AN INVESTIGATION CONCERNING THE PRESERVATION OF STOOL SPECIMENS SUSPECTED OF CONTAINING ORGANISMS OF THE ENTERIC GROUP, WITH SPECIAL EMPHASIS UPON THE SHIGELLAE.

by

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I. INTRODUCTION.

THE PROBLEM AND ITS JUSTIFICATION.

A. The Problem.

The practice employed among public health laboratories is to make available mailing containers for use by physicians for sending, via mail, stool specimens suspected of being infected with members of either the Eberthella, Salmonella or Shigella groups of bacteria. These mailing containers usually include one or more stoppered bottles in which a preservative solution has been placed and to the bottom side of the stopper had been affixed a piece of wooden applicator or small metallic spatula with which to inoculate the bottle. The preservative solution used is not the same throughout this country. It varies as to use and substance among the various laboratories from a single solution for use for all three groups of specimens to no preservative for any of the groups. Instructions for use usually accompany the containers in order that an "overdose" of inoculum is not made into them. The

inoculation of too large a quantity of feces usually results in an overgrowth of the non-pathogenic forms, while the specimen is in transit, thereby making difficult the easy isolation of the pathogens (25).

It was felt that there was a need for the development of a single preservative solution for all three groups of bacteria thereby making possible the elimination of the necessity for two or more different solutions. With the development of such a solution, there should be effected a simplification and improvement to the laboratory services both in the field and laboratory as well as affecting an economy of both time and money.

In a review of the literature concerning the subject, little or no work had been reported pertaining to a single preservative for all three groups of specimens although there had been some work reported for members of the Eberthella and Salmonella groups as a unit and the Shigella groups as another unit (20)(48). In an attempt to eliminate possible duplication of effort in research, a survey was made of all State Public Health Laboratories in this country, Hawaii and Puerto Rico. All laboratory directors were circularized with a questionaire containing six questions. Information was requested concerning the availability of specimen containers for specimens of these three groups; the preservative solution used for each group and whether the solution used was yielding satisfactory results from a "recovery of organism" standpoint; whether research was being conducted concerning the line of thought suggested and whether the development of such a solution would help to simplify and improve their

laboratory services.

B. Analysis of Survey Data and Justification for the Study.

Table 1 indicates in tabulated manner an analysis of the results of the survey undertaken among the various directors of State Public Health Laboratories in this country, Hawaii and Puerto Rico. From a perusal of this table, the following deductions may be made:

1. Replies were received from 35(72.9%) of the 48 questionaires sent out.

2. 25(71.4%) of the laboratories reporting employed the same preservative solution for specimens of the three groups. Of this number there were but 6(24.0%) of the directors that were satisfied and 19(76.0%) dissatisfied with the results obtained from its use.

3. 27(77.1%) of the directors reported favorable opinions as to whether a single preservative solution, if developed, for the three groups of specimens would help simplify and improve their laboratory services, 2(5.7%) reported unfavorably; 2(5.7%) expressed doubt and 4 (11.3%) failed to answer the question.

From the above, it may be deducted that

1. There was a need for an investigation in this field of endeavor.

2. Such an investigation undertaken would be both practical and justified.

II. RESUME OF LITERATURE.

It is not the intention of the author to present a comprehensive review of all culture media employed in Bacteriology since such excellant reviews are already available upon the subject (15)(41). It is felt that for the purpose of this dissertation, a brief resume of only those differential culture media more commonly employed by some bacteriologists should be included, some of which have particular application in the isolation and identification of the organisms of the three groups in question. For purposes of convenience, differential culture media may be divided as follows:

A. Differential culture media employed in the isolation or identification of members of the Eberthella, Salmonella and Shigella groups of bacteria.

1. Liquid Media.

a. Enrichment media for their bacteriostatic or inhibitory action upon non-pathogenic coliform bacteria thereby permitting the pathogenic forms to grow unrestricted after transplantation upon media.

(1) Alkalization

Dudgeon in 1919 (16) reported the use of N/33 sodium hydroxide solution in equal volumes with feces as an "enrichment" preservative for stool specimens subjected to delayed examination for

bacillary dysentery incitants. He believed that the acid reaction of the stool specimen was the chief bactericidal factor involved in the inability to recover the pathogens and that with an alkaline reaction effected, viability of the pathogens was prolonged. ..."final concentrations of 1.5 per cent to 3.0 per cent normal sodium hydroxide are still being used by English bacteriologists for preserving dysenteric stools in the tropics"(8).

(2) Bile

Neufeld reported in 1900(53) that bile exerted a lytic action upon some bacteria and non-lytic action with others. The bacteriostatic and bactericidal effects decrease with dilution. Gram negative bacilli are usually more resistant than gram positive bacteria. In lower concentrations bile exerts a stimulating effect upon bacterial growth(6). The action effected is primarily due to the bile acids present. Among them are choleic, desoxycholic, apocholic, dehydrocholic, taurocholic and glycocholic acids, all of which have a lytic action upon certain bacteria except dehydrocholic acid which has little effect as compared to the other acids.

Leifson in 1935 reported that the most striking effect was exhibited by desoxycholic acid and prepared a sodium salt thereof for bacteriological purposes (39).

(3) Desoxycholate Citrate Solution

Bangxang and Eliot in 1940 (8) proposed a solution of 1% sodium citrate, 0.5% sodium desoxycholate in a phosphate buffered saline solution at pH 8.5 as a preservative medium for use with bacillary dysentery stool specimens for delayed examination. The action of this solution was based upon the inability of E.coli to utilize the carbon of the sodium citrate and thereby become inhibited;the sodium desoxycholate inhibited the gram positive cocci. The authors proposed that Endo and MacConkey Agar be the plating media of choice for use with this group of organisms for isolation purposes since gram positive bacteria were suppressed by both media and neither had an inhibitory effect upon the pathogens.

(4) Dyes

Anilin dyes,either acid or basic type,inhibit gram positive more readily than gram negative organisms (67). Brilliant green,in culture media,in 1/150000 concentration will inhibit lactose fermenting organisms (68). Dunham and Schoenlein (17) reported that brilliant green

in a 1/75000 concentration will not inhibit E.coli. Gentian violet in 1/10000 concentration has a selective action for gram positive and not gram negative bacteria (68).

(5) Glycerin Saline Solution

Teague and Clurman in 1916 (66) proposed that a 30% solution of glycerin in 0.6% sodium chloride be used in the preservation of stool specimens for delayed examination for the presence of E.typhosus. The authors reported that the action of glycerin upon the bacteria present was a bacteriostatic one and its lethal action was relatively slow at that dilution. Wu and Sia (74) reported this solution to have a beneficial preserving action upon S.dysenteriae in stools also for delayed examination. The variation in pH of the various grades of glycerin and within different batches of the same grade has contributed materially to the toxic action of this preservative solution upon the pathogenic bacteria in question. (8) In an attempt to overcome this difficulty, Greenfield in 1936 (22) proposed a modification of the original Teague and Clurman solution in that the 30% glycerin solution was prepared in a phosphate buffered solution of sodium chloride with pH adjusted to 7.2.

(6) Lithium Chloride Glycerin Saline Solution

Havens and Mayfield [25][26] reported a modification of Teague and Clurman's solution by the addition of 0.5% lithium chloride as a specific inhibitory substance for the E.coli in stool specimens for delayed examination for E.typhosus. Gray in 1931 [21] proposed a similar modification involving the Salmonella group. However, due to the toxic action of the solution upon members of the Shigella group of bacteria, its use with specimens of this type has been limited [8].

(7) Selenite F. Medium

Leifson in 1936 [40] reported an enrichment medium consisting of sodium hydrogen selenite, peptone and lactose in a buffered phosphate solution. Due to its initial selective inhibitory action, E.coli does not reach a maximum until long after the usual 24 hours incubation period whereas E.typhosus and members of the Salmonella group multiply rapidly during the usual incubation period and greatly outnumber the Escherichia. The inhibitory effect of this enrichment medium upon the dysentery bacilli renders its use therefor impraactical [3].

(8) Tetrathionate Broth

Muller and Malvoz in 1925 (51) described a medium containing calcium carbonate, sodium hyposulfite, potassium iodide and iodine in bouillon for use as an enrichment medium in the examination of feces, urine, water and sewage and other infected materials for the presence of B. typhosus and members of the Salmonella group. This medium inhibits or kills the coliform bacteria and permits the enteric organisms to grow unrestricted.

2. Solid Media.

a. Enrichment media that do not suppress or inhibit, but serve to differentiate on the basis of lactose fermentation as indicated by a change in indicator employed.

(1) Plating Media.

(a) Bromoresol Purple Lactose Agar

This medium as devised by Chesney (13) was essentially the same as Litmus Lactose Agar except that the litmus indicator had been replaced by bromoresol purple, a more stable dye. Its uses are essentially the same as for Litmus Lactose Agar (15).

(b) Litmus Lactose Agar

This medium is the oldest differential medium reported for bacteriological purposes. It was

developed by Hurtz in 1897 (75) and contained lactose, nutrient agar and litmus. The latter was employed as an indicator for the production of acid by bacteria from lactose. It was developed "to differentiate Bact.coli and Eberth's bacillus, in that Bact.coli fermented lactose with the production of acid" (75). The typhoid colonies were colorless and the coli colonies were red.

(2) Tube Media.

(a) Kliger Lead Acetate Agar.

Kliger in 1918 (85) reported a differential medium based upon the combination of Russell Double Sugar Agar and Lead Acetate Agar for the differentiation of gram negative bacilli both on the basis of their ability to ferment dextrose and lactose and their ability to produce hydrogen sulfide. Bailey and Lacy in 1927 (7) reported a modification of the Kliger Lead Acetate Agar in which the Andrade indicator was replaced with phenol red, ferric ammonium citrate replaced lead acetate as hydrogen sulfide indicator and sodium thiosulfate was added. This medium was reported to differentiate the lactose from non-lactose fermenters, to differentiate Eberthella from the Salmonella and Shigella groups and also to differentiate S.paratyphi from S.schottmuelleri

and S.enteriditis by the detection of hydrogen sulfide production.

(b) Krumwiede Triple Sugar Agar

Krumwiede and Kohn (37) proposed a modification of the Russell Double Sugar Agar in which sucrose was incorporated in addition to the dextrose and lactose of Russell. It was claimed that this medium had the advantage over the Russell medium in that a more accurate differentiation between slow lactose fermenting strains of gram negative intestinal bacteria and the Salmonella group was effected. This was made possible by the slow lactose fermenters attacking sucrose more readily than lactose under aerobic conditions and the Salmonella organisms were not influenced by the sucrose. Andrade indicator was recommended by the authors for use as the indicator for the presence of acid production.

(c) Russell Double Sugar Agar

In 1911, Russell (59) described a double sugar medium for the identification of gram negative organisms of the intestinal group, particularly members of the Eberthella, Salmonella and Escherichia groups. The original formula included litmus as an acid indicator. Phenol red has been recently incorporated to replace litmus since the former is more stable and

gives better results (15).

(d) Sucrose Mannitol Agar

Kendall and Ryan in 1919 (34) described a double sugar medium containing sucrose and mannitol which was proposed to supplement Russell Double Sugar Agar in the differentiation and identification of bacteria of the intestinal group. By means of these two media, the fermentation reactions of four sugars were made possible. The original formula contained Andrade indicator as the acid indicator. Phenol red has been recently incorporated to replace the Andrade indicator since the former is more stable and responds more rapidly to changes in reaction (15).

b. Enrichment media that suppress or inhibit gram positive organisms but not members of the gram negative group and on which there is a reasonably sharp differentiation by means of appropriate indicators between lactose and non-lactose fermenters.

1. Plating Media.

(a) Desoxycholate Agar

In 1935, Leifson (39) described a differential medium for the isolation of intestinal pathogens and for the direct enumeration of coliform bacilli in milk and water. This medium contained lactose, peptone, ferric ammonium citrate, sodium chloride, a potassium phosphate buffer and sodium desoxycholate in water with pH 7.3-7.5. This med-

ium may be considered comparable to MacConkey Agar in that there was present a bile salt, but in a purer state, in a concentration (0.1%) sufficient to have a bacteriostatic action upon gram positive organisms but exerted little or no action upon gram negative intestinal bacteria[39] except S.gallinarum which it completely inhibited. Colonies of lactose fermenters were usually red while those from non-lactose fermenters were colorless.

(b) Endo Agar

In 1904, Endo described a medium[19] in which a fuchsin sulfite indicator was used to differentiate between lactose and non-lactose fermenters of the intestinal tract. Coliform organisms fermenting lactose became red, usually demonstrated a metallic sheen and colored the surrounding medium; non-lactose fermenters were colorless, clear and appeared usually as glistening drops upon the pink background of the medium. The typical reaction by the lactose fermenters was caused by the intermediate product-acetaldehyde- formed from lactose during the fermentation of that sugar and becoming fixed by the sodium sulfite[46][52]. This medium has been subjected to many modifications due largely to variations in the available dyes and sulfites and to new uses for the medium advocated by individual investigators[15].

(c) Eosin Methylene Blue Agar

Holt-Harris and Teague[29] employed a combination of eosin and methylene blue as an indicator in the presence of lactose, sucrose and peptone. In this medium lactose fermenting colonies gave a distinct differential from non-lactose fermenters. Lactose fermenters of the coli-aerogenes group developed blackish colonies or possessed dark centers with transparent colorless peripheries whereas non-lactose fermenters remained colorless. This medium has been subjected to a number of modifications and variations[15].

(d) MacConkey Agar

This medium devised by MacConkey as a differential medium[43][44][45] has been generally used in Great Britain for differentiating members of the Eberthella, Salmonella and Shigella groups from the non-pathogenic coliforms following a number of modifications by both MacConkey and other investigators. The differential action has been reported to be effected by colonies of coliform bacteria becoming brick red in color and usually surrounded by a zone of precipitated bile salts. This reaction was due to the action of the acids, produced by the fermentation of lactose, upon the bile salts and the subsequent adsorption of neutral red. Non-lactose fer-

menters did not alter the appearance of the medium and were uncolored and transparent, Its use as a differential medium has met with favorable results by some investigators (8) and unsatisfactory results by others (30)(32).

e. Enrichment media that contain chemicals to delay or completely suppress the development of gram negative non-pathogenic coliform and other groups and permits the growth of gram negative intestinal pathogens.

1. Plating Media.

(a) Bismuth Sulfite Agar

Wilson (69)(72) and Wilson and Blair (70)(71)(73) described a selective medium by which S.typhosus and sulfite reducing bacteria in water might be detected. In a medium containing sodium sulfite, glucose and an iron salt, reduction of the sulfite to sulfide and the formation of FeS (the blackening of the colony) was effected by S.typhosus and various members of the Salmonella group. The bismuth sulfite indicator in the presence of an excess of sodium sulfite suppressed the growth of most coliform bacilli; and in the presence of brilliant green the selective action was intensified. This medium has been subjected to a number of modifications by in-

vestigators in an effort to effect a more sensitive medium for special purposes. This medium has been reported to yield satisfactory results in the isolation of members of the Eberthella and Salmonella groups but members of the Shigella group become either inhibited or donot grow well upon it (47).

(b) Desoxycholate Citrate Agar

This selective medium was reported by Leifson in 1934 (38) to contain 3% sodium citrate, 0.2% ferric ammonium citrate, 0.5% sodium desoxycholate, 1% lactose, 1/150000 lead acetate, 1/50000 neutral red in beef infusion agar containing 1% peptone and 0.5% sodium chloride at pH 7.4 In this medium the bile salt inhibited gram positive organisms and supplemented by sodium citrate inhibited to a considerable extent members of the Aerobacter, Escherichia, Citrobacter, and Proteus with ocassional strains of each encountered that were resistant. The ferric ammonium citrate served a double function- it lessened whatever inhibiting effect the medium may have had for the intestinal pathogens and enhanced the inhibiting effect of the medium upon the colon bacilli (39). This medium was modified by Leifson in 1935 to contain 1% lactose, 2.5% sodium citrate, neutral red 1/50000, lead chloride 1/300000(the use of which was

left optional to the user) in a pork infusion peptone agar without sodium chloride. He reports that in the use of a pork infusion he always obtained better results, from a growth standpoint, than from infusions made from other mammalian muscle tissue. This medium was proposed for either streak or plating purposes. Due to its strong inhibitory action, this medium has been reported unsatisfactory for the isolation of members of the Shigella group other than those of the S. paradysenteriae (Flexner) [8][23].

(c) SS Agar

This selective medium was recently developed by the Difco Laboratories [14] for the isolation of members of the Shigella, Salmonella and Eberthella groups from stool and other materials suspected of containing these organisms. It has been reported to differentiate between lactose and non-lactose fermenters and has also been reported to give maximum inhibition of coliform organisms with minimum restriction of growth of the pathogenic gram negative intestinal bacilli. This medium contains 0.5% beef extract, 0.5% proteose peptone, 1% lactose, 0.85% bile salts, 0.85% sodium citrate, 0.85% sodium thiosulfate,

0.1% ferric citrate,brilliant green 1/3 million, 0.0025% neutral red. In this medium,as in Desoxycholate Citrate Agar,advantage has been taken of a bile salt to inhibit gram positive bacteria; sodium and ferric citrates to inhibit lactose fermenting organisms;thiosulfate was employed in conjunction with lactose and neutral red to detect the lactose fermenters from non-lactose fermenters by a reddish colored colony formed by the lactose fermenters whereas the non-lactose fermenters were usually colorless, This medium has been reported to give a more defined differentiation between fecal and pathogenic colonies than is rendered by Desoxycholate Citrate Agar (47).

(d) Simmons Citrate Agar

This medium for differentiating fecal from non-fecal coliform organisms was developed in 1926 by Simmons (61). The selective differentiation of this medium was based upon the inability of fecal coliforms(E.coli) to develop in a medium with an inorganic ammonium salt as the only source of nitrogen and with citrate as sole source of carbon for energy,whereas strains of aerogenes(non-fecal)

grow quite unrestricted. This medium was developed by the author to overcome the disadvantage usually encountered at times with a turbidity when large inocula are used in Koser's Citrate Broth. Bromthymol blue was used as indicator.

B. Differential culture media employed in the isolation or identification of bacteria other than the Eberthella, Salmonella and Shigella groups of bacteria.

1. Liquid Media.

a. Enrichment media that do not suppress or inhibit bacteria, but serves to differentiate bacteria on the basis of lactose fermentation.

(1) Eijkman Broth

This medium was devised in 1904 by Eijkman (18) for the separation of strains of E.coli originating from the feces of warm blooded animals. The original formula contained 10% glucose, 10% peptone and 0.5% sodium chloride. Tubes of the sample in question inoculated with 1/8 its volume of broth were incubated at 46°C. for 24 hours. Perry and Hajna (56) replaced glucose with 0.3% lactose. In 1939, Perry and Hajna (57) replaced peptone with 1.5% tryptone and added a phosphate buffer. Its present

use is mostly in Sanitary Bacteriology.

(2) Lactose Broth

The active ingredients in this media are lactose in beef extract-peptone solution (4). Lactose fermenting organisms produce acidity which may be detected by means of an indicator added to the medium. Lactose fermenters may or may not produce gas in addition to acidity.

b. Enrichment media that suppress or inhibit gram positive but not gram negative bacteria and with which there is a reasonably sharp differentiation by means of appropriate indicators between lactose and non-lactose fermenting bacteria.

(1) Brilliant Green Bile Lactose Broth

This medium was devised by Muer and Harris (50) in 1920 to inhibit organisms other than members of the coliform group. Its active ingredients are lactose, oxbile and brilliant green; the former for the fermentation by coliform organisms, oxbile to depress the surface of both the media and bacteria present (usually the anaerobes) and brilliant green to suppress gram positive bacteria. Dunham and Schoenlein (17) recommended a reduced concentration of bile from 5% to 2% and increased the dye from 0.01% to 0.13% thereby improving the conditions for growth of B.coli. Its use is primarily as a confirmatory medium for the presumptive test for the presence of col-

iform bacteria in water [4] and milk samples [5] and is also used in parallel with lactose broth in the control of water filtration plants [4].

(2) Crystal Violet Lactose Broth

This medium is a buffered lactose broth containing crystal violet in a concentration sufficient to inhibit gram positive organisms and other bacteria that may be responsible for false positive tests. The concentration of the bacteriostatic agent in the medium was not sufficient to effect the growth of members of the coliform group. This medium was developed by Salle [60] in 1930. Its present use is primarily as a confirmatory medium for the presumptive test for the presence of coliform bacteria in water samples [4] and is also used in parallel with lactose broth in the control of water filtration plants [4].

(3) Formate Ricinoleate Broth

The active ingredients of this medium are lactose, sodium formate, sodium ricinoleate and peptone. It was developed by Stark and England in 1935 [64] for the detection of coliform bacteria in water and milk. The sodium ricinoleate inhibits the gram positive organisms usually responsible for false positive results. Sodium formate accelerates growth and gas production of the coliform bacteria from lactose. Its present

use is primarily as a confirmatory medium for the presumptive test, in lactose broth, for the presence of coliform bacteria in water samples [4] and is also used in parallel with lactose broth in the control of water filtration plants [4].

(4) Fuchsin Lactose Broth

Ritter devised this medium in 1932 [58] to reduce the number of false positive reactions occuring in the examination of water samples for B.coli. The dye- basic fuchsin- inhibits gram positive organisms and other bacteria that may be responsible for the false positive tests. Lactose is the fermentable sugar available to detect coliform bacteria.

(5) MacConkey Broth

MacConkey in 1901 [42] developed this medium with peptone, glucose, a bile salt and litmus. In 1905, he replaced litmus with neutral red and glucose with lactose [43][44]. This medium is extensively used in Great Britain. The litmus served as indicator for the presence or absence of acidity and the bile salt to suppress the gram positive organisms and other bacteria that might produce false positive reactions. The neutral red was incorporated in the medium as an indicator.

c. Enrichment media that contain chemicals designed to delay or completely suppress the development of certain gram negative bacteria at the expense of other gram negative organisms and the principle of which was based upon the ability of the organisms to utilize certain organic substances.

(1) Koser Citrate Broth

Koser in 1923 (56) developed this medium to differentiate between E.coli and A.aerogenes. The principle upon which it worked was based upon the inability of E. coli to utilize the carbon of the sodium citrate for energy in the presence of an ammonium salt as source of nitrogen whereas A.aerogenes was able to utilize the carbon for energy.

2. Solid Media.

a. Enrichment media that do not suppress or inhibit bacteria but serve to differentiate colonies on the basis of lactose fermentation and as indicated by a change in the indicator employed.

(1) Bromcresol Purple Lactose Agar

This medium as devised by Chesney (13) was described on Page 9.

(2) Litmus Lactose Agar

This medium as devised by Hurtz (75) was described on Page 9.

b. Enrichment media that suppress or inhibit gram positive but not gram negative organisms and on which there is a reasonably sharp differentiation by means of appropriate indicators between lactose and non-lactose fermenters.

(1) Brilliant Green Bile Lactose Agar

This medium was developed by Noble and Tonney in 1935 (54) for determining the relative density of coliform bacteria in water and sewage. The active ingredients were lactose, bile salts, sodium sulfite, ferric chloride, orioglaucine, a phosphate buffer and two dyes-basic fuchsin and brilliant green. The dyes were the gram positive bacterial inhibitors. Colonies of coliform bacteria were deep red at the center with a pink halo of precipitated bile salts sharply outlined against a uniformly blue background.

(2) Endo Agar

This medium was described on Page 13.

(3) Eosin Methylene Blue Agar

This medium was described on Page 14.

(4) MacConkey Agar

This medium was described on Page 14.

c. Enrichment media that contain chemicals which encourage selective bacterial multiplication.

(1) Beef Infusion Lactose Agar

In 1935 [2] this medium was devised, with the addition of skin milk at the time of use. It was employed for determining the total bacterial count in the microbiological analysis of butter and the detection of proteolytic colonies.

(2) Blood Agar

This medium has been used in the isolation and cultivation of many fastidious pathogenic organisms. The principle upon which its action was based was that certain pathogens had the ability to hemolyse red blood corpuscles when cultivated upon its surface. This characteristic was considered a diagnostic criterion among strains of pathogenic streptococci and strains of staphylococci encountered in food poisonings. This medium was first reported by Brown and Croutt in 1920 [9], and consisted of a veal infusion base to which had been added defibrinated blood of an indefinite volume. Many modifications of this medium have been reported using either a beef infusion or a beef extract base and to which was added defibrinated blood of either human, dog, horse, sheep, ox, pigeon or rabbit [41].

(3) Bromthymol Blue Lactose Agar

Chapman et al (10) developed this medium for the detection and isolation of pathogenic staphylococci. Upon it approximately 94% of the non-pathogenic staphylococci willnot grow whereas 98% of the pathogenic strains would grow luxuriantly. In 1938 Chapman et al (11)(12) advocated that Phenol Red Mannitol Agar be carried in parallel with their medium for the primary isolation of pathogenic staphylococci and that Crystal Violet Agar be employed for confirmation.

(4) Nutritive Caseinate Agar

Ayers and Mudge in 1920 reported a differential medium consisting of nutrient extract agar and skim milk powder extractives for use in milk control. (1) This medium, as reported, could be used to obtain differential plate counts including both acid forming and peptonising colonies. The original medium has been modified by a number of investigators.

(5) Tomato Juice Agar

This medium consisted of tomato juice, peptone, peptonized milk and agar and was advocated by Mickle and Breed (49) for use in direct plate counts of milk and the cultivation of Lactobacilli. The use of pep-

tonized milk in culture media for the isolation of lactic acid producing bacteria was reported by Kayser in 1894 (33) and Orla Jensen in 1898 (31).

(6) Stone's Extract Gelatin Agar

Stone (85) described a medium in which enterotoxic strains of staphylococci liquified gelatin while cultures not producing the enterotoxic substance did not liquify gelatin. He revised his original formula. His final medium consisted of 3% beef extract, 3% gelatin and 1.5% agar at pH 6.8. In using this medium defibrinated blood may be added to observe the hemolytic activity of the organisms suspected; the incubated plate containing colonies of staphylococci is "developed" by flooding the surface with a saturated solution of ammonium sulfate and permitting the plate to stand for approximately five minutes or until the reaction had become well developed, which consisted of a zoning about the colonies of the enterotoxic strains. Colonies are usually fished from this plate to other media for additional study. The following interpretation has been recommended by Stone for the results obtained.

No hemolysis,no zoning -considered "potentially non-toxic".

No hemolysis,moderate to marked zoning- considered negative .

Hemolysis,no zoning - considered "potentially toxic".

