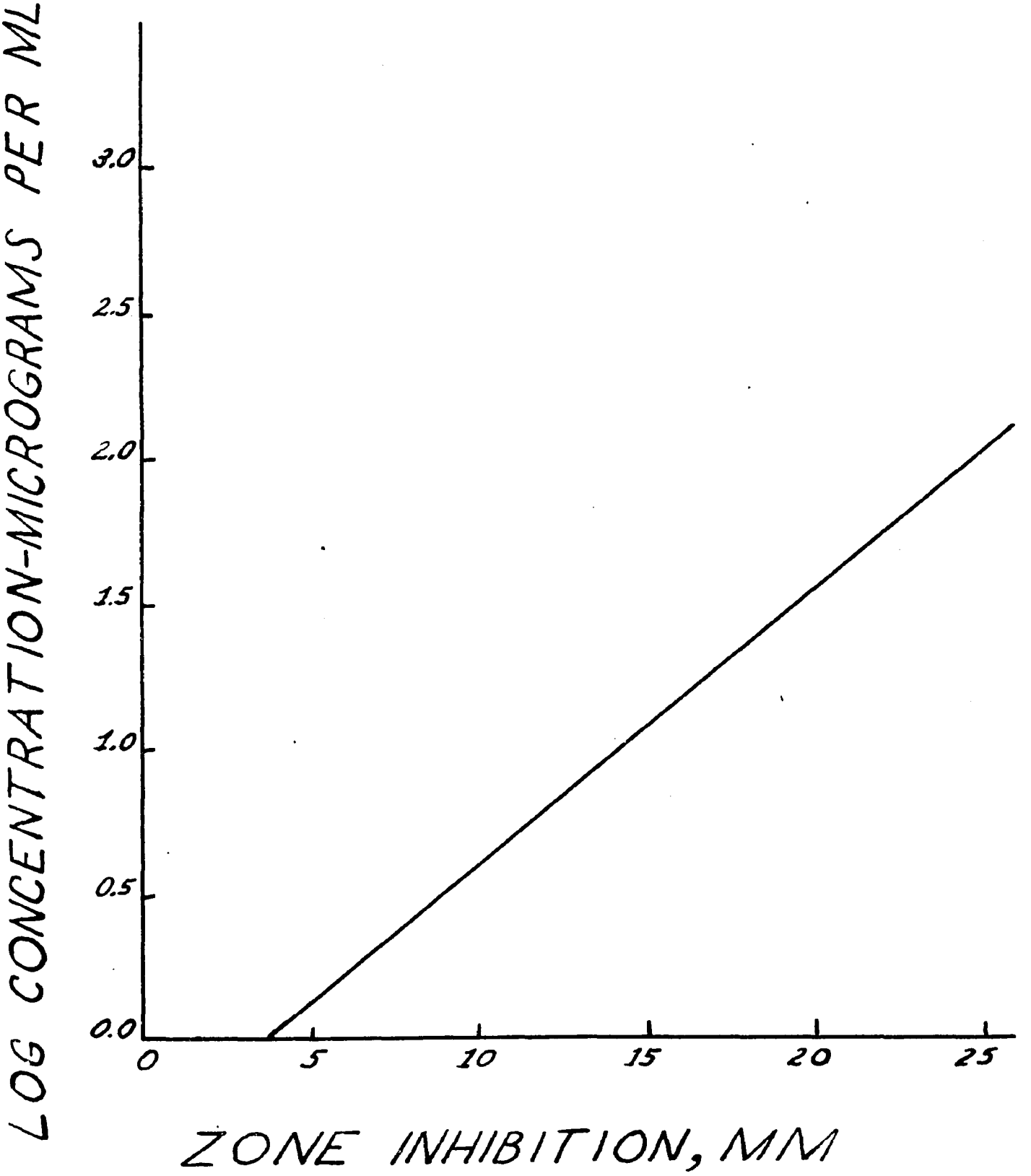


FIG. 3 AUREOMYCIN-REFERENCE CURVE FOR SALMONELLA PULLORUM (1 YEAR)

