STUDIES ON THE BACTERIOSTATIC AND BACTERICIDAL ACTION OF STREPTOMYCIN AND SULFADIAZINE ON SALMONELLA PULLORUM

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REVIEW OF LITERATURE

I. STREPTOMYCIN

Streptomycin was first isolated by Waksman and his coworkers (1) from Streptomyces griseus in 1944. Recently, Johnstone and Waksman (2) reported that a new streptomycin-like antibiotic tentatively designated as streptomycin II, has been obtained from a new species of Streptomyces, Streptomyces bikiniensis, from Bikini soil.

Since the discovery of this antibiotic many reports on in vitro and in vivo tests of streptomycin against various organisms have been published. Robinson, Graessle and Smith (3) found that streptomycin was active in vitro against a variety of gram-negative and gram-positive bacteria. The former included Eberthella, Salmonella, Escherichia, Shigella, Klebsiella, Brucella, Pasteurella, and Proteus; the gram-positive organisms were strains of Streptococcus hemolyticus, Staphylococcus aureus and Diplococcus pneumoniae. Buggs, Bronstein, Hirshfeld and Pilling (4) stated that the majority of the strains of Escherichia coli, Proteus vulgaris, Aerobacter aerogenes, staphylococci and streptococci were susceptible in the vitro to the blood concentration of streptomycin that can be maintained in the average patient over a four-hour period when given in 500,000 unit doses. The blood concentration was 5 to 8 units per ml of blood. Keefer et al. (5) reported the results of treatment with streptomycin in one thousand cases. Streptomycin was most effective in the treatment of tularemia, Hemophilus influenzae infections, urinary tract infections due to gram-negative bacilli, bacteremia and meningitis due to gram-negative bacilli. The results in typhoid fever, brucellosis

and Salmonella infections have been disappointing and inconclusive. Hinshaw and Feldman (6) reported that experimental tuberculosis of guinea pigs may be arrested by prolonged treatment with streptomycin. This antibiotic did not exert a rapidly curative effect on clinical tuberculosis, although it did appear to modify the course of the disease in a favorable manner and exert a suppressive effect on previously progressive tuberculosis.

Some experimental results on the antibacterial activity of streptomycin on different species of the Salmonella group have been reported. West, Doll and Edwards (7) studied the in vitro inhibition by streptomycin of 412 Salmonella culture, including 154 distinct serological types. Of the commonly occurring types, Salmonella paratyphi A and Salmonella typhi were the most sensitive, while Salmonella pullorum and Salmonella enteritidis were somewhat less susceptible. The remainder were more resistant. Seligmann and Wassermann (8) found that 266 Salmonella strains representing 60 different types were sensitive to streptomycin in a concentration of 4 to 8 units. Oral or oral and subcutaneous administration of streptomycin resulted in the suppression of the normal fecal flora and the pathogens in experimental mice. After termination of the treatment the fecal flora and salmonellae reappeared. Foley and Rubenstein (9) reported that 57 strains of Salmonella covering 15 species, isolated from patients and carriers, were inhibited by streptomycin in concentrations ranging from 0.004 to 0.064 µg per ml of medium. Slanetz (10) found that streptomycin was effective in controlling S. enteritidis in mice as judged by fecal culture and by absence of contamination of vaccine for which the brains of the mice were used. Santivanez (11) reported

that in the in vitro tests streptomycin had a definite bacteriostatic effect against Brucella abortus and Pasteurella avicida. Its activity against S. pullorum, Salmonella typhimurium and Salmonella bredeney was less pronounced. Coles (12) found that Salmonella was comparatively more resistant to the action of streptomycin than other susceptible organisms. Benson (13) reported that streptomycin reduced mortality from pullorum disease in baby chicks.

The mode of action of streptomycin is not fully understood. The possibility that streptomycin might affect the oxidation-reduction system in the bacterial cell has received considerable attention. Bondi, Dietz and Spaulding (14) found that the antibacterial activity of streptomycin was reduced in the presence of various reducing agents such as cysteine, sodium thiglycollate, stannous chloride, sodium bisulfite, sodium hydrosulfite, sodium formate and sodium thiosulfate. They believed that the antibacterial action of streptomycin may be due to its ability to block some oxidative enzyme system. Denkelwater, Cook and Tishler (15) reported that streptomycin was inactivated by cysteine. The cysteine inactivation can be reversed by iodine. Geiger, Green and Waksman (16) found that the reducing agents such as cysteine and cevitamic acid diminished the effectiveness of streptomycin. They proposed that the agents may block an active group in the molecule of streptomycin. Donovick and Rake (17) believed that the interfering action of sodium thioglycollate may be due to its role in reducing the oxidation-reduction potential of the medium. Fitzgerald and Bernheim (18) found that the oxidation of benzoic acid by nonpathogenic mycobacteria was inhibited by very small amounts of streptomycin. Since there was a parallelism between the ability of the drug to inhibit this oxidation and the growth of the organism,

this inhibition may be one of the important mechanisms in the bacteriostatic action of streptomycin. Recently they (19) stated that the inhibition of benzoic acid oxidation by streptomycin may not be due to the inhibition of the benzoic acid oxidase but to the inhibition of the formation of the enzyme. On the other hand, Van Dolah and Christenson (20) studied the effect of various oxidizing and reducing agents on antibiotic activity of streptomycin and concluded that inactivation of streptomycin may not logically be ascribed to an oxidizing nor reducing mechanism.

In the study of the mode of action of streptomycin, Rhymer and Wallace (21) found that brain tissue possessed great ability to inhibit the action of streptomycin. Certain peptones, especially phytone from plant proteins also had this ability. Hobby and Lenert (22) reported that the presence of 1 to 5% or more horse or human serum enhanced the resistance of Str. hemolyticus, Streptococcus viridans, D. pneumoniae and certain strains of Straph. aureus. The same concentration of serum, however, had no effect on the sensibility of Esch. coli, Eberthella typhosa, Klebsiella pneumoniae or A. aerogenes to streptomycin. Higher concentration of serum amy at times enhance the action of streptomycin on certain of these organisms.

The changes in morphology of organisms due to streptomycin might be associated with the changes in their physiological function. Smith and Waksman (23) reported that the principal effects of streptomycin on the morphology of tubercle bacilli were loss of acid-fastness, increase in granulation, and, in highly bacteriostatic concentrations, shortening of the rods.

The antibacterial activity of streptomycin against a given organism varies with different factors. Some of them might relate to the mode of action of streptomycin. The sensitivity of an organism to streptomycin was influenced by the age of the culture, size of inoculum, composition of medium, length of incubation period, different strains and even different cells, and other factors (23, 24, 25, and 28).

The effect of pH value on the activity of streptomycin has been studied by many workers. Waksman, Bugie and Schatz (26), Wolinsky and Steenken (27), Berkman, Henry and Housewright (28), Abraham and Duthie (29) and Lee, et al. (33) found that the activity of streptomycin diminished with decreasing pH.

Glucose has been found to reduce the activity of streptomycin (26). The interfering action may be due to the reducing property of glucose or to the production of acid from glucose fermented by the test organism. Geiger, Green and Waksman (16) reported that the influence of various sugars on the potency of streptomycin depended on whether acid was produced by the particular organism tested in the sugar used. The possible mechanism of the reducing agents upon the activity of streptomycin has been discussed above. Sykes and Lumb (30), however, reported that using Esch. coli as test organism, bile-salt-lactose agar as medium, the addition of glucose to the medium did not result in any apparent change in potency of streptomycin. When, however, nutrient agar was substituted for bile-salt agar a decline in potency was observed. If glucose was added to the solution of streptomycin instead of to the culture medium, the activity of streptomycin was reduced when assayed against Bacillus subtilis. In the tests with Esch. coli, no reduction was observed

either in nutrient agar or in bile-salt-lactose agar. They concluded that the effect of glucose on the potency of streptomycin depended on the organism and the culture medium used.

The experiments on the effect of salts upon the antibacterial activity of streptomycin produced conflicting results. Berkman, Henry and Housewright (28) reported that sodium chloride, potassium chloride, sodium sulfate, sodium tartrate, Soerensen's buffer and ammonium acetate markedly decreased the activity of streptomycin against certain strains of Staph. areus, P. vulgaris, Shigella dysenteriae, E. typhosa, Bacillus cereus, Bacillus anthracis and B. subtilis. The degree of antagonism was directly proportional to the concentration of the salts. Klein and Kimmelman (31) found that 0.5% sodium chloride diminished the activity of streptomycin. Green (32) reported that salts of various acids protected Esch. coli against the antibiotic action of streptomycin. On the other hand, Loo et al. (33) found that the addition of phosphate to the streptomycin solution caused a marked increase in the size of the zone of inhibition. An enhancing effect was also shown by sodium chloride, acetate, bicarbonate and sulfate, potassium chloride and bicarbonate, and lithium chloride and sulfate. Recently, Quan (34) reported that the enhancing action of salt depended on the salt concentration in the agar medium as well as on that in the solution of the antibiotic. When the salt was added to the antibiotic solution the activity of the latter was increased. An increased salt concentration in the agar medium decreased the zone of inhibition produced by streptomycin and lessened the enhancing action of salts in the antibiotic solution.

Donovick, Bayan, Canales and Pansy (35) studied the differential effects of various electrolytes on the activity of streptomycin. Of the cations studied, sodium, lithium and potassium ions had little effect on the ability of streptomycin to inhibit bacterial growth. Magnesium and calcium caused the greatest interference with streptomycin activity. Among the anions studied, acetate and pyruvate caused little if any interference, whereas, in increasing order, the following interferred with streptomycin activity: nitrate, chloride, lactate, phosphate, tartrate, citrate and sulfate.

Treffers (36) reported that iodoacetic acid, sodium floride, sodium azide, merthiolate, cetyl pyridinium bromide, crystal violet and mapharsin increased the inhibiting action of streptomycin on Salmonella dysenteriae, Salmonella ambigua, Esch. coli, Staph. aureus and B. cereus. He believed that these chemicals acted as enzyme inhibitors.

The experimental results on the effect of body fluids on the activity of streptomycin were not conclusive. The difference in composition of different body fluids, or even of the same fluid from different origins, probably is responsible for the discrepancy. Wolinsky and Steenken (27) reported that streptomycin was not destroyed, nor were its bacteriostatic and bactericidal powers appreciably influenced by serous body fluid, pus, or normal tissue juices. Schoenbach and Chandler (37) found that the activity of streptomycin on a susceptible strain of Staph. aureus was not changed in the presence of serum or whole blood. The addition of fresh whole blood with or without immune serum did not augument streptomycin activity. On the other hand, the fact that the presence of serum did influence the sensitivity of certain organisms has been mentioned above (22).

The reports on the combined action of two or more antibiotics, and an antibiotic and a sulfa drug are conflicting. This is probably due to the fact that the numerous factors which influence the activity of individual drugs become much more complicated when several drugs are combined. Klein and Kimmelman (38) reported that the combined action of sulfadiazine and streptomycin, or penicillin and streptomycin was greater than that of either drug alone. Klein and Kalter (39) found that the in vitro combination of sulfathiazole, sulfadiazine, or sulfapyrazine and penicillin resulted in an increase in the penicillin titer only if both agents were in inhibitory concentrations. Mayson and McMahon (40) reported that streptomycin, sulfadiazine and sulfapyrazine had definite value in the treatment of experiment animals infected with plague. The use of a combination of streptomycin and sulfadiazine did not seem to add to the effectiveness of either.

There are many methods of assay for streptomycin. Stebbins and Robinson (41) developed a cylinder plate method using Staph. aureus as the test organism. The diameters of the zones of inhibition on the agar plate, produced by the antibacterial action of the sample assayed and standard streptomycin solution, were measured. Loo et al. (35) introduced a paper-disc plate method. B. subtilis was used as the test organism. Waksman and Reilly (42) used an agar-streak method for quick testing of antibiotic substances against a large number of organisms. Price, Nielsen and Welch (43) developed a serial dilution method for determining the presence of streptomycin in body fluids, using Bacillus circulans as the test organism. Donovick, Hamre, Kavanagh and Rake (44) used a broth dilution method for assaying streptomycin. K. pneumoniae was chosen as the test organism.

The degree of growth as affected by the antibacterial activity of streptomycin was measured by visible turbidity. Heilman (45) produced a specific method for the determination of streptomycin content in body fluids. The smallest concentration of streptomycin that completely prevented hemolysis caused by the presence of Bacillus megatherium was chosen as the end point. A colorimetric method for the determination of streptomycin was developed by Boxer, Jelinek and Leghorn (46). Horne and Pollard (47) developed a qualitative colorimetric method for the identification of streptomycin.

II. SODIUM SULFADIAZINE

In studying the therapeutic value of sulfonamides in pullorum disease of poultry, Severens, Roberts and Card (48) found that out of seven sulfonamides, sulfadiazine and sulfamerazine (in feed) were the most effective, judged by both mortality and rate of gain of the surviving chicks. There existed a close agreement between the amount of free sulfonamide in the blood and the effectiveness of the drug. That the use of sulfamerazine in drinking water or in mash is effective in reducing the mortality due to pullorum disease in chicks or poults has been reported by many workers (49, 50, 51, 52 and 53). Bottorff and Kiser (54) found that sulfamethazine, sulfadiazine or sulfamerazine caused a reduction in mortality among chicks. Recently, Pomeroy, Fenstermacher and Roepke (55) reported that sulfadiazine, sulfamerazine, sulfapyrazine, sulfaquinoxaline and sulfamethazine were effective in reducing the mortality from pullorum disease in chicks. Sulfadiazine sulfapyrazine, sulfaguanidine and sulfamerazine had little or no value in poults.

Huddleson (56) found that the presence of sulfadiazine and fresh, normal rabbit serum in a culture medium or in the blood of an infected animal brought about a bactericidal action against Brucella. Sulfadiazine and normal or immune serum, used simultaneously, could terminate experimentally produced brucellosis in guinea pigs. He believed that the bactericidal activity was due to a complex formed by sulfadiazine and the antibody-complement system.

A method for quantitative determination of sulfanilamide has been developed by Bratton and Marshall (57).

MATERIALS AND METHODS

Streptomycin* solution

Streptomycin (calcium chloride complex) 1.5 gm, which is equivalent to 1.0 gm of pure streptomycin base, was diluted with 19 ml of sterile saline. One ml of this solution contains 50 mg of streptomycin base.

Sodium sulfadiazine** solution

One per cent solution of sodium sulfadiazine was made as stock solution and sterilized by passing through a D_6 Hormann filter pad*** in a Seitz filter. The solution must be made freshly on the day when the experiment is performed, since a precipitate will be formed on standing overnight. The desired dilution was then made from the stock solution.

Bacterial suspension

The strain of S. pullorum, used in this experiment, was isolated from a naturally infected chicken. Twentyfour-hour cultures on tryptose agar (Difco) slants were washed off with a diluting fluid consisting of 0.05% tryptose (Difco) and 0.5% sodium chloride. Its turbidity was then adjusted to No. 2 MacFarland nephelometer with the same fluid. One ml of this suspension contained approximately 35×10^7 bacterial cells. The desired dilution was then made and 0.1 ml of the final suspension used as inoculum unless otherwise mentioned.

* Streptomycin used in this experiment was suppled through the courtesy of Merck & Co., Inc., Rahway, N. J.

**Sodium sulfadiazine (Lederle Laboratories, Inc., New York) was kindly supplied through the courtesy of Dr. I. F. Huddleson.

***Hormann & Co., Brooklyn 2, N. Y.

Serum

Blood of chickens and rabbits was collected aseptically by cardiac puncture. Turkey blood was obtained from the wing vein. The collected blood was allowed to clot at room temperature. The serum was then separated by centrifugation.

Determination of bacteriostatic and bactericidal action in vitro

The activity of streptomycin or sodium sulfadiazine against S. pullorum was assayed as follows: Two-fold serial dilutions of the drug assayed were made in 5 ml of tryptose broth. A certain amount of normal serum was added to each broth tube when its enhancing action on the drug was studied. One-tenth ml of a suitable dilution of bacterial suspension was finally added. All tubes were incubated at 37°C and the degree of growth was recorded at intervals by the signs -, + and Ag (agglutination of the organisms). The highest dilution of streptomycin in which the bacteria failed to grow for at least 96 hours of incubation was defined as the bactericidal titer; while that in which the inhibition of growth lasted for less than 96 hours was designated as the bacteriostatic titer for the particular period of time during which the results were observed. The reason for designating a 96-hour incubation period as the dividing line between bactericidal and bacteriostatic activity was that the organisms which failed to grow at the end of 4 days rarely showed growth when the period of incubation was extended beyond that length of time. Sulfadiazine usually inhibited the growth of the organism for a short period of time.

In order to study the enhancing action of certain substances

on streptomycin and sulfadiazine, two sets of serial dilutions of the drug assayed were made in tryptose broth. To one set, a certain amount of the enhancing substance which was previously proved to be unable to inhibit the growth of the organism, was added; the other set, to which this substance was not added, was used as control. The bacterial suspension was then added and the mixture placed in the incubator. Because the antibacterial titer of the drug against S. pullorum changed at times, in spite of efforts to keep every condition constant, it was necessary to include a control in each experiment. The same technic was employed in studying the effect of certain substances which were antagonistic to the drug.

For studying the effect of serum of infected birds on the action of streptomycin and sulfadiazine against S. pullorum, two-fold serial dilutions of such sera were made. To these dilutions, a certain amount of streptomycin or sulfadiazine, with or without normal serum, and bacterial suspension were added, and the mixture was then incubated at 37°C.

The in vivo tests

A. Streptomycin assay. Chickens and turkeys were injected intravenously with 0.5 to 1.0 ml, according to their body weight, of a suspension of a 24-hour culture of S. pullorum (turbidity No. 2, MacFarland nephelometer). After one week, when the agglutination titer was at the highest level, the birds were injected intramuscularly with streptomycin daily (30 to 45 mg per kg of body weight) for two weeks. Blood samples were taken 1, 2, 3 and 6 hours after injection for determining the change in antibacterial activity

of streptomycin in the serum. The birds were then killed for isolation of the organism from various organs.

B. Sulfadiazine assay. Chickens were infected as indicated before. After one week the birds were fed with sodium sulfadiazine at the rate of 0.4 gm per kg of body weight daily for 3 days. Fifteen ml of normal chicken serum was then injected intravenously. Blood samples were taken while the feeding of sulfadiazine was continuing. The bacteriostatic action of the serum from these birds against S. pullorum was observed.