Hemolysis,marked zoning- considered "potentially toxic".

III. EXPERIMENTAL.

A. Methods Employed.

1. Cultures used in this investigation; their source and identification.

The cultures used in this investigation and the sources from which they were obtained were as follows:

a. Maryland Department of Public Health

S.dysenteriae #161: (designated as "161" in this investigation). This culture had been received from the London School of Hygiene in 1937. No other information was available.

S.dysenteriae #639: (designated as "639" in this investigation). No information was available concerning this stock culture.

S.dysenteriae #640: (designated as "640" in this investigation). No information was available concerning this stock culture.

S.paradysenteriae #19: (designated as "19" in this investigation). This culture had been received in 1930 from Parke, Davis Co. Laboratories and numbered as 01654(J64). No other information was available.

S.paradysenteriae #21: (designated as "21" in this investigation). This culture had been received in 1930 from Parke, Davis Co. Laboratories and numbered 01652(J63). No other information was available.

S.paradysenteriae #35451: (designated as "35451" in this investigation). This organism had been isolated from feces of a case on July 12, 1939.

S.sonnei #10: (designated as "10" in this investigation). This culture had been received from Dr.Koser's Collection.Dr.Koser originally obtained it from the British Typo Collection.

S.sonnei #11: (designated as "11" in this investigation). This culture had been received from Dr.Koser's Collection.Dr.Koser originally obtained it from the British Type Collection.

S.sonnei #191: (designated as "191" in this investigation). This culture had been received from Armour Laboratories in 1939. No other information was available.

b. Mississippi Department of Public Health

E.typhosus #5334-41: (designated as "Mayfield" in this investigation). This organism had been isolated from the feces of a typhoid release case in 1941.

S.dysenteriae #5656-41: (designated as "DysMay" in this investigation). This organism had been isolated from the feces of a case in 1941.

S.paradysenteriae : (designated as "ParaUC" in this investigation). This stock culture had been received from Dr.Jordan's Collection at the University of Chicago with date of receipt unknown.

S.paradysenteriae #1-3DM :(designated as "1-3DM" in this investigation). This stock culture had been received from Dr.G.A.Denison of Birmingham,Alabama who isolated it from the feces of a case,date unknown.

S.sonnei : (designated as "SonUC" in this investigation). This stock culture had been received from Dr.Koser's Collection at the University of Chicago. Date of receipt or isolation unknown.

S.typhimurium : (designated as "TM" in this investigation). This stock culture had been received from Dr.Jordan's Collection at the University of Chicago. Date of receipt or isolation unknown.

c. University of Maryland

E.typhosus(Rawlings): (designated as "Rawlings" in this investigation). This organism was a stock culture. Date of receipt unknown.

E.typhosus #58: (designated as "58" in this investigation). This organism was a transplant from the Panama #58 strain used at the Army Medical School for Typhoid Vaccine manufacture.

S.serytryoke #30: (designated as "Ser" in this investigation). This organism was a stock culture whose original transplant was received from the Army Medical School. Date of receipt unknown.

S.enteriditis #36: (designated as "Ent" in this investigation). This organism was a stock culture whose original transplant had been received from the Baltimore City Health Department. Date of receipt was unknown.

S.paratyphi #34: (designated as "A34" in this investigation). This organism was a stock culture obtained from an autopsy with date of isolation unknown.

S.schottmuelleri #35: (designated as "S" in this investigation). This organism was a stock culture obtained from an autopsy with date of isolation unknown.

d. Isolated from fresh stool specimen

E.coli : This organism was isolated on June 8,1941.

Cultural Characteristics

All cultures were purified upon receipt. They were streaked upon Endo Agar and the plates incubated at 37°C. for 24 hours. Typical colonies were fished and inoculations were made in duplicate into Russell Double Sugar Agar tubes. The tubes were incubated at 37°C. for 24 hours after which

their reactions were read and recorded. Inoculations were made from the Russell Double Sugar Agar tubes into duplicate tubes of gelatin, tryptone water (for indole production and testing by the Gore Test (63)), litmus milk, lead acetate agar and the following semi solid carbohydrate media (28): dextrose, dulcitol, inositol, lactose, maltose, mannitol, sucrose and xylose. All tubes were incubated for 24 hours at 37°C. except tryptone water which was incubated for 5 days; gelatin tubes were incubated 10 days at 37°C. and immersed in ice water for several minutes, removed and examined for the presence of liquifaction; the lactose tubes inoculated with S. sonnei strains were incubated for 5 days at 37°C. with daily observations made for acidity and gas production. Motility testing was made from an 18 hour broth culture of each organism. A Gram stained smear was made of each organism.

The results of these tests are displayed in Table 2.

1. The Determination of the pH Range for Growth of Each Organism.

A series of three consecutive daily transplants was made for each organism upon Beef Extract Agar (pH 7.2-7.4) (63) and incubated at 37°C. for 24 hours. From each third transplant a homogeneous suspension of the organism was made in sterile glass distilled water by filtering the suspension through a sterile filter paper. The density of the suspension was standardized against a recently prepared Barium Sulfate Standard (62) #3 using sterile glass distilled water as diluent. From each suspension 0.1 cc. inocula were made into each of two tubes of Beef Extract Broth (pH 3.5) (63).

Similarly 0.1 cc. inocula were made into two tubes each of Beef Extract Broth with pH ranging from 4.0 to 10.1 at approximately 0.5 intervals. Each tube contained 12 cc. of medium. The density of suspension of the inoculated tubes was determined by means of a photoelectric nephthelometer. Uninoculated tubes of broth from each pH used served as controls. All p H determinations were performed with a Beckman pH Meter. All inoculated tubes were incubated at 37°C. for 24 hours. After incubation, the density of suspension in the tubes was again determined. The average of the readings for both tubes of each pH was considered the result for that pH. The pH at which the greatest deflection was observed upon the ammeter was considered the pH for optimum growth for that organism.

The results obtained are displayed in Table 3.

Deductions:

The following deductions may be made from the results obtained.

1. The pH range for optimum growth for 19 of the 22 organisms studied was between 6.85 and 7.2, of which 12 gave maximum deflection in the pH 7.2 tubes.

2. The optimum pH for two of the remaining organisms was 6.2; both organisms showed good growth at 6.85. The optimum pH for the other organism was 8.0 and it yielded a fair growth at 7.2

3. pH 7.2 was considered the optimum for the three groups of bacteria studied.

3. Determination of pH of Chemicals and Reagents reported as incorporated in enrichment or preservative Solutions and differential culture Media used in the Isolation of Organisms of the Eberthella, Salmonella and Shigella groups.

Chemicals and reagents reported to have been used in both enrichment or preservative solutions for stool specimens and differential culture media used in the isolation of members of the three groups of organisms were tested for pH in separate solutions in varying concentrations and in combinations. Glass distilled water was used as solvent. All pH determinations were made with a Beckman pHMeter in duplicate upon the same sample and the average of the two readings considered as the final reaction.

The chemicals employed were the following:

Chemicals

Acetate, sodium, cp.
Chloride, lithium, cp.
" , sodium, cp.
" , " , usp.
Citrate, ferric ammonium, cp.
" , sodium, cp.

Desoxycholate, sodium, pure.
Glycerin, cp.
" , usp.
" , rgt.
Oxbile
Phosphate, potassium (dibasic), cp.
" , " (monobasic), cp.
" , sodium (dibasic), cp.
" , " (monobasic), cp.

Phthalate, potassium, cp.
Sulfate, ferrous, cp.
Sulfite, sodium, cp.
Tellurite, potassium, cp.
Water, distilled (stock)
" , " (glass)

Dyes

Eosin,Y (National Aniline and Chemical Co.)
Fuchsin, acid (Coleman and Bell Co.)
" , basic (" " " ")
Green,brilliant (" " " ")
Thionin (National Aniline and Chemical Co.)
Violet,crystal (Coleman and Bell Co.)

B. DATA.

1. Chemical Solutions Tested and their pH.

The results obtained are displayed in Tables 4,5 and 6.

2. Chemical Solutions yielding pH similar to Optimum pH for Growth for the 3 Groups of Organisms.

Brilliant Green (1:50000) pH 7.2
Crystal Violet (1:50000) pH 7.25
Potassium tellurite (0.01%) in Sodium chloride (0.85%) pH 7.25
Sodium acetate (0.3%) in Sodium chloride (1.0%) pH 7.2
" desoxycholate(0.5%) + Ferric ammonium citrate(0.1%) in Sodium citrate(0.5%) pH 7.2
Thionin (1:100000) pH 7.16

3. Testing of Chemical Solutions of 2(above) Against Organisms of the 3 Groups.

The chemical solutions listed above were tested against the pure cultures,listed previously,to ascertain their effect upon the organisms. The following was the technique employed : All organisms were transplanted to Beef Extract Broth Agar slants (pH 7.2-7.4) and incubated at 37°C.for 24 hours. From these slants,two additional consecutive daily transplants were

made from each organism and similarly incubated. A suspension was prepared from each third daily transplant in sterile glass distilled water (pH 6.8). The suspensions were filtered through sterile filter paper to effect homogeneity. The density of suspension of the filtrates were made approximately uniform with sterile glass distilled water employing the least dense suspension as "standard". Plate counts were made of each of the suspensions, after dilution, upon Endo Agar. 0.1cc. of each suspension was inoculated aseptically into separate one ounce "snap-cap" glass specimen bottles each containing 19.9 cc. of sterile test solution. All solutions had been previously sterilized in the containers for 20 minutes at 15 pounds steam pressure and tested for sterility. The bottle and contents were agitated to insure a good mixture. After 1, 4, 8, 24, 48, 72 and 96 hours incubation at room temperature(21°-24°C.) 1.0 cc. of the contents of each bottle was plated, after dilution, upon Endo Agar. The plates were incubated at 37°C. for 24 hours and the number of typical surface colonies present were counted and recorded. Typical surface colonies only were counted since such procedure made readily possible not only the enumeration but differentiation of these pathogens from other organisms in subsequent experiments. The differentiation between lactose and non-lactose fermenting subsurface colonies is rendered difficult due to the close similarity of both types of colonies, when viewed with a Quebec Colony Counter. Occasional fishings of typical surface colonies were made into Russell Double Sugar Agar with subsequent transplants into carbohydrate media for verification purposes. Gram stained smears were also made from the growth upon Russell Double Sugar Agar. From the law of

averages it becomes evident that the above procedure in counting only surface colonies is a sound procedure since the relationship between surface and subsurface colonies throughout not only these but subsequent experiments approximate throughout since the same technique was employed. This procedure was used with all test solutions listed in B 2 above and in addition several other solutions.

The results obtained are tabulated in Tables 7 through 28 and are graphically displayed in Figures 1 through 22.

Deductions:

From the above screening procedure, the following solutions yielded the results desired upon the organisms tested:

Solution 11 : Sodium desoxycholate(0.5%) + Ferric ammonium citrate (0.1%) in Sodium citrate (0.5%).

Solution 15 : Sodium acetate(0.3%) in Sodium chloride(1.0%).

4. (1). A Study to Determine which of the 2 "desireable" solutions was the better Selective Medium.

In a manner similar to that described in B 3 above, Solutions 11 and 15 were again tested against the 21 pathogenic strains but this time simulated "infected" stool specimens were used instead of pure cultures. Plate counts were made in duplicate upon Endo Agar.

The results obtained are tabulated in Tables 29 and 30 and graphically displayed in Figures 23, 24 and 25.

Deduction:

From the above data, it may be deducted that Solution 11 was the better selective medium.

(2). A Study to Determine the approximate maximum Stool Inoculation that might be employed and yield reasonably good Recovery of the pathogenic Organisms.

In a manner similar to that employed in B 4 (1) above, Solution 11 was again tested against the 21 pathogenic strains in four different series but with the following modifications.

Series A.
To the test solution was added a "normal" (*) inoculum of fresh uninfected stool. The bacterial inocula were in reasonably large numbers (Results obtained from 4 B (1) used here).

Series B.
To the test solution was added a "normal" inoculum of fresh uninfected stool. The bacterial inocula were in reasonably small numbers.

Series C.
To the test solution was added twice the "normal" inoculum of fresh uninfected stool. The bacterial inocula were in reasonably small numbers.

Series D.
To the test solution was added approximately three times the "normal" inoculum of fresh uninfected stool. The bacterial inocula were in reasonably small numbers.

The results obtained are tabulated in Tables 29,31,32 ans 33 and graphically displayed in Figures 23,25,26,27,28,29 and 30.

(*) A "normal" inoculum of fresh uninfected stool= that amount of stool that will readily adhere to the end of a wooden applicator stick(approximately the size of a very small pea).

Deduction:

From the above date, it may be deducted that the maximum stool inoculation that would yield reasonably good recovery of the test organisms was twice the "normal" inoculation.

5. A Comparative Study to Determine the Best Plating Medium for Use in the Isolation of the Organisms.

Transplants of the 21 pathogenic strains of organisms were made upon 2% Human Blood Agar plates and incubated at 37°C. for 24 hours. A transplant of each organism was made from the plates to two Human Blood Agar slants. The inoculated slants were incubated at 37°C. for 24 hours. From one slant of each organism, inoculations were made into two tubes each of Russell Double Sugar Agar, tryptone water, and the following semi-solid carbohydrate media : dextrose, lactose, maltose, mannitol and sucrose. A Gram stain (Hucker Modification) was made upon smears from each organism. All tubes were incubated at 37°C. for 24 hours except the tryptone water, which were incubated 5 days and then tested for indole production by the Gore Test, and the lactose tubes inoculated with S. sonnei strains, which were incubated 4 days at 37°C. with daily observation for fermentation. Motility testing was made from an 18 hour broth culture of each organism. All organisms except S. sonnei (191) gave results similar to those obtained in A 1 . The results obtained from this strain upon Russell Double Sugar Agar were acid butt and slant with gas and in lactose semi-solid media there was produced an acid reaction with no gas within 24 hours. All other characteristics were similar to those obtained for

this organism in A 1. Because of its acquired characteristic for lactose, this organism was eliminated from the ensuing series of experiments.

The second Human Blood Agar slant of each organism was placed into a refrigerator until the tests described above had been completed. Upon completion, a transplant of each organism, except S. sonnei(191) was made upon Human Blood Agar slants and incubated at 37°C. for 24 hours. The growth was suspended in sterile glass distilled water. The suspensions were filtered through sterile filter paper to insure homogeneity. The density of the suspensions were made approximately uniform in a manner similar to that described in B 3 above. "Snap-cap" stoppered specimen bottles, similar to those used in B 3 above, containing 19.9 cc. of Solution 11 were prepared and sterilized at 15 pounds steam pressure for 20 minutes. After cooling to room temperature, the stoppers were snapped into position and the bottles tested for sterility. A plat count in duplicate was made of each bacterial suspension, after dilution, upon Beef Extract Agar. A 0.1 cc. inoculum of each organism was made into separate bottles and the contents agitated to insure a good mixture. After 1, 8, 24, 48, 72 and 96 hours incubation at room temperature (21°-24°C.), 1.0 cc. inocula from each bottle, after dilution, were plated in duplicate upon each of four differential media carried in parrallel. The media were Desoxycholate Citrate Agar, Endo Agar, MacConkey Agar and SS Agar. All plates were incubated at 37°C. for 24 hours and typical surface colonies of each organism were counted.

The results obtained are tabulated in Table 34.

Deduction:

Under the conditions of the experiment, it may be deducted that Endo Agar was the best plating medium for use in the isolation of the members of the three groups of organisms tested.

IV. DISCUSSION.

The assurance of a satisfactory specimen from which dependable laboratory results may be anticipated is *sine qua non* upon which a dependable and accurate laboratory service is based. It therefore behooves the laboratory to insist upon care and thought in the collection of specimens for submission for examination. By a reasonable compliance with suggestions of the laboratory, the percentage of accurate results obtained by the physician may be relatively increased. This is true for all types of specimens and particularly those containing feces. Since feces contains a rich and variable bacterial flora, the isolation of the particular organisms for which search is made will depend, in a large measure, upon the choice and intelligent use of selective and differential media. For selection, it has been customary to use a fluid medium containing substances that will inhibit the growth of bacteria other than the pathogenic incitants for which the search has been made so that the pathogens will remain in either a condition of status quo or proliferate en route to the laboratory and become readily isolated shortly after arrival. For differentiation, there has been usually employed a solid plating medium containing lactose and an indicator that enables the bacteriologist to differentiate reasonably sharply between lactose and non-lactose fermenting organisms.

(67)

It has been reported that among the organisms more commonly found in the large intestine of man are the coliform bacilli, enterococci, staphylococci of both the aureus and albus variety, anaerobic spore-bearing organisms such as Cl.welchii and Cl.putrificum, aciduric bacteria including L.acidophilus, thermophilic bacteria, spirochaetes and yeasts. Less frequent are Proteus bacilli, Ps.pyocyanea, organisms of the Friedlander group, and aerobic spore-bearers such as B.subtilis and B.mesentericus. Due to the rich organic substrate upon which these bacteria have available in the large intestine for proliferation purposes, it becomes evident that the flora of feces would be both abundant in numbers and varied in nature.

In general, the three groups of bacteria concerned in this investigation may be considered "non-lactose fermenters", in that they donot ferment this carbohydrate in broth within twenty four hours when incubated at 37°C. The Shigella, however, include the sonnei strains which usually ferment lactose after several days incubation and are termed "slow lactose fermenters". The sonnei strains, in the absence of a more adequate classification, have been included with the Shigella and from a practical standpoint of primary isolation may be considered among the "non-lactose fermenters." As "non-lactose fermenters", these gram negative pathogenic organisms may be readily distinguished from the more common gram negative non-pathogenic coliform bacilli that ferment lactose; from the gram positive enterococci, staphylococci and anaerobic spore-formers by the Gram stain and morphology and their ability to ferment lactose; and from the gram positive aerobic spore-formers by the Gram stain and morphology.

Because of their characteristic for lactose, these three groups of organisms may become readily differentiated from lactose fermenters upon such differential media as Endo, MacConkey, SS., Desoxycholate, Desoxycholate Citrate, Eosin Methylene Blue and Bismuth Sulfite Agars that contain indicators in addition to lactose, that make possible reasonably sharp differentiation. Lactose fermenters usually are detectable as reddish colored colonies whereas non-lactose fermenters are colorless. Upon Bismuth Sulfite Agar the Shigella organisms grow poorly at best and because of this fact, this medium has not been indicated where the isolation of the Shigella has been concerned. Non-lactose fermenters upon this medium usually develop a blackish colored colony due to the deposition of FeS which had been effected by the reduction of the sulfite present in the medium to sulfide in the presence of glucose and an iron salt.

Since these organisms are usually present in the feces of individuals infected with them, it becomes evident that in the search for these organisms, stool specimens be subjected to bacteriological examination as well as other types of specimens. The manner in which these specimens arrive at the laboratory is largely dependent upon the nearness of the laboratory to the source of material. Frequently where the interval of time involved in transit is small, the specimens are submitted in a fresh state to the laboratory, as is done in hospitals and clinics where laboratory facilities are at the institution. In these institutional laboratories, there is usually employed a selective enrichment medium upon

receipt of the specimen. Among the enrichment media more commonly used are Selenite F Broth and Tetrathionate Broth, in which the usual coliforms and gram positive cocci are inhibited thereby permitting the pathogenic organisms sought to grow unrestricted upon transplantation to a differential plating medium and thereby become readily detected. The use, however, of the former named medium is impractical for use where an examination for the Shigella is indicated because of its toxic action upon these organisms (3).

When the interval of time from source of material to laboratory is not reasonably short, the specimen is considered one for a delayed examination. Since feces contains readily putrescible material and the more common intestinal organisms quickly outgrow the pathogens sought (25) while in transit, there is indicated for use a solution that will "preserve" the specimen until it arrives at the laboratory. The use of "preservative" solutions in this country, among State Public Health Laboratories, varies from a single solution for specimens of all three groups of bacteria, to no preservative for any group (Table 1.).

Among the "preservative" solutions most commonly employed in this country are the following in the order of their appearance in the literature: Teague and Clurman (Glycerin saline) (66), Havens and Mayfield (Lithium chloride- glycerin saline) (26) and Greenfield (Phosphate buffered glycerin saline) (22). In all three solutions glycerin was recommended for its bacteriostatic action upon the non-pathogenic coliform organisms. Unfortunately this action doesnot stop there but becomes general throughout

the specimen and the pathogens sought become subjected to it resulting frequently in the inability to recover them upon the application of the technique for their isolation. Because of its variation in pH, not only among the various grades of the substance but among the various batches within the same grade, from pH 4.0 to pH 5.0 (8), this chemical can be quite toxic for the pathogenic organisms concerned. This general bacteriostatic action is an undesireable feature since the fastidious strains from the three groups, especially from the Shigella, become reduced either in numbers so few as to elude isolation or become completely suppressed or inhibited (Tables 15, 16, 17, 18 and 20). Of the three solutions, Greenfield's solution may be considered as approximating the desireable medium in but one respect, i.e. the adjustment of the pH of the solution to 7.2 before use. This pH was found to be the optimum pH for growth for the members of the three groups in question (Table 3.). The addition of lithium chloride by Havens and Mayfield as a selective bacteriostatic substance for the coliform bacteria has not yielded satisfactory results where members of the Shigella are concerned due to its toxic action upon these organisms (8). Then, too, the dissociative action of the salt upon members of both the coliform and enteric groups of bacteria, has increased the possibilities for failure to readily detect the organisms upon differential plating media thereby decreasing its popularity for use.

In the development of a selective preservative solution for use upon stools for delayed examination, it is evident that since the pH of the suspending or culture medium plays such an important role in the maintainance of optimum growth conditions for bacteria in general (67), it should be equally as important for preservative solutions to have a pH

similar to the optimum for growth of the organisms upon which it is to act in order that the organisms might have as near optimum conditions as possible for reaction. This fact has been emphasized by Greenfield (22) and others (8)(25)(26)(55). It therefore becomes a logical approach to a solution of the problem that the optimum pH for growth of members of the Eberthella, Salmonella and Shigella groups should be determined and that in its process as many strains of each group be employed as possible to make possible a wide range for information. Due to limited facilities, it was felt that the use of a total of 22 selected strains(21 pathogens and E. coli) would be adequate for this investigation.

Among the criteria for a reasonably satisfactory selective preservative solution for members of these groups may be included the following:

1. The medium should have at least a bacteriostatic effect upon those groups of organisms, the presence of which might interfere with the recovery of the individual pathogenic incitants sought. The medium should not be too inhibitory for the undesireable organisms as to effect the desireable organisms in a similar manner (25)(67).

2. In being bacteriostatic for the "undesireable"organisms, it should make possible the maintainance, at least of the desireable organisms in either a condition of status quo while in transit or permit them to proliferate thereby making possible their ready isolation upon receipt in the laboratory.

3. The medium should have a pH similar to the optimum for growth of the organisms for which it was devised.

4. The ingredients should be readily available, the solution comparatively easy to prepare and the cost thereof should not be prohibitive.

5. A reasonably satisfactory medium should make possible the recovery of not only the sturdier members of the groups of bacteria involved but those that are more fastidious since the prestige established by a laboratory for accurate and dependable results is to a large degree predicated by the number of isolations made in "borderline" cases or cases in which the clinical diagnosis has been in doubt.

6. There should be available information concerning the maximum stool inoculum to be used that will yield good recovery of the organisms sought.

From the data submitted in this dissertation, it may be deducted that a solution of Sodium desoxycholate(0.5%) + Ferric ammonium citrate (0.1%) in Sodium citrate(0.5%) at pH 7.2 and prepared in glass distilled water fulfills the above criteria in that

1. In general, the solution maintained a condition at least of status quo for the pathogenic strains used and acted in a "stimulatory" manner upon others, particularly the fastidious strains(S.dysenteriae 161 and 639 and S.paradysenteriae 21) when employed in both pure culture and similated "infected" stool specimens(Tables 7 through 28, and Tables 29, 31, 32 and 33).

2. It exerted a bacteriostatic action upon the normal fecal flora over a period of 4 days, the usual maximum period for keeping spec-

imens in Public Health Laboratories (Tables 29,31,32 and 33).

3. The fact that the solution chosen while selective for the Shigellae is not too highly toxic for the coliforms,a closely related group,would indicate that it might not inhibit the most fastidious Shigellae.

4. The solution had a pH 7.2 which was similar to the optimum for growth of members of the three groups of bacteria involved.

5. The ingredients employed in the solution are readily available, the solution is easily prepared and the cost thereof is not prohibitive.

6. In the use of this solution,information is available concerning the maximum stool inoculum that will yield good recovery of the pathogenic incitants sought (Tables 29,31,32 and 33).

It is strongly felt that any selective preservative solution proposed for use upon stool specimens for delayed examination should be given an opportunity for evaluation against other methods already in use. Such a procedure was included in the original plan for this investigation,but owing to circumstances beyond control of the author,it was not possible to conduct this part of the study. It is the intent of the author that if and when an opportunity should present itself in his new capacity,a field study will be undertaken.

Leifson reported that "the effect of sodium desoxycholate on the growth of bacteria was similar to that of bile but much more powerful" (39). He showed that in a broth or agar medium at pH lower than 7.5,gram positive bacteria failed to grow during the first 24 hours after inoculation,if the medium contained at least a 0.1% concentration of the bile salt. If the pH

was raised above 7.5 (7.6 -9.0) many types of gram positive bacteria grew, and gram negative bacilli became inhibited. The range between 6.5-7.5 was the optimum for selection of gram negative and inhibition of gram positive bacteria. Leifson also showed that a concentration of 0.5% sodium citrate when added to an infusion agar containing 0.5% sodium desoxycholate had a bacteriostatic action upon members of the Aerobacter, Escherichia, Citrobacter and Proteus and as the concentration of the citrate salt was increased to 3% the inhibitory effect was increased. It has been reported that media containing 2.5-3.0% sodium citrate have a marked deliterious effect not only upon undesired microorganisms but also some of the desired ones, particularly members of the Shigella group (3)(24)(47). In the presence of ferric ammonium citrate the toxicity of the medium for some of the intestinal pathogens becomes reduced and the inhibitory action on the colon bacilli becomes enhanced (22). A solution of 0.5% sodium desoxycholate and 0.1% ferric ammonium citrate in 0.5% sodium citrate, in glass distilled water, at pH 7.2 exerted a bacteriostatic effect upon the bacterial flora of normal feces and had little or no deliterious effect on the pathogenic organisms studied(Tables 23, 25, 26 and 27).