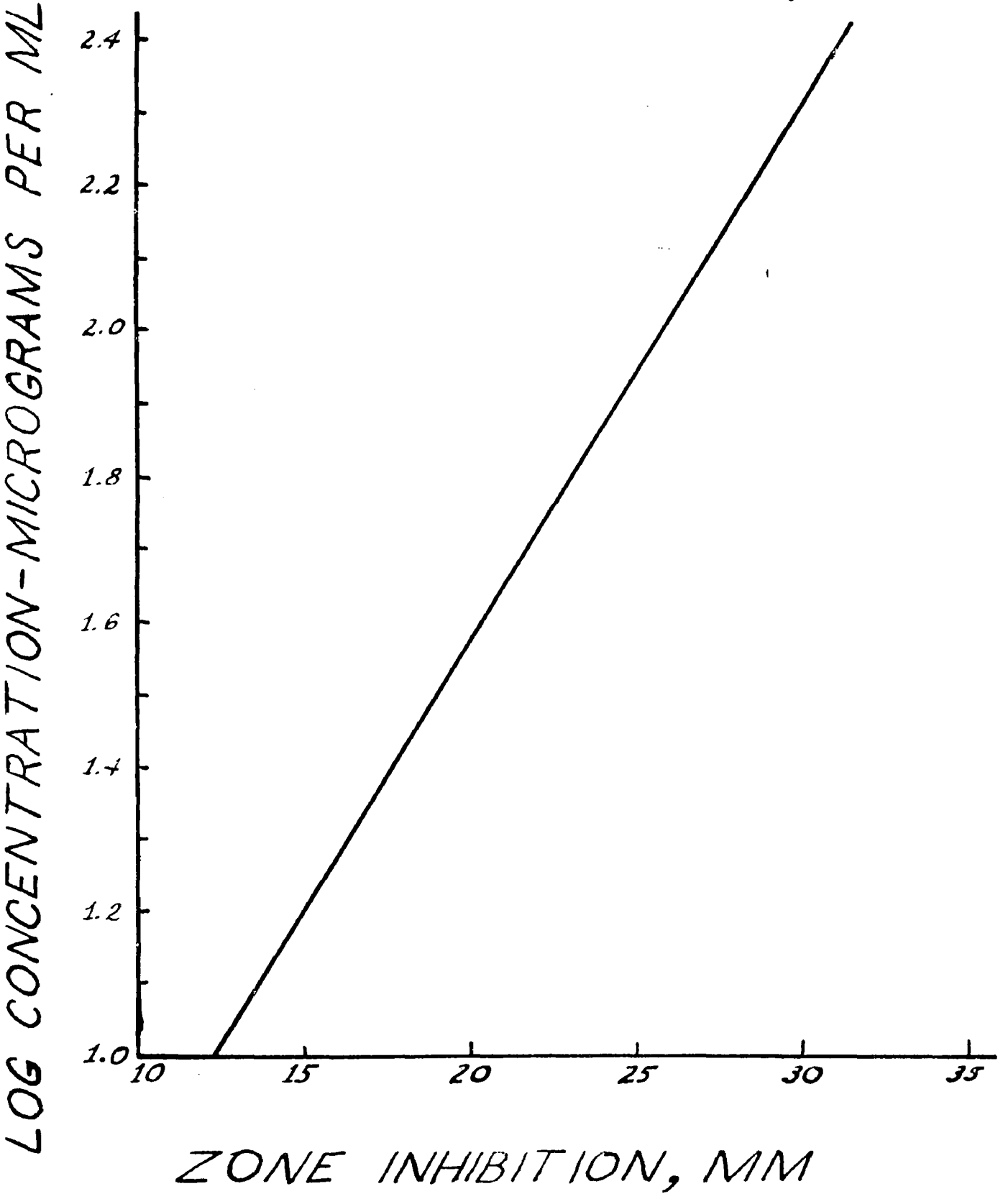


FIG. 4 CHLOROMYCETIN-REFERENCE CURVE FOR SALMONELLA PULLORUM (1 month)