Determination of sodium sulfadiazine in serum

A certain amount of serum, depending upon the concentration of sulfadiazine present, was diluted with 20 ml of 15% trichloroacetic acid and water up to 100 ml. The mixture was then filtered. To 10 ml of the filtrate and to 10 ml of a blank (20 ml of 15% trichloroacetic acid plus 80 ml of water), 1 ml of 0.1% sodium nitrite was added. After standing for 3 minutes, 1 ml of 0.5% ammonium sulfamate was added. Then after 3 more minutes, 1 ml of 0.1% N-(1-naphthyl)-ethylenediamine dihydrocholride was added. The readings were taken in a lumetron. The concentration of sodium sulfadiazine in serum was determined by comparing the reading of an unknown sample with that of a known concentration of sulfadiazine recorded in a standard curve previously established.

Isolation and identification of S. pullorum

The treated and control birds were killed, and inoculations were made on SS agar (Difco), Bismuth sulfite agar (Difco) and in tetrathionate broth (Difco) from various organs. The broth was incubated at 37°C for about 20 hours; then smears were made on SS agar and bismuth sulfite agar. Colonies which resembled those of S. pullorum

were transplanted to lactose motility medium*. If there was any growth with no motility and no fermentation of lactose, transfers were made to single sugar broths (glucose, lactose, sucrose, maltose and mannite) and gramstain made for the identification of S. pullorum.

The course of the agglutination titer of the serum during infection and treatment

The agglutination titer of serum taken from turkeys, infected intravenously with S. pullorum, was determined by the test tube method during the treatment with streptomycin. The birds were also tested before the treatment was begun and after the treatment was discontinued.

*Lactose motility medium:

Water	100.0 %
Motility test medium (Difco)	1.8 %
Beef extract (Difco)	0.2 %
K_2HPO_4	0.05 %
Andrade's indicator	1.0 %
Lactose	1.0 %

RESULTS AND DISCUSSION

I. STREPTOMYCIN

The antibacterial activity of streptomycin against S. pullorum and the influence of serum

In in vitro tests, the bacteriostatic and bactericidal action of streptomycin against an organism can be performed by various methods (33, 41, 42, 43, 44, 45, 46 and 47). The broth dilution method was used in this experiment due to its simplicity and convenience. Two-fold serial dilutions of streptomycin were made in 5 ml of tryptose broth. One-tenth ml of bacterial suspension (350 cells per ml) was added. Table 1 shows that the bactericidal concentration of streptomycin was 0.157 mg* in 5 ml of medium, or 0.031 mg per ml of medium for S. pullorum. This figure was somewhat higher than that obtained by West, Doll and Edwards (7). They found that the amount of streptomycin, completely inhibitive for most cultures of S. pullorum, was 4 to 8 units per ml of medium by the agar-streak method. Obviously the difference in strains, culture media, method of assay and other experimental conditions could account for the discrepancy. Moreover these workers have also reported that the point of complete inhibition varied from test to test with a given culture.

The presence of normal chicken serum did not affect the titer, although a little better bacteriostatic activity was observed (table 1-B) during the first 4 days of incubation. Similar results were obtained by other workers. Schoenbach and Chandler (37) reported that the presence of serum or whole blood did not influence the activity of strepto-

*One mg of streptomycin = 1,000 units

mycin. Wolinsky and Steenken (27) also found that the antibacterial action of streptomycin was not affected by serous body fluid, pus or normal tissue juices. On the other hand, Rhymer and Wallace (21) reported that different peptones showed different ability to affect the activity of streptomycin. Hobby and Lenert (22) found that the presence of serum enhanced the resistance of certain organisms but not that of others. Obviously, the discrepancy is due to the different composition and characteristics of various proteins. Besides, the different amounts of various crystalloids present in various body fluids make the conditions more complicated.

In order to study the effect of serum from artificially infected chickens on the antibacterial activity of streptomycin, serial dilutions of serum from the infected birds were made. The bactericidal amount of streptomycin (0.157 mg in 5 ml of medium) and bacterial suspension were added. No growth was observed at the end of 7 days of incubation as shown in table 2. This indicates that the presence of serum of infected birds did not interfere with the antibacterial activity of streptomycin.

The effect of age of culture and amount of inoculum on the antibacterial activity of streptomycin against S. pullorum

Various amounts, 0.1, 0.5, and 1.0 ml, of culture of S. pullorum at different ages, 24, 48, 72, 96 and 120 hours were inoculated into tryptose broth containing serial dilutions of streptomycin. The results recorded in table 3 show that the older the culture and the smaller the inoculum, the higher was the bactericidal titer of streptomycin (higher dilution). Apparently the older cells were less active in their metabolism, and thus they were less resistant to the drug than the younger ones. The reason why larger amount of streptomycin was required to kill

a large number of organisms was probably that certain cells in the larger inoculum were more resistant to the antibiotic. The effect of amount of inculum on the activity of streptomycin was especially prominent in quantities between 0.1 ml and 0.5 ml of older culture. However, as regards 24-hour-old cultures, the size of inoculum made no difference in the bactericidal titer of streptomycin. A likely explanation might be that the vigorous younger cells were more resistant so that the "all or none law" can be applied within a certain range of amount of inoculum. The bactericidal titers of streptomycin for cultures of various ages and amounts are shown in table 3-F.

The effect of pH on the antibacterial activity of streptomycin

Seven lots of tryptose broth with different pH values, pH 4, 5, 6, 6.9, 8, 9 and 10, were prepared by adding sodium hydroxide or hydrocholoric acid to the medium, the original pH of which was 6.9. The measurement of the pH value was made with Beckman pH meter. Serial dilutions of streptomycin were made and bacterial suspension was added. The data in table 4 and accompanying figure show that at pH 4 and 10, the organisms did not grow, due to the unsuitable acidity, as demonstrated in the control tube. The activity of streptomycin was completely destroyed at pH 5. The organism grew in all dilutions of streptomycin at that pH. At pH 6, streptomycin maintained its bacteriostatic action in the lowest dilution (1:16,000) for 96 hours only. Streptomycin showed its highest bactericidal activity at pH 6.9 and 8. The bactericidal titer at these pH values was 1:64,000. At pH 9, the bactericidal titer was reduced to 1:32,000. Generally, on the acid side, the lower the pH value of the medium, the lower was the bactericidal titer (lower dilution) of the antibiotic. Similar results have been

reported by many workers (26, 27, 28, 29 and 33). The most likely explanation is that the basic property of streptomycin, which probably is essential to its activity, is neutralized by acid. Abraham and Duthie (29) assumed that the ionized forms of basic streptomycin compete with hydrogen ions for position on the cell surface.

The effect of some sugars on the antibacterial activity of streptomycin

Glucose has been found by many workers (16, 17 and 30) to have the ability to diminish the activity of streptomycin. This may be due to the reducing property of glucose, or to the production of acid from glucose fermented by organisms, or both, or some other reasons. Geiger Green and Waksman (16) reported that dextrose, levulose and sucrose which yield acid during incubation diminished the activity of streptomycin; while non-acid-producers, mannose, galactose, and lactose had little effect on the activity of streptomycin against B. subtilis. However, Sykes and Lumb (30) believed that the effect of glucose on the antibacterial action of streptomycin varied with the kinds of organism tested and the culture medium used. In this experiment an attempt has been made to find out whether there was any relationship between acid produced from the fermentation of sugars and the antibacterial activity of streptomycin. Four different sugars, glucose, mannite, lactose and sucrose, were incorporated in the medium in a concentration of one per cent. The first two sugars were fermented by S. pullorum; the last two were not. Streptomycin and bacterial suspension were added as before. The pH of all media before incubation was 6.9. The results recorded in table 5 and accompanying fig. show that glucose reduced the bactericidal titer of streptomycin from 1:64,000 to 1:32,000. The final pH of glucose broth after 7 days of

incubation was 4.7. The decrease in titer might be related to the acid formed by the fermentation of glucose which lowered the pH value of the medium. However, the nature of the sugar also played a part in the alteration of streptomycin activity. As shown in table 4, the antibacterial activity of streptomycin was completely destroyed at pH 5. In this experiment, streptomycin still retained its bactericidal titer of 1:32,000 even though the pH of the medium was as low as 4.7. This indicates that some metabolic products, other than acid, have something to do with the activity of streptomycin, since glucose itself was inactive, as far as antibacterial activity was concerned, as shown in the control tube (table 5-B). On the other hand, mannite increased the bactericidal titer of the antibiotic from 1:64,000 to 1:256,000, although the final pH of the medium was reduced to 5.6. This further proves that the nature of individual sugars or their by-products plays some part in changing the antibacterial activity of streptomycin. Lactose increased the titer somewhat. Little change in titer was observed in sucrose medium. The changes in pH of the sugar media after incubation with S. pullorum are shown in table 5-F.

The effect of some reducing agents on the antibacterial activity of streptomycin

It is well known that reducing agents, organic and inorganic, diminish the effectiveness of streptomycin. Two reducing agents, ascorbic acid and sodium thiosulfate, were used in this experiment. Table 6 and accompanying fig. show that the bactericidal titer of streptomycin was greatly reduced by ascorbic acid. The more of the vitamin added to the medium, the lower was the bactericidal titer of streptomycin. The addition of the vitamin in a concentration of 0.5

mg per tube (5 ml of medium) or 0.1 mg per ml of medium reduced the titer from 1:128,000 to 1:32,000 (tables 6-A and 6-C). One mg of ascorbic acid per ml of medium completely destroyed the bactericidal activity of streptomycin (table 6-B). On the other hand, the addition of sodium thiosulfate in a concentration of 4 mg per ml of medium had no effect on the bactericidal titer (table 6-E). In lower concentration, 2 mg per ml of medium, however, the titer was somewhat increased (table 6-F).

There was much evidence indicating that the antibacterial action of streptomycin might relate to the oxidation-reduction system of the bacterial cell. Bondi, Dietz and Spaulding (14) found that the antibacterial activity of streptomycin was diminished by anaerobic incubation, and by the presence of reducing agents, and that streptomycin showed its greatest activity against bacteria which grew better aerobically than anaerobically. They concluded that the antibacterial action of streptomycin may be due to its ability to block some enzyme system, oxidative in nature, which is essential only to the growth of susceptible aerobic bacteria. Donovick and Rake (17) proposed that the interfering action of sodium thioglycollate may be due to its role in reducing the oxidation-reduction potential of the medium. The fact that the inactivation of streptomycin by cysteine can be reversed by iodine (15) further indicates the relation between the activity of streptomycin and the oxidation-reduction reaction. On the other hand, Van Dolah and Christenson (20) found that the oxidizing agents, potassium permanganate and potassium periodate, were very specific in the elimination of the antibiotic action of streptomycin. One of the reducing agents, sodium hypophosphite in high concentration caused essentially

complete inactivation of streptomycin. Sodium thiosulfate had no effect. They believed that the inactivation of streptomycin may not have to do with the oxidizing nor reducing mechanism, but rather with a specific reaction or an interference mechanism. The results in this experiment support the latter theory.

The effect of some dyes on the antibacterial activity of streptomycin

Dyes themselves possess bacteriostatic activity against certain organisms. An attempt has been made to see if there is any synergistic action between streptomycin and certain dyes. Two dyes, crystal violet and brilliant green, were tested for their bactericidal titer. The titer of the former against S. pullorum was 1:80,000 or 0.013 mg per ml of medium (table 7-C) and that of the latter was 1:320,000 or 0.003 mg per ml of medium (table 7-F). To two sets of serial dilutions of streptomycin, crystal violet in concentrations of 0.01 mg and 0.006 mg per ml of medium (which were below its bactericidal titer) was added. The results in table 7 and accompanying fig. show that crystal violet increased the bactericidal titer of streptomycin. A mixture of 0.01 mg of crystal violet per ml of medium and streptomycin in a dilution of 1:4, 096,000, which alone was unable to kill the bacteria, completely destroyed the organism. All tubes showed no growth after 7 days of incubation. The presence of 0.006 mg of crystal violet per ml of medium increased the bactericidal titer of streptomycin from 1:128,000 to 1:2,048,000 (tables 7-A and 7-B). Brilliant green had higher bactericidal titer (1:320,000), than streptomycin (1:128,000) and crystal violet (1:80,000), but the addition of brilliant green did not increase the bactericidal titer of streptomycin. It appears that certain dyes may act with streptomycin as specific synergists.

The combined action of streptomycin and sulfadiazine

Sodium sulfadiazine in a dilution of 1:1,000 inhibited the growth of S. pullorum for only 24 hours. However, when a smaller amount of sulfadiazine was added to the serial dilutions of streptomycin, the bactericidal titer of the latter was increased (table 8 and accompanying fig.). That the combined action of sulfadiazine and streptomycin was greater than that of either drug alone was found by Klein and Kimmelman (38). They believed that the effect was not a simple additive one since sulfadiazine in concentration less inhibitory than penicillin, was more effective as a synergist than penicillin when combined with streptomycin. On the other hand, Wayson and McMahon (40) reported that the combination of streptomycin and sulfadiazine did not enhance the antibacterial activity of either agent against experimental plague. The difference in results was probably due to the differences in the organisms, which varied greatly in their sensitivity to streptomycin, in the medium used and in other factors.

The duration of antibacterial activity of streptomycin in the body of infected birds.

Three artificially infected chickens were given streptomycin at the rate of 45 mg per kg of body weight intremuscularly. Blood samples were collected 1, 2, 3 and 6 hours after injection. Two-fold serial dilutions of the sera were made and the bacterial suspension was added. The results in table 9 show that in 1:5 dilution, all sera taken from the 3 infected chickens 1 hour after injection possessed bactericidal activity. Of the samples taken 2 hours after injection, one had bactericidal properties, the other two showed bacteriostatic activity for 48 and 72 hours. Of the samples taken 3 hours after injection, one

possessed bactericidal activity, the other two maintained bacteriostatic action for only 24 hours. Of the samples taken 6 hours after injection one possessed bacteriostatic activity for 24 hours, the other two showed no bacteriostatic action at all. In 1:10 dilution, the bacteriostatic action lasted only for a short time. Similar results were observed when working with infected turkeys (table 10). These results indicate that it will be necessary in treating poultry to inject streptomycin every 3 to 4 hours in order to maintain a proper blood level of streptomycin to insure complete control of the organism. Such a procedure is not practical in poultry practice.

The therapeutic value of streptomycin against pullorum disease

There are few reports on the clinical value of streptomycin against pullorum disease. Benson (13) reported that streptomycin was effective in reducing mortality from pullorum disease in baby chicks. S. pullorum was recovered from the bone marrow of 90% of all the chicks which died. An attempt was made to study the therapeutic value of streptomycin on pullorum disease in adult chickens and poults using one injection daily intramuscularly. Three groups of chickens and three groups of turkeys, all artificially infected, five in each group, were used. Group 1 and 2 were given 30 mg and 45 mg respectively per kg of body weight daily for 14 days. Group 3 served as untreated control. One week after the discontinuation of the injections the birds were killed for isolation of the organism from the internal organs. It was isolated from the spleen, ovary, lungs and intestine of one control chicken which was very sick. It was not isolated from the other con-

trol birds nor from the infected birds. The isolation of S. pullorum was easier in baby chicks and poults (13, 58, 59, 60 and 61), especially in naturally infected birds which showed severe symptoms, than in artificially infected adult birds which usually suffered from subacute infection. The only evidence of infection in the artificially infected birds was a high agglutination titer. The failure to isolate the organism in this experiment was probably due to the fact that the adult chickens were more resistant to the infection than the baby chicks; and the poults were perhaps more resistant to S. pullorum strain of chicken origin.

The effect of streptomycin on the course of the agglutination titer of infected turkey serum

Five turkeys were inoculated intravenously with 0.5 ml of a suspension of a 24-hour agar culture of S. pullorum, turbidity No. 2 MacFarland nephelometer. Agglutinins were detected by the tube test after infection. The results in fig. A-1, A-2, A-3 and A-4 show that the presence of agglutinins in the serum of infected birds can usually be detected on the fourth or fifth day after inoculation. The titer reached a maximum on the sixth to ninth day after inoculation, remained at this level for 6 to 8 days and then went down. It rose as high as 1:1600. Treatment with streptomycin had little influence on the course of the agglutination titer. Reinoculation with 1 ml of the same concentration of bacterial suspension made the titer rise again in a curve similar to that of the primary infection. These results were similar to those obtained by Corpron, Bivins and Stafseth (62). They reported that agglutinins were detected by the tube test 3 days after intravenous injection of S. pullorum into turkeys. The maximum titers of intra-

venously infected and orally infected birds occurred on about the ninth day, after which the titer decreased in fluctuating descent. The results in this experiment show that the change in agglutination titer does not necessarily indicate a change in severity of the infection nor effectiveness of the treatment.

II. SODIUM SULFADIAZINE

The antibacterial activity of sodium sulfadiazine against S. pullorum and the influence of normal serum

In order to study the effect of sodium sulfadiazine on the growth of S. pullorum, 0.1 ml of the bacterial suspension was added to serial dilutions of the drug in 5 ml of tryptose broth in the presence and absence of 0.5 ml of normal rabbit or chicken serum. The results in table 11-A show that sodium sulfadiazine inhibited the growth of S. pullorum for the first 24 hours of incubation. The presence of 0.5 ml of normal rabbit serum made little difference in the bacteriostatic activity of sulfadiazine (tables 11-A and 11-B), while 0.5 ml of normal chicken serum greatly increased its antibacterial action (table 11-C). Huddleson (56) found that the presence of fresh normal rabbit serum in the solution of sodium sulfadiazine, which showed growth retarding effect against Brucella for less than 24 hours of incubation, brought about a remarkable bactericidal activity of the drug. He believed that the increased action of sulfadiazine in the presence of a sufficient amount of fresh normal serum, which contained complement and a small amount of natural Brucella antibody, could be attributed to the activity of the complex formed by sulfadiazine and the antibody-complement system. In this experiment the combined action of sulfadiazine and normal chicken serum was not an additive one, since the growth inhibiting action of normal chicken serum continued for only 16 hours as shown in the control tube in table 11-C; that of sulfadiazine lasted for only 24 hours. The combined action of these agents increased the antibacterial action up to at least 96 hours. Since the normal

chicken serum contained complement and a small amount of natural antibody against S. pullorum, as shown in the control tube in table 11-C, Huddleson's theory could be applied in explanation of the results. The fact that normal rabbit serum, which contained complement but no natural antibody against S. pullorum (control tube in table 11-B), did not enhance the activity of sulfadiazine, further supports his theory.