In the use of the four differential media carried in parallel for the isolation of the pathogens from simulated specimens, it was observed that with Endo Agar the best recovery of organisms was obtained. Upon this medium all pathogens studied were encountered in goodly numbers and readily detected upon the surface of the medium. All organisms were recovered upon

SS Agar but generally in smaller numbers than upon Endo Agar(Table 34). In the use of MacConkey Agar, 10 or 50% of the organisms studied were not recovered whereas with Desoxycholate Citrate Agar,2 strains of S. sonnei and a fastidious strain of S.dysenteriae(161) were not recovered upon the surface of the media. Other members of the Shigella,except S. paradysenteriae,grew poorly at best thereby confirming the results obtained by other investigators[8][23][24].

V. SUMMARY AND CONCLUSIONS.

A survey was made among State Public Health Laboratory Directors in this country, Hawaii and Puerto Rico to ascertain whether specimen containers for stool specimens suspected of being infected with members of the Eberthella, Salmonella and Shigella groups of bacteria were made available to the medical profession; the preservative solution used in these containers for each group and whether the solution used was yielding satisfactory results from a "recovery of organism" standpoint; whether research was being conducted concerning the development of a single preservative solution for all three groups of specimens and whether the development of such a solution would help to simplify and improve their laboratory services.

From an analysis made from the data compiled, the following facts became established: Replies had been received from 35(72.9%) of the 48 questionaires sent out; 25(71.4%) of the laboratory directors reporting were employing the same preservative solution for specimens of the three groups and that 19(76%) of them were dissatisfied with the results obtained from its use; 27(77.1%) of the directors reported favorable opinions as to whether a single preservative solution would simplify and improve their laboratory services, 2 (5.7%) reported unfavorably, 2(5.7%) expressed doubt and 4(11.3%) failed to answer the question. From this analysis, it was evident that there was a need for such a preservative solution and the undertaking of this investigation was justified and practical.

A resume of the literature pertaining to the subject was made, particularly as it related to the enrichment or selective media reported as employed in the isolation of members of the three groups of bacteria from stool specimens for delayed examination.

Twenty one strains of organisms, members of the three groups, were received from the Maryland and Mississippi Departments of Public Health and University of Maryland. A strain of B.coli was isolated from a fresh uninfected stool specimen. The organisms were purified and checked for their cultural characteristics. The pH range for growth for each organism was determined in Beef Extract Broth ranging from pH 3.5 to 10.1 at approximately 0.5 intervals. All pH determinations were made with a Beckman pHMeter. The density of growth for each organism, at each pH used, was determined by means of a photoelectric nephthelometer. The pH at which there was the greatest deflection upon the ammeter was considered the optimum pH for growth of that organism. The optimum range for 19 of the 22 strains used was pH 6.85 to 7.2 of which 12 showed maximum deflection in the pH 7.2 tubes. Of the remaining three strains, two gave maximum deflection at pH 6.2 with reasonably good growth at pH 6.85 and 7.2; the third culture gave maximum deflection at pH 8.0 and with but fair growth at pH 7.2 . pH 7.2 was therefore considered the optimum for growth for members of the three groups of bacteria.

The pH of both chemicals and reagents reported as the principle ingredients in preservative solutions and differenital media used in the isolation of the members of the three groups of bacteria were determined

in single solutions of varying concentration and in combinations thereof. Glass distilled water was employed as the solvent in each case. Solutions that yielded a pH of 7.2 were as follows:

Brilliant green (1:50000)
Crystal violet (1:50000)
Potassium tellurite(0.01%) in Sodium chloride(0.85%)
Sodium acetate(0.3%) in Sodium chloride (1.0%)
Sodium desoxycholate(0.5%) + Ferric ammonium citrate (0.1%) in Sodium citrate (0.5%)
Thionin (1:100000)

These solutions in addition to several others, making a total of 18, were tested against the pure cultures to ascertain their effect upon the organisms at the end of 1, 4, 8, 24, 48, 72 and 96 hours incubation at room temperature (21°-24°C) by enumerating the typical surface colonies of each organism observed upon Endo Agar plates. Two solutions were obtained that yielded, in general, a "stimulatory" effect upon the pathogens, particularly the fastidious strains of S. dysenteriae (161 and 639) and S. paradysenteriae (21). These solutions were:

Solution 11
Sodium desoxycholate(0.5%) + Ferric ammonium citrate(0.1%) in Sodium citrate(0.5%).

Solution 15
Sodium acetate(0.3%) in Sodium chloride(1.0%).

To determine the better selective solution, the two solutions were again tested against the 21 pathogenic strains in a manner similar to that employed previously except that in addition there had been added to each bottle a "normal" inoculum of fresh uninfected stool. Solution 11 was observed to effect the better selective action in exerting a bacteriostatic action upon the coliform organisms over a period of four days

thereby permitting the pathogens to grow reasonably unrestricted. Solution 15 didnot exert as marked a bacteriostatic action upon the coliform organisms.

Solution 11 was again tested against the pathogenic strains to determine the maximum stool inoculation that would yield reasonably good recovery of the organisms. In these experiments,both the bacterial and stool inoculations were varied in concentration as follows:

Series A
: to the test solution was added a "normal" inoculum of fresh uninfected stool.The bacterial inocula were in reasonably large numbers.

Series B
: to the test solution was added a "normal"inoculum of fresh uninfected stool. The bacterial inocula were in reasonably small numbers.

Series C
: to the test solution was added approximately twice the "normal" inoculum of fresh uninfected stool. The bacterial inocula were in reasonably small numbers.

Series D
: to the test solution was added approximately three times the "normal" inoculum of fresh uninfected stool, The bacterial inocula were in reasonably small numbers.

It was observed that Solution 11 yielded good recovery of the test organisms in the presence of twice the "normal" inoculation of stool. This volume was considered the maximum limit for inoculation purposes.

To determine the best selective plating medium to be used for the isolation of the organisms from simulated "infected" stool specimens,Series C was repeated and in addition to Endo Agar as plating medium,Desoxycholate Citrate Agar,MacConkey Agar and SS Agar were carried in parallel. All sim-

ulated specimens were plated upon the four media in duplicate, after the necessary dilutions were made. An enumeration of the typical surface colonies of each organism was made in duplicate upon each medium and the average of the two readings was considered the final plate count. Endo Agar was observed to be the best selective medium for the recovery of all organisms of the three groups of bacteria tested.

CONCLUSIONS.

The following conclusions may be arrived at from the above data:

1. A solution of Sodium desoxycholate(0.5%) + Ferric ammonium citrate(0.1%) in Sodium citrate(0.5%) gave satisfactory results as a selective preservative solution for simulated stool specimens "infected" with members of the Enteric groups of bacteria.

2. Endo Agar was the best selective plating medium used, under the experimental conditions, for the isolation of the organisms of these three groups of bacteria.

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VII. EXHIBITS.

TABLE 1.

Analysis of Data Compiled from Survey of State Public Health Laboratories (*).

Number of Questionaires sent out	48
" " " received	35(72.9%)
1. Laboratories providing services for all 3 groups of bacteria	32(91.3%)
" " " " Eberthella and Salmonella groups	1(2.9%)
" " " " " group only	1(2.9%)
" " no services for any group	1(2.9%)-35
2. Preservative Solutions used for Eberthella group (by laboratories) :	
30% Glycerin in Physiological Saline	10(28.5%)
" " " " " (buffered)	2(5.7%)
" " " 0.6% NaCl (buffered)	1(2.9%)
" " " Distilled water	5(14.2%)
" " " 0.6% NaCl	2(5.7%)
" " " Distilled water + Brilliant Green(1:400)	2(5.7%)
" " " Physiological Saline + 0.5% $LiCl_3$	3(8.5%)
50% " " " " "	1(2.9%)
10% Oxbile " Distilled water	1(2.9%)
" " + 0.5% $LiCl_3$	2(5.7%)
Undiluted Oxbile	2(5.7%)
No Preservative Used	4(11.3%)-35
Laboratories satisfied with present preservative solution:	
yes	25(71.4%)
no	7(20.0%)
doubtful	1(2.9%)
no reply	2(5.7%)
3. Preservative Solutions used for Salmonella group (by laboratories) :	
30% Glycerin in Physiological Saline	9(25.7%)
" " " " " (buffered)	1(2.9%)
" " " 0.6% NaCl	2(5.7%)
" " " " " (buffered)	1(2.9%)
" " " Distilled water	5(14.2%)
" " " " " + Brilliant Green(1:400)	2(5.7%)
50% " " Physiological Saline	1(2.9%)
10% Oxbile " Distilled water	1(2.9%)
" " " 0.5% $LiCl_3$	2(5.7%)
Undiluted Oxbile	2(5.7%)
No Preservative Used	4(11.3%)
No Reply or Not Performed	2(5.7%)-35
Laboratories satisfied with present preservative solution :	
yes	21(60.0%)
no	6(17.1%)
doubtful	2(5.7%)
no reply	6(17.1%)-35
4. Preservative Solutions used for Shigella group (by laboratories) :	
30% Glycerin in Physiological Saline	10(28.5%)
" " " " " (buffered)	2(5.7%)
" " " " " + Na Desoxycholate (buffered)	1(2.9%)
" " " Distilled water + Brilliant Green (1:400)	1(2.9%)
" " " 0.6% NaCl	3(8. 5%)
" " " Distilled water	3(8.5%)
50% " " Physiological Saline	1(2.9%)
30% " " 0.5% $LiCl_3$	2(5.7%)
10% Oxbile + 0.5% $LiCl_3$	2(5.7%)
Na Desoxycholate-Citrate (Bangxang)	1(2.9%)
No Preservative Used	5(14.2%)
No Reply or Not Performed	4(11.3%)-35
Laboratories satisfied with present preservative solution :	
yes	11(31.7%)
no	11(31.7%)
doubtful	5(14.2%)
no reply	8(22.8%)-35
5. Laboratories engaged in research concerning the replacement of present preservatives with a more satisfactory one	5(14.2%)
Discontinued or Indefinite 2	
Contemplated 3	
Laboratories not engaged in research	30(85.7%)-35
Laboratories undertaking research for a single preservative for 3 groups but which had been discontinued	2(5.7%)
6. Laboratory Directors' reaction re effect of single preservative for 3 groups upon laboratory service rendered :	
help to simplify and improve service	27(77.1%)
will not help to simplify and improve service	2(5.7%)
doubtful as to effect	2(5.7%)
no reply	4(11.3%)-35

(*) Date questionaires mailed : February 21,1941.

TABLE 2.

Cultural Characteristics of Organisms.

Organisms	Russell Double Sugar Agar		Dextrose	Dulcitol	Inositol	Lactose	Lead Acetate Agar	Litmus Milk	Maltose	Mannitol	Sucrose	Xylose	Gram Stain	Motility	Indole	Gelatin Liquifaction	Endo Agar
	B	Sl.															
E.typhosus(58)	A	Alk.	A	-	-	-	±	-	A	A	-	A	- rods	+	-	-	typ.
E. " (Rawlings)	A	Alk.	A	-	-	-	±	-	a	A	-	A	- rods	+	-	-	typ.
E. " (Mayfield)	A	Alk.	A	-	-	-	-	-	A	A	-	A	- rods	+	-	-	typ.
S.aerytryoke (Aer)	AG	Alk.	AG	AG	AG	-	+	a	AG	AG	A	AG	- rods	+	-	-	typ.
S.enteriditis (Ent)	A	Alk.	Ag	AG	AG	-	+	-	-	Ag	A	AG	- rods	+	-	-	typ.
S.paratyphi (A34)	AG	Alk.	A	AG	-	A	-	a	-	-	-	-	- rods	+	-	-	typ.
S.schottmuelleri (B)	AG	Alk.	AG	AG	AG	-	+	-	AG	AG	-	AG	- rods	+	-	-	typ.
S.typhimurium (T8)	AG	Alk.	AG	-	AG	-	+	a	AG	AG	-	-	- rods	+	-	-	typ.
S.dysenteriae (161)	A	Alk.	A	-	-	-	-	a	-	-	-	-	- rods	-	-	-	typ.
S. " (639)	A	Alk.	A	-	-	-	-	a	A	-	-	-	- rods	-	-	-	typ.
S. " (640)	A	Alk.	A	-	-	-	-	a	A	-	-	-	- rods	-	-	-	typ.
S. " (DysMay)	A	Alk.	A	-	-	-	-	a	-	-	-	-	- rods	-	-	-	typ.
S.paradysenteriae (19)	A	Alk.	A	-	-	-	-	-	A	A	-	-	- rods	-	-	-	typ.
S. " (21)	A	Alk.	A	-	-	-	-	a	-	A	-	-	- rods	-	-	-	typ.
S. " (35451)	A	Alk.	A	-	-	-	-	-	A	A	-	-	- rods	-	-	-	typ.
S. " (ParaUC)	A	Alk.	A	-	-	-	-	a	A	A	-	-	- rods	-	-	-	typ.
S. " (1-3DE)	A	Alk.	A	-	-	-	-	a	-	A	-	-	- rods	-	-	-	typ.
S.sonnei (10)	A	Alk.	A	-	-	a 4da	-	-	A	A	-	-	- rods	-	-	-	typ.
S. " (11)	A	Alk.	A	-	-	a 3da	-	a	a	A	-	-	- rods	-	-	-	typ.
S. " (191)	A	Alk.	A	-	-	a 3da	-	a	A	A	A	-	- rods	-	-	-	typ.
S. " (SonUC)	A	Alk.	A	-	-	a 4da	-	-	A	A	-	-	- rods	-	-	-	typ.
E.coli	AG	A	AG	AG	a	AG	-	A clot	AG	AG	a	AG	- rods	+	+	-	typ.

AG = Acid + Gas; Ag = Acid + slight gas; A = Acid; a = slight acid; a 3da = slight acid at end of 3 days; - = no growth or reaction; rods = gram negative bacilli; typ.= typical colony formation for the group; + = positive; ± = slight H_2S production.

TABLE 3.

Nephthelometer Readings of Growth Densities at Varying pH in Extract Broth.

Organisms	Uninoculated Broth	Inoculated Broth	3.5	4.0	4.45	5.0	5.6	6.2	6.85	7.2	7.6	8.0	8.6	9.0	9.5	10.1
E.typhosus (58)	50.0	49.5	48.5	48.5	48.5	38.0	32.5	29.5	30.5	33.0	38.0	40.0	42.5	45.0	48.5	49.5
E. " (Rawlings)	50.0	49.5	49.5	48.5	48.5	45.	37.	33.	31.5	28.	34.	34.	38.	43.5	46.5	49.5
E. " (Mayfield)	50.	49.5	48.5	48.5	46.	43.	40.	35.	30.	27.	32.	39.	45.	48.5	49.	49.
S.aerytryoke (Aer)	50.	49.	48.5	45.	40.	37.5	34.	32.5	30.	28.5	33.5	36.5	40.	43.5	46.	49.5
S.enteriditis (Ent)	50.	49.	49.	48.5	46.	43.5	40.	33.5	29.	32.5	36.	38.	42.	46.	49.	49.
S.paratyphi (A34)	50.	49.5	49.5	49.	49.5	45.5	40.	37.5	34.	38.	40.	42.	46.	48.5	49.5	49.5
S.schottmuelleri (B)	50.	49.5	49.	48.5	46.	42.	35.	32.	31.	28.5	32.5	37.5	40.	46.	49.5	49.5
S.typhimurium (TM)	50.	49.5	48.5	48.5	45.	36.	32.5	31.	30.	28.	32.	36.5	39.	43.	46.5	49.5
S.dysenteriae (161)	50.	49.5	49.5	49.	48.5	48.5	45.	40.5	38.5	35.	39.	43.5	48.5	49.5	49.5	49.5
S. " (639)	50.	49.5	49.5	48.5	48.5	43.	41.5	39.	37.5	35.	31.	28.	36.	44.	48.5	49.5
S. " (640)	50.	49.5	49.5	48.5	48.5	44.	41.5	36.	31.	35.5	39.	41.5	46.	48.5	49.	49.5
S. " (DysMay)	50.	49.5	49.5	48.5	46.	44.	38.	35.5	34.	31.	38.5	40.	46.5	48.5	49.5	49.5
S.paradysenteriae(19)	50.	49.5	48.5	48.5	48.5	46.	42.	39.	35.	33.	38.5	42.5	45.	47.	49.5	49.5
S. " (21)	50.	49.5	49.5	48.5	48.5	45.	41.5	39.	37.5	35.	39.5	42.5	45.5	48.5	49.5	49.5
S. " (35451)	50.	49.5	49.5	48.5	48.	45.	40.	36.	32.5	36.	40.	42.	44.5	48.5	49.5	49.5
S. " (1-3DE)	50.	49.5	49.	48.5	48.5	45.5	42.	38.5	35.	32.	36.	41.5	46.	48.5	49.5	49.5
S. " (ParaUC)	50.	49.5	49.5	49.	49.	45.	41.5	40.	37.	32.	39.5	43.5	49.	49.5	49.5	49.5
S.sonnei (10)	50.	49.5	49.5	48.	48.5	42.	36.5	33.	31.	33.5	36.	37.5	43.5	46.5	49.	49.5
S. " (11)	50.	49.5	49.5	48.5	48.5	43.	36.	32.5	34.	38.	40.	43.	46.	47.	48.5	49.5
S. " (191)	50.	49.5	49.5	49.	48.5	43.5	39.	35.	31.	34.	41.	44.	46.5	48.5	49.5	49.5
S. " (SonUC)	50.	49.5	49.5	48.5	48.5	43.	39.	34.	30.	35.	40.	43.	46.	48.5	49.5	49.5
E.coli	50.	49.	49.	48.5	48.5	43.5	37.5	33.	29.	24.	28.5	34.	36.	40.	45.	49.

TABLE 4.

Reactions of Chemicals in Solution.

Chemicals	Concentration(*)																		
	.0001%	.001%	.002%	.004%	.005%	.01%	.02%	.03%	.05%	.1%	.2%	.3%	.4%	.5%	.85%	1.0%	2.0%	5.0%	10.0%	dry or undiluted
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
Acetate, sodium, cp.										6.4	6.5	6.6	6.8	6.85		6.95				
Chloride, lithium, cp.										6.3	6.35			6.3		6.5				3.3
" , sodium, cp.															6.3	6.35		6.2	6.0	6.4
" , " , usp.															6.15	6.1		6.0	5.9	5.0
Citrate, Ferric Ammonium, cp.										3.95		3.0		2.75		2.5				4.7
" , sodium, cp.														6.05		6.65	7.0			5.6
Desoxycholate, sodium										6.35		6.35		7.0		7.45				8.3
Oxbile										7.5				7.65						7.0
Phosphate, potassium (dibasic) cp.										5.5	5.2	5.05	4.85	4.9		4.7				6.45
" " (monbasic) cp.										8.2	8.3	8.4	8.45	8.5		8.6				9.0
" , sodium, (dibasic) cp.										5.4	5.3	5.35	5.15	5.1		4.8				6.9
" , " , (monbasic) cp.										8.1	8.15	8.2	8.3	8.35		8.4				8.9
Phthalate, Potassium, cp.										4.0	3.95	3.9	3.85	3.8		3.7				4.65
Sulfate, ferrous, cp.						5.4	5.3	5.1	4.75	4.4										5.8
Sulfite, sodium, cp.	6.9	7.1	7.5	8.1			8.65			9.0	9.2					9.35				8.5
Tellurite, potassium, cp.		6.9			7.5	8.8														9.55

(*) diluent of glass distilled water pH-6.9

TABLE 4 (Continued).

Reactions of Chemicals in Solution.

Chemicals	Concentration (*) 5.0%	10.0%	25.0%	50.0%	50.0%	75.0%	Undiluted
	pH	pH	pH	pH	pH	pH	pH
Glycerin, cp.	5.6	5.85	5.65	5.85	6.2	6.0	5.1
" , rgt.	6.6	6.7	6.9	6.8	6.8	6.8	6.8
" , usp.	5.8	5.95	6.0	6.2	5.0	5.95	5.9
Water, distilled (stock)							4.8
" , " (glass)							6.9

(*) diluent of glass distilled water pH 6.9.

TABLE 6.

Reactions of Chemicals in Combined Solutions (*)

Chemicals	pH
Acetate, sodium, cp.	
0.1% in 0.85% NaCl, cp.	6.9
1.0% "	6.6
5.0% "	6.5
10.0% "	6.45
0.3% in 0.85% "	7.5
1.0% "	7.2
5.0% "	7.0
10.0% "	7.1
0.5% in 0.85% "	7.65
1.0% "	7.15
5.0% "	7.2
10.0% "	7.3
1.0% in 0.85% "	7.75
1.0% "	7.6
5.0% "	7.8
10.0% "	8.0
0.1% in 0.1% KH_2PO_4, cp.	5.7
.3% "	5.6
.5% "	5.5
1.0% "	5.25
0.3% in 0.1% "	6.1
.3% "	5.75
.5% "	5.65
1.0% "	5.4
0.5% in 0.1% "	6.2
.3% "	5.9
.5% "	5.9
1.0% "	5.6
0.1% in 0.1% K_2HPO_4, cp.	7.7
.3% "	8.15
.5% "	8.3
1.0% "	8.4
0.3% in 0.1% "	7.75
.3% "	8.15
.5% "	8.3
1.0% "	8.4
0.5% in 0.1% "	7.7
.3% "	8.1
.5% "	8.3
1.0% "	8.45
1.0% in 0.1% "	7.75
.3% "	8.1
.5% "	8.3
1.0% "	8.4
0.1% in 0.1% NaH_2PO_4, cp.	6.15
.3% "	5.8
.5% "	5.65
1.0% "	5.4
0.3% in 0.1% "	6.3
.3% "	5.9
.5% "	5.75
1.0% "	5.65
0.5% in 0.1% "	6.35
.3% "	6.05
.5% "	5.9
1.0% "	5.7
1.0% in 0.1% "	6.55
.3% "	6.15
.5% "	6.0
1.0% "	5.85

Chemicals	pH
Acetate, sodium, cp.	
0.1% in 0.1% Na_2HPO_4, cp.	7.8
.3% "	8.3
.5% "	8.4
1.0% "	8.55
0.3% in 0.1% "	7.85
.3% "	8.3
.5% "	8.45
1.0% "	8.55
0.5% in 0.1% "	7.8
.3% "	8.25
.5% "	8.4
1.0% "	8.55
1.0% in 0.1% "	7.85
.3% "	8.25
.5% "	8.4
1.0% "	8.45
Chloride, lithium, cp.	
0.1% in 0.85% NaCl, cp.	5.05
1.0% "	5.35
5.0% "	5.95
10.0% "	5.75
0.5% in 0.85% "	5.25
1.0% "	6.26
5.0% "	5.9
10.0% "	5.75
1.0% in 0.85% "	5.2
1.0% "	5.25
5.0% "	5.35
10.0% "	5.0
0.1%+0.85% NaCl, cp. in 5% Glycerin, mt.	5.8
.5%+ " " " " " "	5.7
1.0% " " " " " "	5.6
0.1%+1.0% " " " " "	5.85
.5%+ " " " " "	5.6
1.0%+ " " " " "	5.5
0.1%+5.0% " " " " "	5.55
.5%+ " " " " "	5.45
1.0%+ " " " " "	5.4
0.1%+10.0% " " " " "	5.15
.5%+ " " " " "	5.2
1.0%+ " " " " "	5.25
0.1%+0.85% " " " " "	5.95
.5%+ " " " " "	6.15
1.0%+ " " " " "	6.4
0.1%+1.0% " " " " "	6.45
.5%+ " " " " "	6.6
1.0%+ " " " " "	6.7
0.1%+5.0% " " " " "	6.55
.5%+ " " " " "	6.0
1.0%+ " " " " "	6.5
0.1%+10.0% " " " " "	6.55
0.5%+ " " " " "	6.4
1.0%+ " " " " "	6.3
0.1%+0.85% " " " " "	5.7
.5%+ " " " " "	6.0
1.5%+ " " " " "	6.2
0.1%+1.0% " " " " "	6.2
.5%+ " " " " "	6.45
1.0%+ " " " " "	6.3
0.1%+5.0% " " " " "	6.05
.5%+ " " " " "	5.9
1.0%+ " " " " "	6.15
0.1%+10.0% " " " " "	5.7
.5%+ " " " " "	5.8
1.0%+ " " " " "	5.95

Chemicals	pH
Chloride, sodium, cp.	
0.85% in 0.1% Ox gall	5.77
.5% "	6.35
1.0% "	6.6
1.0% in 0.1% "	5.65
.5% "	6.1
1.0% "	6.25
5.0% in 0.1% "	5.65
.5% "	6.3
1.0% "	6.05
10.0% in 0.1% "	6.0
.5% "	6.6
1.0% "	6.3
0.85% in 0.001% Potassium tellurite, cp	5.15
.005% "	6.6
.01% "	7.25
1.0% in 0.001% "	5.85
.005% "	6.7
.01% "	7.45
5.0% in 0.001% "	5.8
.005% "	6.75
.01% "	7.5
10.0% in 0.001% "	5.65
.005% "	6.85
.01% "	6.75
0.85% in 0.0001% Na_2O_2, cp.	6.3
.001% "	6.4
.002% "	6.42
.004% "	6.4
.02% "	6.8
.1% "	7.05
.2% "	7.55
1.0% "	8.4
1.0% in 0.0001% "	6.2
.001% "	6.25
.002% "	6.2
.004% "	6.15
.02% "	6.3
.1% "	6.3
.2% "	7.5
1.0% "	8.1
5.0% in 0.0001% "	6.0
.001% "	6.05
.002% "	6.0
.004% "	6.0
.02% "	6.0
.1% "	7.0
.2% "	7.5
1.0% "	8.1
10.0% in 0.0001% "	5.75
.001% "	5.95
.002% "	5.95
.004% "	6.0
.02% "	5.85
.1% "	6.66
.2% "	7.05
1.0% "	7.7
0.85% in 0.1% K_2HPO_4, cp.	8.65
.3% "	9.05
.5% "	9.2
1.0% "	9.1
1.0% in 0.1% "	8.35
.3% "	8.9
.5% "	8.85
1.0% "	8.95
5.0% in 0.1% "	8.15
.3% "	8.5
.5% "	8.65
1.0% "	8.7
10.0% in 0.1% "	7.9
.3% "	8.4
.5% "	8.5
1.0% "	8.55

(*) diluent was glass distilled water pH 6.8

TABLE 5 (Continued)