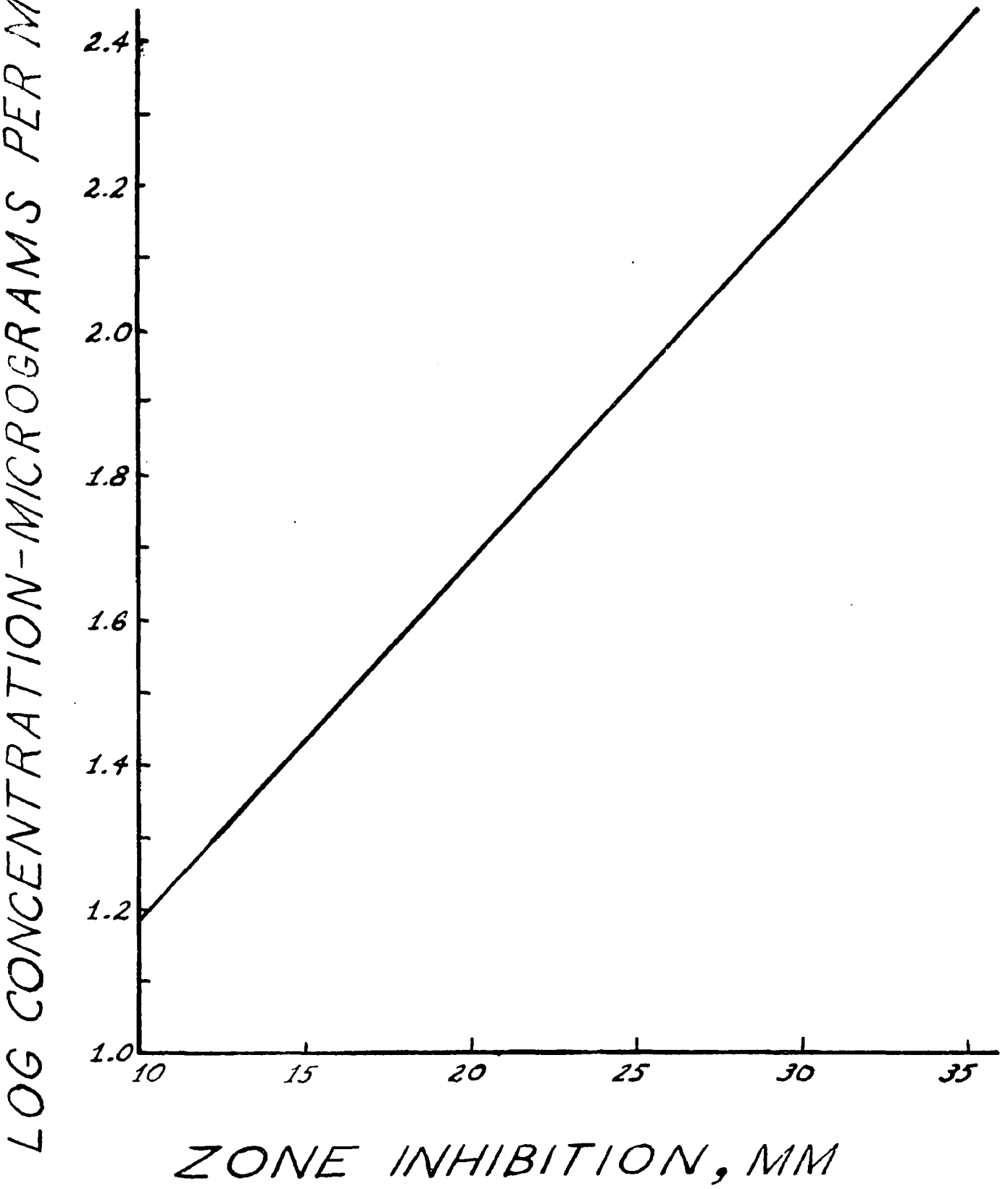


FIG. 5 CHLOROMYCETIN-REFERENCE CURVE FOR SALMONELLA PULLORUM (1 YEAR)