The effect of amount of normal chicken serum on the antibacterial action of sulfadiazine

Various amounts of normal chicken serum were added to the two-fold dilutions of sodium sulfadiazine. The bacterial suspension was then added. It is clear from the data in the table 12 that the more serum added, the higher was the enhancing activity of serum on sulfadiazine. Less than 0.3 ml of normal chicken serum alone showed no inhibitory action against S. pullorum, but 0.4 and 0.5 ml of serum inhibited the growth of the organism for 16 hours (control tubes in tables 12-D and 12-E). However, 0.2 ml and 0.3 ml of normal chicken serum in sulfadiazine solution increased the growth-inhibiting period of sulfadiaine from 24 hours to 5 and 7 days respectively in a dilution of 1 part of sulfadiazine to 5,000 parts of tryptose broth (tables 12-A, 12-B and 12-C). In the presence of 0.4 and 0.5 ml of normal chicken serum, dilutions of sulfadiazine lower than 1:20,000 completely destroyed the organism (tables 12-D and 12-E).

The influence of heating on the bacteriostatic activity of normal chicken serum and on its enhancing effect on sulfadiazine

An attempt was made to find out whether heating had any effect on the activity of normal chicken serum. The fresh normal chicken serum was heated in a water bath at 56°C for 30 minutes. It was then added to the serial dilutions of sulfadiazine solution. Bacterial suspension

Was then introduced. Unheated serum was tested in the same manner as control. The results in table 13 clearly show that heating greatly diminished both the enhancing activity of normal chicken serum on sulfadiazine and its own bacteriostatic action. The unheated serum from the same pool inhibited the growth of S. pullorum for 24 hours (control tube in table 13-C), while the heated serum lost this inhibitory activity (control tube in table 13-B). Again, in a dilution of 1:80,000, the unheated serum increased the period of growth-inhibiting activity of sulfadiazine from 16 hours to at least 72 hours (tables 13-A and 13-C), while the heated serum increased the period only to 24 hours (table 13-B). It is well known that the complement in normal serum is inactivated at 56°C for 30 minutes. Therefore, removal of or reduction in complement activity may account for the reduction in antibacterial activity of serum and of the serum-sulfadiazine complex.

The influence of storage on the bacteriostatic activity of normal chicken serum and on its enhancing effect on sulfaziazine

The complement of normal chicken serum became gradually inactivated on standing. Collection of a large amount of chicken blood and separation of serum require a considerable period of time. It would be quite useful if normal serum could be preserved for a longer period without serious detriment to its bacteriostatic action and its enhancing effect on sulfadiazine. Normal chicken serum from the same source as that used in the previous experiment (table 13-C) was stored in the refrigerator room for 24 hours before the addition of sulfsdiazine and bacteria. The date in table 14 show that there was a little decrease in the bacteriostatic activity of the serum after storage and that there was practically no decrease in the enhancing activity on sulfadiazine except in the first

24 hours of incubation (tables 14-B and 14-C). Both fresh and stored normal chicken sera were bacteriostatic against S. pullorum and increased the bacteriostatic titer of sulfadiazine.

The combined action of infected chicken serum and different proportion of sulfadiazine and normal chicken serum

Since serum taken from chickens infected with S. pullorum contained complement and a large amount of specific pullorum antibodies, an experiment was set up to determine if greater antibacterial activity could be obtained when various amounts of normal chicken serum and sulfadiazine were added to serial dilutions of serum of infected birds. The data in table 15-A show that serum of infected birds alone in a dilution of one part of serum to twenty parts or more of tryptose broth possessed no antibacterial activity against S. pullorum. The organisms were agglutinated by the agglutinins in the serum, but they were still alive as proved by subcultures in broth tubes. The addition of 0.1 mg of sulfadiazine, which was not bacteriostatic by itself (control tube in table 15-B), to the serial dilutions of serum of infected birds did inhibit the growth of S. pullorum for 24 hours in a serum dilution of 1:2,560 or lower and for 48 hours in a dilution of 1:1,280 or lower. There was no bacteriostatic activity in a dilution of 1:5,120, nor in any dilutions at the end of 96 hours of incubation. A possible explanation for the combined action of these agents may be a potentiating effect of sulfadiazine on the complement or antibody-complement system in the serum of infected chickens.

The addition of 0.3 ml of normal chicken serum which possessed no antibacterial activity by itself (control tube in table 15-C) to serial dilutions of serum of infected birds inhibited the growth of the organism

im dilutions of 1:160 to 1:5,120 (table 15-C) for 24 hours. No bacteriostatic activity was observed in dilutions of serum of infected birds between 1:20 to 1:80. It seemed that a small amount of serum on infected birds was necessary to enhance the growth-inhibiting activity of normal chicken serum. This may be a prozone phenomenon.

It was interesting to note that the addition of 0.1 mg of sodium sulfadiazine and 0.5 ml of normal chicken serum to serial dilutions of infected chicken serum (either of these three agents being bacteriostatic alone) made the antibacterial activity of the mixture pronounced (table 15-D). In dilutions of 1:640 or higher of serum of infected birds, the mixture showed bactericidal effect, while in lower dilutions only bacteriostatic action was observed.

An attempt was made to determine what would be the better proportion of sodium sulfadiazine and normal chicken serum in serial dilutions of serum from the infected birds, as far as the bacteriostatic activity of the mixture against S. pullorum is concerned. Three kinds of combination, 0.1 mg of sulfadiazine and 0.5 ml of serum, 0.1 mg of sulfadiazine and 0.4 ml of serum, and 0.05 mg of sulfadiazine and 0.5 ml of serum, were used. The data in tables 15-D, 15-E and 15-F show that in dilutions lower than 1:320 of serum from infected birds, the combination of 0.1 mg of sulfadiazine and 0.5 ml of normal serum showed a bacteriostatic effect similar to that of the combination of 0.1 mg of sulfadiazine and 0.4 ml of normal serum; and that the combination of 0.05 mg of sulfadiazine and 0.5 ml of normal serum was somewhat less effective. However, when the dilutions of serum from infected birds were 1:640 or higher, all tubes showed no growth at the end of 7 days of incubation (bactericidal effect).

The effect of the amount of serum of infected birds on the combined action of sulfadiazine and normal chicken serum

The importance of the amount of serum from infected chickens on the antibacterial activity of normal chicken serum, and of combination of sulfadiazine and normal chicken serum has been mentioned above (tables 15-C, 15-D, 15-E and 15-F). Five more higher dilutions of the serum from infected chickens were made. Sulfadiazine, normal chicken serum and the organism were added either alone or in combination (table 16). The results show that serum of infected chickens alone possessed no bacteriostatic activity (table 16-A), that sulfadiazine showed bacteriostatic action for 24 hours in dilutions of 1:20 to 1:160 of serum of infected birds (table 16-B), and that normal chicken serum needed certain amounts of serum of infected birds for enhancement of its bacteriostatic activity. Dilutions of serum of infected birds between 1:320 amd 1:10,240 were needed to maintain bacteriostatic action of 0.3 ml of normal chicken serum for 16 hours (table 16-C). In the presence of 0.1 mg of sulfadiazine and 0.3 ml of normal chicken serum, no growth was observed at the end of 96 hours of incubation in the dilutions of infected chicken serum between 1:640 and 1:1,512. Higher and lower dilutions caused a decrease in the antibacterial activity of the mixture (table 16-D). Similar results were obtained with serum from infected turkeys (table 17).

The results in tables 16-C, 16-D, 17-C and 17-D were typical of prozone phenomena which have also been observed in studies on the potentiating action of sulfonamides on the Brucella antibody-complement system by Huddleson (56).

Sodium sulfadiazine level in chicken serum

The concentration of sulfadiazine in blood was generally proportional to the amount of the drug administered. Severens, Roberts and Card (48) found that when chicks were given sulfadiazine, 2% in feed, the concentration of free sulfadiazine in 100 ml of blood was 35.4 mg. Pomeroy, Fenstermacher and Roepke (55) reported that in amounts of 0.5 1.0 and 2.0% of sulfadiazine in feed, the concentrations of the drug in 100 ml of blood were 12, 21 and 29 mg respectively. Since the consumption of feed was not even throughout the 24 hours of the day and some feed was wasted, in this experiment, the sodium sulfadiazine, instead of being mixed in mash, was dissolved in water and administered to the chickens per os 3 times daily in order to make sure that the drug reached the alimentary tract. Three hours after administration blood samples were taken for analysis. The average concentrations of sulfadiazine in 100 ml of blood were 11.2 (3.1-22.3) and 40.1 (17.2-63.5) mg resulting from daily doses of 0.5 and 1.0 gm per kg of body weight respectively. The results indicate that sulfadiazine did get into the blood stream in proportion to the amount of the drug administered. However, the amount of drug found in the blood varied greatly for a given dose. This was probably due to variation in rate of absorption and excretion of the drug in individual birds.

The antibacterial activity of sulfadiazine in serum of normal chickens

Sodium sulfadiazine was given to normal chickens at the rate of 1 gm per kg of body weight daily for 3 days. Blood samples were then taken and the serum separated. The concentration of sodium sulfadiazine was 18.9 mg per 100 ml of serum. Two sets of two-fold serial dilutions of

the serum were made, starting with 1:50,000 in terms of sulfadiazine to culture medium. One set received S. pullorum alone, the other 0.3 ml of normal chicken serum and S. pullorum. The data in table 18-A show that the serum containing sulfadiazine inhibited the growth of S. pullorum in a dilution of 1:200,000 or lower for the first 16 hours of incubation; in 1:100,000 or lower for 24 hours; and in 1:50,000 for 48 hours. In the presence of 0.3 ml of normal chicken serum the bacteriostatic titer was greatly increased (table 18-B). In a dilution of 1:200,000 or lower, the growth of the organism was inhibited for the first 48 hours; in 1:100,000 or lower for 72 hours; and in 1:50,000 for 7 days, which was practically bactericidal. For the purpose of comparing the bacteriostatic activity of sulfadiazine passed through a bird's body and that of the drug in an aqueous solution, serial dilutions of sodium sulfadiazine were tested for activity against S. pullorum. Table 18-C show that without normal chicken serum added to the broth tubes, sulfadiazine inhibited the growth of the organism in a dilution of 1:80,000 or lower for the first 16 hours of incubation; in 1:40,000 or lower for 24 hours; and in 1:5,000 for 48 hours. In the presence of 0.3 ml of normal chicken serum (table 18-D), the growth was inhibited in a dilution of 1:160,000 or lower for the first 24 hours of incubation; in 1:40,000 or lower for 72 hours; and in 1:5,000 for 7 days. Generally, the drug offered better bacteriostatic activity in serum than in aqueous solution. Obviously, this was due to the additional activity of serum which, as discussed above, possessed bacteriostatic activity and an enhancing action on sulfadiazine against S. pullorum.

The combined action of sulfadiazine and normal chicken serum in the body of infected birds.

For the purpose of studying the enhancing action of normal chicken serum on sulfadiazine in the body of infected birds, 15 ml of normal chicken serum was injected intravenously into artificially infected chickens that had been given 0.4 gm of sulfadiazine per kg of body weight daily for 3 days before the injection of normal serum. Blood samples were taken from the treated birds one hour before and 24 hours after the injection, while the feeding of sulfadiazine was continuing. The concentrations of sulfadiazine in the serum before and after the injection were 35.3 and 56.3 mg per 100 ml of serum respectively. This sulfadiazine-containing serum was then diluted serially and inoculated with S. pullorum. The results recorded in table 19 show that the injection of 15 ml of normal chicken serum caused an increase in bacteriostatic titer of sulfadiazine from 1:120,000 (dilution of sulfadiazine in tryptose broth) before injection to 1:160,000 after injection for the first 16 hours of incubation; and from nil to 1:40,000 for 24 hours. Apparently, this short growth-inhibiting period would not be sufficient to insure the complete control of the organism in the body of infected birds. The great enhancing activity of normal chicken serum on the bacteriostatic action of sulfadiazine in the in vitro test has been mentioned above. The inefficiencey of normal chicken serum in the body of infected birds may be due to the dilution of the serum in the blood stream or to reactions which bring about conjugation of the serum component with compounds in the tissues of the body.

SUMMARY

Streptomycin showed bactericidal action against S. pullorum in a concentration of 0.031 mg per ml of tryptose broth medium.

Serum from normal or infected chickens did not influence the antibacterial activity of streptomycin.

The older the culture and the smaller the inoculum, the higher was the bactericidal titer of streptomycin.

Streptomycin showed the highest bactericidal activity at pH 6.9 and 8.0. On the acid side, the lower the pH value of the medium, the lower was the bactericidal activity.

Different sugars produced different effects on the bactericidal activity of streptomycin with no direct relation to the amount of acid produced from the fermentation of the sugars by S. pullorum.

The inactivation of streptomycin by certain reducing agents may not relate to the reducing nor the oxidizing mechanism but rather to certain specific reactions between the drug and the reducing agent or its by-products.

Different dyes showed different effects on the bactericidal activity of streptomycin. The effect was not parallel with their own bacteriostatic action against S. pullorum.

Sodium sulfadiazine enhanced the bactericidal activity of streptomycin.

The antibacterial activity of streptomycin in serum from streptomycin treated birds lasted about 3 hours (less than 6 hours) after intramuscular injection of the antibiotic. The activity was lost gradually.

The therapeutic value of streptomycin against S. pullorum could

not be evaluated due to failure to isolate the organism in all treated and most of untreated birds. The failure was probably due to the fact that the turkeys and adult chickens used in this experiment possessed a high degree of resistance to infection by the S. pullorum strain employed. This strain had been isolated from a chicken.

The treatment with streptomycin had little influence on the course of the agglutination titer of the artificially infected turkeys.

Sodium sulfadiazine showed bacteriostatic activity against S. pullorum.

Normal chicken serum possessed bacteriostatic action against S. pullorum and greatly enhanced the antibacterial activity of sulfadiazine. No such properties were observed in normal rabbit serum.

These bacteriostatic and enhancing activities of normal chicken serum were diminished by heating at 56°C for 30 minutes, and were only slightly reduced by storage in the refrigerator for 24 hours.

Serum from artificially infected birds showed no bacteriostatic activity. The addition of sulfadiazine to the serum in a concentration below its bacteriostatic titer caused bacteriostasis.

When normal chicken serum was added to certain dilutions of serum from infected birds, the mixture became bacteriostatic but not bactericidal; when sulfadiazine was added to a mixture of normal chicken serum and certain dilutions of serum of infected birds, a bactericidal effect was produced. In the dilutions employed none of these agents was bacteriostatic when used alone.

The bacteriostatic activity of sulfadiazine-containing serum from normal chickens was better than that of aqueous solution of sulfadiazine.

The simultaneous injection of normal chicken serum and oral administration of sulfadiazine to infected birds showed a little better bacteriostatic activity than resulted from oral administration of sulfadiazine alone.

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Table 1. The antibacterial activity of streptomycin on *Salmonella pullorum* and the influence of norum chicken serum

A. Streptomycin alone

Tube No.		1	2	3	4	5	6	7	8	9	10	con.
Streptomycin, mg		*2.500	1.250	0.625	0.313	0.157	0.079	0.040	0.020	0.010	0.005	-
Bact. susp., 10^{-6}		0.1 ml to each tube										
Incubation period, hours	16	-	-	-	-	-	-	-	+	3+	3+	3+
	24	-	-	-	-	-	-	±	3+	3+	3+	3+
	48	-	-	-	-	-	3+	3+	4+	4+	4+	4+
	72	-	-	-	-	-	4+	4+	4+	4+	4+	4+
	96	-	-	-	-	-	5+	5+	5+	5+	5+	5+
	120	-	-	-	-	-	5+	5+	5+	5+	5+	5+
	144	-	-	-	-	-	5+	5+	5+	5+	5+	5+
	168	-	-	-	-	-	5+	5+	5+	5+	5+	5+

Bact. susp. ----bacterial suspension, 35 x 10^7 bacterial cells per ml
Con. ----------control
- = no visible growth, + = growth
*2.5 mg in 5 ml of medium = dilution of 1:2,000, and so on

B. Streptomycin plus normal chicken serum

Tube No.		1	2	3	4	5	6	7	8	9	10	con.
Streptomycin, mg		2.500	1.250	0.625	0.313	0.157	0.079	0.040	0.020	0.010	0.005	-
Normal chicken serum		0.3 ml to each tube										
Bact. susp., 10^{-6}		0.1 ml to each tube										
Incubation period, hours	16	-	-	-	-	-	-	-	-	2+	3+	3+
	24	-	-	-	-	-	-	-	2+	3+	3+	3+
	48	-	-	-	-	-	-	3+	5+	5+	5+	5+
	72	-	-	-	-	-	-	5+	5+	5+	5+	5+
	96	-	-	-	-	-	-	6+	6+	6+	6+	6+
	120	-	-	-	-	-	5+	6+	6+	6+	6+	6+
	144	-	-	-	-	-	5+	6+	6+	6+	6+	6+
	168	-	-	-	-	-	5+	6+	6+	6+	6+	6+

Table 2. The effect of infected chicken serum on the antibacterial activity of streptomycin

A. Infected chicken serum alone

Tube No.		1	2	3	4	5	6	7	8	control
Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	-
Bact. susp., 10^{-6}				0.1 ml to each tube						
Incubation period, hours	16	-	-	±	+	3+	3+	3+	3+	3+
	24	2Ag	3+	3+	3+	3+	3+	3+	3+	3+
	48	3Ag	3+	4+	4+	4+	4+	4+	4+	4+
	72	3Ag	4+	4+	4+	4+	4+	4+	4+	4+
	96	3Ag	4+	4+	4+	4+	4+	4+	4+	4+
	120	3Ag	4+	4+	4+	4+	4+	4+	4+	4+
	144	5+	5+	5+	5+	5+	5+	5+	5+	5+
	168	6+	6+	6+	6+	6+	6+	6+	6+	6+

Ag.---------- agglutination of living organism

B. Infected chicken serum plus streptomycin

Tube No.		1	2	3	4	5	6	7	8	control
Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	-
Streptomycin				0.157 mg to each tube						
Bact. susp., 10^{-6}				0.1 ml to each tube						
Incubation period, hours	16	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	-	-	-
	72	-	-	-	-	-	-	-	-	-
	96	-	-	-	-	-	-	-	-	-
	120	-	-	-	-	-	-	-	-	-
	144	-	-	-	-	-	-	-	-	-
	168	-	-	-	-	-	-	-	-	-

Table 3. The effect of age of culture and amount of inoculum on the antibacterial activity of streptomycin

A. Twenty-four-hour culture

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}					0.1 ml to each tube					
	16	-	-	-	-	4+	4+	4+	4+	4+
	24	-	-	-	2+	4+	4+	4+	4+	4+
Incubation	48	-	-	-	3+	4+	4+	4+	4+	4+
period,	72	-	-	3+	4+	4+	4+	4+	4+	4+
hours	96	-	-	3+	4+	4+	4+	4+	4+	4+
	120	-	-	4+	4+	4+	4+	4+	4+	4+
	144	-	-	5+	5+	5+	5+	5+	5+	5+
	168	-	-	5+	5+	5+	5+	5+	5+	5+
	T ------thousands									
Bact. susp., 10^{-6}					0.5 ml to each tube					
	16	-	-	-	-	4+	4+	4+	4+	4+
	24	-	-	-	4+	4+	4+	4+	4+	4+
Incubation	48	-	-	4+	4+	4+	4+	4+	4+	4+
Period,	72	-	-	4+	4+	4+	4+	4+	4+	4+
hours	96	-	-	4+	4+	4+	4+	4+	4+	4+
	120	-	-	4+	4+	4+	4+	4+	4+	4+
	144	-	-	5+	5+	5+	5+	5+	5+	5+
	168	-	-	5+	5+	5+	5+	5+	5+	5+
Bact. susp., 10^{-6}					1.0 ml to each tube					
	16	-	-	-	-	4+	4+	4+	4+	4+
	24	-	-	-	4+	4+	4+	4+	4+	4+
Incubation	48	-	-	4+	4+	4+	4+	4+	4+	4+
period,	72	-	-	4+	4+	4+	4+	4+	4+	4+
hours	96	-	-	4+	4+	4+	4+	4+	4+	4+
	120	-	-	4+	4+	4+	4+	4+	4+	4+
	144	-	-	5+	5+	5+	5+	5+	5+	5+
	168	-	-	5+	5+	5+	5+	5+	5+	5+

Table 3. The effect of age of culture and amount of inoculum on the antibacterial activity of streptomycin (continued).