Chemicals	pH
Chloride, sodium, cp.	
0.85% in 0.1% KH_2PO_4, cp.	4.7
.3% "	4.5
.5% "	4.5
1.0% "	4.4
1.0% in 0.1% "	4.85
.3% "	4.8
.5% "	4.65
1.0% "	4.6
5.0% in 0.1% "	4.75
.3% "	4.45
.5% "	4.4
1.0% "	4.35
10.0% in 0.1% "	4.55
.3% "	4.55
.5% "	4.55
1.0% "	4.55
0.85% in 0.1% Na_2HPO_4, cp.	8.5
.3% "	8.85
.5% "	8.85
1.0% "	8.9
1.0% in 0.1% "	8.4
.3% "	8.75
.5% "	8.8
1.0% "	8.85
5.0% in 0.1% "	8.1
.3% "	8.6
.5% "	8.6
1.0% "	8.75
[illegible]	[illegible]
0.85% in 0.1% NaH_2PO_4, cp.	4.75
.3% "	4.7
.5% "	4.8
1.0% "	4.9
1.0% in 0.1% "	5.1
.3% "	5.0
.5% "	5.1
1.0% "	4.8
5.0% in 0.1% "	4.9
.3% "	4.75
.5% "	4.95
1.0% "	4.7
10.0% in 0.1% "	5.15
.3% "	4.6
.5% "	4.85
1.0% "	4.55
0.85% in 0.1% Na desoxycholate	6.9
.3% "	7.2
.5% "	7.3
1.0% "	7.65
1.0% in 0.1% "	6.6
.3% "	7.0
.5% "	7.45
1.0% "	7.6
5.0% in 0.1% "	6.8
.3% "	6.9
.5% "	7.3
1.0% "	7.55
0.85% in 0.5% Na citrate, cp.	6.75
1.0% "	6.85
2.0% "	7.0
1.0% in 0.5% "	6.7
1.0% "	6.85
2.0% "	7.0
5.0% in 0.5% "	6.5
1.0% "	6.6
2.0% "	6.75

Chemicals	pH
Chloride, sodium, cp.	
0.85% in 0.1% $Fe(NH_4)$ citrate, cp	3.5
.3% "	2.85
.5% "	2.8
1.0% "	2.4
1.0% in 0.1% "	3.5
.3% "	2.85
.5% "	2.65
1.0% "	2.45
5.0% in 0.1% "	3.85
.3% "	2.8
.5% "	2.8
1.0% "	2.4
0.85% in 5% Glycerin, rgt.	5.1
10% "	5.6
25% "	5.9
30% "	5.95
50% "	6.1
75% "	6.1
1.0% in 5% "	6.0
10% "	5.7
25% "	5.6
30% "	5.6
50% "	5.65
75% "	5.15
5.0% in 5% "	5.8
10% "	5.15
25% "	5.25
30% "	5.5
[illegible]	[illegible]
[illegible]	[illegible]
10% "	5.8
25% "	6.45
30% "	5.3
50% "	5.45
75% "	5.8
Citrate, ferric, ammonium, cp.	
0.1% in 0.5% Na Citrate, cp.	6.85
1.0% "	7.0
2.0% "	7.1
0.3% in 0.5% "	6.1
1.0% "	6.45
2.0% "	6.6
0.5% in 0.5% "	5.75
1.0% "	6.15
2.0% "	6.35
1.0% in 0.5% "	4.95
1.0% "	5.65
2.0% "	6.0
0.1% in 0.1% Na desoxycholate	insoluble
.3% "	
.5% "	
1.0% "	
0.3% in 0.1% "	
.3% "	
.5% "	even with
1.0% "	
0.5% in 0.1% "	
.3% "	
.5% "	prolonged
1.0% "	
1.0% in 0.1% "	
.3% "	
.5% "	boiling
1.0% "	

Chemicals	pH
Citrate, ferric, ammonium, cp.	
0.1% + 0.5% Na citrate, cp. in 0.1% Na desoxycholate	
(cloudy)	6.7
.3% " (clear)	6.9
.5% " (clear)	7.20
1.0% " (clear)	7.45
0.3% + 0.5% Na citrate in 0.1% Na desoxycholate	
(ppt.)	6.4
.3% " (ppt.)	6.65
.5% " (ppt.)	6.9
1.0% " (jelled)	7.25
0.5% + 0.5% Na citrate in 0.1% Na desoxycholate	
(h.ppt.)	6.1
.3% " (jelled)	6.3
.5% " (ppt.)	6.5
1.0% " (jelled)	7.0
1.0% + 0.5% Na citrate in 0.1% Na desoxycholate	
(h.ppt.)	5.2
.3% " (ppt.)	5.5
.5% " (h.ppt.)	5.8
1.0% " (jelled)	6.5
0.1% + 1.0% Na citrate in 0.1% Na desoxycholate	
(clear)	6.95
.3% " (ppt.)	7.4
.5% " (ppt.)	7.35
1.0% " (ppt.)	7.5
[illegible]	[illegible]
0.1% + 1.0% Na citrate in 0.1% Na desoxycholate	
(ppt.)	6.6
.3% " (sl.jell)	7.3
.5% " (jelled)	7.05
1.0% " (jelled)	7.1
0.5% + 1.0% Na citrate in 0.1% Na desoxycholate	
(ppt.)	6.3
.3% " (ppt.)	7.0
.5% " (ppt.)	6.7
1.0% " (jelled)	7.0
1.0% + 1.0% Na citrate in 0.1% Na desoxycholate	
(h.ppt.)	5.9
.3% " (h.ppt.)	6.2
.5% " (jelled)	6.45
1.0% " (jelled)	6.6
0.1% + 2.0% Na citrate in 0.1% Na desoxycholate	
(clear)	7.05
.3% " (sl.jell)	7.1
.5% " (clear)	7.2
1.0% " (clear)	7.35
0.3% + 2.0% Na citrate in 0.1% Na desoxycholate	
(jelled)	6.8
.3% " (jelled)	6.75
.5% " (jelled)	6.9
1.0% " (jelled)	7.0
0.5% + 2.0% Na citrate in 0.1% Na desoxycholate	
(ppt.)	6.5
.3% " (h.ppt.)	6.6
.5% " (s.jelled)	6.75
1.0% " (jelled)	6.9
1.0% + 2.0% Na citrate in 0.1% Na desoxycholate	
(h.ppt.)	5.85
.3% " (h.ppt.)	6.75
.5% " (h.ppt.)	6.0
1.0% " (h.ppt.)	6.2

TABLE 5 (continued)

Chemicals	pH
Citrate, ferric, ammonium, cp.	
0.1% in 0.1% Na desoxycholate (ppt.)	5.95
.3% " (ppt.)	6.7
.5% " (ppt.)	6.9
1.0% " (ppt.)	7.2
0.3% in 0.1% Na desoxycholate (ppt.)	3.0
.3% " (ppt.)	4.5
.5% " (ppt.)	6.2
1.0% " (ppt.)	6.8
0.5% in 0.1% Na desoxycholate (ppt.)	2.7
.3% " (ppt.)	3.0
.5% " (ppt.)	3.7
1.0% " (ppt.)	6.9
1.0% in 0.1% Na desoxycholate (ppt.)	2.4
.3% " (ppt.)	2.5
.5% " (ppt.)	2.7
1.0% " (ppt.)	3.0
Citrate, sodium, cp.	
0.5% in 0.1% Na desoxycholate	7.5
.3% "	7.55
.5% "	7.7
1.0% "	7.9
1.0% in 0.1% Na desoxycholate	7.8
.3% "	7.6
.5% "	7.7
1.0 "	7.9
3.0 in 0.1 "	7.7
.3 "	7.75
.5% "	7.75
1.0% "	7.95
Glycerin, rgt.	
5.0% + 0.85% NaCl, cp. +	
0.1% $NaSe_2$, cp.	7.3
.3% "	8.3
.5% "	8.5
1.0% "	8.7
5.0% + 1.0% NaCl +	
0.1% $NaSO_2$	7.3
.3% "	8.25
.5% "	8.5
1.0% "	8.7
10.0% + 0.85% NaCl +	
0.1% $NaSO_2$	7.75
.3% "	8.25
.5% "	8.45
1.0% "	8.65
10.0% + 1.0% NaCl +	
0.1% $NaSO_2$	7.7
.3% "	8.2
.5% "	8.3
1.0% "	8.60
30.0% + 0.85% NaCL +	
0.1% $NaSO_2$	7.6
.3% "	8.15
.5% "	8.3
1.0% "	8.5
30.0% + 1.0% NaCL +	
0.1% $NaSO_2$	7.55
.3% "	8.0
.5% "	8.2
1.0% "	8.45

Chemicals	pH
Phosphate, potassium (dibasic) cp.	
0.1% in 0.1% K_2HPO_4, cp.	6.1
.3% "	6.9
.5% "	7.2
1.0% "	7.45
0.3% in 0.1% "	6.1
.3% "	6.45
.5% "	6.65
1.0% "	7.15
0.5% in 0.1% "	5.9
.3% "	6.45
.5% "	6.65
1.0% "	6.85
1.0% in 0.1% "	5.85
.3% "	6.0
.5% "	6.25
1.0% "	6.8
Phosphate, sodium (dibasic), cp.	
0.1% in 0.1% Na_2HPO_4, cp.	6.5
.3% "	6.7
.5% "	6.85
1.0% "	7.2
0.3% in 0.1% "	6.1
.3% "	6.5
.5% "	6.7
1.0% "	6.9
0.5% in 0.1% "	5.9
.3% "	6.3
.5% "	6.5
1.0% "	6.9
1.0% in 0.1 "	5.8
.3 "	6.0
.5% "	6.1
1.0% "	6.45
0.1% in 0.1% KH_2PO_4, cp.	5.25
.3% "	5.0
.5% "	4.9
1.0% "	4.8
0.3% in 0.1% "	5.05
.3% "	5.2
.5% "	4.9
1.0% "	4.8
0.5% in 0.1% "	5.15
.3% "	5.0
.5% "	4.9
1.0% "	4.85
1.0% in 0.1% "	4.85
.3% "	5.0
.5% "	5.05
1.0% "	4.85

Chemicals	pH
Phosphate, sodium (dibasic) cp.	
0.1% in 0.1% K_2HPO_4, cp.	6.3
.3% "	6.6
.5% "	7.0
1.0% "	7.45
0.3% in 0.1% "	6.5
.3% "	6.6
.5% "	6.7
1.0% "	6.5
0.5% in 0.1% "	6.55
.3% "	6.6
.5% "	6.6
1.0% "	6.45
1.0% in 0.1% "	6.4
.3% "	6.45
.5% "	6.6
1.0% "	6.5
Phosphate, sodium (monobasic) cp.	
0.1% in 0.1% K_2HPO_4, cp.	8.35
.3% "	8.45
.5% "	8.55
1.0% "	8.8
0.3% in 0.1% "	8.65
.3% "	8.7
.5% "	8.78
1.0% "	9.0
0.5% in 0.1% "	8.7
.3% "	8.8
.5% "	8.7
1.0% "	8.95
1.0% in 0.1 "	8.8
.3 "	8.86
.5% "	8.9
1.0% "	9.0
0.1% in 0.1% KH_2PO_4, cp.	7.15
.3% "	6.5
.5% "	6.35
1.0% "	6.0
0.3% in 0.1% "	7.5
.3% "	7.0
.5% "	6.85
1.0% "	6.35
0.5% in 0.1% "	7.75
.3% "	7.05
.5% "	6.8
1.0% "	6.5
1.0% in 0.1% "	8.0
.3% "	7.4
.5% "	7.2
1.0% "	6.9

TABLE 6.

Reactions of Dyes in Solution.

Dye	Concentration(*)								
	1:100	1:500	1:1000	1:5000	1:25000	1:30,000	1:100,000	1:1million	Dry
	pH	pH	pH	pH	pH	pH	pH	pH	pH
Eosin,y	6.75	7.0	7.0	6.9	6.9	6.9	6.9	6.9	6.43
Fuchsin,acid	4.6	5.25	5.8	6.4	6.6	6.7	6.8	6.7	3.4
Fuchsin,basic	5.45	5.9	6.95	7.0	6.95	6.9	6.9	6.9	10.25
Green,brilliant	2.75	3.6	4.15	5.05	6.75	7.2	7.35	7.55	1.8
Thionin	3.6	6.0	6.35	6.85	6.9	7.0	7.15	7.0	1.2
Violet,crystal	4.9	6.6	6.8	7.1	7.2	7.25	7.3	7.35	3.55

(*) diluent of glass distilled water ph=6.9

TABLE 7.

Effect of Test Solutions upon E.typhosus(58).

Solutions(*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1.Distilled Water(stock)	630.	300.	12.	0.2	0.05	0.0	0.0	0.0
2. " " (glass)	12000.	1200.	900.	750.	300.	70.	20.	4.7
3.0.85% NaCl,cp.	24000.	20000.	13000.	12000.	3000.	600.	300.	100.
4.30% Glycerin,cp.	10000.	800.		4600.	1800.	6100.	440.	700.
5.30% " ,rgt.	12750.	6280.	6200.	7400.	3300.	2200.	660.	540.
6.30% " ,rgt. in phosphate buffered saline	9000.	10000.	5500.	5000.	3300.	1680.	910.	280.
7.30% Glycerin,rgt.+ Crystal Violet(1:50000)	10000.	3000.	4000.		6000.	1400.	590.	420.
8.30% Glycerin,rgt.+ Brilliant Green(1:50000)	22750.	4480.	10000.	10000.	2000.	7100.	2440.	2400.
9.Brilliant Green(1:50000)	20500.	13000.	4000.	3000.	380.	1.	0.	0.
10.Thionin(1:100000)	6000.	920.			4.	0.8	0.	0.
11.Na Desoxycholate(0.5%)+ Fe(NH_4) Citrate(0.1%)in Na Citrate(0.5%)	13000.	3900.	12000.	12000.	55400.	19000.	27000.	43800.
12.Na Desoxycholate(0.5%) in Na Citrate(0.5%)	12000.	10000.	18000.		20000.	5300.		3900.
13. (5) + (11)	4000.	3030.	4400.	1200.	900.	220.		81.
14.Na Desoxycholate(0.3%) in NaCl(0.85%)	5500.	4900.	5000.	5460.	1200.	120.	8.	120.
15.Na Acetate(0.3%) in NaCl(1.0%)	24500.	26000.	24000.	28000.	40200.		52000.	40000.
16.KF(0.01%) in NaCl(0.85%)	24500.	10000.	4000.	1000.	12.6	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

Table 8.

Effect of Test Solutions upon E.typhosus(Rawlings).

Solutions(*)	Plate Counts in Thousands							
	Initial	1 hr.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
1. Distilled Water(stock)	720.	5.2	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	4000.	800.	400.	200.	.5	.1	0.	0.
3. 0.85% NaCl,cp.	80000.	50000.	20000.	8000.	330.	90.	40.	1.5
4. 30% Glycerin,cp.	3000.	4000.		6300.	5000.	4700.	600.	9 4.
5. 30% " ,rgt.	6000.	5640.	6100.	5600.	5100.	3400.	10.	4.
6. 30% " ,rgt. in phosphate buffered saline	110000.	60000.	5000.	3000.	1000.	140.	170.	20.8
7. 30% Glycerin,rgt.+ Crystal Violet(1:50000)	1400.	1400.	100.		35.8	12.	0.8	0.3
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	10250.	600.	150.	120.	70.	0.4	0.42	0.0
9. Brilliant Green(1:50000)	24750.	20000.	16000.	5000.	1000.	20.	.008	0.0
10. Thionin(1:100000)	13000.	8000.			38.	0.6	0.0	0.0
11. Na Desoxycholate(0.5%)+ Fe(NH_4) Citrate(0.1%) in NaCitrate(0.5%)	7500.	2080.	6000.	6800.	5300.	720.	210.	15.6
12. Na Desoxycholate(0.3%) in Na Citrate(0.5%)	7000.	9000.	12000.		10000.	900.		13.9
13. (5)+ (11)	3750.	1440.	6200.	3200.	2000.	330.		13.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	31250.	20000.	16000.	9000.	4700.	3460.	2600.	2400.
15. Na Acetate(0.3%) in NaCl(1.0%)	12500.	3000.	2000.	2540.	3400.		3200.	422.
16. KI(0.01%) in NaCl(0.85%)	12500.	320.	40.	3.	0.2	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 9.

Effect of Test Solutions upon B.typhosus(Mayfield).

Solutions(*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	550.	11.0	0.00	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	4000.	900.	300.	270.	9.	.4	.72	0.0
3. 0.85% NaCl,ep.	8000.	1000.	2620.	100.	10.	0.	0.	0.
4. 30% Glycerin,ep.	5000.	7000.		8800.	6000.	5200.	1300.	700.
5. 30% " ,rgt.	6000.	2620.	3700.	3600.	36 0.	2200.	80.	30.
6. 30% " ,rgt.in phosphate buffered saline	8000.	6000.	4000.	1.00.	2000.	600.	710.	80.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	8000.	7300.	3200.		350.	500.	150.	30.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	10000.	280.	500.	120.	70.	2.2	70.	10.
9. Brilliant Green(1:50000)	15750.	8000.	3000.	5000.	800.	10.	0.3	0.
10. Thionin(1:100000)	5500.	1350.	300.	50.	8.	1.2	0.	0.
11. Na Desoxycholate(0.5%)+ Fe(NH_4) Citrate(0.1%) in Na Citrate(0.5%)	2500.	360.	4000.	7100.	4000.	1280.	530.	36.0
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	13250.	20000.	17000.		16000.	7500.		1700.
13. (5) + (11)	6000.	7000.	3800.	3450.	5100.	430.		51.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	10000.	2500.	2620.	2300.	5200.	2000.	1100.	600.
15. Na Acetate(0.3%) in NaCl(1.0%)	14000.	10000.	12000.	4000.	4200.		2100.	6600.
16. KI(0.01%) in NaCl(0.85%)	14500.	40.	2.	0.4	0.0	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 10.

Effect of Test Solutions upon S.aerytrycke(Aer).

Solutions(*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	800.	260.	3.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	2000.	100.	2.	.7	0.	0.	0.	0.
3. 0.85% NaCl,cp.	6000.	10000.	1940 .	330.	100.	3.	1.	.2
4. 30% Glycerin,cp.	1280.	2000.		2500.	2000.	800.	.8	.003
5. 30% " ,rgt.	10500.	9000.	4900.	8400.	2800.	800.	300.	90.
6. 30% " ,rgt. in phosphate buffered saline	92000.	100000.	83000.	70000.	4100.	3.6	110.	70.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	8000.	9000.	14900.		1180.	1000.	300.	80.
8. 30% Glycerin,rgt.+ Brilliant Green(1:50000)	20000.	20000.	10000.	1610.	1050.	24.	20.	.068
9. Brilliant Green(1:50000)	7500.	5000.	30000.	1000.	200.	.3	0.	0.
10.Thionin(1:100000)	6000.	580.		12.	.8	0.	0.	0.
11.Na Desoxycholate(0.5%)+ $Fe(NH_4)$Citrate(0.1%) in Na Citrate(0.5%)	8000.	2100.	2000.	4300.	9800.	45000.	38000.	68100.
12.Na Desoxycholate(0.5%) in Na Citrate(0.5%)	8500.	9000.	6000.		12000.	3000.		1700.
13. (5)+(11)	4000.	790.	3900.	1800.	10000.	4000.	39.	.9
14.Na Desoxycholate(0.5%) in NaCl(0.85%)	14000.	4260.	6000.	4120.	3900.	4100.	4600.	5100.
15.Na Acetate(0.3%) in NaCl (1.0%)	9250.	9000.	4920.	2000.	3100.	5100.	8200.	18400.
16.KT(0.01%) in NaCl(0.85%)	9250.	2580.	580.	180.	13.4	.033	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 11.

Effect of Test Solutions upon S.enteriditis(Ent).

Solutions(*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	6hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	750.	133.	0.6	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	1120.	80.	61.	2.	.8	0.	0.	0.
3. 0.85% NaCl,cp.	14000.	1950.	720.	20.	1.	.9	.5	.1
4. 30% Glycerin,cp.	8000.	10000.	10000.	3900.	3000.	220.	10.	1.9
5. 30% " ,rgt.	4000.	630.	1900.	103.	200.	300.		48.
6. 30% " ,rgt. in phosphate buffered saline	8800.	9800.	8800.	7200.	5100.	1480.	1450.	180.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	6000.	5000.	3700.		200.	20.	20.	30.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	13500.	7000.	900.	600.	280.	1.2	.019	0.
9. Brilliant Green(1:50000)	20500.	30000.	20000.	9000.	110.	.2	0.	0.
10. Thionin(1:100000)	10750.	800.		80.	.9	0.	0.	0.
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	14500.	3580.	8000.	8100.	8000.	18000.	20000.	20200.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	9500.	10000.	12000.		14000.	8000.		800.
13. (5)+(11)	4000.	630.	1900.	103.	200.	300.		48.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	12000.	13000.			4300.	1800.	1400.	1100.
15. Na Acetate(0.3%) in NaCl(1.0%)	11000.	1000.	1430.		1.	9.	72.	600.
16. KT(0.01%) in NaCl(0,85%)	11000.	1380.	2020.	480.	42.8		4.	6.

(*) diluent was glass distilled water except as indicated.

TABLE 12

Effect of Test Solutions upon S.paratyphi(A34).

Solutions(*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1.Distilled Water(stock)	600.	210.	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	4760.	650.	10.	2.5	0.	0.	0.	0.
3. 0.85% NaCl,cp.	6000.	2000.	800.	200.	1.	.02	.002	0.
4. 30% Glycerin,cp.	45000.	40000.	21000.	13200.	480.	200.	130.	23.
5. 30% " ,rgt.	10000.	10000.	9600.	5900.	4900.	14.	4.8	1.
6. 30% " ,rgt.in phosphate buffered saline	8600.	10000.	8200.	7000.	4400.	580.	510.	20.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	20000.	20000.	12000.		790.	300.	220.	20.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	5000.	800.	800.	520.	640.	51.	31.	3.
9. Brilliant Green(1:50000)	10500.	6000.	5000.	3000.	400.	.2	.008	0.
10.Thionin(1:100000)	9500.	650.	210.	30.	2.	0.	0.	0.
11.Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	14000.	4200.	9000.	8900.	13000.	23000.	18000.	2000.
12.Na Desoxycholate(0.5%) in Na Citrate(0.5%)	18000.	15000.	7000.		45000.	3300.		3400.
13. (5)+(11)	6000.	3150.	4300.	2100.	1600.	190.		.1
14.Na Desoxycholate(0.3%) in NaCl(0.85%)	11000.	8000.	1030.	1120.	1600.	58.	6.8	6.6
15.Na Acetate(0.3%) in NaCl(1.0%)	20500.	17000.	23000.		5000.	7100.	6200.	2800.
16.KT(0.01%) in NaCl(0.85%)	20500.	3960.	880.	6.	.4	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 13.

Effect of Test Solutions upon S.schottmuelleri(B).

Solutions (*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	554.	100.	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	2480.	1000.	295.	10.	.8	.2	0.	0.
3. 0.85% NaCl,cp.	30000.	20000.	15000.	3000.	40.	10.	5.3	.1
4. 30% Glycerin,cp.	55000.	41000.	12500.	6000.	880.	300.	202.	15.
5. 30% " ,rgt.	8000.	6000.	7100.	6000.	6200.	15.4	1.1	.6
6. 30% " ,rgt.in phosphate buffered saline	6400.	5300.	6500.	5000.	4100.	880.	920.	640.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	6000.	7600.	23400.		10000.	2500.	370.	260.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	13250.	2320.	3120.	2490.	1110.	1.8	.182	0.
9. Brilliant Green(1:50000)	9000.	2000.	2000.	910.	28.	.3	.009	0.
10. Thionin(1:100000)	12250.	430.	330.	120.	4.	.3	0.	0.
11. Na Desoxycholate(0.5%)+ Fe(NH_4)Citrate(0.1%) in Na Citrate(0.5%)	12250.	830.	736.	600.	2400.	660.	1110.	21000.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	17500.	16000.	9000.		2000.	3000.		1100.
13. (5)+(11)	5000.	1690.	1900.	1000.	600.	400.		10.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	20000.	22000.	16000.	2610.	1800.	1320.	4600.	4300.
15. Na Acetate(0.3%) in NaCl(1.0%)	15250.	12000.	6000.	570.	15.2	200.	680.	15000.
16. KT(0.01%) in NaCl(0.85%)	15250.	410.	50.	5.	1.6	.023	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 14.

Effect of Test Solutions upon S.typhimurium(TM).

Solutions (*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	600.	80.	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	4200.	800.	100.	4.	.1	0.	0.	0.
3. 0.85% NaCl,ep.	12000.	15000.	6000.	1000.	130.	20.	0.	0.
4. 30% Glycerin,ep.	5000.	5000.		9000.	16000.	18200.	16100.	376.
5. 30% " ,rgt.	15750.	12000.	7000.	3800.	2000.	100.	.9	.062
6. 30% " ,rgt.in phosphate buffered saline	192000.	120000.	112000.	81000.	9600.	4000.	1350.	50.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	12000.	18000.	15000.	12000.	4000.	4900.	990.	460.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	10000.	4030.	3000.	10000.	2000.	6.8	.148	.008
9. Brilliant Green(1:50000)	16500.	6000.	5000.	4000.	1000.	.6	.003	0.
10. Thionin(1:100000)	9000.	800.		80.	1.	3.2	5.3	.6
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	15500.	17000.	19000.	8900.	9800.	9000.	18000.	22200.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	19250.	13000.	13000.		23000.	22600.		13900.
13. (5)+(11)	9000.	8000.	5700.	4200.	1500.	580.		60.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	12000.	11000.	8000.	6620.	5300.	1980.	1200.	800.
15. Na. Acetate(0.3%)in NaCl(1.0%)	11250.	10000.	6000.	8000.	30600.		50200.	10600.
16. KT(0.01%) in NaCl(0.85%)	11250.	320.	50.	5.	.6	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 15.

Effect of Test Solutions upon S.dysenteriae(161).