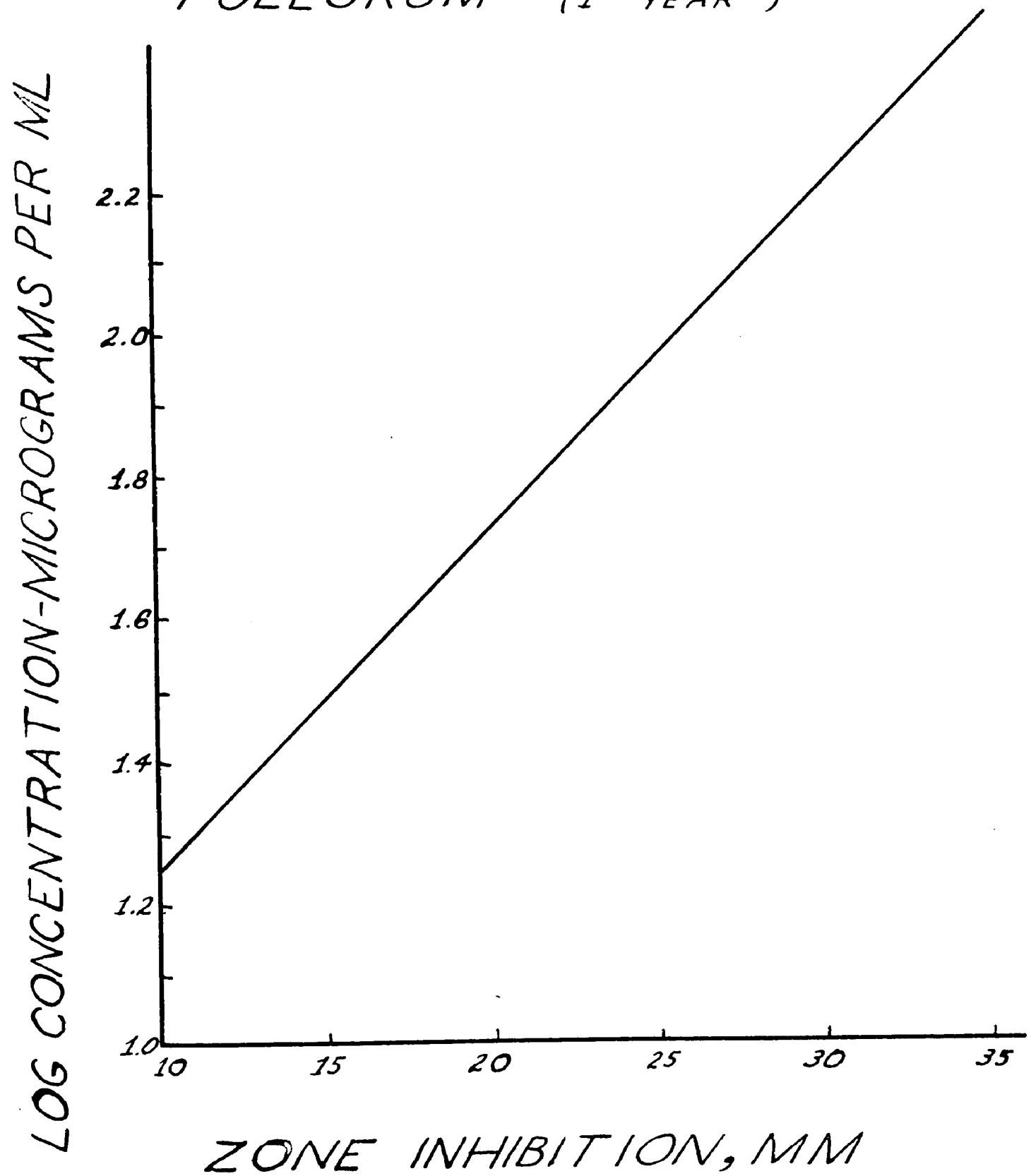


FIG. 6 STREPTOMYCIN-REFERENCE CURVE FOR SALMONELLA PULLORUM (1 month)

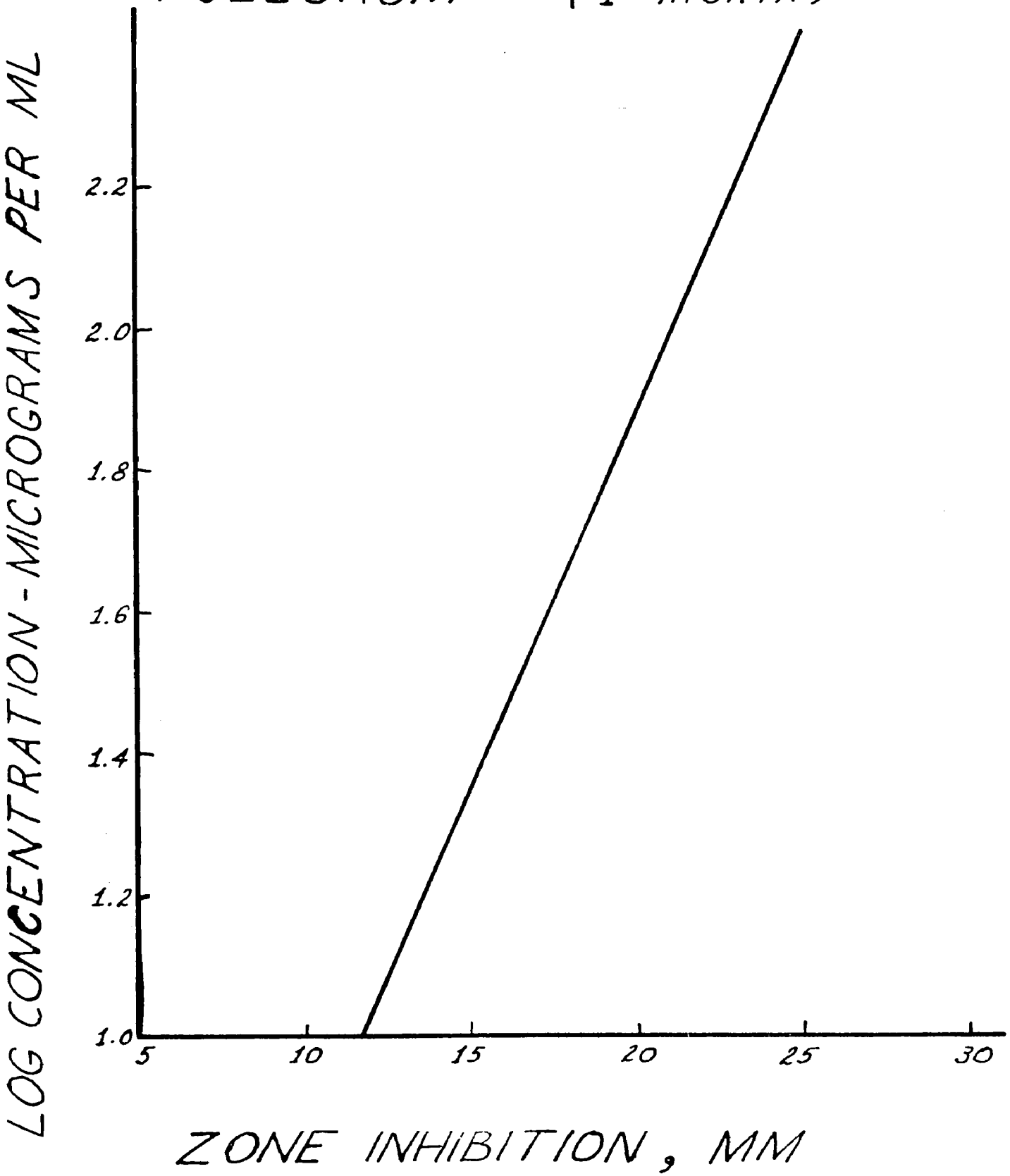


FIG. 7 STREPTOMYCIN-REFERENCE CURVE FOR SALMONELLA PULLORUM (1 YEAR)