B. Forty-eight-hour culture

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}		0.1 ml to each tube								
	16	-	-	-	-	3+	4+	4+	4+	4+
	24	-	-	-	-	4+	4+	4+	4+	4+
Incubation	48	-	-	-	4+	4+	4+	4+	4+	4+
period,	72	-	-	-	4+	4+	4+	4+	4+	4+
hours	96	-	-	-	4+	4+	4+	4+	4+	4+
	120	-	-	-	4+	4+	4+	4+	4+	4+
	144	-	-	-	5+	5+	5+	5+	5+	5+
	168	-	-	-	5+	5+	5+	5+	5+	5+
Bact. susp., 10^{-6}		0.5 ml to each tube								
	16	-	-	-	-	3+	4+	4+	4+	4+
	24	-	-	-	3+	4+	4+	4+	4+	4+
Incubation	48	-	-	-	4+	4+	4+	4+	4+	4+
period,	72	-	-	3+	4+	4+	4+	4+	4+	4+
hours	96	-	-	4+	4+	4+	4+	4+	4+	4+
	120	-	-	4+	4+	4+	4+	4+	4+	4+
	144	-	-	5+	5+	5+	5+	5+	5+	5+
	168	-	-	5+	5+	5+	5+	5+	5+	5+
Bact. susp., 10^{-6}		1.0 ml to each tube								
	16	-	-	-	-	4+	4+	4+	4+	4+
	24	-	-	-	4+	4+	4+	4+	4+	4+
Incubation	48	-	-	+	4+	4+	4+	4+	4+	4+
period,	72	-	-	4+	4+	4+	4+	4+	4+	4+
hours	96	-	-	4+	4+	4+	4+	4+	4+	4+
	120	-	-	4+	4+	4+	4+	4+	4+	4+
	144	-	-	5+	5+	5+	5+	5+	5+	5+
	168	-	-	5+	5+	5+	5+	5+	5+	5+

Table 3. The effect of age of culture and amount of inoculum on the antibacterial activity of streptomycin (continued)

C. Seventy-two-hour culture

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}					0.1 ml to each tube					
	16	-	-	-	-	-	4+	4+	4+	4+
	24	-	-	-	-	4+	4+	4+	4+	4+
Incubation	48	-	-	-	-	4+	4+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+	4+
	144	-	-	-	-	5+	5+	5+	5+	5+
	168	-	-	-	-	5+	5+	5+	5+	5+
Bact. susp., 10^{-6}					0.5 ml to each tube					
	16	-	-	-	-	-	4+	4+	4+	4+
	24	-	-	-	-	4+	4+	4+	4+	4+
Incubation	48	-	-	-	2+	4+	4+	4+	4+	4+
period,	72	-	-	-	3+	4+	4+	4+	4+	4+
hours	96	-	-	-	3+	4+	4+	4+	4+	4+
	120	-	-	-	4+	4+	4+	4+	4+	4+
	144	-	-	-	4+	5+	5+	5+	5+	5+
	168	-	-	-	4+	5+	5+	5+	5+	5+
Bact. susp., 10^{-6}					1.0 ml to each tube					
	16	-	-	-	-	-	4+	4+	4+	4+
	24	-	-	-	-	4+	4+	4+	4+	4+
Incubation	48	-	-	-	2+	4+	4+	4+	4+	4+
period,	72	-	-	-	3+	4+	4+	4+	4+	4+
hours	96	-	-	-	4+	4+	4+	4+	4+	4+
	120	-	-	-	4+	4+	4+	4+	4+	4+
	144	-	-	-	5+	5+	5+	5+	5+	5+
	168	-	-	-	5+	5+	5+	5+	5+	5+

Table 3. The effect of age of culture and amount of inoculum on the antibacterial activity of streptomycin (continued)

D. Ninty-six-hour culture

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}		0.1 ml to each tube								
	16	-	-	-	-	-	3+	4+	4+	4+
	24	-	-	-	-	4+	4+	4+	4+	4+
Incubation	48	-	-	-	-	4+	4+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+	4+
	144	-	-	-	-	5+	5+	5+	5+	5+
	168	-	-	-	-	5+	5+	5+	5+	5+

E. One-hundred-twenty-hour culture

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}		0.1 ml to each tube								
	16	-	-	-	-	-	-	4+	4+	4+
	24	-	-	-	-	4+	4+	4+	4+	4+
Incubation	48	-	-	-	-	4+	4+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+	4+
	144	-	-	-	-	5+	5+	5+	5+	5+
	168	-	-	-	-	5+	5+	5+	5+	5+

Table 3. The effect of age of culture and amount of inoculum on the antibacterial activity of streptomycin (continued)

F. The bactericidal titers of streptomycin for various ages and amounts of cultures

Age of culture	Amount of inoculum	Bactericidal titer	Amount of inoculum	Bactericidal titer
hours	ml	dilution	ml	dilution
24	0.1	1:32T	0.5 & 1.0	1:32T
48	"	1:64T	"	1:32T
72	"	1:128T	"	1:64T
96	"	1:128T	-	-
120	"	1:128T	-	-

Table 4. The effect of pH on the antibacterial activity of streptomycin

A. At pH 4 and pH 10

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10-6		0.1 ml to each tube								
Incubation period, hours	24	-	-	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	-	-	-
	72	-	-	-	-	-	-	-	-	-
	96	-	-	-	-	-	-	-	-	-
	120	-	-	-	-	-	-	-	-	-
	144	-	-	-	-	-	-	-	-	-
	168	-	-	-	-	-	-	-	-	-

B. At pH 5

Incubation period, hours	24	2+	2+	3+	3+	3+	3+	3+	3+	3+
	48	3+	3+	5+	5+	5+	5+	5+	5+	5+
	72	4+	4+	5+	5+	5+	5+	5+	5+	5+
	96	4+	5+	5+	5+	5+	5+	5+	5+	5+
	120	5+	5+	6+	6+	6+	6+	6+	6+	6+
	144	5+	5+	6+	6+	6+	6+	6+	6+	6+
	168	5+	5+	6+	6+	6+	6+	6+	6+	6+

C. At pH 6

Incubation period, hours	24	-	-	-	2+	4+	4+	4+	4+	4+
	48	-	-	3+	5+	5+	5+	5+	5+	5+
	72	-	-	4+	5+	5+	5+	5+	5+	5+
	96	-	5+	5+	5+	5+	5+	5+	5+	5+
	120	5+	5+	5+	5+	5+	5+	5+	5+	5+
	144	5+	5+	5+	6+	6+	6+	6+	6+	6+
	168	5+	5+	5+	6+	6+	6+	6+	6+	6+

Table 4. The effect of pH on the antibacterial activity of streptomycin (continued)

D. At pH 6.9

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10-6		0.1 ml to each tube								
Incubation period, hours	24	-	-	-	-	+	4+	4+	4+	4+
	48	-	-	-	-	5+	5+	5+	5+	5+
	72	-	-	-	4+	5+	5+	5+	5+	5+
	96	-	-	-	4+	5+	5+	5+	5+	5+
	120	-	-	-	5+	6+	6+	6+	6+	6+
	144	-	-	-	5+	6+	6+	6+	6+	6+
	168	-	-	-	5+	6+	6+	6+	6+	6+

E. At pH 8

Incubation period, hours	24	-	-	-	-	-	2+	2+	2+	2+
	48	-	-	-	-	4+	4+	4+	4+	4+
	72	-	-	-	-	4+	4+	4+	4+	4+
	96	-	-	-	4+	4+	4+	4+	4+	4+
	120	-	-	-	4+	4+	4+	4+	4+	4+
	144	-	-	-	5+	5+	5+	5+	5+	5+
	168	-	-	-	5+	5+	5+	5+	5+	5+

F. At pH 9

Incubation period, hours	24	-	-	-	-	-	+	+	+	+
	48	-	-	-	2+	4+	4+	4+	4+	4+
	72	-	-	3+	4+	4+	4+	4+	4+	4+
	96	-	-	4+	4+	4+	4+	4+	4+	4+
	120	-	-	4+	4+	4+	4+	4+	4+	4+
	144	-	-	5+	5+	5+	5+	5+	5+	5+
	168	-	-	5+	5+	5+	5+	5+	5+	5+

Fig. for table 4

Effect of pH on the antibacterial activity of streptomycin against S. pullorum

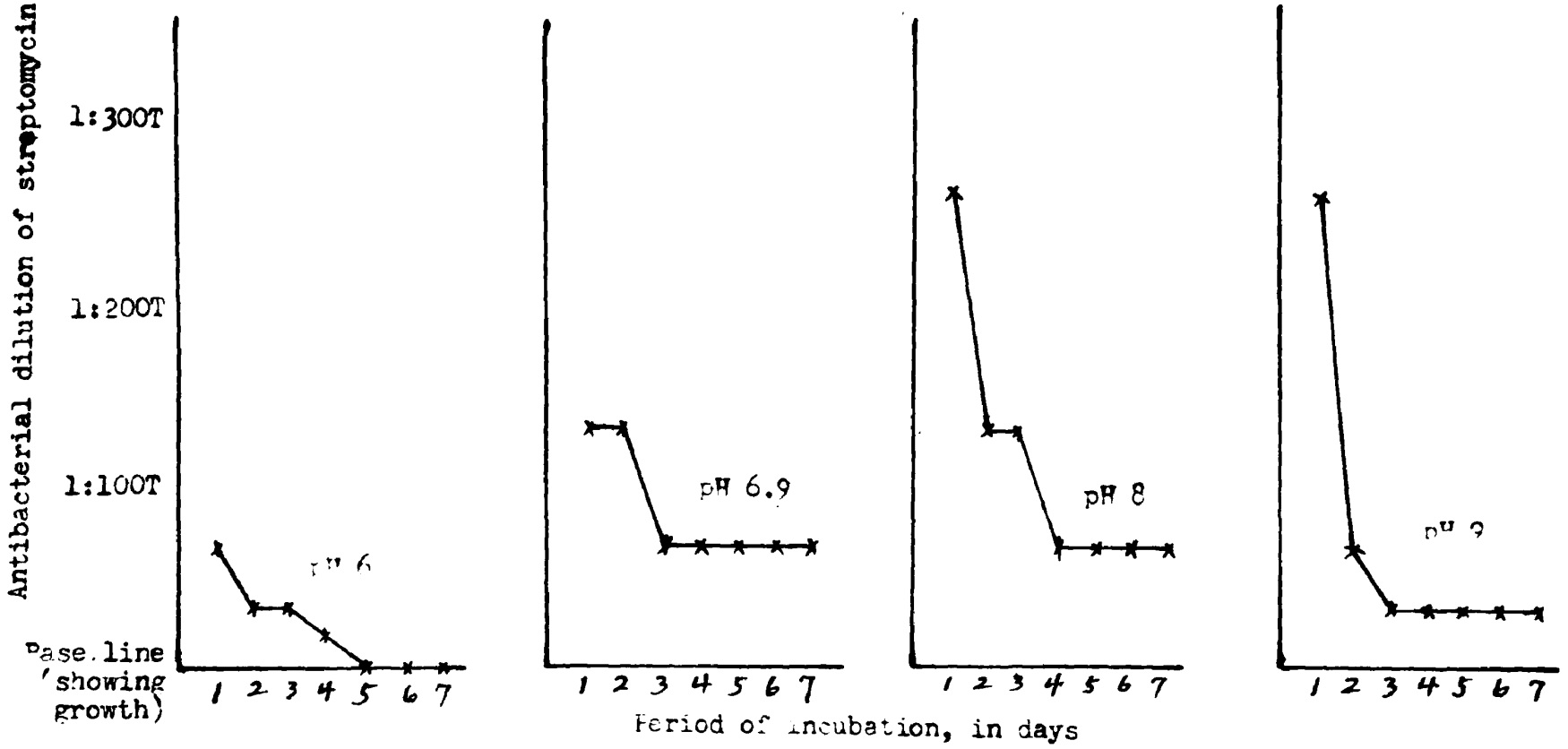

Table 5. The effect of some sugars on the antibacterial action of streptomycin

A. Streptomycin alone

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}		0.1 ml to each tube								
Incubation period, hours	24	-	-	-	-	+	4+	4+	4+	4+
	48	-	-	-	-	5+	5+	5+	5+	5+
	72	-	-	-	4+	5+	5+	5+	5+	5+
	96	-	-	-	4+	5+	5+	5+	5+	5+
	120	-	-	-	5+	6+	6+	6+	6+	6+
	144	-	-	-	5+	6+	6+	6+	6+	6+
	168	-	-	-	5+	6+	6+	6+	6+	6+

B. Streptomycin plus glucose (1% in medium)

Incubation period, hours	24	-	-	-	-	5+	5+	5+	5+	5+
	48	-	-	-	5+	5+	5+	5+	5+	5+
	72	-	-	5+	6+	6+	6+	6+	6+	6+
	96	-	-	6+	6+	6+	6+	6+	6+	6+
	120	-	-	6+	6+	6+	6+	6+	6+	6+
	144	-	-	6+	6+	6+	6+	6+	6+	6+
	168	-	-	6+	6+	6+	6+	6+	6+	6+

C. Streptomycin plus mannite (1% in medium)

Incubation period, hours	24	-	-	-	-	-	-	5+	5+	5+
	48	-	-	-	-	-	5+	5+	5+	5+
	72	-	-	-	-	-	6+	6+	6+	6+
	96	-	-	-	-	-	6+	6+	6+	6+
	120	-	-	-	-	-	6+	6+	6+	6+
	144	-	-	-	-	-	6+	6+	6+	6+
	168	-	-	-	-	-	6+	6+	6+	6+

Table 5. The effect of some sugars on the antibacterial action of streptomycin (continued)

D. Streptomycin plus lactose (1% in medium)

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}					0.1 ml to each tube					
Incubation period, hours	24	-	-	-	-	-	4+	4+	4+	4+
	48	-	-	-	-	5+	5+	5+	5+	5+
	72	-	-	-	-	5+	5+	5+	5+	5+
	96	-	-	-	-	5+	5+	5+	5+	5+
	120	-	-	-	-	5+	5+	5+	5+	5+
	144	-	-	-	-	6+	6+	6+	6+	6+
	168	-	-	-	-	6+	6+	6+	6+	6+

E. Streptomycin plus sucrose (1% in medium)

Incubation period, hours	24	-	-	-	-	-	4+	4+	4+	4+
	48	-	-	-	-	5+	5+	5+	5+	5+
	72	-	-	-	3+	5+	5+	5+	5+	5+
	96	-	-	-	5+	6+	6+	6+	6+	6+
	120	-	-	-	5+	6+	6+	6+	6+	6+
	144	-	-	-	6+	6+	6+	6+	6+	6+
	168	-	-	-	6+	6+	6+	6+	6+	6+

F. The change in pH value of the sugar media after incubation With S. pullorum

Media	Before incubation	After incubation	
		Without S. pullorum	With S. pullorum
	pH	pH	pH
Tryptose broth	6.9	6.9	7.0
Tryptose broth plus glucose	6.9	6.5	4.7
Tryptose broth plus mannite	6.9	6.9	5.6
Tryptose broth plus lactose	6.9	6.7	7.0
Tryptose broth plus sucrose	6.9	6.9	7.2