(*) Solutions	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1.Distilled Water(stock)	400.	0.4	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	2680.	650.	1.	.08	0.	0.	0.	0.
3. 0.85% NaCl,ep.	1000.	300.	410.	220.	30.	0.	0.	0.
4. 30% Glycerin, ep.	30000.	15000.	90.	10.	0.	0.	0.	0.
5. 30% " ,rgt.	9000.	520.	3.	1.6	4.1	0.	0.	0.
6. 30% " ,rgt.in phosphate buffered saline	80000.	50000.	350.	22.	.8	0.	0.	0.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	4000.	1500.	10.	2.	.2	0.	0.	0.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	4580.	680.	510.	350.	180.	.008	.002	0.
9. Brilliant Green(1:50000)	10000.	7000.	9000.	4000.	800.	1.5	.2	0.
10.Thionin(1:100000)	10250.	8900.			8.	.012	0.	0.
11.Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	5000.	120.	90.	12.	600.	200.	120.	8.6
12.Na Desoxycholate(0.6%) in Na Citrate(0.5%)	8000.	9000.	7000.		8000.	30.	6.	0.
13. (5)+(11)	13000.	12000.	8000.	800.	5.	.8	0.	0.
14.Na Desoxycholate(0.5%) in NaCl(0.85%)	10000.	8000.	3000.	130.	1000.	4.	0.	0.
15.Na Acetate(0.3%) in NaCl(1.0%)	17000.	90.	23.	.8	.039	.4	3.	400.
15.Kt(0.01%) in NaCl(0.85%)	17000.	12.	.009	0.0	0.0	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 16.

Effect of Test Solutions upon S.dysenteriae(639).

Solutions (*)	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	300.	0.9	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	3500.	820.	27.	.9	1.	0.	0.	0.
3. 0.85% NaCl,cp.	8000.	7000.	80.	10.	.3	.01	0.	0.
4. 30% Glycerin,cp.	50000.	40000.		3000.	1250.	100.	10.	.4
5. 30% " ,rgt.	4000.	330.	2600.	2900.	250.	.21	.007	0.
6. 30% " ,rgt.in phosphate buffered saline	20000.	80.	65.	31.	1.	.084	.002	.000
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	1560.	900.	93.		2.3	3.	.6	.4
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	7500.	290.	190.	200.	12.	.32	.012	.003
9. Brilliant Green(1:5000)	5000.	4000.	3000.	1000.	39.	.2	0.	0.
10. Thionin(1:100000)	5000.	2000.		120.	10.	.2	0.	0.
11. Na Desoxycholate(0.5%)+ Fe(NH_4) Citrate(0.1%) in Na Citrate(0.5%)	3000.	800.	12.	2.	6.8	5800.	7000.	2300.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	8000.	980.	810.	620.	900.	12.	0.	0.
13. (5)+(11)	12000.	260.	50.	65.8	38.9	20.1		.86
14. Na Desoxycholate(0.3% in NaCl(0.85%)	8000.	9000.	13000.	5300.	1800.	33.4	29.4	4.2
15. Na Acetate(0.3%) in NaCl(1.0%)	10000.	720.	600.	620.	500.		66.	68.
16. Kt(0.01%) in NaCl(0.85%)	10000.	15.	0.0	0.0	0.0	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 17.

Effect of Test Solutions upon S.dysenteriae(640).

(*) Solutions	Plate Counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	700.	100.	0.01	0.2	0.0	0.0	0.0	0.0
2. " " (glass)	15000.	650.	1.0	.5	.008	0.	0.	0.
3. 0.85% NaCl,cp.	12000.	10000.	80.	2.	.6	.04	0.	0.
4. 30% Glycerin,cp.	38000.		8300.	3600.	1260.	300.	250.	90.
5. 30% " ,rgt.	7000.	970.	1600.	800.	1100.	2.	.8	.007
6. 30% " ,rgt.in phosphate buffered saline	80000.	65000.	2900.	2200.	1300.	.034	.102	2.
7. 30% Glycerin,rgt.+ Crystal Violet(1:50000)	6000.	7000.	1600.		2480.	2000.	1050.	270.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	7500.	1100.	630.	420.	250.	9.2	.38	.018
9. Brilliant Green(1:50000)	7000.	6000.	8000.	4000.	300.	.8	.002	0.
10. Thionin(1:100000)	3000.	900.			3.	.6	0.	0.
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	5000.	120.	100.	96.	4300.	980.	1100.	200.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	14000.	13000.	8000.		630.	2.4		.8
13. (5)+(11)	175.	170.	2400.	2500.	2500.	52.5		.285
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	2500.	1540.	860.		600.	520.	9.8	6.2
15. Na Acetate(0.3%) in NaCl(1.0%)	3000.	3080.	2100.	2200.	3200.		384.	400.
16. KT(0.01%) in NaCl(0.85%)	3000.	570.	390.	20.	.052	0.0	0.0	0.0

(*) Diluent was glass distilled water except as indicated.

TABLE 13.

Effect of Test Solutions upon S.dysenteriae(DysMay).

Solutions (*)	Plate Counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1.Distilled Water(stock)	500.	61.	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	60000.	300.	8.	.2	.006	0.0	0.0	0.0
3. 0.85% NaCl,cp.	12000.	11000.	1000.	72.	.024	0.0	0.0	0.0
4. 30% Glycerin,cp.	25000.	20000.		4200.	1400.	1370.00	2100.	259.
5. 30% " ,rgt.	7000.	850.	1300.	2000.	1800.	34.	3.	.022
6. 30% " ,rgt.in phosphate buffered saline	80000.	50000.	2100.	800.	19.	.061	0.0	0.0
7. 30% Glycerin,rgt.+Crystal Violet)1:50000)	6000.	9000.	12000.	1300.	290.	44.	2.	.8
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	5750.	1500.	300.	140.	20.	2.2	3.	.3
9. Brilliant Green(1:50000)	7750.	6000.	3000.	920.	500.	.041	.004	0.0
10.Thionin(1:100000)	8000.	820.	610.		5.	.1	0.0	0.0
11.Na Desoxycholate(0.5%)+ Fe(NH_4) Citrate(0.1%) in Na Citrate(0.5%)	4500.	220.	1240.	3300.	3600.	480.	1400.	600.
12.Na Desoxycholate(0.5%) in Na Citrate(0.5%)	8000.	9000.	1750.	2200.	8000.	33.8		1.9
13. (5)+(11)	400.	460.	40.	72.	46.	4.5		.078
14.Na Desoxycholate(0.5%) in NaCl(0.85%)	8000.	9000.	1200.	640.	4200.	2900.	29.4	13.
15.Na Acetate(0.3%) in NaCl(1.0%)	7500.	6000.	2000.	3540.	21400.		406.	500.
16.KI(0.01%) in NaCl(0.85%)	7500.	1040.	210.	9.	.041	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 19.

Effect of Test Solutions upon S.paradysenteriae(19).

Solutions (*)	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	800.	140.	8.	.3	0.0	0.0	0.0	0.0
2. " " (glass)	1660.	40.	23.	12.	2.	0.0	0.0	0.0
3. 0.85% NaCl,cp.	2200.	2000.	890.	550.	70.	0.0	0.0	0.0
4. 30% Glycerin,cp.	4000.	3000.	2000.	3300.	850.	200.	500.	400.
5. 30% " ,rgt.	8000.	4300.	5900.	7400.	5800.	1200.	150.	17.6
6. 30% " ,rgt.in phosphate buffered saline	2600.	1500.	2000.	1800.	400.	90.	1.	1.8
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	3480.	3000.	6000.		510.	500.	50.	30.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	6750.	1520.	1380.	1020.	410.	33.5	100.	.086
9. Brilliant Green(1:50000)	9000.	8000.	6000.	5000.	1200.	.8	0.0	0.0
10. Thionin(1:10000)	6000.	590.		65.	16.	.032	0.0	0.0
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	4500.	2070.	9000.	8200.	9700.	960.	9000.	1800.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	10000.	11000.	12000.		9000.	7200.		800.
13. (5)+(11)	1200.	1180.	2800.	1500.	1000.	1300.		44.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	1500.	490.	460.	310.	300.	89.	2.2	2.1
15. Na Acetate(0.3%) in NaCl(1.0%)	8625.	8000.	2000.	6000.	6400.		8200.	14600.
16. KT(0.01%) in NaCl(0.85%)	8625.	50.	4.	.6	.041	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 20.

Effect of Test Solutions upon S.paradysenteriae(21).

Solutions (*)	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1.Distilled Water(stock)	450.	4.	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	4000.	60.	2.	0.0	0.0	0.0	0.0	0.0
3. 0.85% NaCl,cp.	6000.	4000.	800.	300.	8.	.06	.02	0.0
4. 30% Glycerin,cp.	7200.	6000.		100.	50.	100.	368.	40.
5. 30% " ,rgt.	11000.	5600.	5600.	5400.	3900.	11.6	8.8	.072
6. 30% " ,rgt.in phosphate buffered saline	6000.	6000.	4300.	3200.	1400.	230.	110.	10.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	1800.	2000.	8000.		34.5	11.	10.	.8
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	4500.	100.	30.	130.	2.	.8	.012	0.0
9. Brilliant Green(1:50000)	9000.	8000.	7000.	5000.	1200.	.4	.004	0.0
10.Thionin(1:100000)	16250.	6000.			320.	1.1	0.0	0.0
11.Na Desoxycholate(0.2%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	4500.	1700.	5000.	4000.	9100.	620.	5000.	1920.
12.Na.Desoxycholate(0.5%) in Na Citrate(0.5%)	11000.	15000.	13000.		8000.	15.1		1.8
13. (5)+(11)	2300.	570.	2800.	200.	95.	110.		230.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	2000.	480.	610.	580.	1200.	2150.	15200.	13800.
15.Na Acetate(0.3%) in NaCl(1.0%)	5000.	5360.	2000.	1510.	3400.		550.	310.
16.RT(0.01%) in NaCl(0.85%)	5000.	800.	410.	40.	.061	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 21.

Effect of Test Solutions upon S. paradysenteriae (35451).

(*) Solutions	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	700.	500.	51.	12.6	0.5	0.003	0.0	0.0
2. " " (glass)	4000.	100.	2.	.3	.3	0.0	0.0	0.0
3. 0.85% NaCl,cp.	16000.	9980.	6000.	2000.	.95	8.	6.6	.8
4. 30% Glycerin,cp.	10000.	10000.	5000.	2700.	1200.	1430.	3400.	2500.
5. 30% " ,rgt.	5500.	5400.	5400.	5300.	3200.	30.6	4.8	.8
6. 30% " ,rgt.in phosphate buffered saline	6200.	6000.	5000.	5100.	3000.	1520.	1840.	510.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	14000.	11000.	3200.		230.	200.	30.	4.3
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	11250.	1520.	900.	420.	160.	100.	.2	.011
9. Brilliant Green(1:50000)	20250.	20000.	12000.	10000.	400.	.6	0.0	0.0
10. Thionin(1:100000)	10250.	8000.			7.	.2	0.0	0.0
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	8000.	1080.	1500.	2500.	6000.	2000.	1800.	2300.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	15400.	15000.	17000.		920.	102.4		1.1
13. (5)+(11)	9240.	10000.	2600.	1300.	1500.	400.		145.
14. Na Desoxycholate(0.3%) in NaCl(0.85%)	8000.	890.	920.	870.	4200.	460.	500.	600.
15. Na Acetate(0.3%) in NaCl(1.0%)	12000.	5000.	820.	430.	9.4		5.	.008
16. KT(0.01%) in NaCl(0.85%)	12000.	1000.	220.	40.	0.0	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 22.

Effect of Test Solutions upon S.paradysenteriae(1-SDH).

Solutions (*)	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	700.	300.	2.4	0.3	0.0	0.0	0.0	0.0
2. " " (glass)	250.	170.	10.	.4	0.0	0.0	0.0	0.0
3. 0.85% NaCl,cp.	5000.	1700.	1000.	1510.	210.	80.	.295	0.0
4. 30% Glycerin,cp.	7800.	8000.	6000.	5300.	1460.	1490.	700.	900.
5. 30% " ,rgt.	11500.	15000.	9800.	7000.	6800.	6.0	4.1	.246
6. 30% " ,rgt. in phosphate buffered saline	7300.	6000.	4300.	4100.	2000.	920.	750.	620.
7. 30% Glycerin,rgt.+ Crystal Violet(1:50000)	8000.	6000.	1300.		130.	98.	20.	1.2
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	8250.	1820.	3000.	1000.	420.	4.2	.682	.022
9. Brilliant Green(1:50000)	10500.	1500.	420.	630.	300.	.4	0.0	0.0
10. Thiamin(1:100000)	5500.	1050.	360.	120.	3.	.012	0.0	0.0
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	13250.	640.	780.	1100.	4800.	5000.	6000.	800.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	16750.	14000.	11000.		19000.	3000.		1000.
13. (5)+(11)	2750.	490.	1000.	1300.	900.	170.		85.
14. Na Desoxycholate(0.5%) in NaCl(0.85%)	16000.	17000.	13000.	8000.	4800.	2360.	2000.	1400.
15. Na Acetate(0.3%) in NaCl(1.0%)	15250.	11000.	12000.	6150.	3200.		4200.	2200.
16. MT(0.01%) in NaCl(0.85%)	15250.	390.	210.	90.	.031	0.0	0.0	0.0

(*) diluent was glass distilled except as indicated.

TABLE 23.

Effect of Test Solutions upon S.paradysenteriae(para UC).

(*) Solutions	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	420.	3.	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	1600.	82.	8.	.09	0.0	0.0	0.0	0.0
3. 0.85% NaCl,cp.	8000.	8000.	8000.	1600.	800.	500.	140.	20.
4. 30% Glycerin,cp.	42000.	36000.	9000.	2700.	630.	1700.	800.	700.
5. 30% " ,rgt.	10000.	8000.	3000.	1300.	800.	1.	.008	.151
6. 30% " ,rgt.in phosphate buffered saline	5800.	2700.	2500.	2100.	1700.	640.	810.	310.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	15000.	5500.	7100.		1280.	2500.	280.	140.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	10000.	500.	150.	130.	30.	.9	.1	.002
9. Brilliant Green(1:50000)	9750.	7000.	4500.	3620.	1200.	.3	0.0	0.0
10. Thionin(1:100000)	17500.	12000.	6200.	430.	8.	.5	0.0	0.0
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	9000.	3540.	5000.	3900.	4200.	2010.	11000.	900.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	15400.	18000.	8000.		24000.	1900.		15.6
13. (5)+(11)	6500.	800.	3800.	1300.	96.	150.		52.
14. Na Desoxycholate(0.5%) in NaCl(0.85%)	16000.	13000.	11000.	4120.	1200.	280.	300.	42.2
15. Na Acetate(0.3%) in NaCl(1.0%)	16250.	9000.	5000.	3000.	4200.		8400.	1800.
16. XT(0.01%) in NaCl(0.85%)	16250.	4000.	720.	200.	2.5	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 24.

Effect of Test Solutions upon S.sonnei(10).

(*) Solutions	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	800.	51.	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	4000.	4000.	1300.	250.	1.	.007	.0	.0
3. 0.85% NaCl,cp.	10000.	10000.	15000.	6000.	1000.	600.	420.	60.
4. 30% Glycerin,cp.	5000.	8000.	6000.	3000.	2000.	1400.	590.	520.
5. 30% " ,rgt.	13000.	7150.	12600.	10000.	8700.	3100.	530.	170.
6. 30% " ,rgt.in phosphate buffered saline	15000.	20000.	1300.	1250.	9500.	4000.	1390.	300.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	4250.	4800.	5100.		0.8	0.482	0.2	0.018
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	15400.	2250.	1200.	930.	260.	11.1	.61	.012
9. Brilliant Green(1:50000)	11000.	8000.	7000.	4000.	100.	3.	.004	0.0
10. Thionin(1:100000)	16500.	1600.			3.	.02	0.0	0.0
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	14750.	6020.	12000.	15000.	27100.	10000.	13000.	16800.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	13250.	14000.	8000.		11000.	4400.		300.
13. (5)+(11)	12000.	11000.	3800.	1800.	1000.	600.		78.
14. Na Desoxycholate(0.5%) in NaCl(0.85%)	13000.	1000.	680.	420.	1200.	80.	5.2	3.6
15. Na Acetate(0.3%) in NaCl(1.0%)	17250.	8000.	3000.	4000.	1200.		.8	1160.
16. KT(0.01%) in NaCl(0.85%)	17250.	680.	100.	80.	.8	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 25.

Effect of Test Solutions upon S.sonnei(11).

Solutions (*)	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water(stock)	400.	3.2	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	12000.	300.	153.	90.	3.	.4	.037	0.0
3. 0.85% NaCl,ep.	10000.	10000.	5200.	800.	470.	60.	4.0	1.0
4. 30% Glycerin,ep	9000.	7000.	2000.	1700.	500.	700.	68.	6.
5. 30% " ,rgt.	11000.	9000.	12800.	9400.	9000.	3800.	1610.	830.
6. 30% " ,rgt.in phosphate buffered saline	7000.	6000.	5000.	3000.	5000.	960.	390.	120.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	9000.	6000.	10000.		13.2	3.	.8	.062
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	21250.	18000.	890.	620.	280.	2.	.018	.002
9. Brilliant Green(1:50000)	10750.	8500.	5000.	4000.	120.	.8	0.0	0.0
10. Thionin(1:100000)	11500.	7000.			32.	.7	0.0	0.0
11. Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	10000.	4070.	6000.	8900.	7300.	6000.	5000.	6800.
12. Na Desoxycholate(0.5%) in Na Citrate(0.5%)	14000.	20000.	18000.		6000.	4100.		1700.
13. (5)+(11)	8250.	6000.	3800.	1200.	800.	330.		12.3
14. Na Desoxycholate(0.5%) in NaCl(0.85%)	8000.	8000.	180.	120.	56.	24.8	1.2	1.2
15. Na Acetate(0.3%) in NaCl(1.0%)	6750.	1420.	130.	50.	.082		.2	211.
16. KT(0.01%) in NaCl(0.85%)	6750.	480.	180.	100.	.072	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 26.

Effect of Test Solutions upon S.sonnei(191)

(*) Solutions	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1.Distilled Water(stock)	400.	12.	5.	2.	.012	0.0	0.0	0.0
2. " " (glass)	4060.	600.	400.	120.	27.	1.8	.049	0.0
3. 0.85% NaCl,cp.	6000.	5000.	1000.	150.	30.	7.	4.7	2.
4. 30% Glycerin,cp.	7200.	8000.	810.	2.	.012	0.0	0.0	0.0
5. 30% " ,rgt.	6000.	330.	1700.	58.	38.	.9	.008	0.0
6. 30% " ,rgt. in phosphate buffered saline	8040.	9000.	750.	500.	.8	0.0	0.0	0.0
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	4000.	3000.	1000.		.09	.012	.008	0.0
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	2000.	180.	80.	50.	50.	.9	0.0	0.0
9. Brilliant Green(1:50000)	5500.	4000.	3000.	2000.	200.	.8	0.0	0.0
10.Thionin(1:100000)	6000.	1500.			7.	.3	0.0	0.0
11.Na Desoxycholate(0.5%)+ $Fe(NH_4)$ Citrate(0.1%) in Na Citrate(0.5%)	10000.	400.	40.	110.	1200.	890.	100.	.093
12.Na Desoxycholate(0.5%) in Na Citrate(0.5%)	13000.	15000.	11000.		1000.	3.		0.0
13. (5)+(11)	6000.	940.	4900.	2800.	8.	1.2	0.0	0.0
14.Na Desoxycholate(0.3%) in NaCl(0.85%)	13000.	1200.	780.	320.	9.	0.0	0.0	0.0
15.Na Acetate(0.3%) in NaCl(1.0%)	8500.	860.	740.	32.	.2	0.0	0.0	0.0
16.KT(0.01%) in NaCl(0.85%)	8500.	60.	2.	.9	0.0	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 27.

Effect of Test Solutions upon S.sonnei(Son UC).

(*) Solutions	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1.Distilled Water(stock)	720.	95.4	0.3	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	3060.	600.	100.	80.	12.	.8	.077	0.0
3. 0.85% NaCl,cp.	26000.	20000.	3000.	1470.	170.	86.	25.	5.0
4. 30% Glycerin,cp.	10000.	11000.	10000.	9200.	4750.	7600.	3000.	3300.
5. 30% " ,rgt.	18000.	17000.	13300.	12200.	8200.	1000.	720.	190.
6. 30% " ,rgt.in phosphate buffered saline	7600.	8000.	7700.	7100.	5100.	620.	340.	120.
7. 30% Glycerin,rgt.+Crystal Violet(1:50000)	5000.	7000.	3000.		2310.	800.	100.	160.
8. 30% Glycerin,rgt.+Brilliant Green(1:50000)	13500.	2320.	1730.	1050.	920.	28.9	.087	.006
9. Brilliant Green(1:50000)	15700.	10000.	6000.	4000.	1900.	.6	.002	0.0
10.Thionin(1:100000)	11750.	8200.			2.	.3	.5	.226
11.Na Desoxycholate(0.5%)+ Fe(NH_4) Citrate(0.1%) in Na Citrate(0.5%)	12250.	3980.	6000.	4500.	6400.	3650.	8000.	5500.
12.Na Desoxycholate(0.5%) in Na Citrate(0.5%)	14000.	14000.	10000.		17000.	6800.		4700.
13. (5)+(11)	5250.	6000.	6300.	4200.	800.	340.	8.	0.6
14.Na Desoxycholate(0.3%) in NaCl(0.85%)	16000.	13000.	6210.	4620.	8900.	9400.	15800.	15500.
15.Na Acetate(0.3%) in NaCl(1.0%)	12000.	15000.	8000.	6000.	17300.		31000.	31200.
16.KT(0.01%) in NaCl(0.85%)	12000.	9.	.021	0.0	0.0	0.0	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 28.

Effect of Test Solutions upon E. coli.

(*) Solutions	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
1. Distilled Water (stock)	560.	17.8	0.0	0.0	0.0	0.0	0.0	0.0
2. " " (glass)	12000.	160.	110.	90.	7.	.8	1.75	.9
3. 0.85% NaCl, cp.	14000.	1660.	3000.	4000.	6000.	8200.	9800.	40.
4. 30% Glycerin, cp.	40000.	32000.	14000.	7700.	4000.	4500.	2500.	2100.
5. 30% " , rgt.	12000.	10000.	11600.	11600.	5800.	500.	869.	320.
6. 30% " , rgt. in phosphate buffered saline	18000.	14000.	13000.	8000.	6600.	3000.	1020.	580.
7. 30% Glycerin, rgt. + Crystal Violet (1:50000)	6000.	8900.	10000.		5000.	1000.	760.	320.
8. 30% Glycerin, rgt. + Brilliant Green (1:50000)	11250.	15000.	10000.	2400.	2310.	2500.	290.	180.
9. Brilliant Green (1;50000)	15000.	2420.	1120.	490.	10.	.061	.008	0.0
10. Thionin (1:100000)	12000.	410.			2.	.9	.6	.004
11. Na Desoxycholate (0.5%) + $Fe(NH_4)$ Citrate (0.1%) in Na Citrate (0.5%)	8000.	2160.	1880.	920.	330.	19.	2.7	.8
12. Na Desoxycholate (0.5%) in Na Citrate (0.5%)	9000.	6000.	6000.		8000.	3200.		2600.
13. (5) + (11)	6000.	7000.	3400.	2800.	300.	330.		86.
14. Na Desoxycholate (0.3%) in NaCl (0.85%)	15600.	13000.	7000.	6000.	6600.	7600.	9800.	9400.
15. Na Acetate (0.3%) in NaCl (1.0%)	47000.	48000.	68000.	60000.	69000.	78000.	82000.	90000.
16. KT (0.01%) in NaCl (0.85%)	47000.	40000.	41000.	10000.	3.4	.2	0.0	0.0

(*) diluent was glass distilled water except as indicated.

TABLE 29.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series A.

Organisms	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
E.typhosus(T58)	430.	360.		1670.	30800.	51200.	56000.	35000.
E. " (Rawlings)	590.	400.		680.	40000.	24200.	102000.	42000.
E. " (Mayfield)	250.	260.		240.	11000.	31600.	24000.	10200.
S.aerytycke (aer)	1280.	900.		900.	6800.	16600.	30000.	26000.
S.enteriditis(Ent)	670.	520.		41.2	1400.	25400.	82000.	12600.
S.paratyphi (A34)	1020.	1600.		1900.	1800.	14200.	22000.	31200.
S.schottmuelleri (B)	1270.	620.		488.	1800.	16800.	28000.	22000.
S.typhimurium (TM)	920.	820.		620.	4800.	26600.	34000.	20200.
S.dysenteriae(161)	160.	80.		10.6	7400.	30000.	48000.	42000.
S. " (639)	160.	60.		7.	1300.	10800.	16000.	12000.
S. " (640)	170.	41.2		100.	11800.	24400.	48000.	40000.
S. " (DysMay)	290.	280.		300.	600.	12000.	20000.	22000.
S.paradysenteriae(19)	1100.	820.		760.	1800.	19400.	56400.	32000.
S. " (21)	210.	23.5		130.	17200.	48400.	52000.	86.
S. " (35451)	210.	210.		210.	32200.	22400.	42000.	25800.
S. " (1-3DE)	480.	280.		420.	3400.	15400.	30000.	6800.
S. " (ParaUC)	600.	510.		680.	4000.	13600.	44000.	8600.
S.sonnei (10)	880.	610.		2100.	21400.	7000.	66000.	52000.
S. " (11)	865.	700.		13400.	9200.	33000.	44000.	20000.
S. " (191)	280.	21.2		13.1	2600.	10400.	32000.	3120.
S. " (SonUC)	1360.	680.		820.	9600.	18200.	28000.	3600.
Control(uninoculated stool) (on Extract Agar pH 7.2-7.4)		9.		10.1	19.	47.	22.	19.

TABLE 30.

Effect of Solution 15 upon Organisms in Simulated Specimens.

Organisms	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
E.typhosus(T58)	430.	390.		1000.	10800.	22600.	30000.	21000.
E. " (Rawlings)	590.	340.		980.	6000.	15200.	24000.	18000.
E. " (Mayfield)	250.	360.		420.	5400.	13000.	28000.	84000.
S.aerytycke(Aer)	1280.	1040.		1160.	4000.	18000.	24000.	6000.
S.enteriditis(Ent)	670.	620.		42.6	100.	6000.	4000.	3000.
S.paratyphi(A34)	1020.	1780.		1810.	1600.	9100.	10000.	8000.
S.schottmuelleri(B)	1270.	980.		560.	12800.	20200.	10000.	6000.
S.typhimurium(TM)	920.	980.		1000.	14200.	980.	17000.	10000.
S.dysenteriae(161)	160.	80.		20.	1200.	21000.	29000.	19000.
S. " (639)	160.	60.		60.	3200.	11000.	13000.	6300.
S. " (640)	170.	37.1		29.	1400.	5200.	42000.	20000.
S. " (DysMay)	290.	180.		53.	800.	8800.	38000.	18000.
S.paradysenteriae(19)	1100.	710.		88.	2000.	10400.	8000.	2000.
S. " (21)	210.	32.2		3.6	2.	.8	62.	72.
S. " (35451)	210.	170.		1000.	8800.	24600.	12000.	10400.
S. " (1-3DE)	480.	400.		380.	9600.	6800.	28000.	10800.
S. " (ParaUC)	600.	360.		210.	300.	12400.	20000.	9800.
S.sonnei (10)	880.	530.		960.	2000.	1800.	122.	900.
S. " (11)	865.	620.		1220.	10400.	12800.	20000.	11200.
S. " (191)	280.	1000.		2.2	400.	8800.	22000.	21600.
S. " (SonUC)	1360.	780.		920.	1000.	5800.	22000.	8000.
Control(uninoculated stool) (on Extract Agar pH 7.2-7.4)		6.9		8.2	220.	810.	3100.	7900.