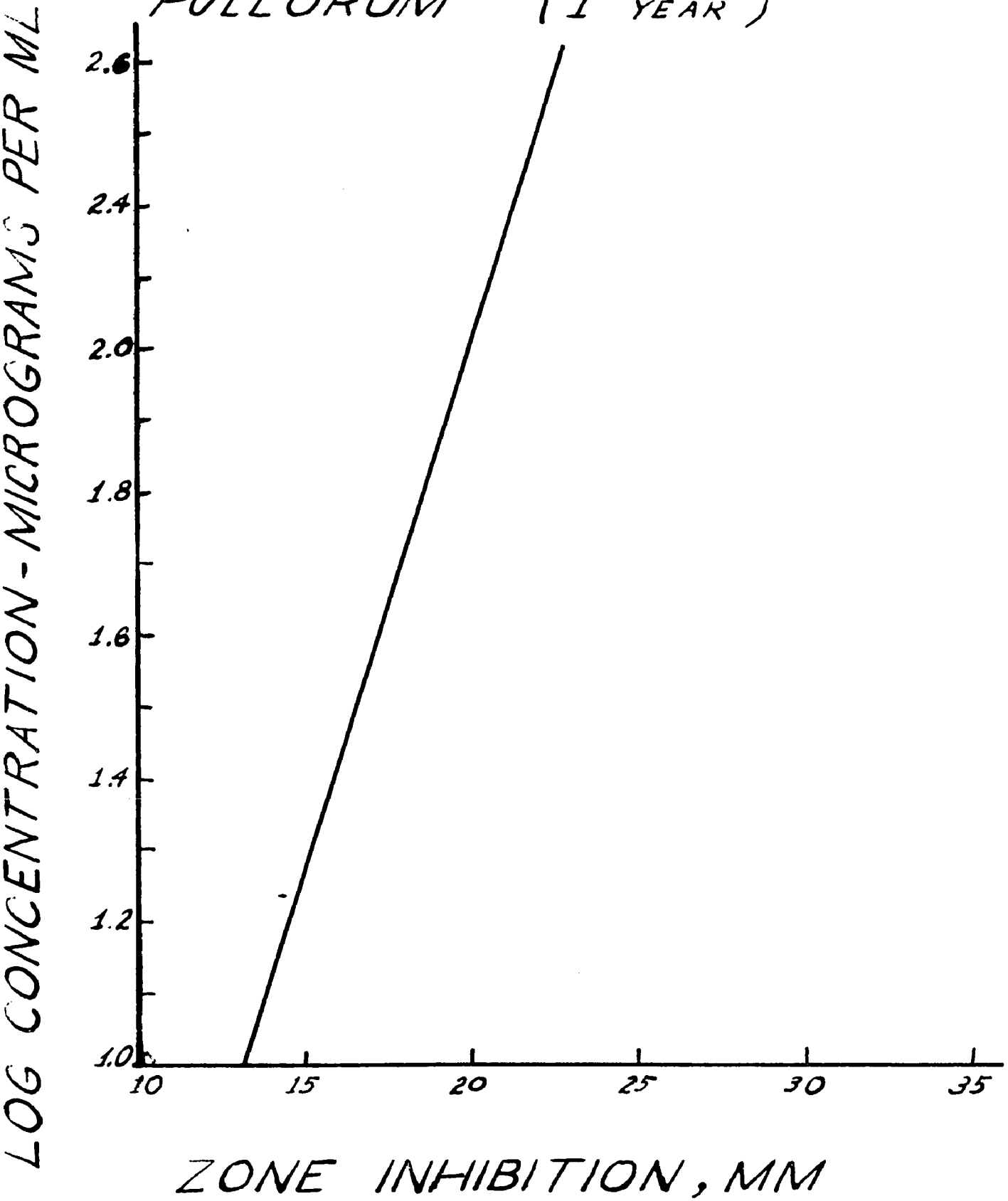


FIG. 8 PENICILLIN-REFERENCE CURVE FOR MICROCOCCUS PYOGENES VAR. AUREUS (1 month)