Fig. for table 5

Effect of some sugars on the antibacterial activity of streptomycin

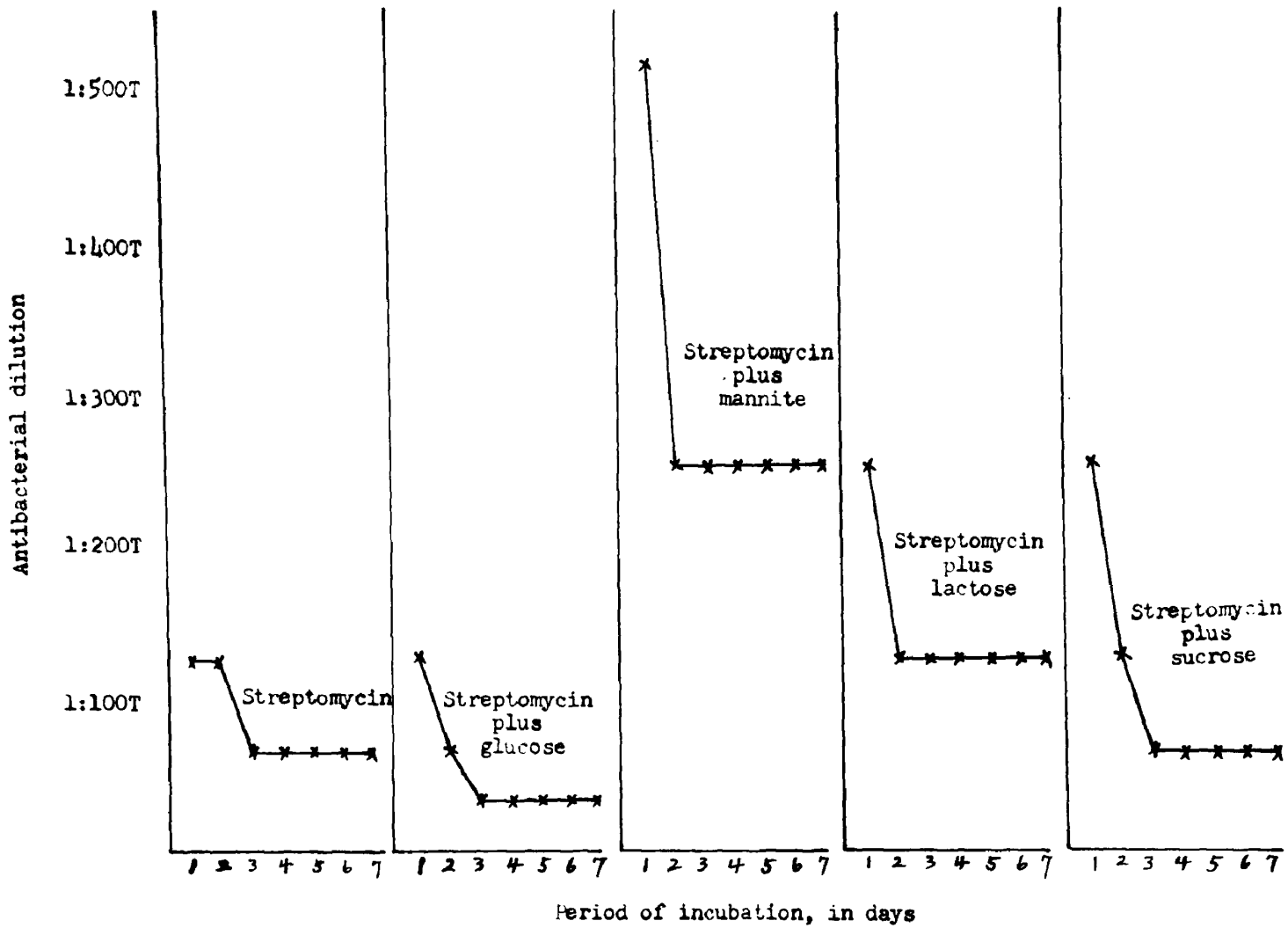

Table 6. The effect of some reducing agents on the antibacterial activity of streptomycin

A. Streptomycin alone

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}					0.1 ml to each tube					
	16	-	-	-	-	-	-	+	3+	3+
	24	-	-	-	-	-	+	3+	3+	3+
Incubation	48	-	-	-	-	4+	4+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+	4+
	144	-	-	-	-	4+	4+	4+	4+	4+
	168	-	-	-	-	4+	4+	4+	4+	4+

B. Streptomycin plus 5 mg of ascorbic acid

Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Ascorbic acid				5.0 mg to each tube						
Bact. susp., 10^{-6}					0.1 ml to each tube					
	16	-	-	-	-	-	-	-	-	-
	24	-	-	2+	3+	3+	3+	3+	3+	3+
Incubation	48	+	4+	4+	4+	4+	4+	4+	4+	4+
period,	72	5+	5+	5+	5+	5+	5+	5+	5+	5+
hours	96	5+	5+	5+	5+	5+	5+	5+	5+	5+
	120	5+	5+	5+	5+	5+	5+	5+	5+	5+
	144	5+	5+	5+	5+	5+	5+	5+	5+	5+
	168	5+	5+	5+	5+	5+	5+	5+	5+	5+

Table 6. The effect of some reducing agents on the antibacterial activity of streptomycin (continued)

C. Streptomycin plus 0.5 mg of ascorbic acid

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Ascorbic acid				0.5 mg to each tube						
Bact. susp., 10^{-6}				0.1 ml to each tube						
Incubation period, hours	16	-	-	-	-	-	+	+	+	+
	24	-	-	-	-	2+	3+	3+	3+	3+
	48	-	-	-	4+	4+	4+	4+	4+	4+
	72	-	-	-	4+	4+	4+	4+	4+	4+
	96	-	-	4+	4+	4+	4+	4+	4+	4+
	120	-	-	4+	4+	4+	4+	4+	4+	4+
	144	-	-	4+	4+	4+	4+	4+	4+	4+
	168	-	-	4+	4+	4+	4+	4+	4+	4+

D. Ascorbic acid alone

Tube No.		1	2	3	4	5	6	7	8
Ascorbic Acid *		1:T	1:2T	1:4T	1:8T	1:16T	1:32T	1:64T	1:128T
Bact. susp., 10^{-6}				0.1 ml to each tube					
Incubation period, hours	16	-	-	-	2+	3+	3+	3+	3+
	24	2+	2+	3+	3+	3+	3+	3+	3+
	48	4+	4+	4+	4+	4+	4+	4+	4+
	72	4+	4+	4+	4+	4+	4+	4+	4+
	96	5+	5+	5+	5+	5+	5+	5+	5+
	120	5+	5+	5+	5+	5+	5+	5+	5+
	144	5+	5+	5+	5+	5+	5+	5+	5+
	168	5+	5+	5+	5+	5+	5+	5+	5+

*Dilution of 1:T = 5 mg in 5 ml of medium, and so on

Table 6. The effect of some reducing agents on the antibacterial activity of streptomycin (continued)

E. Streptomycin plus 20 mg of sodium thiosulfate

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Sod. thiosulfate		20.0 mg to each tube								
Bact. susp., 10^{-6}		0.1 ml to each tube								
	16	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	2+	3+	3+	3+
Incubation	48	-	-	-	-	4+	4+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+	4+
	144	-	-	-	-	4+	4+	4+	4+	4+
	168	-	-	-	-	4+	4+	4+	4+	4+

F. Streptomycin plus 10 mg of sodium thiosulfate

Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	control
Sod. thiosulfate		10.0 mg to each tube								
Bact. susp., 10^{-6}		0.1 ml to each tube								
	16	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	+	+	+	+
Incubation	48	-	-	-	-	-	4+	4+	4+	4+
period,	72	-	-	-	-	-	4+	4+	4+	4+
hours	96	-	-	-	-	-	4+	4+	4+	4+
	120	-	-	-	-	-	4+	4+	4+	4+
	144	-	-	-	-	-	4+	4+	4+	4+
	168	-	-	-	-	-	4+	4+	4+	4+

Table 6. The effect of some reducing agents on the antibacterial activity of streptomycin (continued)

G. Sodium thiosulfate alone

Tube No.		1	2	3	4	5	6	7	8
Sod. thiosulfate		1:200*	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600
Bact. susp., 10-6		0.1 ml to each tube							
Incubation period, hours	16	-	-	-	+	2+	3+	3+	3+
	24	3+	3+	3+	3+	3+	3+	3+	3+
	48	4+	4+	4+	4+	4+	4+	4+	4+
	72	4+	4+	4+	4+	4+	4+	4+	4+
	96	4+	4+	4+	4+	4+	4+	4+	4+
	120	4+	4+	4+	4+	4+	4+	4+	4+
	144	4+	4+	4+	4+	4+	4+	4+	4+
	168	4+	4+	4+	4+	4+	4+	4+	4+

* Dilution of 1:200 = 25 mg in 5 ml of medium, and so on

Fig. for table 6

Effect of some reducing agents on the antibacterial activity of streptomycin

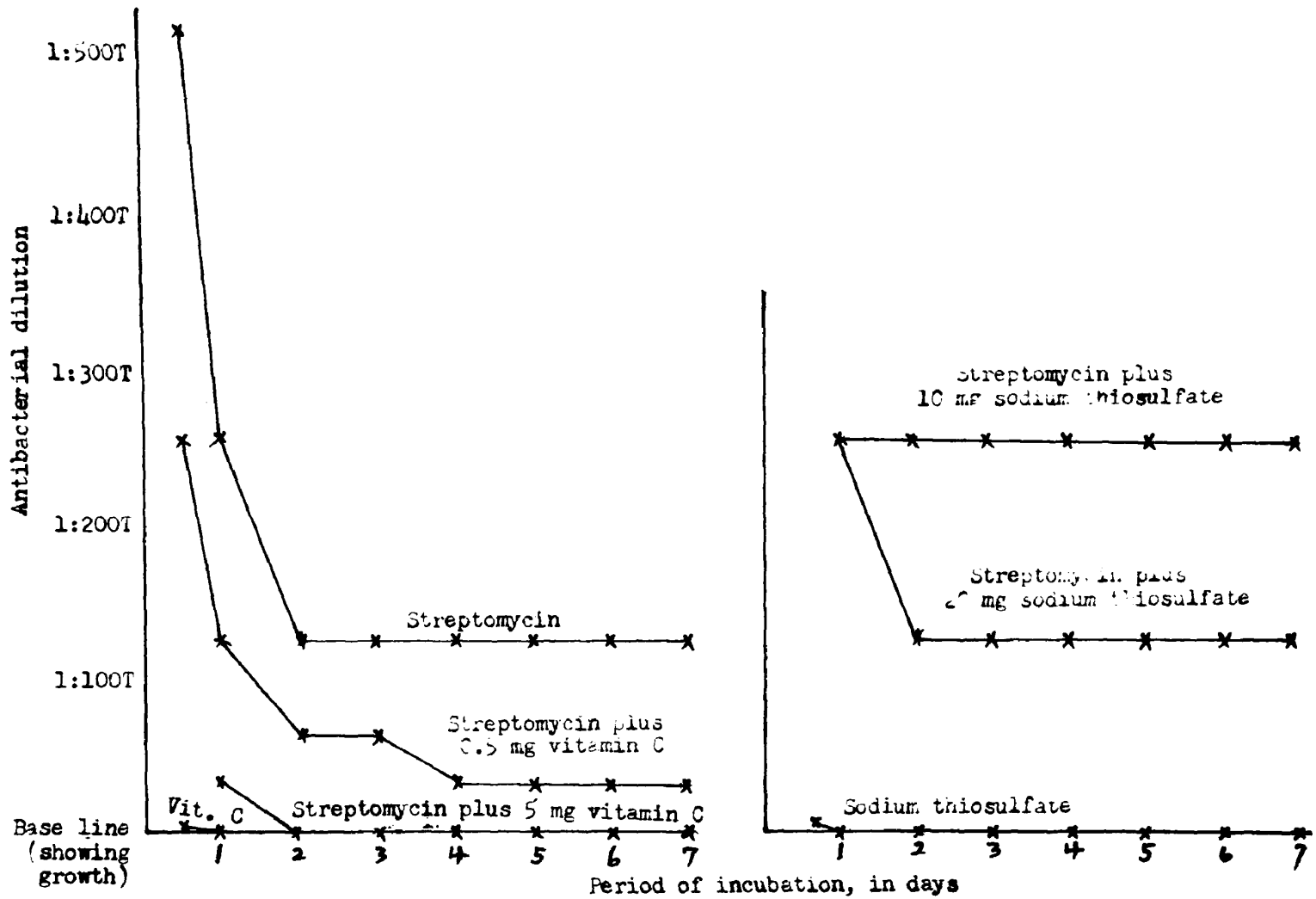

Table 7. The effect of some dyes on the antibacterial action of streptomycin

A. Streptomycin alone

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Bact. susp., 10^{-6}					0.1 ml to each tube					
	16	-	-	-	-	-	-	+	3+	3+
	24	-	-	-	-	-	+	3+	3+	3+
Incubation	48	-	-	-	-	4+	4+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+	4+
	144	-	-	-	-	4+	4+	4+	4+	4+
	168	-	-	-	-	4+	4+	4+	4+	4+

B. Streptomycin plus 0.03 mg of crystal violet

Tube No.		1	2	3	4	5	6	7	8	9	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	1:4096T	-
Crystal violet					0.03 mg to each tube						
Bact. susp., 10^{-6}					0.1 ml to each tube						
	16	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-
Incubation	48	-	-	-	-	-	-	-	-	-	4+
period,	72	-	-	-	-	-	-	-	-	4+	4+
hours	96	-	-	-	-	-	-	-	-	4+	4+
	120	-	-	-	-	-	-	-	-	4+	4+
	144	-	-	-	-	-	-	-	-	4+	4+
	168	-	-	-	-	-	-	-	-	4+	4+

Table 7. The effect of some dyes on the antibacterial action of streptomycin (continued)

C. Crystal violet alone

Tube No.		1	2	3	4	5	6	7	8
Crystal violet		*1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T
Bact. susp., 10^{-6}		0.1 ml to each tube							
	16	-	-	-	-	-	-	+	2+
	24	-	-	-	-	-	3+	3+	3+
Incubation	48	-	-	-	-	4+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+
	144	-	-	-	-	4+	4+	4+	4+
	168	-	-	-	-	4+	4+	4+	4+

*Dilution of 1:10T = 0.5 mg in 5 ml of medium, and so on

D. Streptomycin plus 0.01 mg of brilliant green

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Brilliant green		0.01 mg to each tube								
Bact. susp., 10^{-6}		0.1 ml to each tube								
	16	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-
Incubation	48	-	-	-	-	-	-	4+	4+	4+
period,	72	-	-	-	-	-	2+	4+	4+	4+
hours	96	-	-	-	-	-	4+	4+	4+	4+
	120	-	-	-	-	3+	4+	4+	4+	4+
	144	-	-	-	-	4+	4+	4+	4+	4+
	168	-	-	-	-	4+	4+	4+	4+	4+

Table 7. The effect of some dyes on the antibacterial action of streptomycin (continued)

E. Streptomycin plus 0.002 mg of brilliant green

Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	-
Brilliant green		0.002 mg to each tube								
Bact. susp., 10^{-6}		0.1 ml to each tube								
	16	-	-	-	-	-	-	2+	3+	3+
	24	-	-	-	-	-	-	3+	3+	3+
Incubation	48	-	-	-	-	2+	3+	4+	4+	4+
period,	72	-	-	-	-	4+	4+	4+	4+	4+
hours	96	-	-	-	-	4+	4+	4+	4+	4+
	120	-	-	-	-	4+	4+	4+	4+	4+
	144	-	-	-	-	4+	4+	4+	4+	4+
	168	-	-	-	-	4+	4+	4+	4+	4+

F. Brilliant green alone

Tube No.		1	2	3	4	5	6	7	8
Brilliant green		*1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T
Bact. susp., 10^{-6}		0.1 ml to each tube							
	16	-	-	-	-	-	-	-	+
	24	-	-	-	-	-	-	-	2+
Incubation	48	-	-	-	-	-	-	3+	4+
period,	72	-	-	-	-	-	-	4+	4+
hours	96	-	-	-	-	-	-	4+	4+
	120	-	-	-	-	-	-	4+	4+
	144	-	-	-	-	-	-	4+	4+
	168	-	-	-	-	-	-	4+	4+

*Dilution of 1:10T = 0.5 mg in 5 ml of medium, and so on

Fig. for table 7

Effect of some dyes on the antibacterial activity of streptomycin

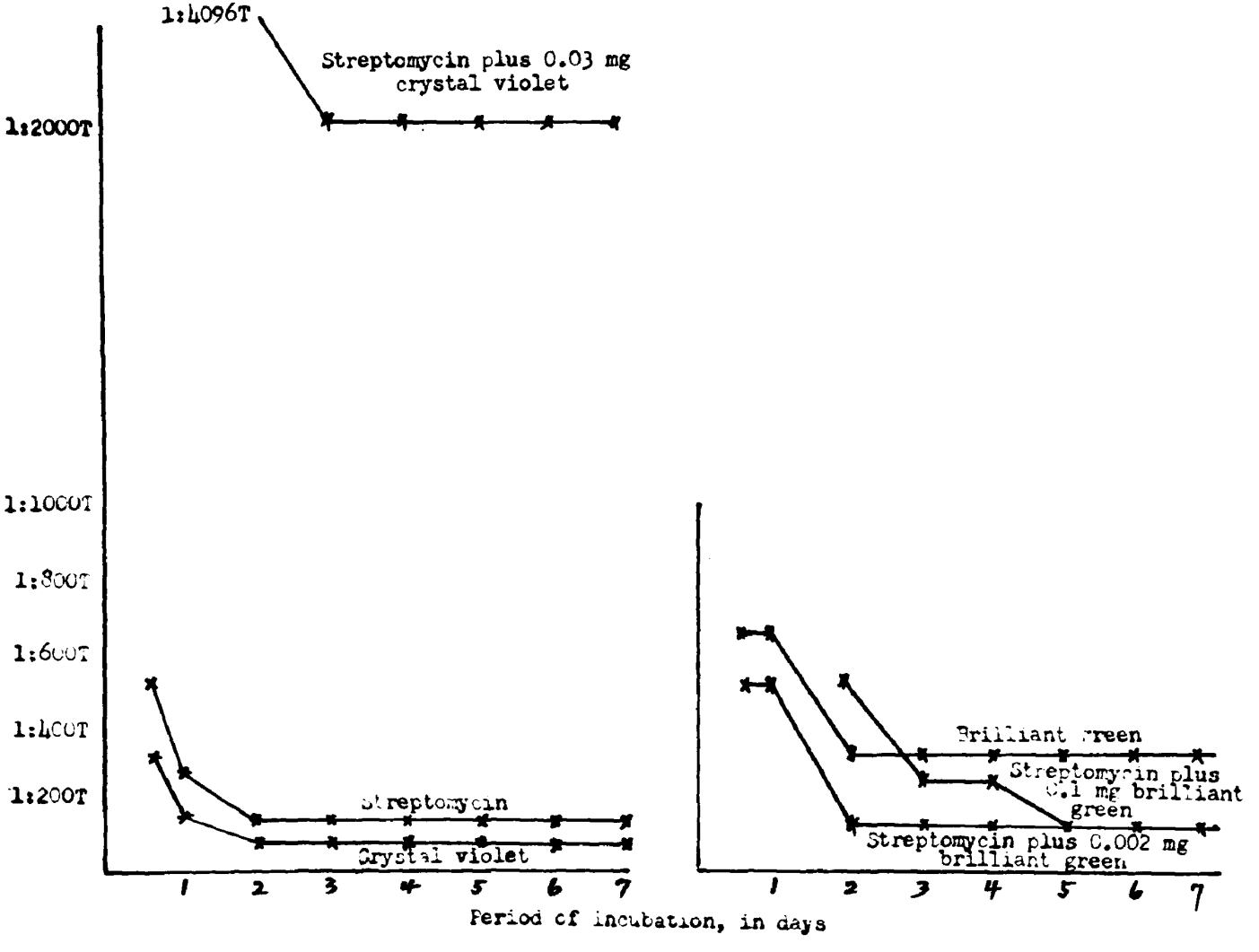

Table 8. The combined action of streptomycin and sulfadiazine

A. Streptomycin alone

Tube No.		1.	2	3	4	5	6	7	8	9	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	1:4096T	-
Bact. susp., 10^{-6}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	3+	3+
	24	-	-	-	-	-	-	-	3+	4+	4+
	48	-	-	-	-	-	-	4+	4+	4+	4+
	72	-	-	-	-	-	-	4+	4+	4+	4+
	96	-	-	-	-	-	5+	5+	5+	5+	5+
	120	-	-	-	-	-	5+	5+	5+	5+	5+
	144	-	-	-	-	-	5+	5+	5+	5+	5+
	168	-	-	-	-	-	5+	5+	5+	5+	5+