TABLE 31.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series B.

Organisms	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
E.typhosus(T58)	69.	80.	32.2	200.	8400.	8700.	12800.	16100.
E. " (Rawlings)	70.	100.	33.2	100.	9600.	8100.	6400.	7300.
E. " (Mayfield)	12.	100.	26.2	200.	7800.	8300.	9400.	9900.
S.aerytrycke (Aer)	71.	100.	10.2	12.	1200.	4900.	13400.	17100.
S.enteriditis(Ent)	126.	3.3	11.8	11.	8600.	8400.	11200.	10100.
S.paratyphi(A34)	58.	20.	9.8	8.6	5200.	6500.	9200.	16900.
S.schott muelleri(B)	68.	9.8	14.2	13.	15000.	12900.	7800.	12600.
S.typhimurium (TM)	69.	40.	21.	80.	7000.	8900.	10200.	8300.
S.dysenteriae(161)	29.	20.	9.4	10.8	580.	9100.	13200.	10100.
S. " (639)	2.63	2.4	9.2	24.	8600.	10800.	10200.	10100.
S. " (640)	17.	10.	12.2	6.8	2800.	3400.	5200.	13700.
S. " (DysMay)	4.	2.2	9.	14.2	12800.	14700.	19400.	10800.
S.paradysenteriae(19)	70.	30.	10.8	200.	10800.	9300.	9600.	12600.
S. " (21)	21.	7.4	14.2	12.2	6600.	7800.	9800.	8700.
S. " (35451)	54.	40.	31.2	26.	3200.	4100.	6800.	5900.
S. " (1-3DE)	101.	100.	220.	200.	10200.	12900.	16600.	12100.
S. " (ParaUC)	35.	58.	11.8	18.4	9600.	9900.	11200.	11900.
S.sonnei (10)	104.	110.	30.	13.2	6200.	6100.	6400.	7500.
S. " (11)	44.	60.	14.6	16.2	860.	6300.	11200.	14100.
S. " (191)	20.	10.	13.4	100.	8600.	9100.	10400.	8700.
S. " (SonUC)	104.	100.	24.2	28.6	4800.	5100.	5200.	8900.
Control(uninoculated stool) (on Extract Agar pH 7.2-7.4)		3.8	20.6	23.	78.	63.	36.	1.9

TABLE 32.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series C.

Organisms	Plate counts in Thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
E.typhosus (T58)	70.5	90.	39.	89.	3100.	4100.	7900.	10100.
E. " (Rawlings)	66.	70.	29.	480.	4300.	6300.	4300.	5700.
E. " (Mayfield)	49.	49.	30.	670.	4700.	5100.	4900.	5100.
S. aerytryoke(Aer)	44.	13.	14.	19.	730.	1100.	2900.	3200.
S. enteriditis(Ent)	51.	8.	23.	45.	3100.	4800.	6200.	5100.
S. paratyphi(A34)	50.	20.	8.	9.	520.	340.	2300.	1200.
S. schottmuelleri(B)	49.	11.	14.	22.	430.	320.	560.	410.
S. typhimurium(TM)	49.	42.	16.	41.	29.	3200.	2700.	4200.
S. dysenteriae (161)	31.	22.	11.	12.	360.	680.	3900.	5200.
S. " (639)	23.	2.3	21.	43.	2100.	4200.	3300.	4900.
S. " (640)	41.	32.	20.	16.	230.	3100.	690.	2100.
S. " (DysMay)	51.	29.	33.	59.	3200.	6600.	6200.	7500.
S. paradysenteriae(19)	49.	36.	22.	30.	860.	4100.	7600.	5100.
S. " (21)	71.	50.	62.	122.	5300.	5200.	7200.	5400.
S. " (35451)	61.	56.	43.	2.9	310.	2900.	3400.	1600.
S. " (1-3DE)	31.5	36.	78.	830.	2300.	4100.	5000.	3300.
S. " (ParaUC)	37.	6.3	12.	29.	4200.	5800.	4100.	5400.
S. sonnei (10)	23.5	16.	5.	9.	2300.	2500.	2900.	3300.
S. " (11)	80.5	100.	27.	36.	210.	900.	2300.	6200.
S. " (191)	31.5	19.	21.	2.2	290.	2900.	4800.	5200.
S. " (SonUC)	52.	50.	130.	210.	320.	900.	2200.	1200.
Control(uninoculated stool) (on Extract Agar pH 7.2-7.4)		6.5	13.	29.	59.	31.	25.	2.

TABLE 33.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series D.

Organisms	Plate counts in thousands							
	Initial	1hr.	4hrs.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
S.typhosus(T59)	113.	130.		9.7	8.9	over	discontinued	
S. " (Rawlings)	91.	170.		.9	over	over	"	
S. " (Mayfield)	62.	40.		73.	over	over	"	
S.aerytrycke(Aer)	34.	30.9		53.	0.9	over	"	
S.enteriditis(Ent)	72.	23.1		13.	over	over	"	
S.paratyphi(A34)	49.	20.1		11.	over	over	"	
S.schottmuelleri(B)	23.	1.9		12.9	1.9	over	"	
S.typhimurium(TM)	114.	110.		190.	over	over	"	
S.dysenteriae(101)	49.	1.6		2.1	over	over	"	
S. " (639)	14.	14.9		17.	over	over	"	
S. " (640)	78.	100.		120.	0.7	over	"	
S. " (Dye May)	22.	8.3		6.1	over	over	"	
S.paradysenteriae(19)	70.	50.		8.3	over	over	"	
S. " (21)	55.	9.8		8.9	over	over	"	
S. " (35451)	99.	90.		20.	over	over	"	
S. " (1-38F)	40.	13.5		13.	over	over	"	
S. " (ParaNC)	84.	30.		3.5	over	over	"	
S.sonnei(10)	91.	150.		650.	90.	over	"	
S. " (11)	98.	190.		17.9	over	over	"	
S. " (191)	4.5	2.9		2.1	over	over	"	
S. " (SonNC)	77.	50.		11.9	over	over	"	
Control(uninoculated stool) (on Extract Agar pH 7.2-7.4)		14.8		21.5	1900.	8900.	discontinued	

Over = an overgrowth of coliforms resulting in no characteristic colonies of the pathogens observed in either low (1-10) or high dilution plates (1-10000).

TABLE 34.

Recovery of Organisms from Simulated Specimens Preserved with Solution 11.

Organisms	Media	Plate count in thousands Initial	1hr.	8hrs.	24hrs.	48hrs.	72hrs.	96hrs.
E.typhosus (88)	Endo Agar	89.0	41.0	50.0	4300.0	2200.0	6100.0	8900.0
	Desoxycholate Citrate Agar	89.	1.8	6.3	580.	320.	140.	210.
	MacConkey Agar	89.	no	non-lactose	fermenters	observed.		
	SS Agar	89.	2.1	5.6	100.	90.	160.	180.
E.typhosus (Rawlings)	Endo Agar	101.	36.	8.	200.	2600.	4100.	3000.
	Desoxycholate Citrate Agar	101.	3.6	6.8	380.	210.	230.	250.
	MacConkey Agar	101.	70.	2.	20.	80.	120.	160.
	SS Agar	101.	11.2	.2	20.	60.	130.	120.
E.typhosus (Mayfield)	Endo Agar	82.	83.	50.	1000.	2800.	4300.	5100.
	Desoxycholate Citrate Agar	82.	6.6	8.7	820.	320.	310.	160.
	MacConkey Agar	82.	6.	.08	630.	800.	200.	800.
	SS Agar	82.	5.1	9.8	440.	1320.	160.	230.
S.aerytrycke (Aer)	Endo Agar	83.	53.	80.	1100.	7800.	8900.	10200.
	Desoxycholate Citrate Agar	83.	8.8	2.6	74.	500.	1020.	1210.
	MacConkey Agar	83.	14.	10.	1200.	2400.	7200.	8700.
	SS Agar	83.	12.2	14.8	120.	500.	112.	168.
S.enteriditis (Ent)	Endo Agar	96.	38.	130.	1300.	4200.	6400.	5700.
	Desoxycholate Citrate Agar	96.	1.6	1.5	22.	600.	380.	430.
	MacConkey Agar	96.	14.	(.1	.10	30.	60.	40.
	SS Agar	96.	1.2	3.	60.	90.	150.	210.
S.paratyphi (B54)	Endo Agar	83.	8.	.8	18.	1300.	4100.	3100.
	Desoxycholate Citrate Agar	83.	.8	.4	10.	60.	100.	80.
	MacConkey Agar	83.	no	non-lactose	fermenters	observed.		
	SS Agar	83.	8.8	(.1	3.2	2.1	4.	3.1
S.schottmueller(B)	Endo Agar	90.	24.	100.	2800.	1600.	2600.	3300.
	Desoxycholate Citrate Agar	90.	1.8	3.8	5.	100.	13.	8.
	MacConkey Agar	90.	no	non-lactose	fermenters	observed.		
	SS Agar	90.	5.4	1.1	2.8	.6	3.3	7.
S.typhimurium (TM)	Endo Agar	99.	88.	104.	970.	8200.	7400.	7900.
	Desoxycholate Citrate Agar	99.	.8	3.9	4.3	8.2	3.3	4.8
	MacConkey Agar	99.	36.	51.	72.	120.	1320.	1020.
	SS Agar	99.	4.8	2.9	160.	20.	80.	150.
S.dysenteriae (161)	Endo Agar	30.	3.	9.	80.	3100.	5900.	5200.
	Desoxycholate Citrate Agar	30.	no	non-lactose	fermenters	observed.		
	MacConkey Agar	30.	"	"	"	"		
	SS Agar	30.	(.1	(.1	.5	2.	4.	9.
S.dysenteriae (639)	Endo Agar	76.	48.	8.	290.	4200.	4300.	3500.
	Desoxycholate Citrate Agar	76.	(.1	(.1	(.1	.2	.3	.5
	MacConkey Agar	76.	no	non-lactose	fermenters	observed.		
	SS Agar	76.	3.6	(.1	1.6	30.	160.	90.
S.dysenteriae (640)	Endo Agar	100.5	93.	20.	280.	5200.	7600.	8100.
	Desoxycholate Citrate Agar	100.5	.4	(.1	2.	2.8	80.	12.
	MacConkey Agar	100.5	no	non-lactose	fermenters	observed.		
	SS Agar	100.5	4.6	2.2	.9	10.	120.	390.
S.dysenteriae (DysMay)	Endo Agar	51.5	41.	90.	210.	800.	4600.	3900.
	Desoxycholate Citrate Agar	51.5	(.1	(.1	(.1	.3	.3	.9
	MacConkey Agar	51.5	4.	20.	40.	200.	300.	800.
	SS Agar	51.5	3.2	.5	1.8	90.	210.	300.
S.paradysenteriae(19)	Endo Agar	46.	88.	90.	320.	180.	3200.	2900.
	Desoxycholate Citrate Agar	46.	11.	9.2	100.	20.	300.	270.
	MacConkey Agar	46.	34.	3.	90.	800.	800.	800.
	SS Agar	46.	6.4	4.3	6.8	3.8	280.	650.
S.paradysenteriae(21)	Endo Agar	98.	89.	40.	800.	8200.	6200.	5100.
	Desoxycholate Citrate Agar	98.	10.2	1.8	140.	6.2	160.	30.
	MacConkey Agar	98.	no	non-lactose	fermenters	observed.		
	SS Agar	98.	26.	3.6	2.2	210.	330.	270.
S.paradysenteriae(35451)	Endo Agar	85.	84.	8.	200.	4800.	6700.	5400.
	Desoxycholate Citrate Agar	85.	12.4	9.1	20.	18.	190.	17.
	MacConkey Agar	85.	no	non-lactose	fermenters	observed.		
	SS Agar	85.	8.6	2.7	1.2	560.	430.	540.
S.paradysenteriae(1-3DK)	Endo Agar	80.	78.	10.	38.	2000.	6100.	5300.
	Desoxycholate Citrate Agar	80.	20.8	5.3	6.	2.9	6.	12.
	MacConkey Agar	80.	28.	9.	80.	300.	600.	10 00.
	SS Agar	80.	21.8	13.6	4.8	3.1	2.8	3.1
S.paradysenteriae(ParaSG)	Endo Agar	69.	28.	30.	620.	4800.	6300.	5500.
	Desoxycholate Citrate Agar	69.	16.2	5.8	740.	20.	60.	180.
	MacConkey Agar	69.	no	non-lactose	fermenters	observed.		
	SS Agar	69.	12.6	7.4	340.	460.	470.	310.
S.sonnei (10)	Endo Agar	93.	34.	120.	1000.	800.	1100.	2000.
	Desoxycholate Citrate Agar	93.	no	non-lactose	fermenters	observed.		
	MacConkey Agar	93.	"	"	"	"		
	SS Agar	93.	10.6	.6	1.1	1.8	22.	80.
S.sonnei (11)	Endo Agar	94.	82.	50.	900.	800.	2300.	5900.
	Desoxycholate Citrate Agar	94.	.2	.8	.5	9.8	100.	8.
	MacConkey Agar	94.	36.	7.	300.	1200.	2400.	2900.
	SS Agar	94.	(.1	(.1	3.1	1.2	21.	180.
S.sonnei (SonUC)	Endo Agar	134.	38.	20.	290.	1000.	2100.	3000.
	Desoxycholate Citrate Agar	134.	no	non-lactose	fermenters	observed.		
	MacConkey Agar	134.	25.	3.	1000.	1200.	700.	1200.
	SS Agar	134.	.2	(.1	4.	9.	28.	22.
Control (un inoculated Feel; SM	Extract Agar (pH 7.2-7.4*		5.	30.	101.	61.	53.	41.

(.1 = less than 100.

FIGURE 1.

Effect of Test Solutions upon E.typhosus(58).

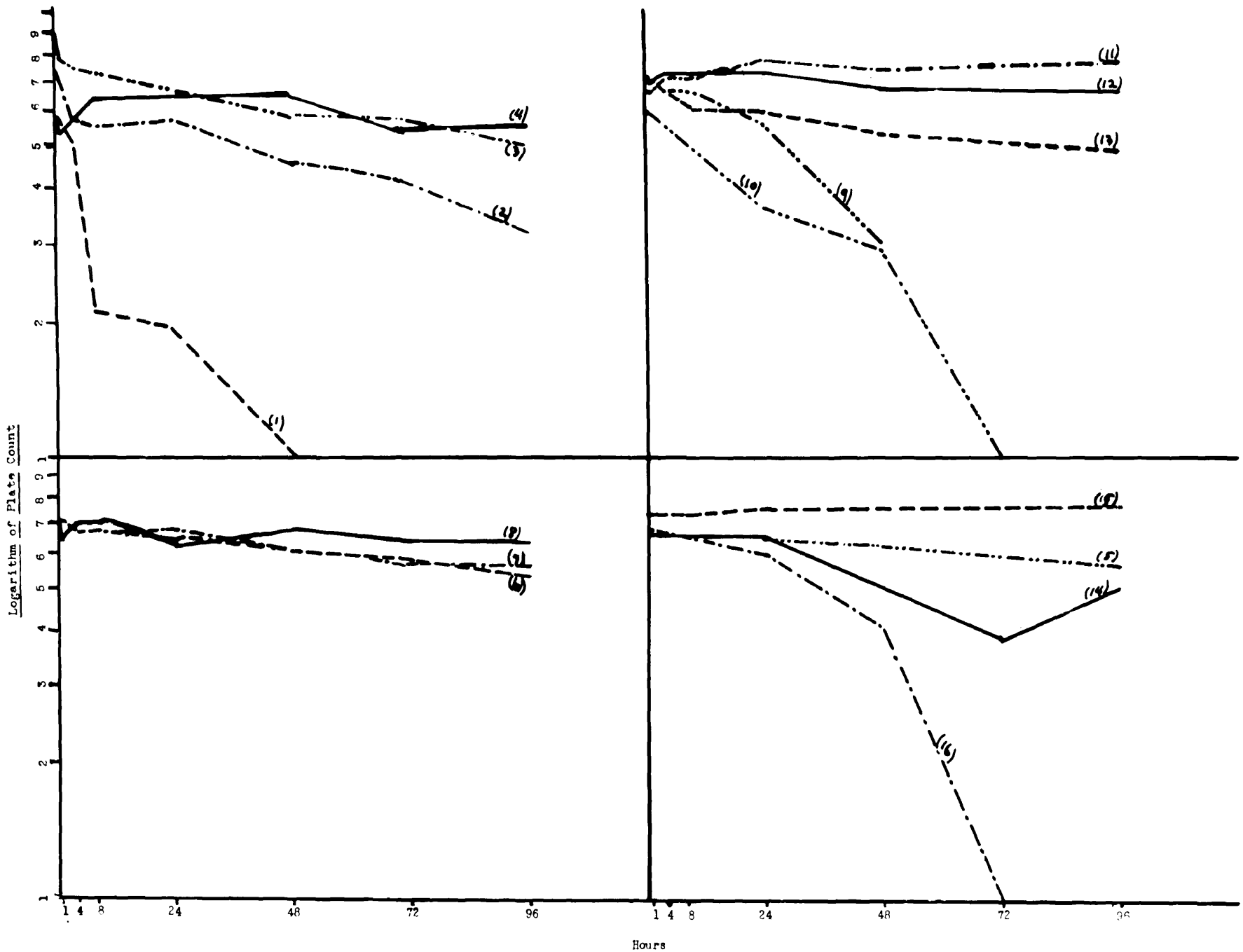

FIGURE 2.

Effect of Test Solutions upon E.typhosus(Rawlings).

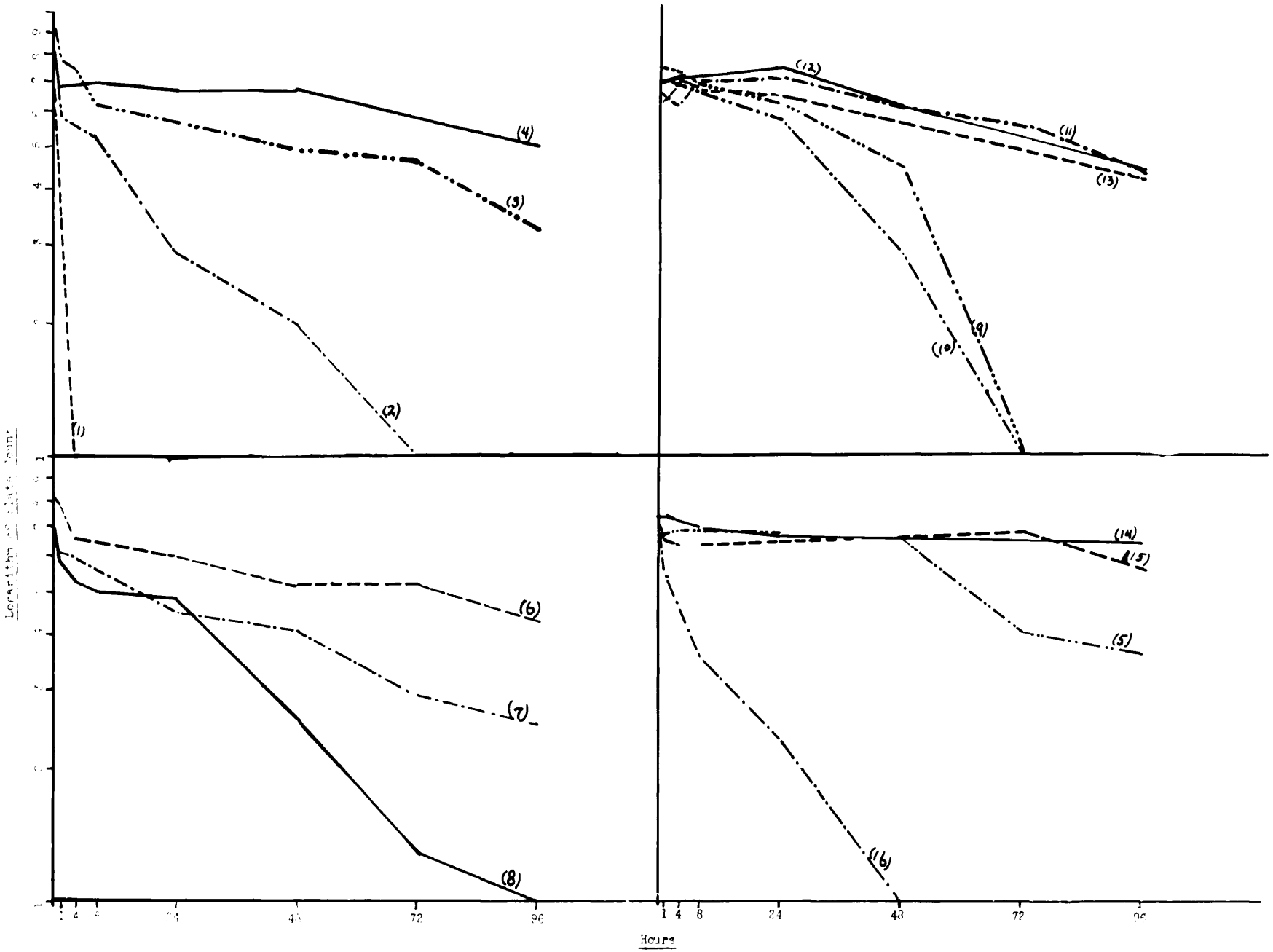

FIGURE 3.

Effect of Test Solutions upon E.typhosus (Mayfield).

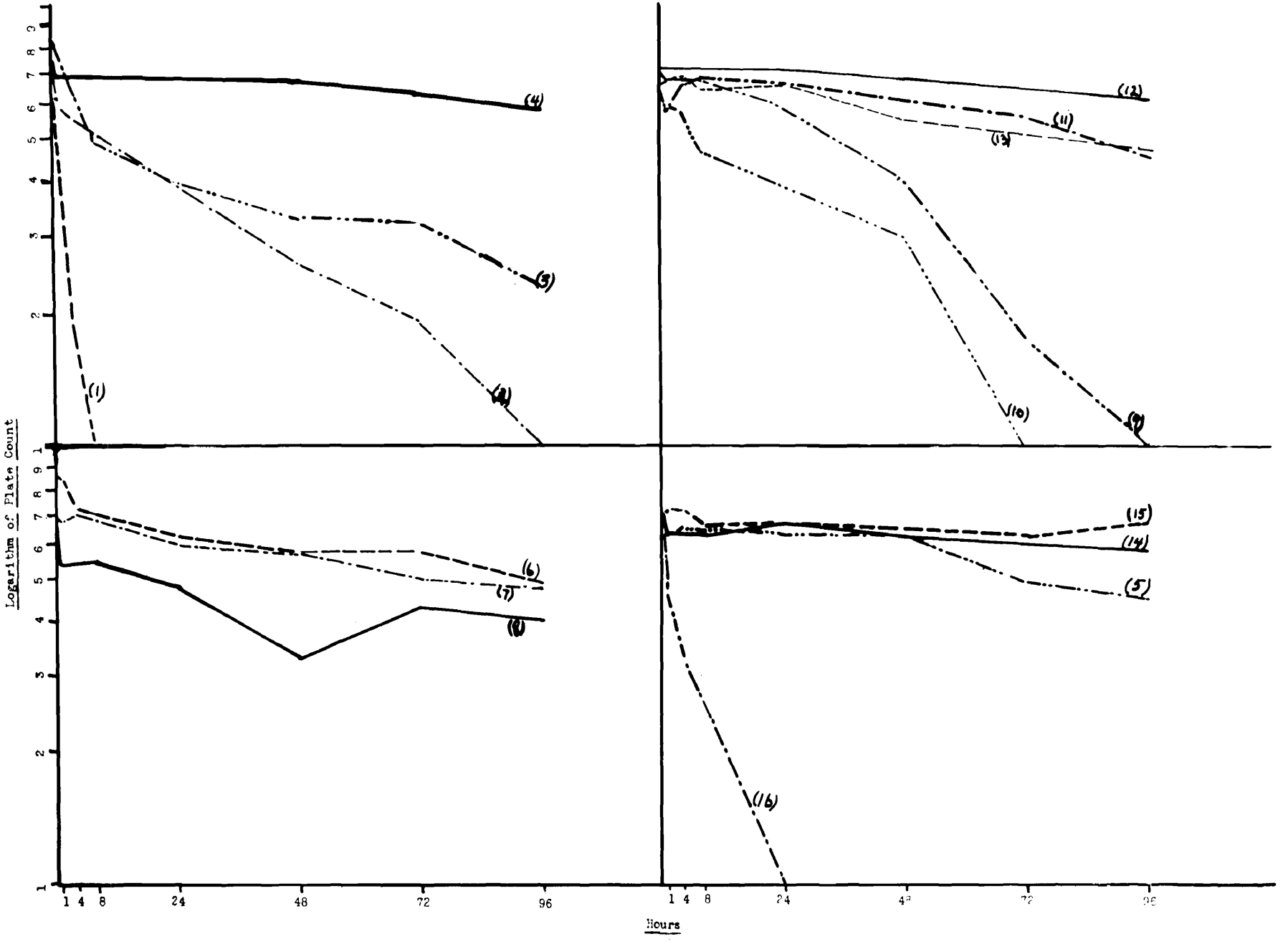

FIGURE 4.

Effect of Test Solutions upon S. aerytryoke(Aer).