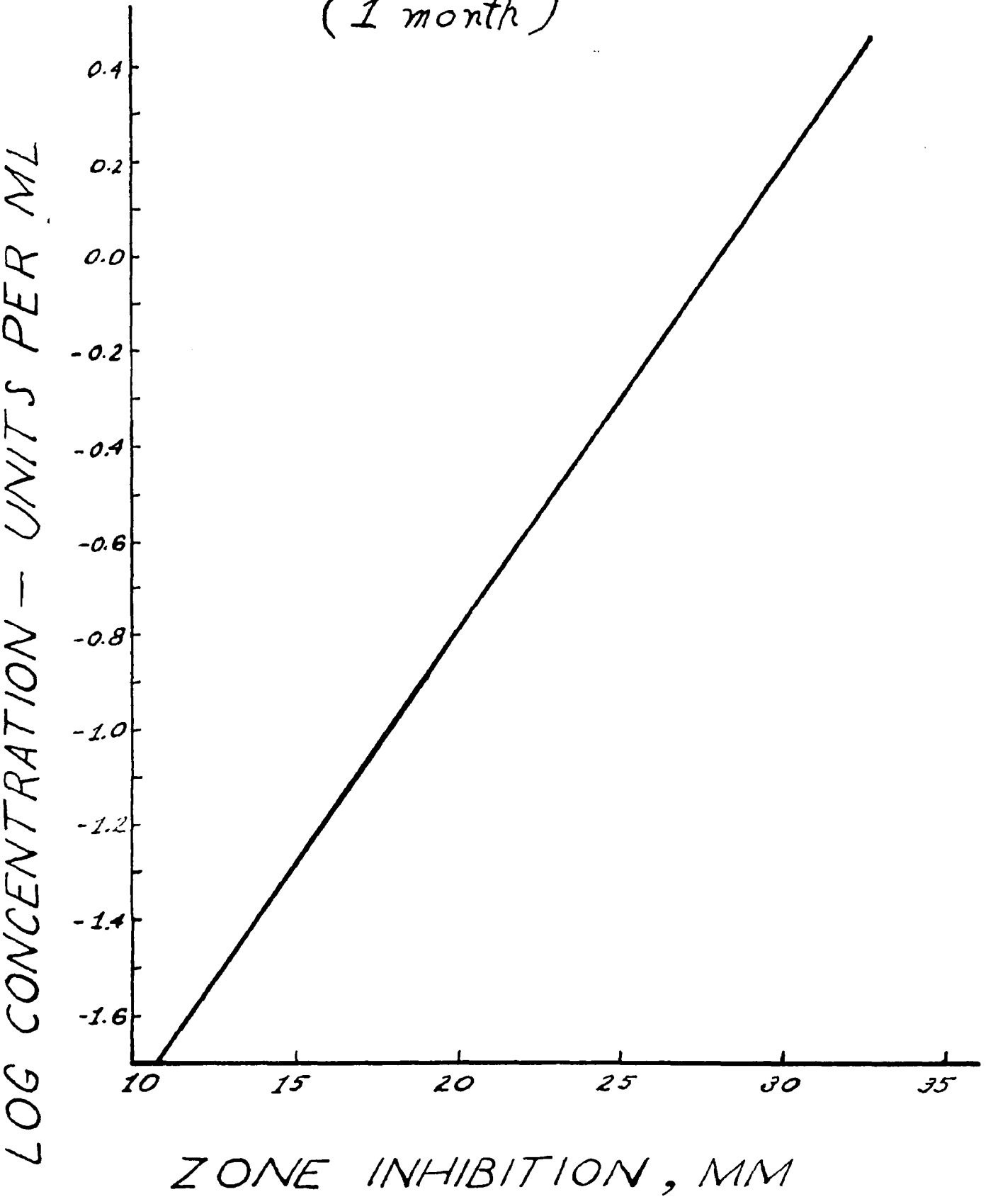


FIG. 9 PENICILLIN-REFERENCE CURVE FOR MICROCOCCUS PYOGENES VAR. AUREUS (6 months)