B. Streptomycin plus 5 mg of sodium sulfadiazine

Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	1:4096T	-
Sod. sulfadiazine		5.0 mg to each tube									
Bact. susp., 10^{-6}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	-	-	-	2+
	72	-	-	-	-	-	-	-	-	-	2+
	96	-	-	-	-	-	-	-	-	+	3+
	120	-	-	-	-	-	-	-	-	3+	4+
	144	-	-	-	-	-	-	-	-	4+	4+
	168	-	-	-	-	-	-	-	-	5+	5+

Table 8. The combined action of streptomycin and sulfadiazine (continued)

C. Streptomycin plus 0.25 mg of sodium sulfadiazine

Tube No.		1	2	3	4	5	6	7	8	9	control
Streptomycin		1:16T	1:32T	1:64T	1:128T	1:256T	1:512T	1:1024T	1:2048T	1:4096T	-
Sod. sulfadiazine		0.25 mg to each tube									
Bact. susp., 10^{-6}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	
	24	-	-	-	-	-	-	-	-	-	3+
	48	-	-	-	-	-	-	-	3+	3+	3+
	72	-	-	-	-	-	-	-	3+	4+	4+
	96	-	-	-	-	-	-	-	5+	5+	5+
	120	-	-	-	-	-	-	-	5+	5+	5+
	144	-	-	-	-	-	-	-	5+	5+	5+
	168	-	-	-	-	-	-	-	5+	5+	5+

D. Sodium sulfadiazine alone

Tube No.		1	2	3	4	5	6	7	8	9
Sod. sulfadiazine		*1:500	1:T	1:2T	1:4T	1:8T	1:16T	1:32T	1:64T	1:128T
Bact. susp., 10^{-6}		0.1 ml to each tube								
Incubation period, hours	16	-	-	-	-	-	-	-	+	2+
	24	-	-	2+	2+	3+	3+	3+	4+	4+
	48	2+	2+	3+	3+	4+	4+	4+	4+	4+
	72	2+	2+	3+	3+	4+	4+	4+	4+	4+
	96	3+	3+	4+	4+	5+	5+	5+	5+	5+
	120	4+	4+	5+	5+	5+	5+	5+	5+	5+
	144	4+	5+	5+	5+	5+	5+	5+	5+	5+
	168	5+	5+	5+	5+	5+	5+	5+	5+	5+

*Dilution of 1:500 = 10 mg in 5 ml of medium, and so on

Fig. for table 6

The combined action of streptomycin and sodium sulfadiazine

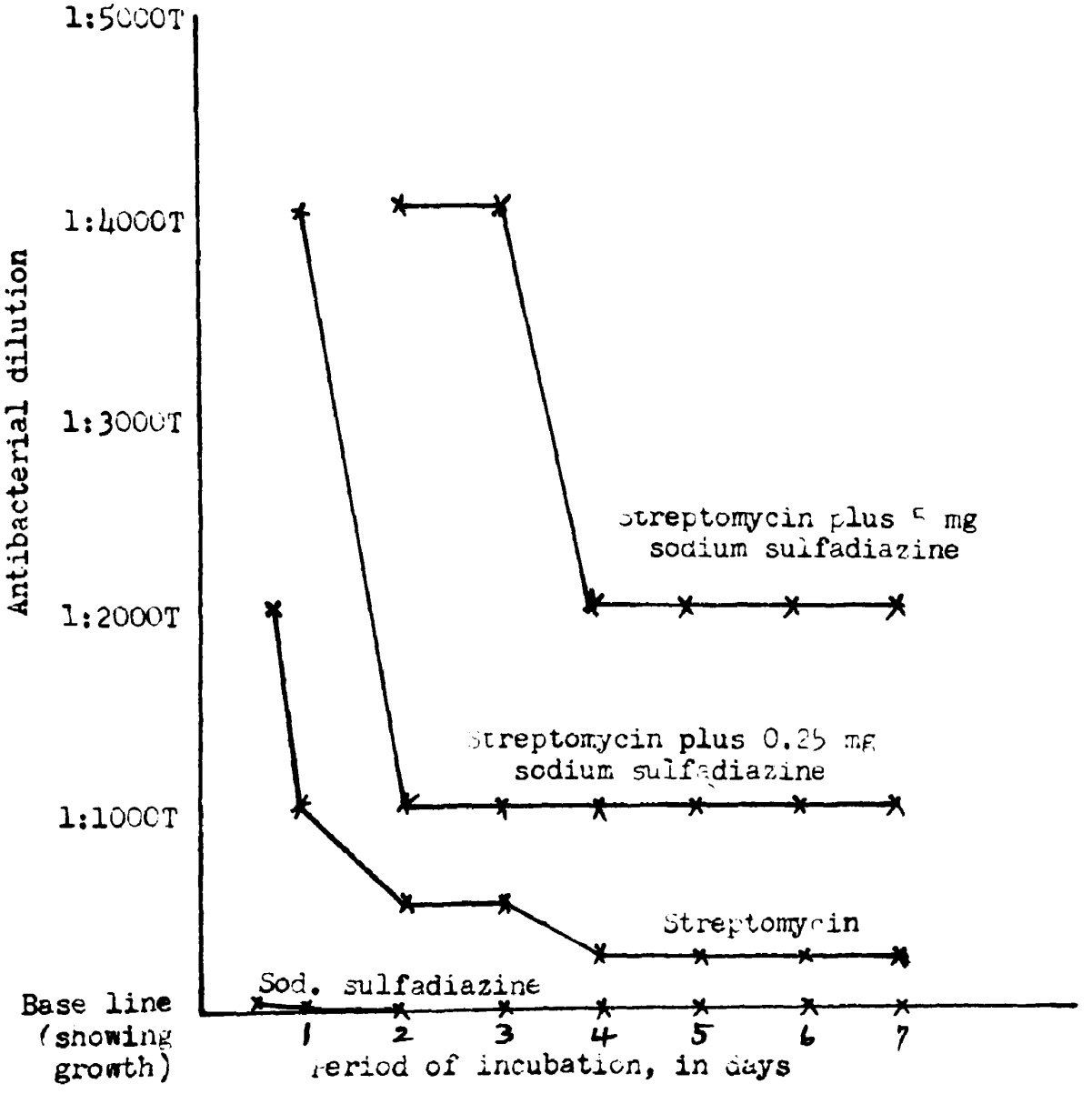

Table 9. The duration of antibacterial activity of streptomycin in the body of infected chickens

Chicken No. 637		A. One hour after injection								
Tube No.		1	2	3	4	5	6	7	8	control
Streptomycin-containing serum		1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	-
Bact. susp., 10^{-6}					0.1 ml to each tube					
Incubation period, hours	16	-	-	±Ag	3Ag	3Ag	2Ag	2Ag	3+	3+
	24	-	-	Ag	3Ag	3Ag	2Ag	2Ag	3+	3+
	48	-	-	4Ag	3Ag	3Ag	4+	4+	4+	4+
	72	-	2Ag	4Ag	3Ag	3Ag	4+	4+	4+	4+
	96	-	5Ag	4Ag	3Ag	3Ag	4+	4+	4+	4+
	120	-	5Ag	4Ag	4Ag	4Ag	4+	4+	4+	4+
		B. Two hours after injection								
Incubation period, hours	16	-	-	3Ag	3Ag	2Ag	2Ag	2Ag	3+	
	24	-	Ag	3Ag	3Ag	2Ag	2Ag	2Ag	3+	
	48	-	4Ag	3Ag	4+	4+	4+	4+	4+	
	72	-	4Ag	3Ag	4+	4+	4+	4+	4+	
	96	3Ag	4Ag	3Ag	4+	4+	4+	4+	4+	
	120	4Ag	4Ag	3Ag	4+	4+	4+	4+	4+	
		C. Three hours after injection								
Incubation period, hours	16	-	Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	
	24	-	3Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	
	48	Ag	3Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	
	72	3Ag	3Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	
	96	4Ag	3Ag	3Ag	3Ag	4+	4+	4+	4+	
	120	4Ag	3Ag	3Ag	4+	4+	4+	4+	4+	
		D. Six hours after injection								
Incubation period, hours	16	3Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag	
	24	3Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag	
	48	4Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag	
	72	4Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag	
	96	4Ag	4Ag	3Ag	3Ag	3Ag	4+	4+	4+	
	120	4Ag	4Ag	4Ag	3Ag	4+	4+	4+	4+	

Table 9. The duration of antibacterial activity of streptomycin in the body of infected chickens (continued)

Chicken No. 612				A. One hour after injection					
Tube No.		1	2	3	4	5	6	7	8
Streptomycin-containing serum		1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Bact. susp., 10^{-6}				0.1 ml to each tube					
Incubation period, hours	16	-	-	±Ag	3Ag	3Ag	2Ag	3+	3+
	24	-	-	Ag	3Ag	3Ag	2Ag	3+	3+
	48	-	-	3Ag	3Ag	4+	4+	4+	4+
	72	-	Ag	3Ag	3Ag	4+	4+	4+	4+
	96	-	3Ag	3Ag	4+	4+	4+	4+	4+
	120	-	4Ag	4+	4+	4+	4+	4+	4+
				B. Two hours after injection					
Incubation period, hours	16	-	-	2Ag	2Ag	2Ag	2+	3+	3+
	24	-	-	3Ag	3Ag	2Ag	3+	3+	3+
	48	-	3Ag	3Ag	4+	4+	4+	4+	4+
	72	2Ag	3Ag	3Ag	4+	4+	4+	4+	4+
	96	4Ag	3Ag	4+	4+	4+	4+	4+	4+
	120	5Ag	4Ag	4+	4+	4+	4+	4+	4+
				C. Three hours after injection					
Incubation period, hours	16	-	-	Ag	3Ag	3Ag	2Ag	2Ag	2+
	24	-	-	3Ag	3Ag	3Ag	2Ag	2Ag	2+
	48	Ag	2Ag	4Ag	3Ag	3Ag	2Ag	3+	3+
	72	2Ag	2Ag	4Ag	3Ag	3Ag	2Ag	3+	3+
	96	4Ag	3Ag	4Ag	3Ag	3Ag	3+	3+	3+
	120	4Ag	3Ag	4Ag	3Ag	4+	4+	4+	4+
				D. Six hours after injection					
Incubation period, hours	16	Ag	2Ag	3Ag	3Ag	2Ag	2Ag	2Ag	2Ag
	24	2Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag	2+
	48	4Ag	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2+
	72	4Ag	4Ag	3Ag	3Ag	3Ag	3Ag	2+	3+
	96	4Ag	4Ag	3Ag	3Ag	3Ag	4+	4+	4+
	120	4Ag	4Ag	3Ag	3Ag	4+	4+	4+	4+

Table 9. The duration of antibacterial activity of streptomycin in the body of infected chickens (continued)

Chicken No. 658		A. One hour after injection							
Tube No.		1	2	3	4	5	6	7	8
Streptomycin-containing serum		1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Bact. susp., 10^{-6}					0.1 ml to each tube				
Incubation period, hours	16	-	-	-	3Ag	3Ag	3Ag	2Ag	2Ag
	24	-	-	Ag	3Ag	3Ag	3Ag	2Ag	2Ag
	48	-	-	4Ag	3Ag	3Ag	3Ag	3Ag	3Ag
	72	-	-	4Ag	3Ag	3Ag	3Ag	3Ag	3Ag
	96	-	3Ag	4Ag	3Ag	3Ag	3Ag	4+	4+
	120	-	4Ag	4+	4+	4+	4+	4+	4+
				B. Two hours after injection					
Incubation period, hours	16	-	-	2Ag	3Ag	3Ag	2Ag	2Ag	2Ag
	24	-	Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag
	48	-	4Ag	3Ag	3Ag	3Ag	3Ag	4+	4+
	72	-	4Ag	3Ag	3Ag	3Ag	4+	4+	4+
	96	-	4Ag	3Ag	4+	4+	4+	4+	4+
	120	-	4Ag	4+	4+	4+	4+	4+	4+
				C. Three hours after injection					
Incubation period, hours	16	-	-	2Ag	3Ag	3Ag	3Ag	3Ag	2Ag
	24	-	Ag	2Ag	3Ag	3Ag	3Ag	3Ag	2Ag
	48	-	3Ag	3Ag	3Ag	3Ag	3Ag	3Ag	2Ag
	72	-	4Ag	3Ag	3Ag	3Ag	3Ag	3Ag	3Ag
	96	-	4Ag	3Ag	3Ag	3Ag	4+	4+	4+
	120	-	4Ag	3Ag	3Ag	3Ag	4+	4+	4+
				D. Six hours after injection					
Incubation period, hours	16	-	Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag
	24	-	3Ag	3Ag	3Ag	3Ag	2Ag	2Ag	2Ag
	48	Ag	4Ag	4Ag	3Ag	3Ag	2Ag	2Ag	2Ag
	72	2Ag	4Ag	4Ag	4Ag	3Ag	2Ag	2Ag	2Ag
	96	4Ag	4Ag	4Ag	3Ag	3Ag	3Ag	4+	4+
	120	4Ag	4Ag	4Ag	3Ag	3Ag	4+	4+	4+

Table 10. The duration of antibacterial activity of streptomycin in the body of infected turkeys

Turkey No. 2664		A. One hour after injection					
Tube No.		1	2	3	4	5	6
Streptomycin-containing serum		1:5	1:10	1:20	1:40	1:80	1:160
Bact. susp., 10^{-6}		0.1 ml to each tube					
Incubation period, hours	16	-	-	-	3Ag	3Ag	3Ag
	24	-	-	2Ag	3Ag	3Ag	3Ag
	48	-	3Ag	3Ag	3Ag	3Ag	3Ag
	72	-	4Ag	4Ag	4Ag	4Ag	4Ag
	96	-	4Ag	4Ag	4Ag	4Ag	4Ag
	120	-	5Ag	5Ag	5Ag	5Ag	5Ag
		B. Two hours after injection					
Incubation period, hours	16	-	-	Ag	2Ag	3Ag	3Ag
	24	-	-	3Ag	3Ag	3Ag	3Ag
	48	-	3Ag	3Ag	3Ag	3Ag	3Ag
	72	-	4Ag	4Ag	4Ag	4Ag	4Ag
	96	-	4Ag	4Ag	4Ag	4Ag	4Ag
	120	-	5Ag	5Ag	5Ag	5Ag	5Ag
		C. Three hours after injection					
Incubation period, hours	16	-	-	2Ag	3Ag	3Ag	3Ag
	24	-	2Ag	3Ag	3Ag	3Ag	3Ag
	48	2Ag	3Ag	3Ag	3Ag	3Ag	3Ag
	72	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	96	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	120	4Ag	5Ag	5Ag	5Ag	5Ag	5Ag
		D. Six hours after injection					
Incubation period, hours	16	-	3Ag	3Ag	3Ag	3Ag	3Ag
	24	-	4Ag	4Ag	4Ag	4Ag	4Ag
	48	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	72	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	96	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	120	4Ag	5Ag	5Ag	5Ag	5Ag	5Ag

Table 10. The duration of antibacterial activity of streptomycin in the body of infected turkeys (continued)

Turkey No. 2651

A. One hour after injection

Tube No.		1	2	3	4	5	6
Streptomycin-containing serum		1:5	1:10	1:20	1:40	1:80	1:160
Bact. susp., 10^{-6}			0.1 ml to each tube				
Incubation period, hours	16	-	-	-	3Ag	3Ag	3Ag
	24	-	-	2Ag	3Ag	3Ag	3Ag
	48	-	3Ag	3Ag	3Ag	3Ag	3Ag
	72	-	4Ag	4Ag	4Ag	4Ag	4Ag
	96	-	4Ag	4Ag	4Ag	4Ag	4Ag
	120	-	5Ag	5Ag	5Ag	5Ag	5Ag
B. Two hours after injection							
Incubation period, hours	16	-	2Ag	2Ag	3Ag	3Ag	3Ag
	24	-	3Ag	3Ag	3Ag	3Ag	3Ag
	48	-	4Ag	4Ag	4Ag	4Ag	4Ag
	72	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	96	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	120	4Ag	5Ag	5Ag	5Ag	5Ag	5Ag
C. Three hours after incubation							
Incubation period, hours	16	-	2Ag	2Ag	3Ag	3Ag	3Ag
	24	-	3Ag	3Ag	3Ag	3Ag	3Ag
	48	3Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	72	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	96	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	120	4Ag	5Ag	5Ag	5Ag	5Ag	5Ag
D. Six hours after injection							
Incubation period, hours	16	-	3Ag	3Ag	3Ag	3Ag	3Ag
	24	-	4Ag	4Ag	4Ag	4Ag	4Ag
	48	3Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	72	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	96	4Ag	4Ag	4Ag	4Ag	4Ag	4Ag
	120	5Ag	5Ag	5Ag	5Ag	5Ag	5Ag