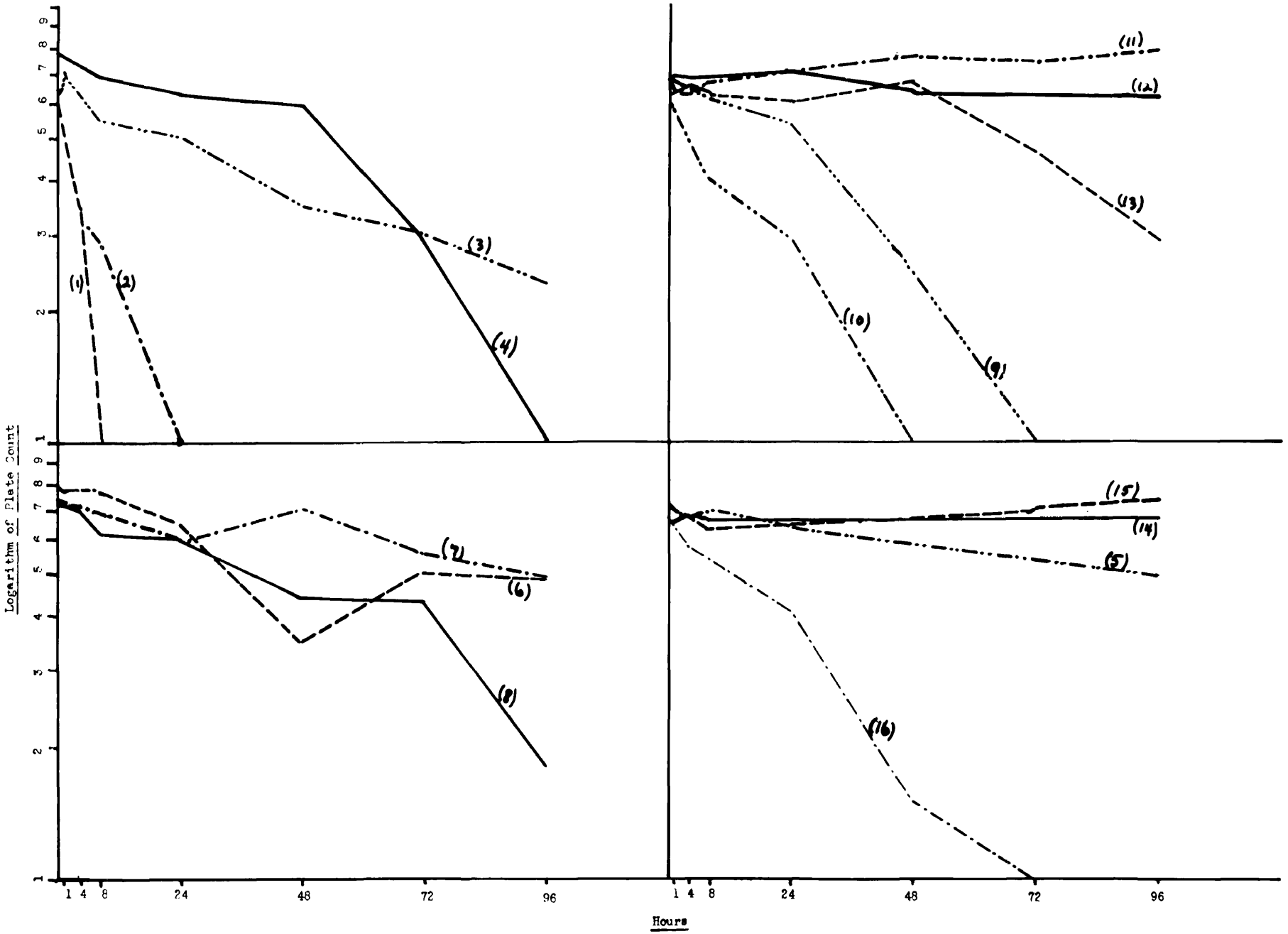

FIGURE 5.

Effect of Test Solutions upon S. enteriditis(Ent).

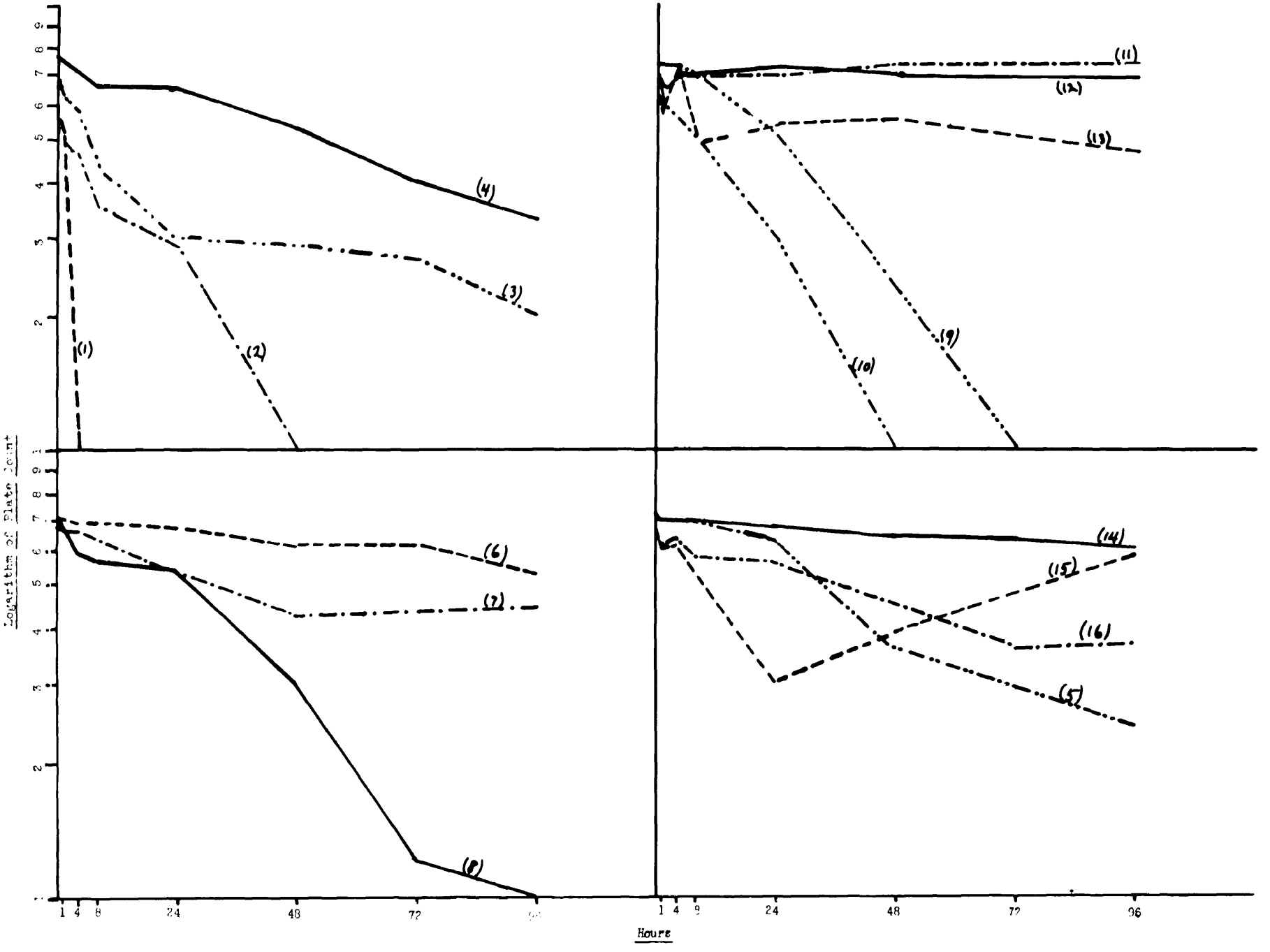

FIGURE 6.

Effect of Test Solutions upon S. paratyphi(A34).

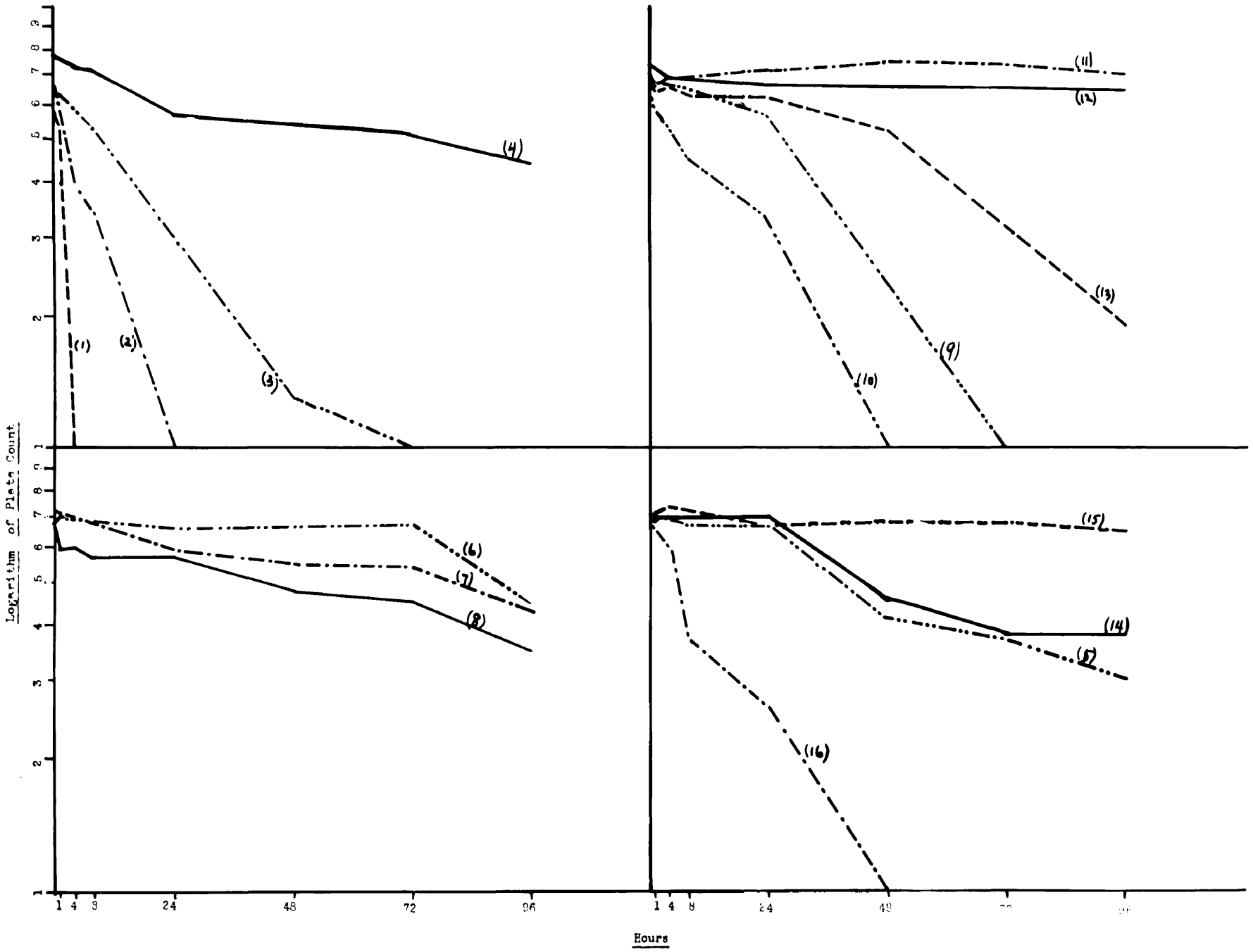

FIGURE 7.

Effect of Test Solutions upon S. schottmuelleri (B).

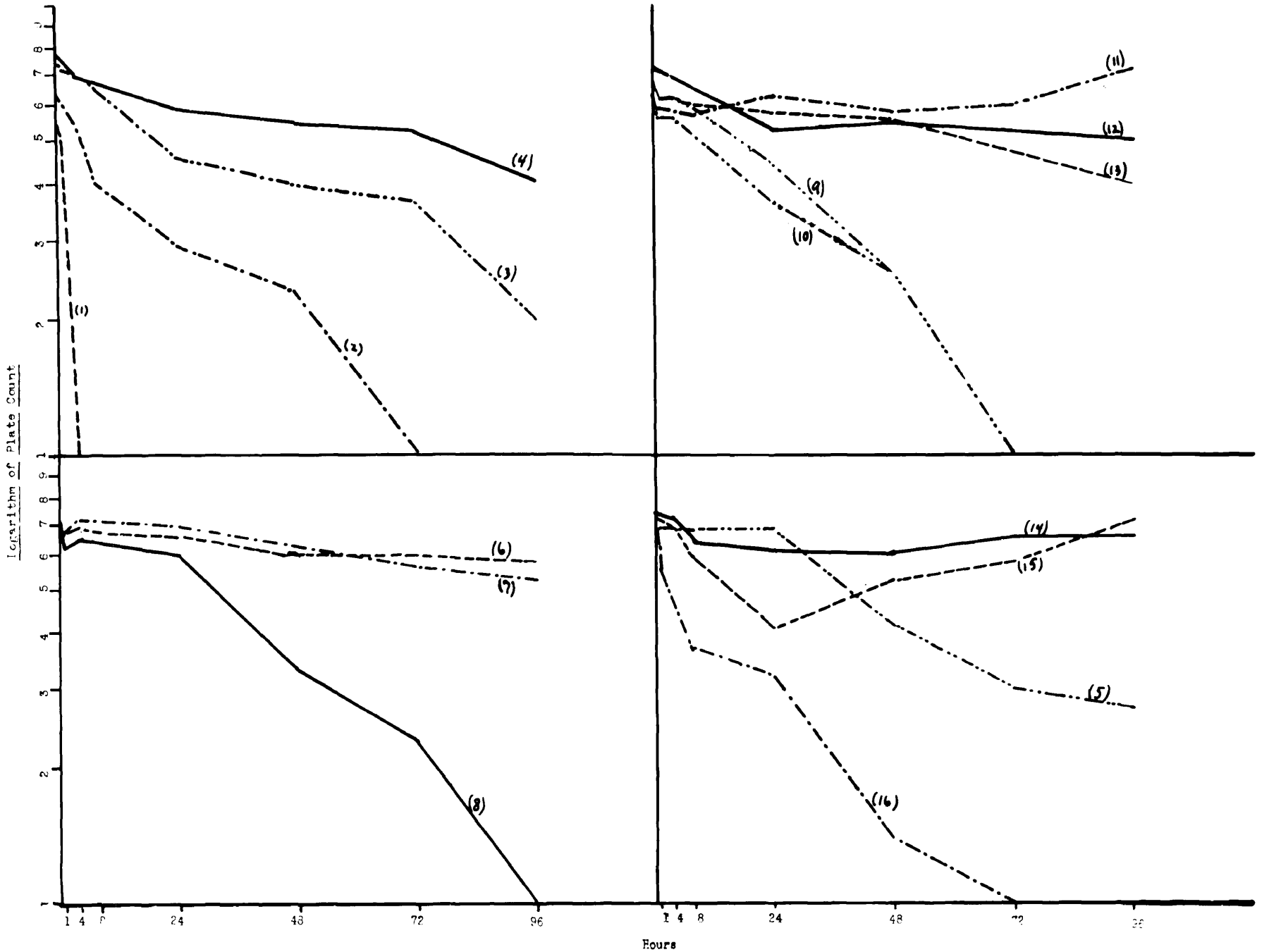

FIGURE 8.

Effect of Test Solutions upon S.typhimurium(TM).

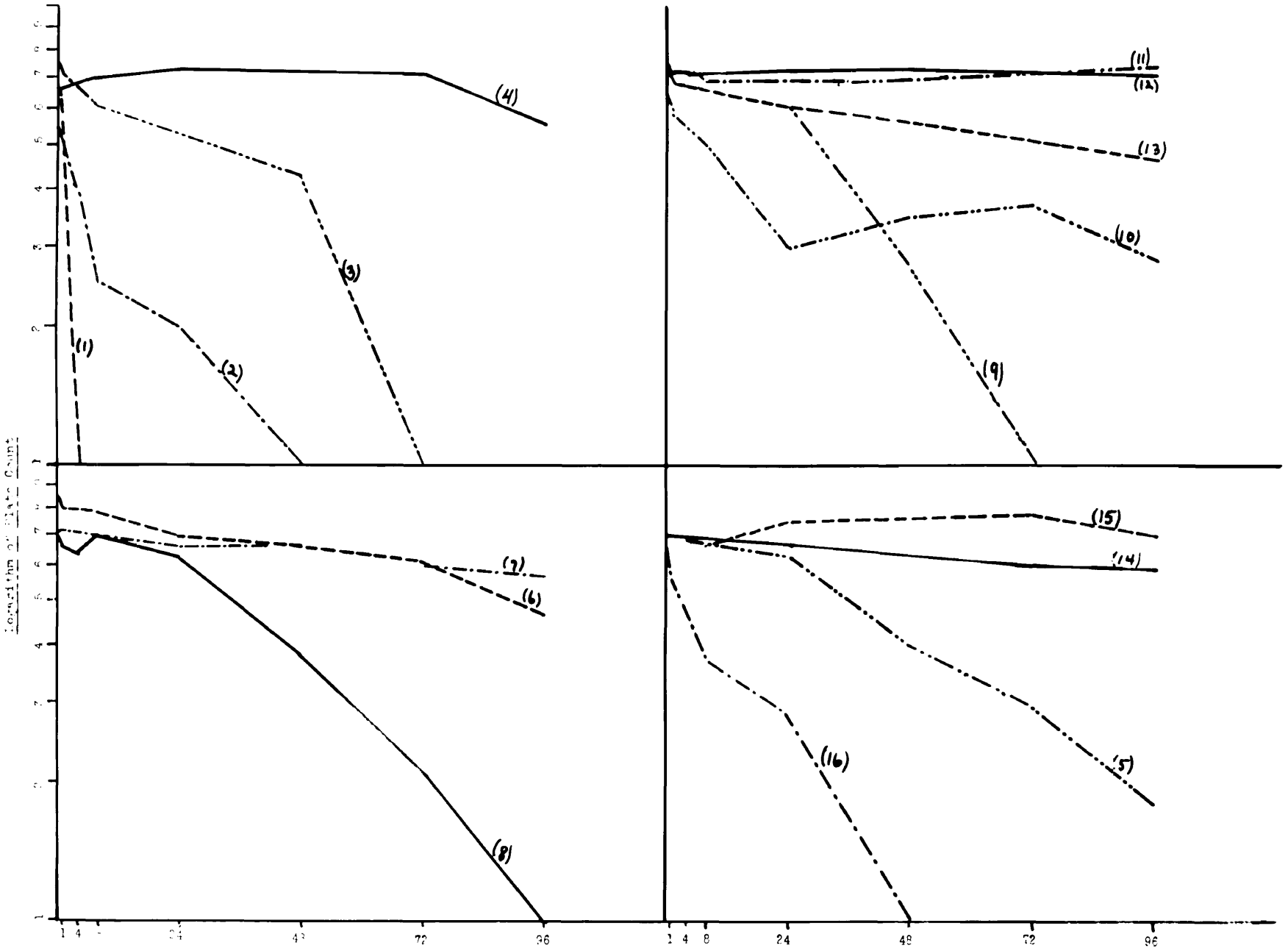

FIGURE 9.

Effect of Test Solutions upon S.dysenteriae(161).

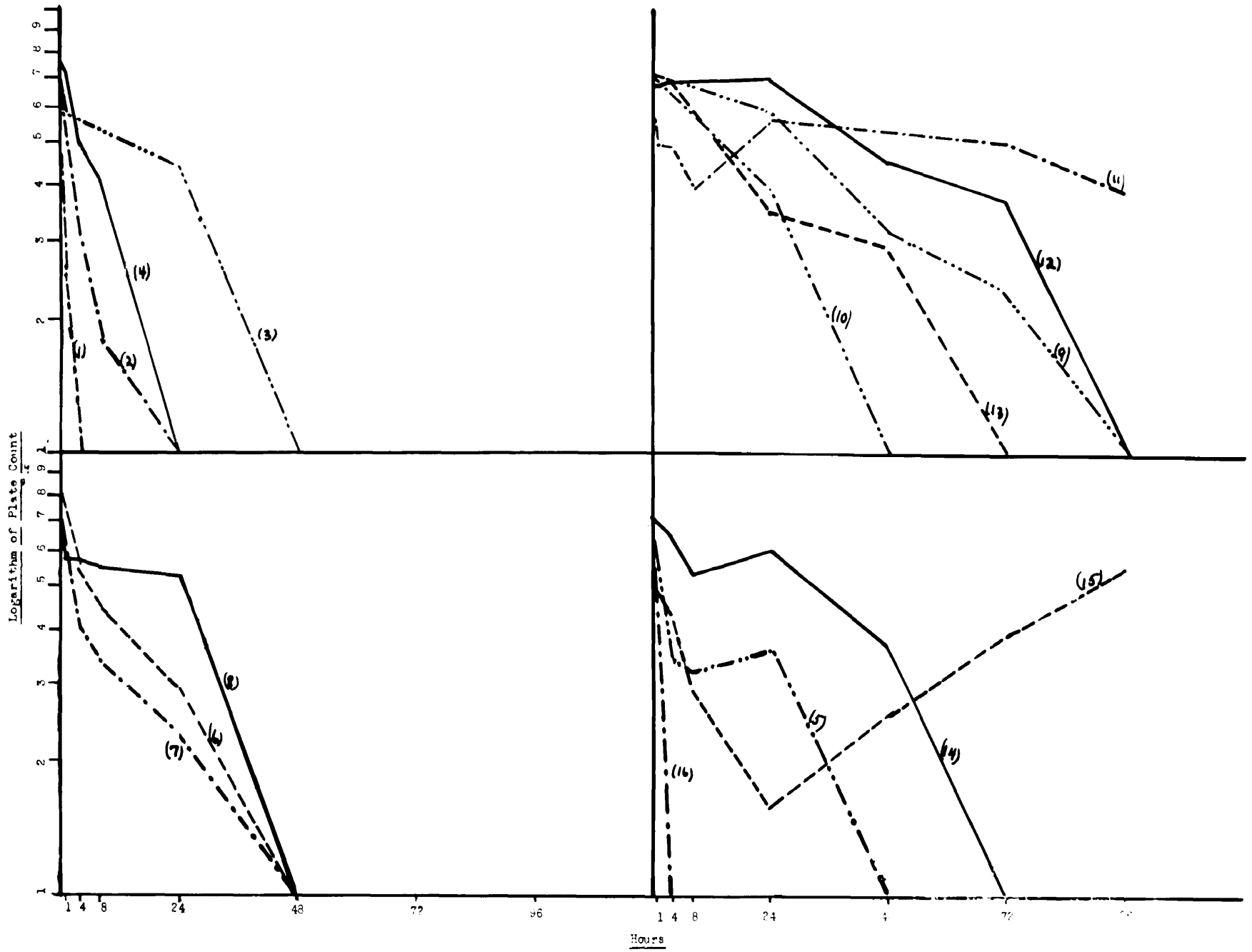

FIGURE 10.

Effect of Test Solutions upon S.dysenteriae(639).

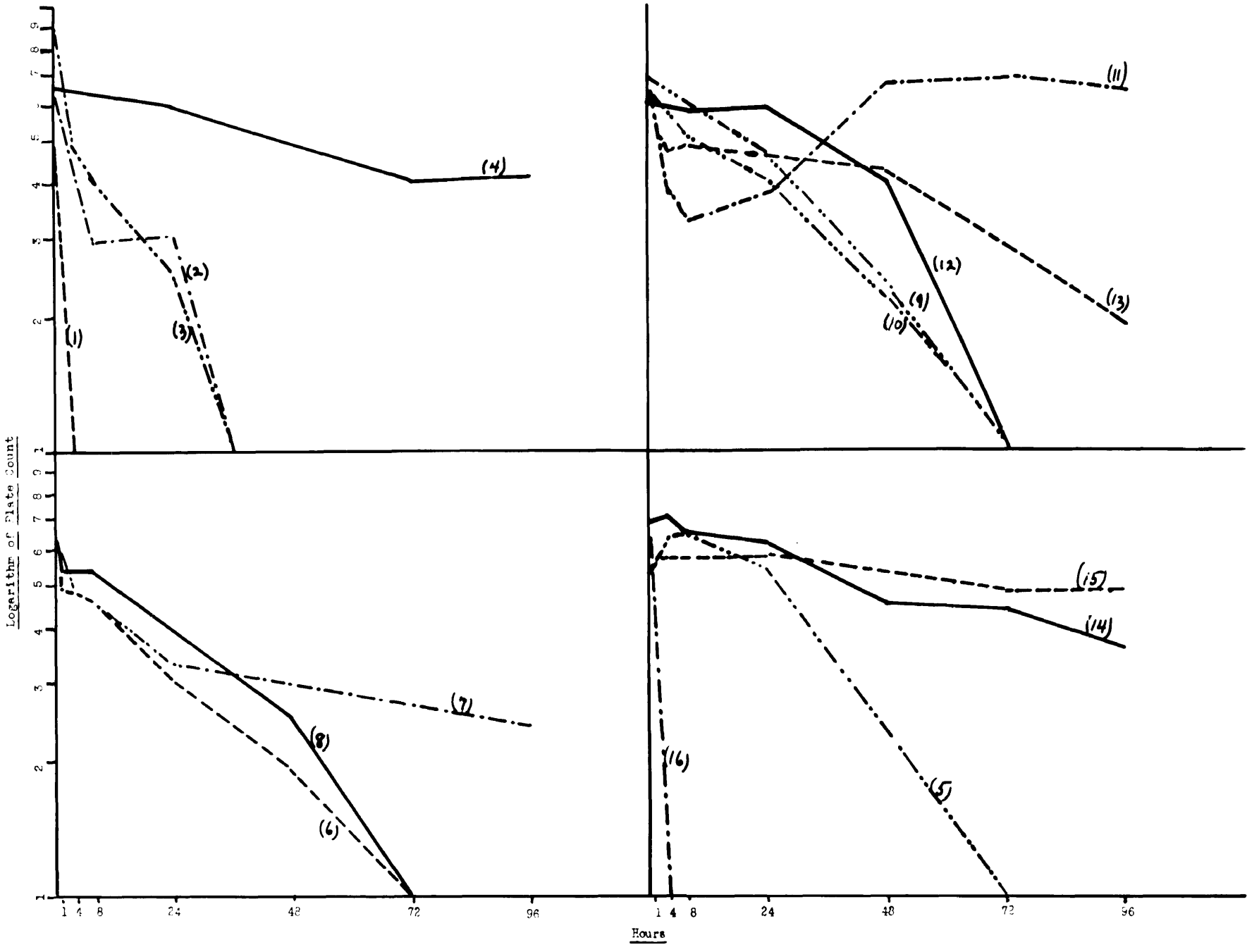

FIGURE 11.

Effect of Test Solutions upon S.dysenteriae(640).