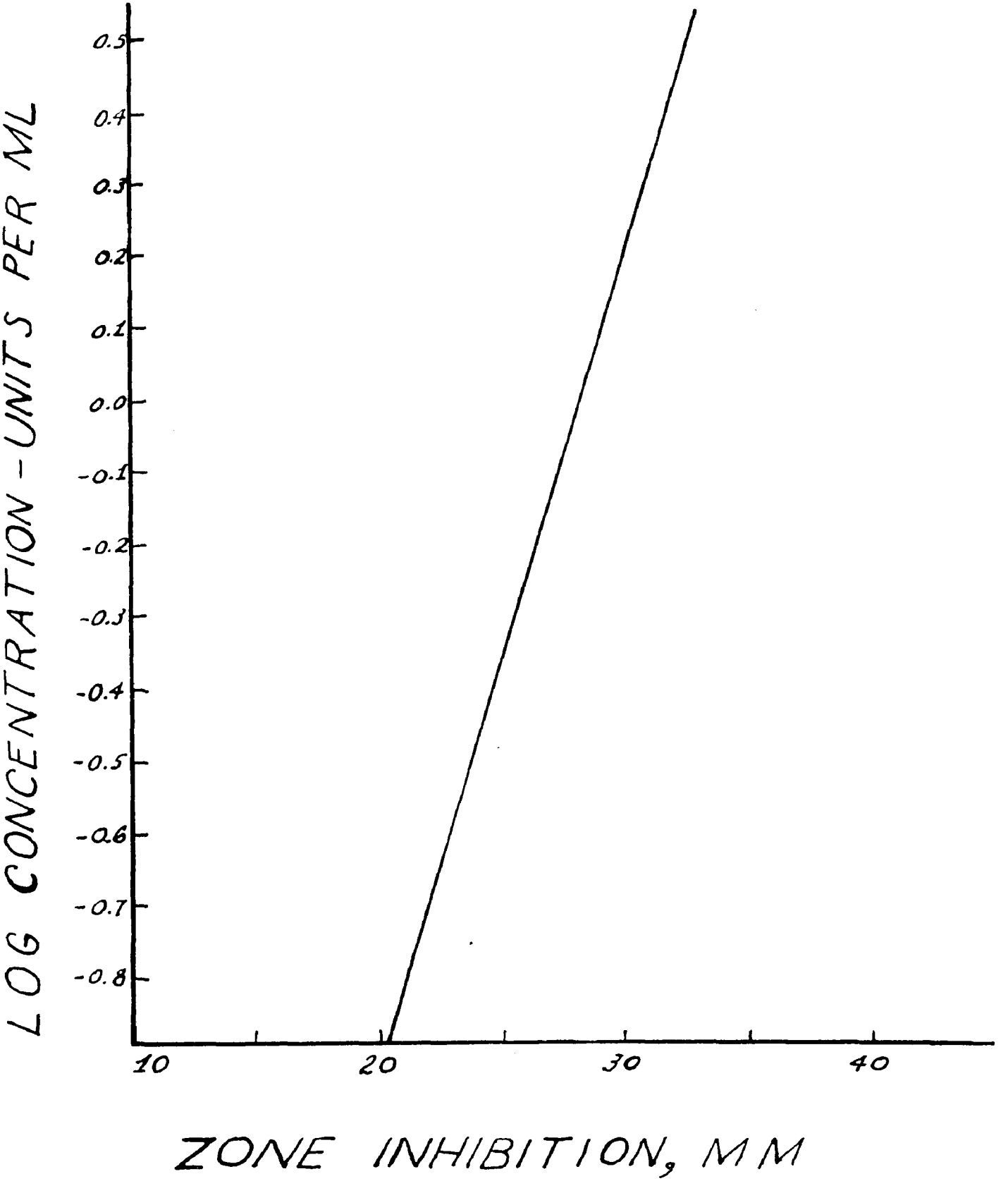


FIG. 10 GARLIC-REFERENCE CURVE FOR SALMONELLA PULLORUM (1 and 12 months)