Fig. A-1 The course of agglutination titer

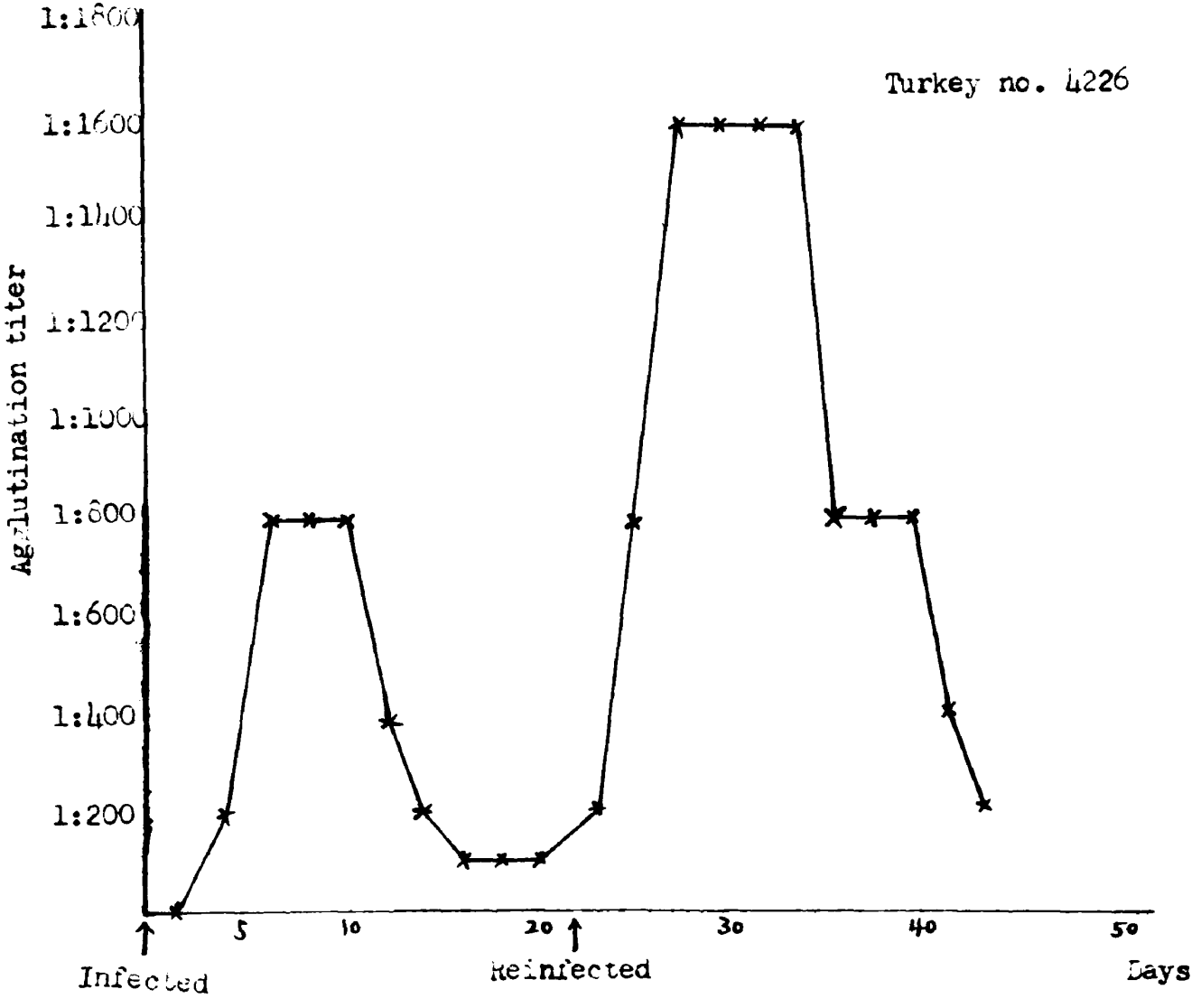

Fig. A-2 The course of agglutination titer

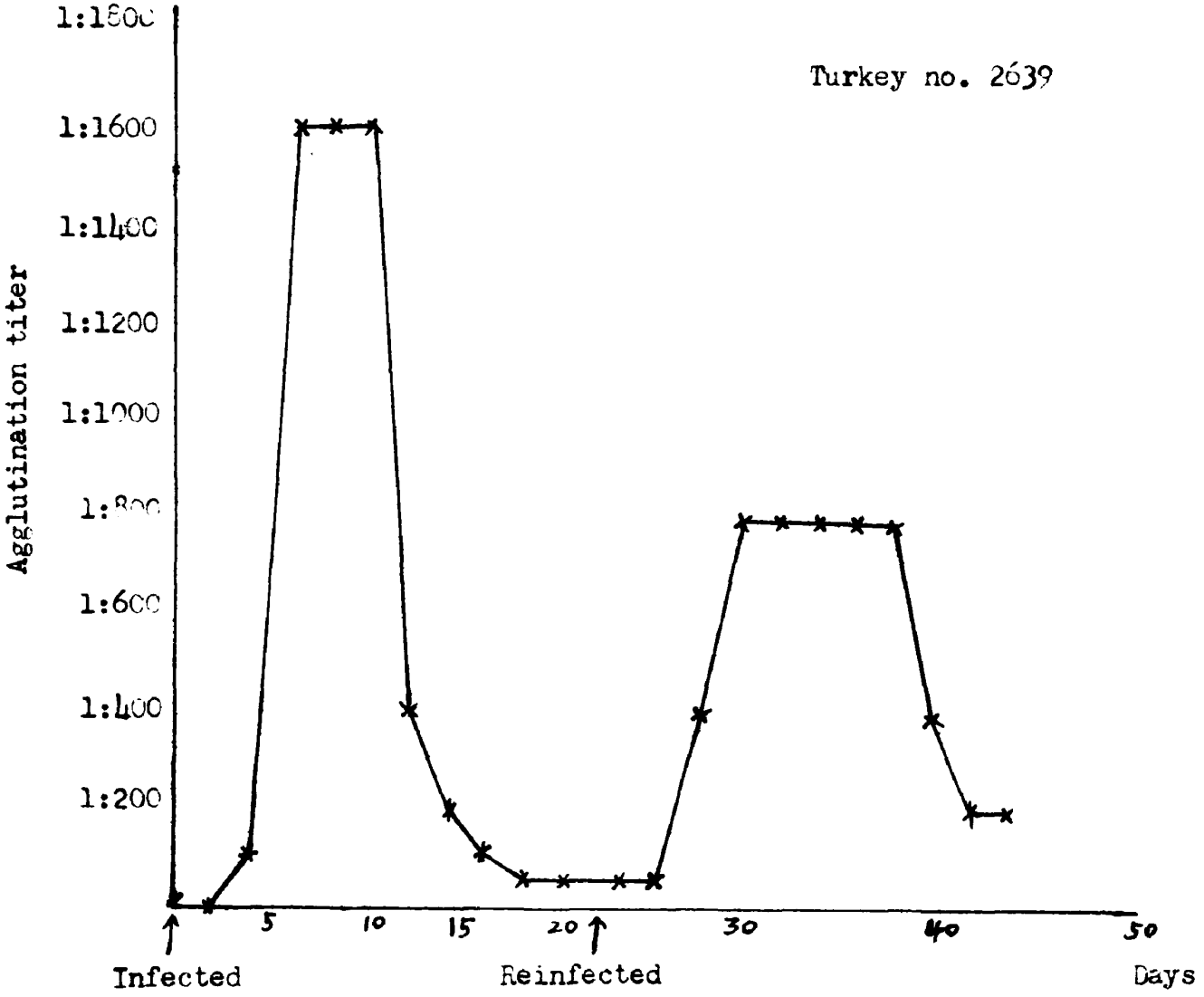

Fig. A-3 The effect of streptomycin on the course of agglutination titer

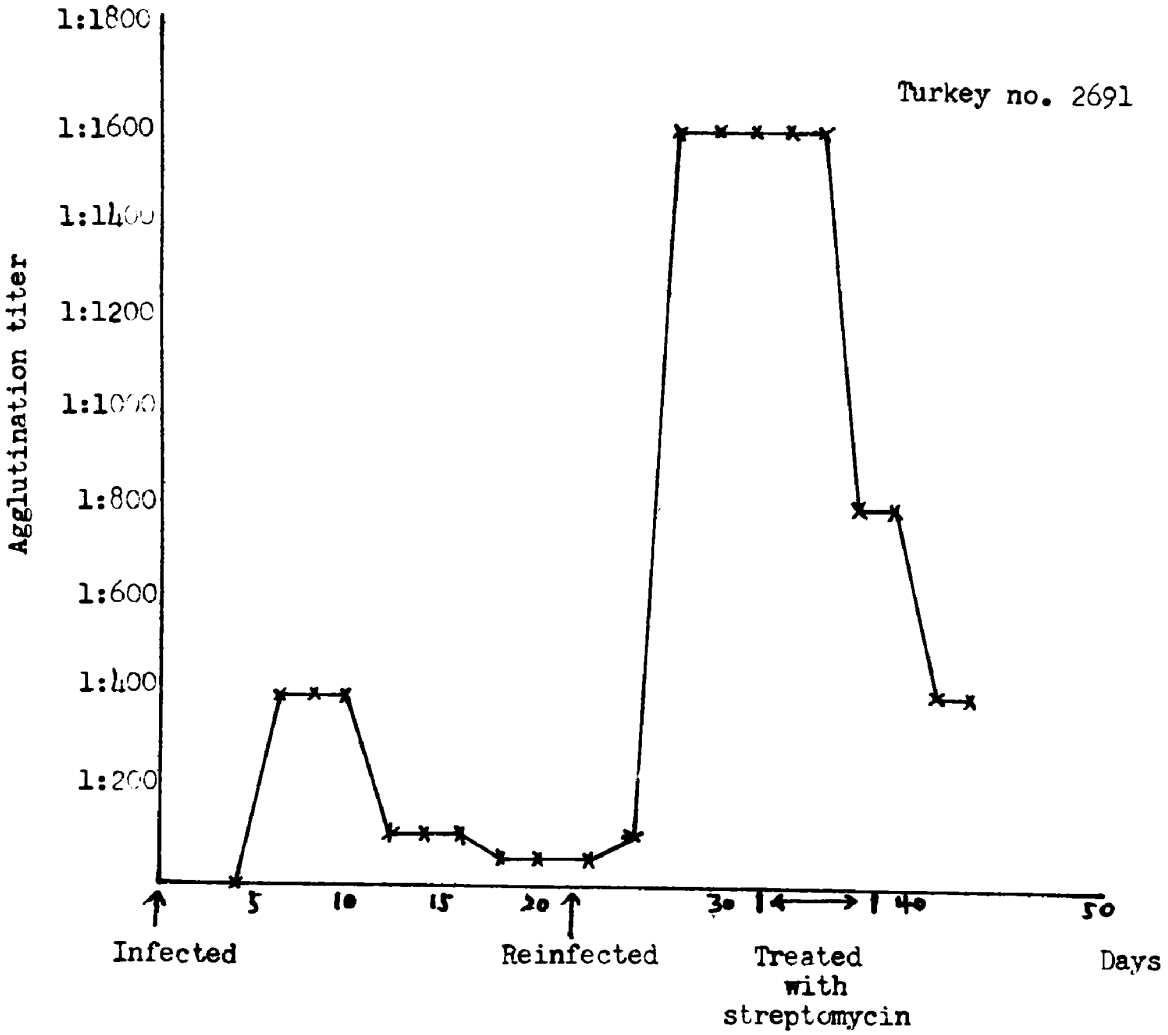

Fig. A-4 The effect of streptomycin on the course of agglutination titer

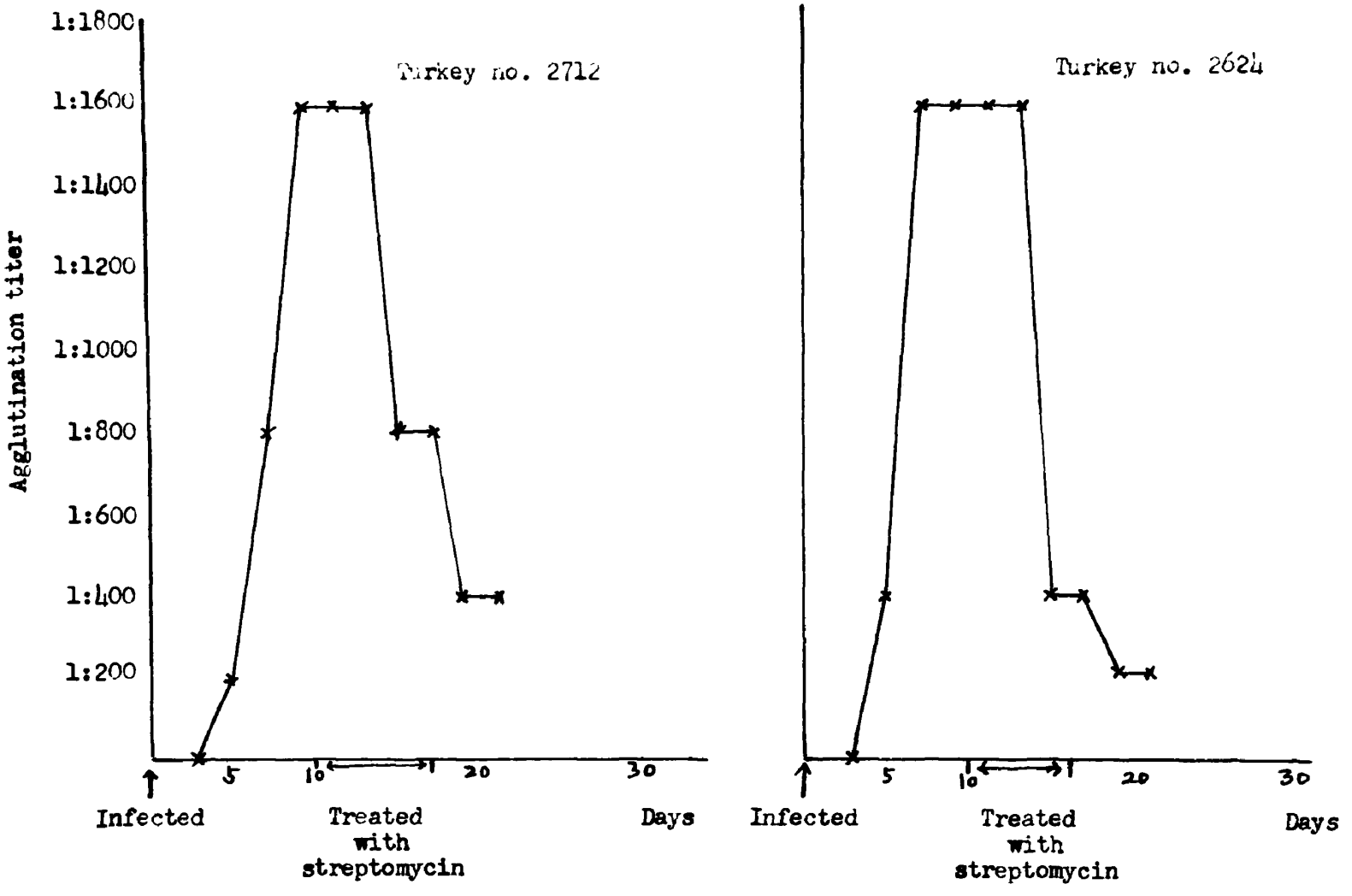

Table 11. The antibacterial action of sodium sulfadiazine on *S. pullorum* and the influence of normal serum

A. Sulfadiazine alone

Tube No.		1	2	3	4	5	6	7	8	control
Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	-
Bact. susp., 10^{-5}					0.1 ml to each tube					
Incubation period, hours	16	-	±	+	+	2+	2+	2+	2+	2+
	24	-	+	+	2+	2+	2+	2+	3+	3+
	48	2+	2+	2+	3+	3+	3+	3+	3+	3+

B. Sulfadiazine plus normal rabbit serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	-
Normal rabbit serum					0.5 ml to each tube					
Bact. susp., 10^{-5}					0.1 ml to each tube					
Incubation period, hours	16	-	-	+	+	2+	2+	2+	2+	2+
	24	-	-	+	2+	3+	3+	3+	3+	3+
	48	2+	3+	3+	3+	3+	3+	3+	4+	4+

C. Sulfadiazine plus normal chicken serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	-
Normal chicken serum					0.5 ml to each tube					
Bact. susp., 10^{-5}					0.1 ml to each tube					
Incubation period, hours	16	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	+	+
	48	-	-	-	-	-	+	+	2+	2+
	72	-	-	-	-	+	2+	2+	3+	3+
	96	-	-	±	2+	3+	3+	4+	4+	4+

Table 12. The effect of amount of normal chicken serum on the antibacterial action of sulfadiazine

A. Sulfadiazine alone

Tube No.		1	2	3	4	5	6	7	8	9	control
Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	±	+	2+	2+	2+	3+	3+
	24	-	-	-	+	3+	3+	3+	3+	3+	3+
	48	+	+	2+	2+	3+	3+	3+	3+	3+	3+
	72	+	2+	2+	2+	3+	3+	3+	3+	3+	3+
	96	2+	3+	3+	3+	3+	3+	3+	3+	3+	3+

B. Sulfadiazine plus 0.2 ml of normal chicken serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Normal chicken serum		0.2 ml to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	4+	4+
	24	-	-	-	-	-	-	-	4+	5+	5+
	48	-	-	-	-	-	2+	5+	6+	6+	6+
	72	-	-	-	+	-	6+	6+	6+	6+	6+
	96	-	-	-	5+	5+	6+	6+	6+	6+	6+
	120	-	-	5+	5+	5+	6+	6+	6+	6+	6+
	144	4+	4+	5+	5+	6+	6+	6+	6+	6+	6+
	168	4+	4+	5+	6+	6+	6+	6+	6+	6+	6+

Table 12. The effect of amount of normal chicken serum on the antibacterial action of sulfadiazine (continued)

C. Sulfadiazine plus 0.3 ml of normal chicken serum

Tube No.		1	2	3	4	5	6	7	8	9	control
Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Normal chicken serum		0.3 ml to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	4+
	24	-	-	-	-	-	-	-	-	5+	5+
	48	-	-	-	-	-	-	5+	5+	6+	6+
	72	-	-	-	-	-	6+	6+	6+	6+	6+
	96	-	-	-	4+	4+	6+	6+	6+	6+	6+
	120	-	-	4+	5+	5+	6+	6+	6+	6+	6+
	144	-	4+	5+	5+	6+	6+	6+	6+	6+	6+
	168	-	5+	5+	5+	6+	6+	6+	6+	6+	6+

D. Sulfadiazine plus 0.4 ml of normal chicken serum

Sod. Sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Normal chicken serum		0.4 ml to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	+	2+	5+
	48	-	-	-	-	-	-	5+	5+	5+	5+
	72	-	-	-	-	-	5+	6+	6+	6+	6+
	96	-	-	-	-	5+	6+	6+	6+	6+	6+
	120	-	-	-	-	6+	6+	6+	6+	6+	6+
	144	-	-	-	5+	6+	6+	6+	6+	6+	6+
	168	-	-	-	6+	6+	6+	6+	6+	6+	6+

Table 12. The effect of amount of normal chicken serum on the antibacterial action of sulfadiazine (continued)

E. Sulfadiazine plus 0.5 ml of normal chicken serum

Tube No.		1	2	3	4	5	6	7	8	9	control
Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Normal chicken serum		0.5 ml to each tube									
Bact. susp., 10-7		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	6+	6+	6+
	48	-	-	-	-	-	5+	6+	6+	6+	6+
	72	-	-	-	-	-	6+	6+	6+	6+	6+
	96	-	-	-	-	5+	6+	6+	6+	6+	6+
	120	-	-	-	4+	5+	6+	6+	6+	6+	6+
	144	-	-	-	5+	6+	6+	6+	6+	6+	6+
	168	-	-	-	6+	6+	6+	6+	6+	6+	6+

Table 13. The influence of heating (at 56° C for 30 minutes) on the bacteriastatic activity of normal chicken serum and on its enhancing effect on sulfadiazine

A. Sulfadiazine alone

Tube No.		1	2	3	4	5	6	7	8	9	control
Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	2+	3+	4+	4+	4+
	24	-	-	-	-	2+	3+	4+	4+	4+	4+
	48	+	+	2+	3+	4+	4+	4+	4+	4+	4+
	72	2+	2+	3+	3+	5+	5+	5+	5+	5+	5+

B. Sulfadiazine plus heated chicken serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Heated chicken serum		0.5 ml to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	3+	4+	4+
	24	-	-	-	-	-	+	2+	4+	4+	4+
	48	-	-	-	2+	3+	4+	4+	5+	5+	5+
	72	+	2+	2+	2+	4+	4+	5+	6+	6+	6+

C. Sulfadiazine plus unheated normal chicken serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Normal chicken serum		0.5 ml to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	2+	5+	5+	5+
	72	-	-	-	-	-	2+	4+	5+	5+	6+

Table 14. The influence of storage (in refrigerator for 24 hours) on the bacteriostatic activity of normal chicken serum and on its enhancing effect on sulfadiazine

A. Sulfadiazine alone

Tube No.		1	2	3	4	5	6	7	8	9	control
Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	+	2+	2+	3+	3+	3+
	24	-	-	-	+	2+	2+	3+	3+	3+	3+
	48	2+	2+	2+	3+	3+	4+	4+	4+	4+	4+
	72	2+	2+	2+	4+	4+	4+	4+	4+	4+	4+

B. Sulfadiazine plus stored chicken serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Stored chicken serum		0.5 ml to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	2+	3+	3+
	48	-	-	-	-	-	-	5+	5+	5+	5+
	72	-	-	-	-	-	5+	5+	5+	5+	5+

C. Sulfadiazine plus fresh normal chicken serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	-
Normal chicken serum		0.5 ml to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	2+	5+	5+	5+
	72	-	-	-	-	-	3+	4+	5+	5+	6+

Table 15. The combined action of infected chicken serum and different proportion of sulfadiazine and normal chicken serum

A. Infected chicken serum alone

Tube No.		1	2	3	4	5	6	7	8	9	control
Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	-
Bact.susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	24	4Ag	4Ag	2Ag	4+	4+	4+	4+	4+	4+	3+
	48	5Ag	5Ag	2Ag	5+	5+	5+	5+	5+	5+	3+
	96	6Ag	6Ag	6+	6+	6+	6+	6+	6+	6+	4+
	144	6+	6+	6+	6+	6+	6+	6+	6+	6+	4+
	168	6+	6+	6+	6+	6+	6+	6+	6+	6+	4+

B. Infected chicken serum plus 0.1 mg of sulfadiazine

Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	-
Sod. sulfadiazine		0.1 mg to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	24	-	-	-	-	-	-	-	-	2+	2+
	48	-	-	-	-	-	-	-	4+	4+	4+
	96	3Ag	3Ag	2Ag	2Ag	2Ag	Ag	+	4+	4+	4+
	144	4Ag	4Ag	3Ag	3+	3+	3+	3+	4+	4+	4+
	168	4Ag	4Ag	3Ag	3+	3+	3+	3+	4+	4+	4+

Table 15. The combined action of infected chicken serum and different proportion of sulfadiazine and normal chicken serum (continued)

C. Infected chicken serum plus 0.3 ml of normal chicken serum

Tube No.		1	2	3	4	5	6	7	8	9	control
Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	-
Normal chicken serum					0.3 ml to each tube						
Bact. susp., 10^{-7}					0.1 ml to each tube						
Incubation period, hours	24	4Ag	3Ag	2Ag	-	-	-	-	-	-	3+
	48	5Ag	5Ag	4Ag	4Ag	4Ag	3Ag	3Ag	3Ag	3Ag	4+
	96	6Ag	5Ag	4Ag	4Ag	4+	4+	4+	4+	4+	4+
	144	6Ag	5Ag	4Ag	4Ag	4+	4+	4+	4+	4+	4+
	168	6Ag	6Ag	4Ag	4Ag	4+	4+	4+	4+	4+	4+

D. Infected chicken serum plus 0.1 mg of sulfadiazine and 0.3 ml of normal chicken serum

Infected chicken serum	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	
Sod. sulfadiazine				0.1 mg to each tube						
Normal chicken serum				0.3 ml to each tube						
Bact. susp., 10^{-7}				0.1 ml to each tube						
Incubation period, hours 24	-	-	-	-	-	-	-	-	-	
48	Ag	-	-	-	-	-	-	-	-	
96	2Ag	2Ag	Ag	-	-	-	-	-	-	
144	4Ag	3Ag	2Ag	2+	+	-	-	-	-	
168	4Ag	4Ag	3Ag	2Ag	2+	-	-	-	-	

Table 15. The combined action of infected chicken serum and different proportion of sulfadiazine and normal chicken serum (continued)

E. Infected chicken serum plus 0.1 mg of sulfadiazine and 0.4 ml of normal chicken serum

Tube No.		1	2	3	4	5	6	7	8	9
Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
Sod. sulfadiazine		0.1 mg to each tube								
Normal chicken serum		0.4 ml to each tube								
Bact. susp., 10^{-7}		0.1 ml to each tube								
Incubation period, hours	24	-	-	-	-	-	-	-	-	-
	48	Ag	3+	-	-	-	-	-	-	-
	96	3Ag	3+	+	-	-	-	-	-	-
	144	4Ag	4Ag	3Ag	2Ag	-	-	-	-	-
	168	4Ag	4Ag	3Ag	2Ag	2+	-	-	-	-

F. Infected chicken serum plus 0.05 mg of sulfadiazine and 0.5 ml of normal chicken serum

Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
Sod. sulfadiazine		0.05 mg to each tube								
Normal chicken serum		0.5 ml to each tube								
Bact. susp., 10^{-7}		0.1 ml to each tube								
Incubation period, hours	24	-	-	-	-	-	-	-	-	-
	48	3Ag	2Ag	Ag	4+	-	-	-	-	-
	96	4Ag	3Ag	2Ag	5+	-	-	-	-	-
	144	4Ag	3Ag	3Ag	5+	2+	-	-	-	-
	168	5Ag	4Ag	3Ag	5+	3+	-	-	-	-

Table 16. The effect of amount of infected chicken serum on the combined action of sulfadiazine and normal chicken serum

A. Infected chicken serum alone

Tube No.		1	2	3	4	5	6	7	8	9	control
Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	-
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	4Ag	3Ag	3Ag	2Ag	2Ag	2Ag	2Ag	2Ag	2Ag	4+
	24	4Ag	3Ag	4+	4+	4+	4+	4+	4+	4+	4+
	48	4Ag	4Ag	4+	4+	4+	4+	4+	4+	4+	4+
	72	4Ag	4+	4+	4+	4+	4+	4+	4+	4+	4+
	96	5+	4+	4+	4+	4+	4+	4+	4+	4+	4+

B. Infected chicken serum plus sulfadiazine

Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	-
Sod. sulfadiazine		0.1 mg to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	-	-	-	-	-	Ag	2Ag	2Ag	2Ag	+
	24	-	-	-	-	Ag	2Ag	3Ag	3Ag	3Ag	2+
	48	3Ag	3Ag	3Ag	3Ag	3Ag	3Ag	4+	4+	4+	3+
	72	4Ag	4Ag	3Ag	3Ag	3Ag	3+	4+	4+	4+	3+
	96	5+	4+	4+	4+	4+	4+	4+	4+	4+	4+

Table 16. The effect of amount of infected chicken serum on the combined action of sulfadiazine and normal chicken serum (continued)

C. Infected chicken serum plus normal chicken serum

Tube No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	control
Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	1:20480	1:40960	1:81920	1:163840	1:327680	-
Normal chicken serum					0.3 ml to each tube												
Bact. susp., 10^{-7}					0.1 ml to each tube												
Incubation period, hours	16	3Ag	3Ag	2Ag	Ag	-	-	-	-	-	-	2+	2+	2+	2+	2+	3+
	24	4Ag	3Ag	3Ag	2Ag	Ag	3+	3+	-	-	4+	4+	4+	4+	4+	4+	4+
	48	4Ag	4Ag	3Ag	3Ag	2Ag	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	72	4Ag	4Ag	3Ag	3Ag	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	96	5Ag	5Ag	4Ag	4Ag	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+

D. Infected chicken serum plus sulfadiazine and normal chicken serum

Infected chicken serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	1:20480	1:40960	1:81920	1:163840	1:327680	-
Sod. sulfadiazine					0.1 mg to each tube												
Normal chicken serum					0.3 ml to each tube												
Bact. susp., 10^{-7}					0.1 ml to each tube												
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	48	3Ag	2Ag	Ag	-	-	-	-	-	-	-	-	-	-	-	-	-
	72	4Ag	4Ag	3Ag	3Ag	-	-	-	-	-	-	-	-	3+	2+	2+	3+
	96	5Ag	5Ag	4Ag	4Ag	3Ag	-	-	-	-	4+	4+	4+	4+	4+	4+	4+

Table 17. The effect of amount of infected turkey serum on the combined action of sulfadiazine and normal chicken serum

A. Infected turkey serum alone

Tube No.		1	2	3	4	5	6	7	8	9	control
Infected turkey serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	-
Bact. susp., 10^{-7}		0.1 ml to each tube									-
Incubation period, hours	16	3Ag	3Ag	3Ag	3Ag	+	2+	3+	3+	4+	4+
	24	4Ag	4Ag	4Ag	4+	4+	4+	4+	4+	4+	4+
	48	4Ag	4Ag	4+	4+	4+	4+	4+	4+	4+	4+
	72	4Ag	4+	4+	4+	4+	4+	4+	4+	4+	4+
	96	5+	5+	4+	4+	4+	4+	4+	4+	4+	4+

B. Infected turkey serum plus sulfadiazine

Infected turkey serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	-
Sod. sulfadiazine		0.1 mg to each tube									
Bact. susp., 10^{-7}		0.1 ml to each tube									
Incubation period, hours	16	2Ag	2Ag	2Ag	2Ag	2Ag	2Ag	2Ag	2Ag	2Ag	+
	24	2Ag	2Ag	2Ag	3Ag	3Ag	3Ag	3Ag	3Ag	3Ag	2+
	48	4Ag	3Ag	3Ag	3Ag	2+	4+	4+	4+	4+	3+
	72	4Ag	3Ag	3Ag	4+	4+	4+	4+	4+	4+	3+
	96	5+	4+	4+	4+	4+	4+	4+	4+	4+	4+

Table 17. The effect of amount of infected turkey serum on the combined action of sulfadiazine and normal chicken serum (continued)

C. Infected turkey serum plus normal chicken serum

Tube No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	control
Infected turkey serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	1:20480	1:40960	1:81920	1:163840	1:327680	-
Normal chicken serum		0.3 ml to each tube															
Bact. susp., 10^{-7}		0.1 ml to each tube															
Incubation period, hours	16	Ag	+	+	-	-	-	-	-	-	-	-	-	-	2+	2+	3+
	24	2Ag	4+	3+	-	-	-	-	-	4+	4+	4+	4+	4+	4+	4+	4+
	48	3Ag	4+	3+	3+	-	-	2+	-	4+	4+	4+	4+	4+	4+	4+	4+
	72	5+	4+	3+	3+	-	-	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	96	5+	5+	5+	5+	-	-	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+

D. Infected turkey serum plus sulfadiazine and normal chicken serum

Infected turkey serum		1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	1:20480	1:40960	1:81920	1:163840	1:327680	-
Sod. sulfadiazine		0.1 mg to each tube															
Normal chicken serum		0.3 ml to each tube															
Bact. susp., 10^{-7}		0.1 ml to each tube															
Incubation period, hours	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	48	2Ag	Ag	-	-	-	-	-	-	-	-	-	-	3+	3+	3+	-
	72	4Ag	3Ag	-	3+	-	-	-	-	-	-	-	-	3+	3+	3+	3+
	96	4+	4+	4+	4+	-	-	-	-	-	4+	4+	4+	4+	4+	4+	3+

Table 18. The antibacterial action of sulfadiazine in serum of normal chickens

A. Sulfadiazine-containing serum (normal chicken) alone

Tube No.		1	2	3	4	5	6	7	8	control
Sulfadiazine-containing serum		*1:50T	1:100T	1:200T	1:400T	1:800T	1:1600T	1:3200T	1:6400T	-
Bact. susp., 10^{-7}		0.1 ml to each tube								
Incubation period, hours	16	-	-	-	2+	3+	3+	3+	3+	3+
	24	-	-	2+	3+	3+	3+	3+	3+	3+
	48	-	4+	4+	4+	4+	4+	4+	4+	4+
	72	4+	5+	5+	5+	5+	5+	5+	5+	5+

B. Sulfadiazine-containing serum (normal chicken) plus normal chicken serum

Sulfadiazine-containing serum		*1:50T	1:100T	1:200T	1:400T	1:800T	1:1600T	1:3200T	1:6400T	-
Normal chicken serum		0.3 ml to each tube								
Bact. susp., 10^{-7}		0.1 ml to each tube								
Incubation period, hours	16	-	-	-	-	-	-	-	-	-
	24	-	-	-	3+	3+	3+	3+	3+	3+
	48	-	-	5+	5+	5+	5+	5+	5+	5+
	72	-	-	5+	5+	5+	5+	5+	5+	5+
	120	-	6+	6+	6+	6+	6+	6+	6+	6+
	168	-	6+	6+	6+	6+	6+	6+	6+	6+

*Dilution of sulfadiazine to medium

Table 18. The antibacterial action of sulfadiazine in serum of normal chickens (continued)

C. Sulfadiazine alone

Tube No.		1	2	3	4	5	6	7	8
Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T
Bact. susp., 10^{-7}		0.1 ml to each tube							
Incubation period, hours	16	-	-	-	-	-	+	3+	3+
	24	-	-	-	-	2+	3+	3+	3+
	48	-	+	3+	4+	4+	4+	4+	4+
	72	+	3+	3+	4+	4+	4+	4+	4+

D. Sulfadiazine plus normal chicken serum

Sod. sulfadiazine		1:5T	1:10T	1:20T	1:40T	1:80T	1:160T	1:320T	1:640T
Normal chicken serum		0.3 ml to each tube							
Bact. susp., 10^{-7}		0.1 ml to each tube							
Incubation period, hours	16	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	2+	3+
	48	-	-	-	-	4+	4+	4+	4+
	72	-	-	-	-	5+	5+	5+	5+
	120	-	4+	4+	4+	5+	6+	6+	6+
	168	-	6+	6+	6+	6+	6+	6+	6+

Table 19. The combined action of sulfadiazine and normal chicken serum in the body of infected chickens

A. Before injection of normal chicken serum

Tube No.		1	2	3	4	5	6	7	8
Sulfadiazine-containing serum		*1:30T	1:60T	1:120T	1:240T	1:480T	1:960T	1:1920T	1:3840T
Bact. susp., 10^{-7}		0.1 ml to each tube							
Incubation period, hours	16	-	-	-	Ag	2Ag	3+	3+	3+
	24	Ag	Ag	2Ag	3Ag	4+	4+	4+	4+
	48	Ag	3Ag	3Ag	4+	4+	4+	4+	4+
	72	3Ag	3Ag	4+	4+	4+	4+	4+	4+
	96	4Ag	4+	4+	4+	4+	4+	4+	4+
	168	5+	4+	4+	4+	4+	4+	4+	4+

B. After injection of normal chicken serum

Sulfadiazine-containing serum		*1:20T	1:40T	1:80T	1:160T	1:320T	1:640T	1:1280T	1:2560T
Bact. susp., 10^{-7}		0.1 ml to each tube							
Incubation period, hours	16	-	-	-	-	Ag	2Ag	3+	3+
	24	-	-	±Ag	Ag	2Ag	3Ag	4+	4+
	48	Ag	Ag	3Ag	3Ag	4+	4+	4+	4+
	72	3Ag	3Ag	3Ag	4+	4+	4+	4+	4+
	96	4Ag	4Ag	4+	4+	4+	4+	4+	4+
	168	4+	4+	4+	4+	4+	4+	4+	4+

*Dilution of sulfadiazine to medium