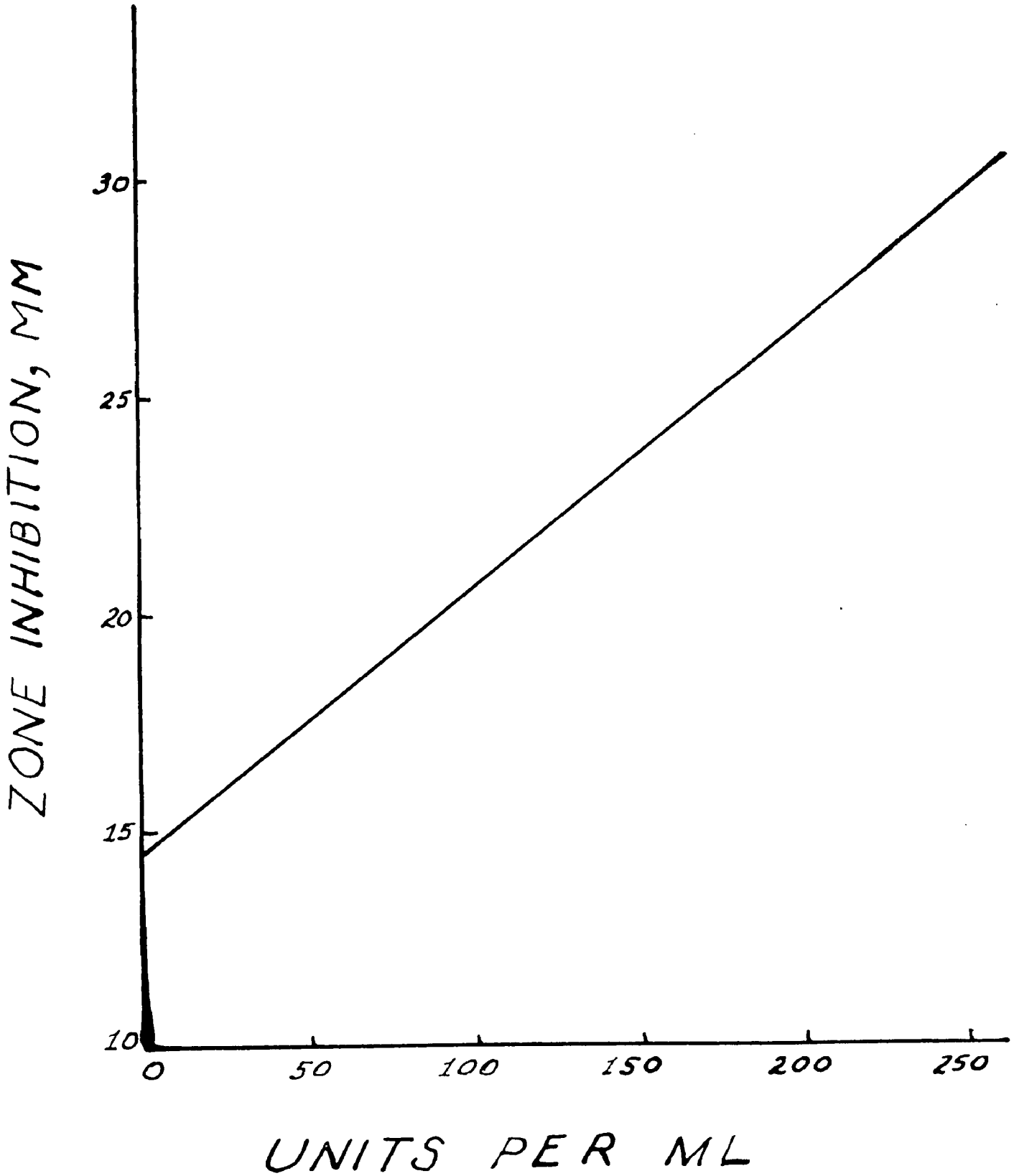
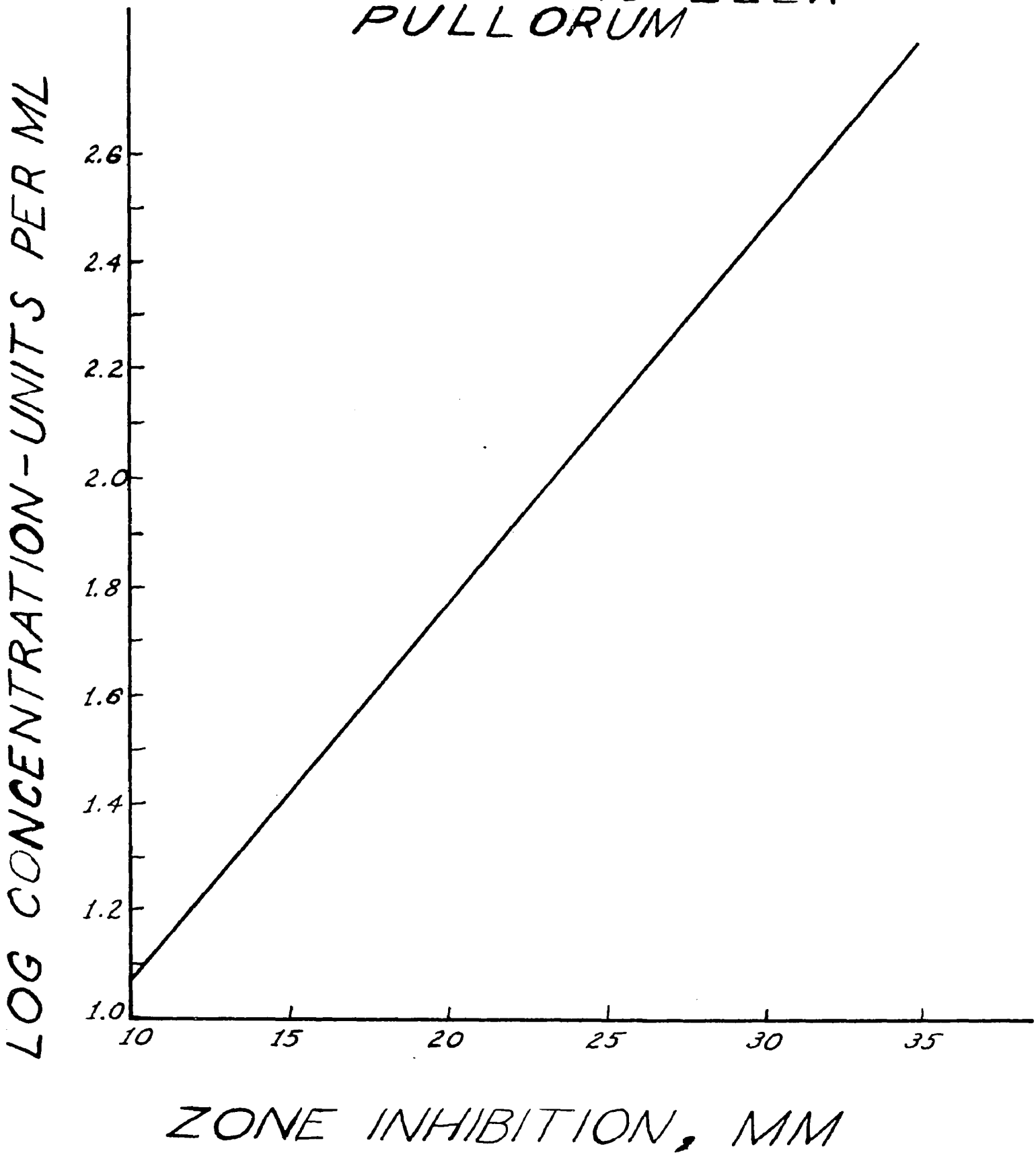


FIG. 11 PENICILLIN-COBALT REFERENCE CURVE FOR SALMONELLA PULLORUM



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