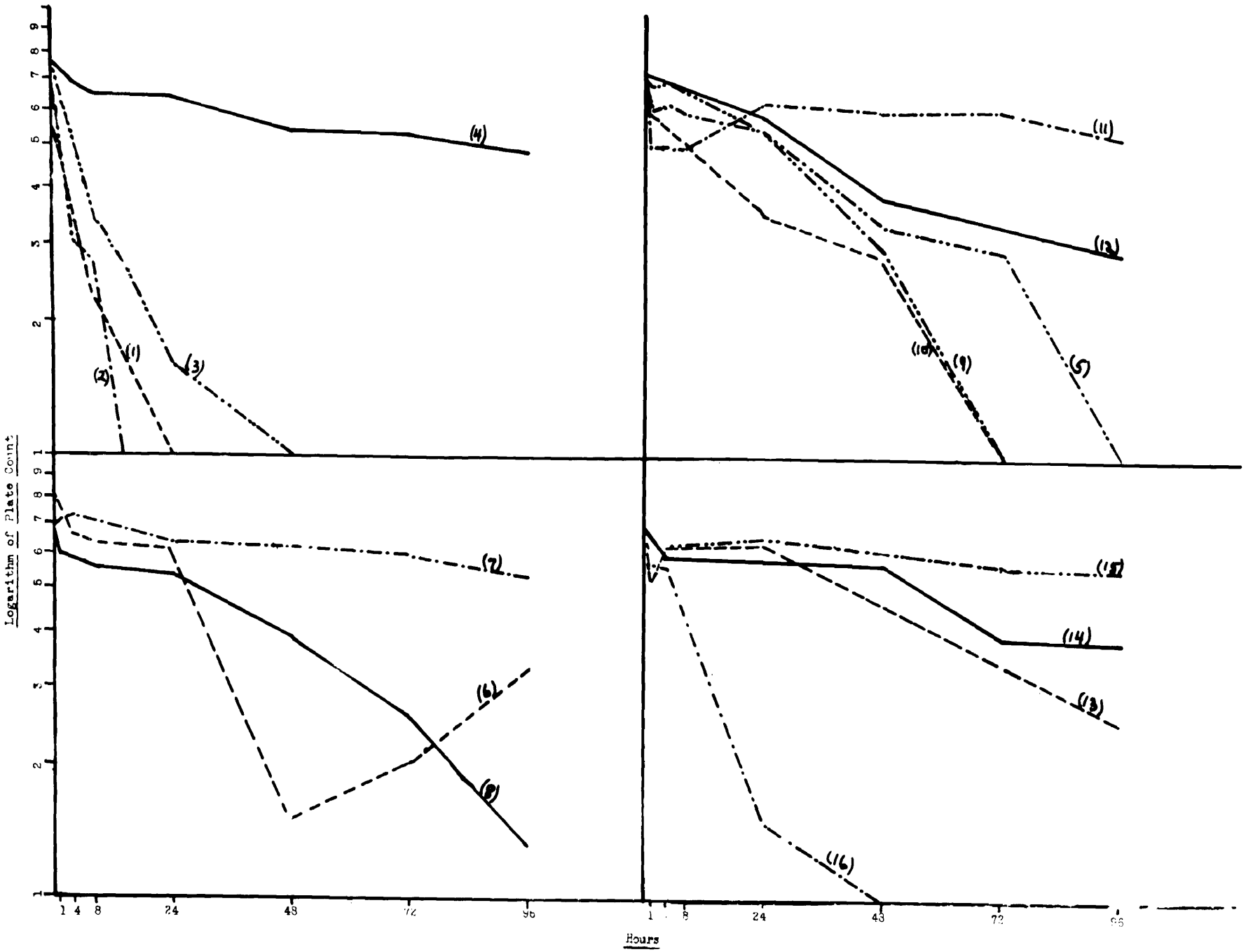

FIGURE 12.

Effect of Test Solutions upon S.dysenteriae(Dysmay).

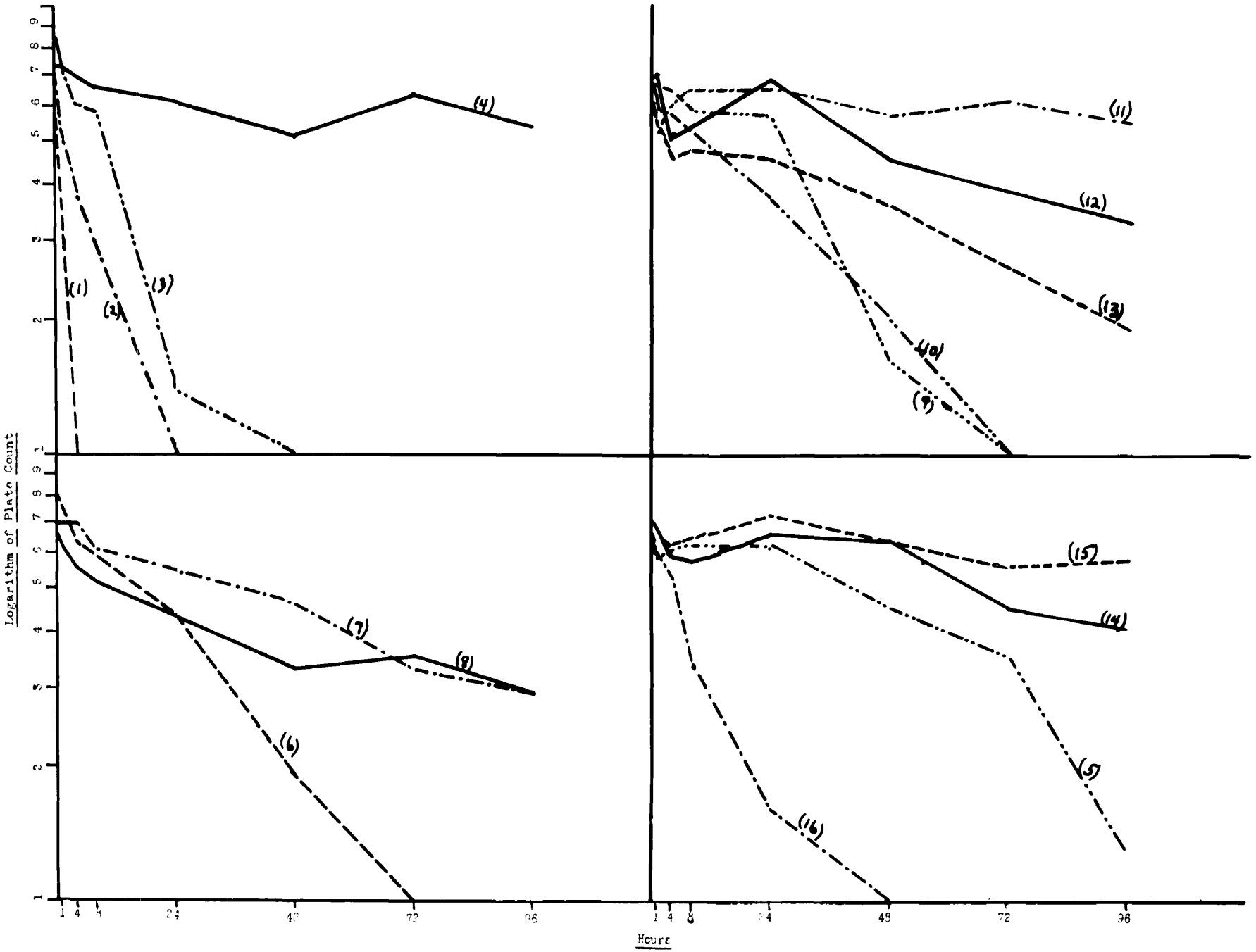

FIGURE 13.

Effect of Test Solutions upon S.paradysenteriae(19).

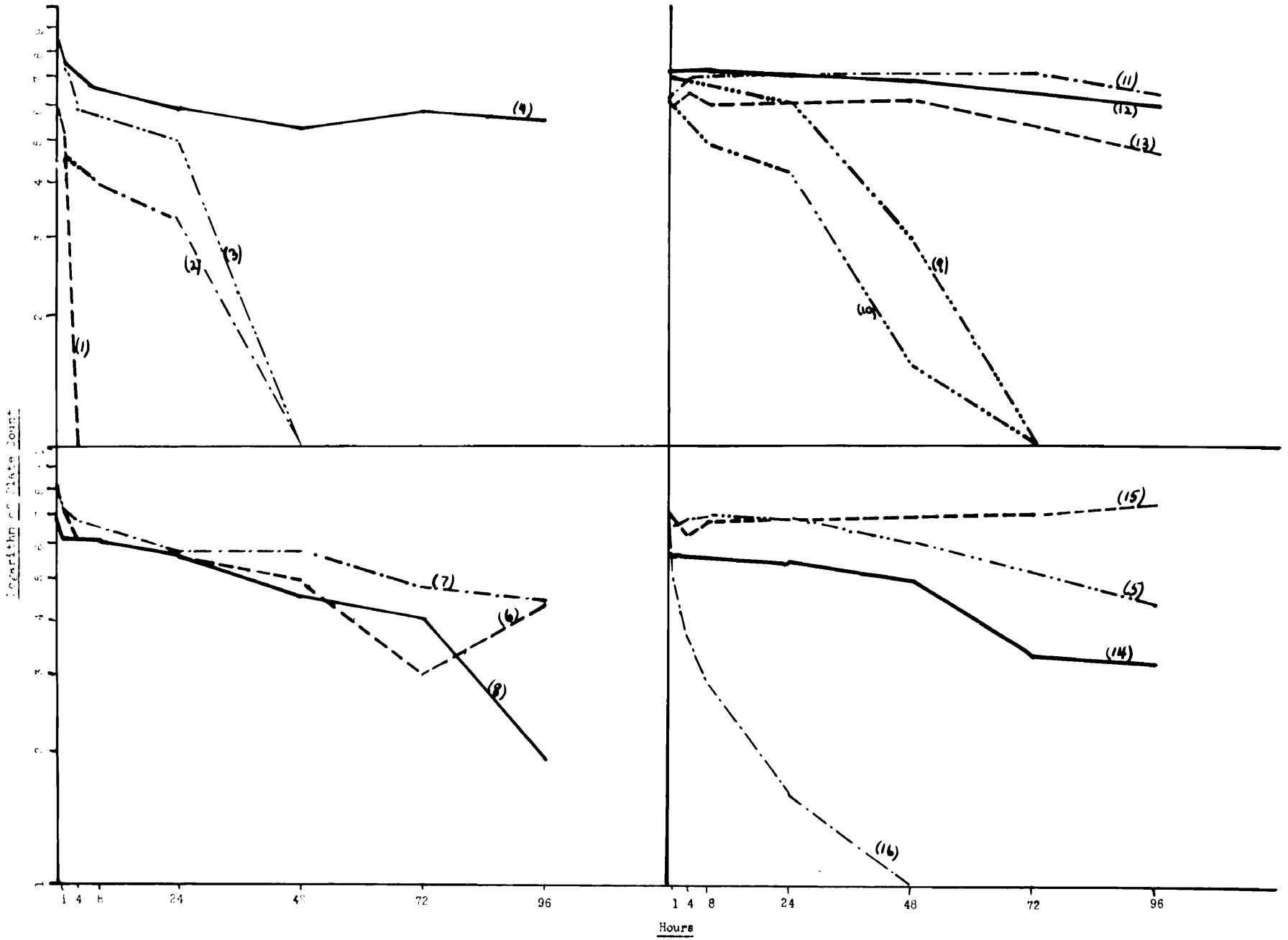

FIGURE 14.

Effect of Test Solutions upon S.paradysenteriae(21).

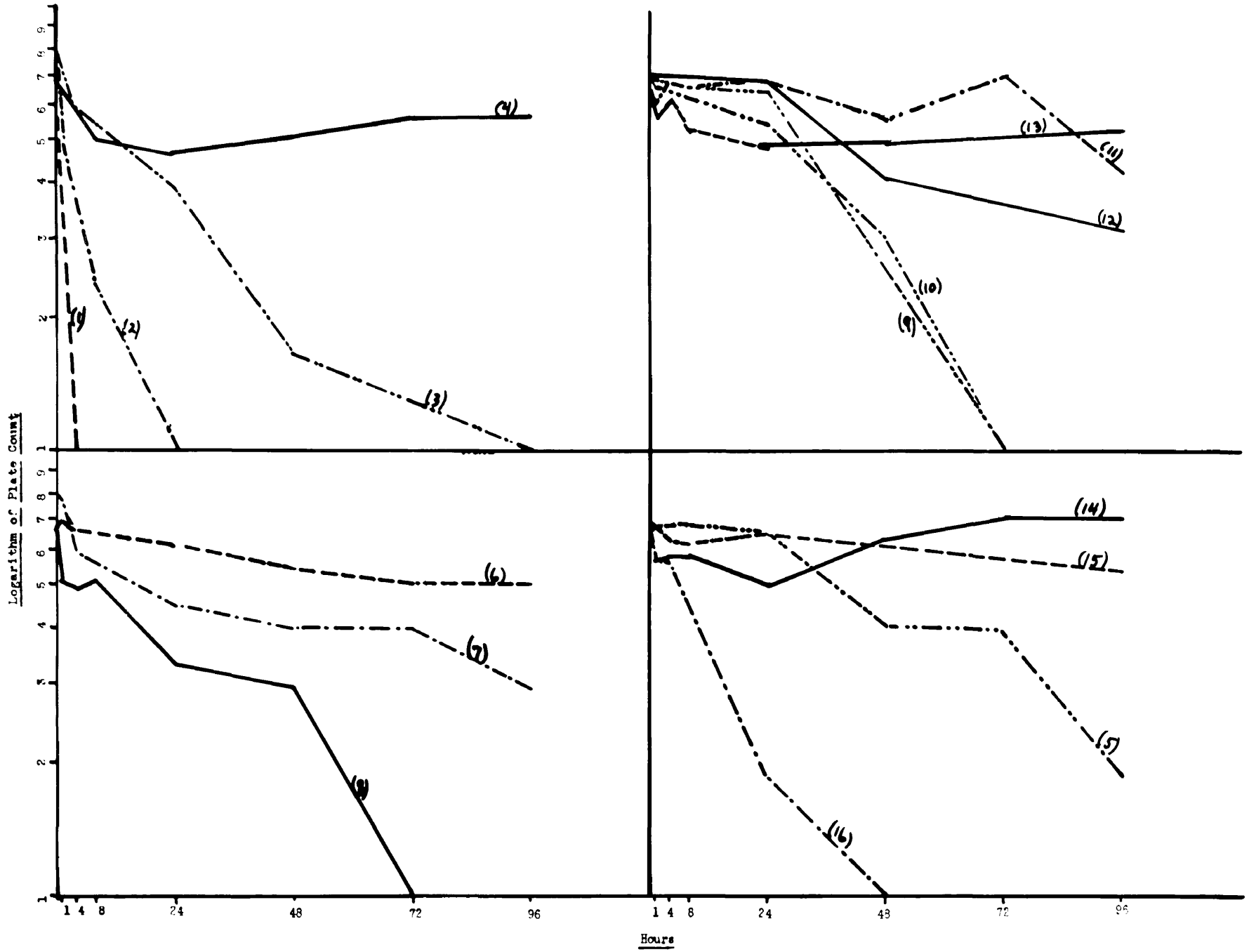

FIGURE 15.

Effect of Test Solutions upon S.paradysenteriae(35451).

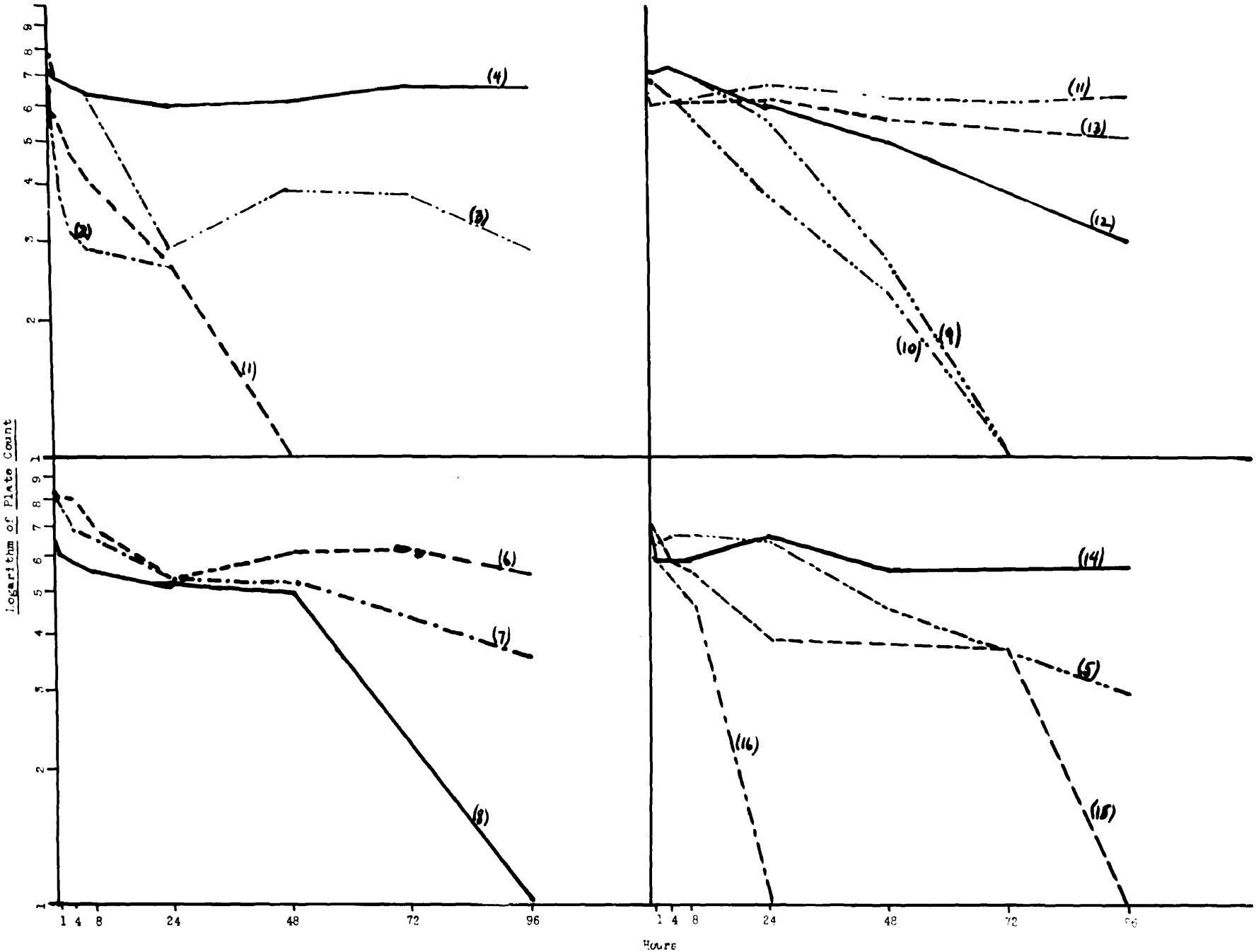

FIGURE 16.

Effect of Test Solutions upon S.paradysenteriae(1-SDE).

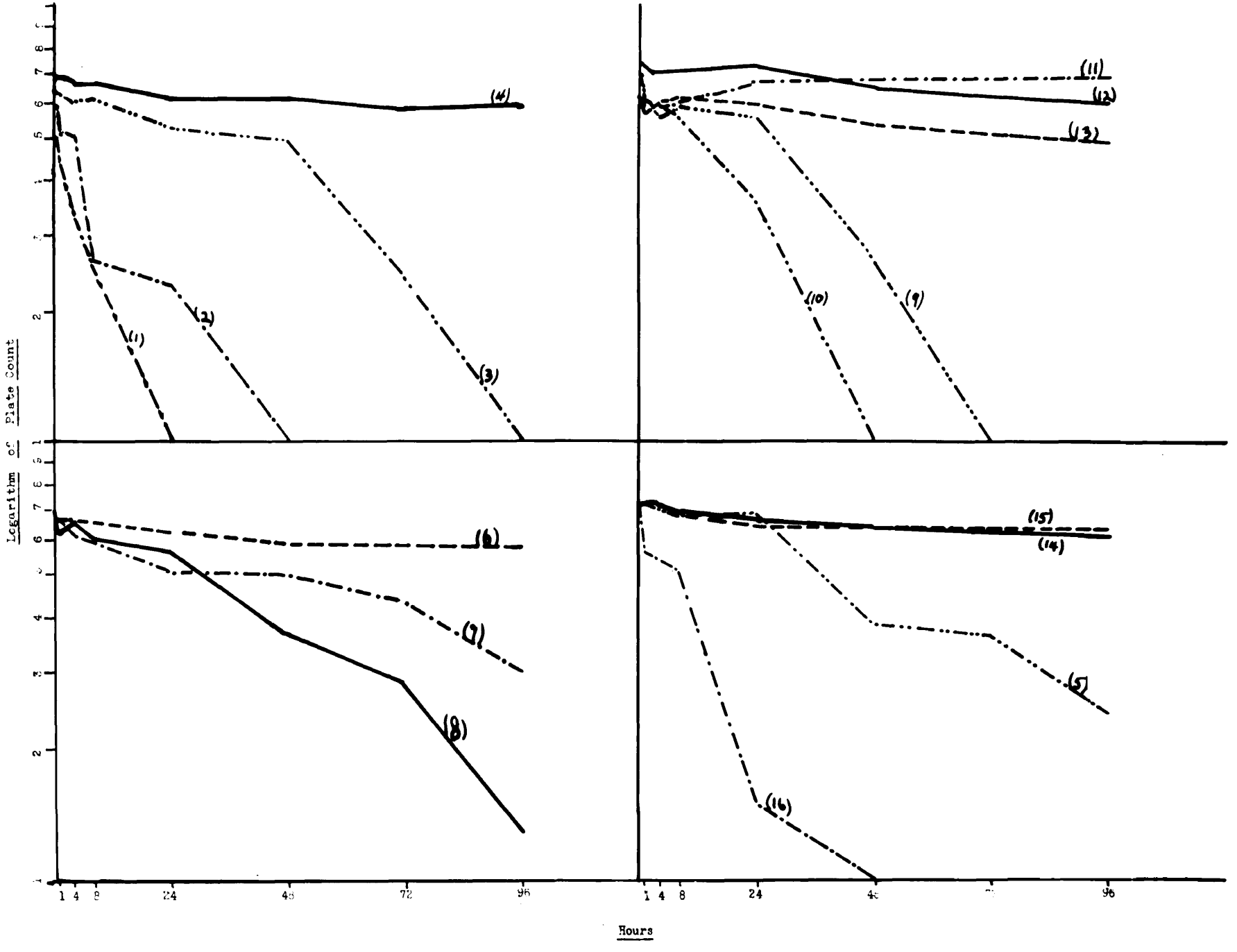

FIGURE 17.

Effect of Test Solutions upon S.paradysenteriae(ParaUC).

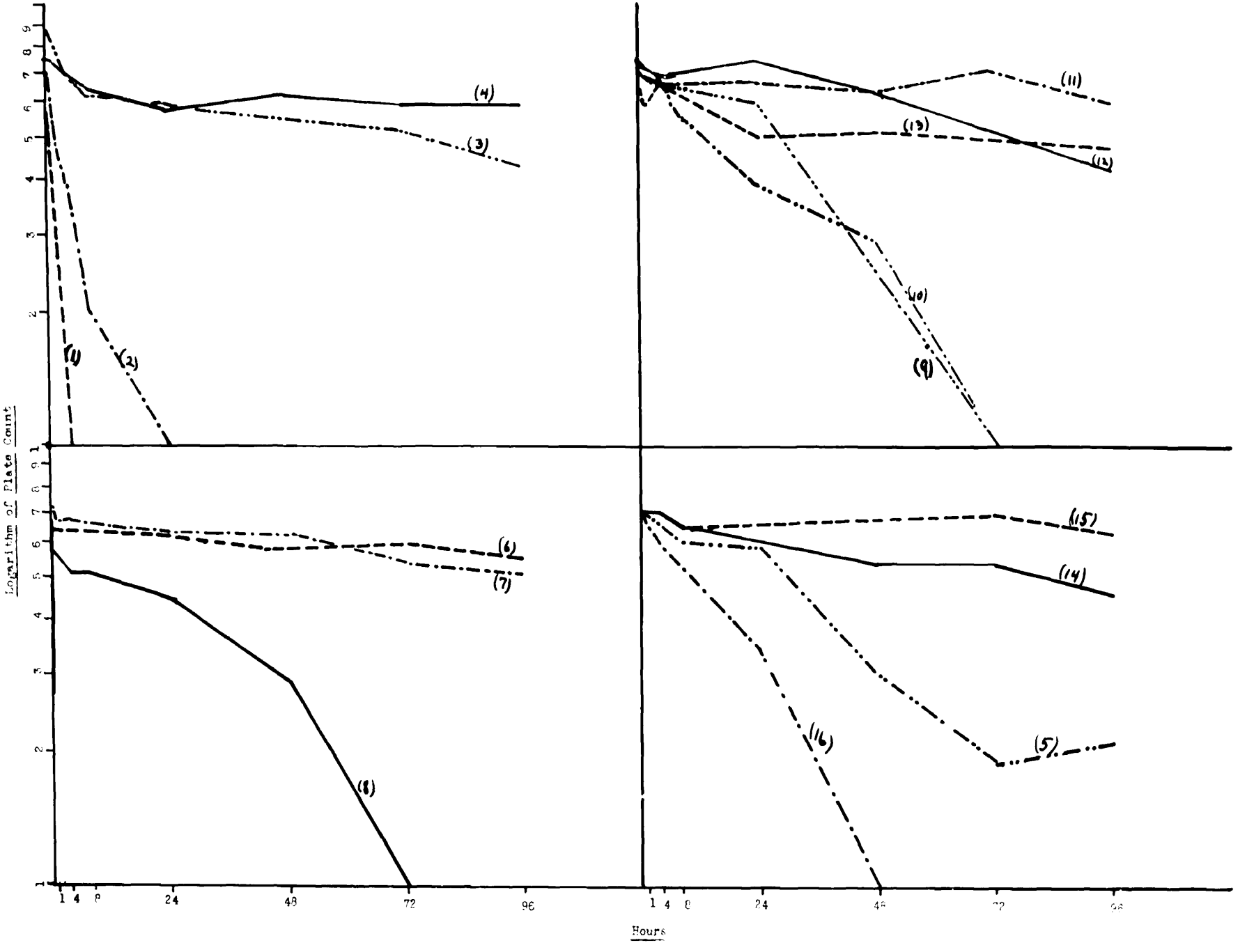

FIGURE 18.

Effect of Test Solutions upon S. sonnei(10).

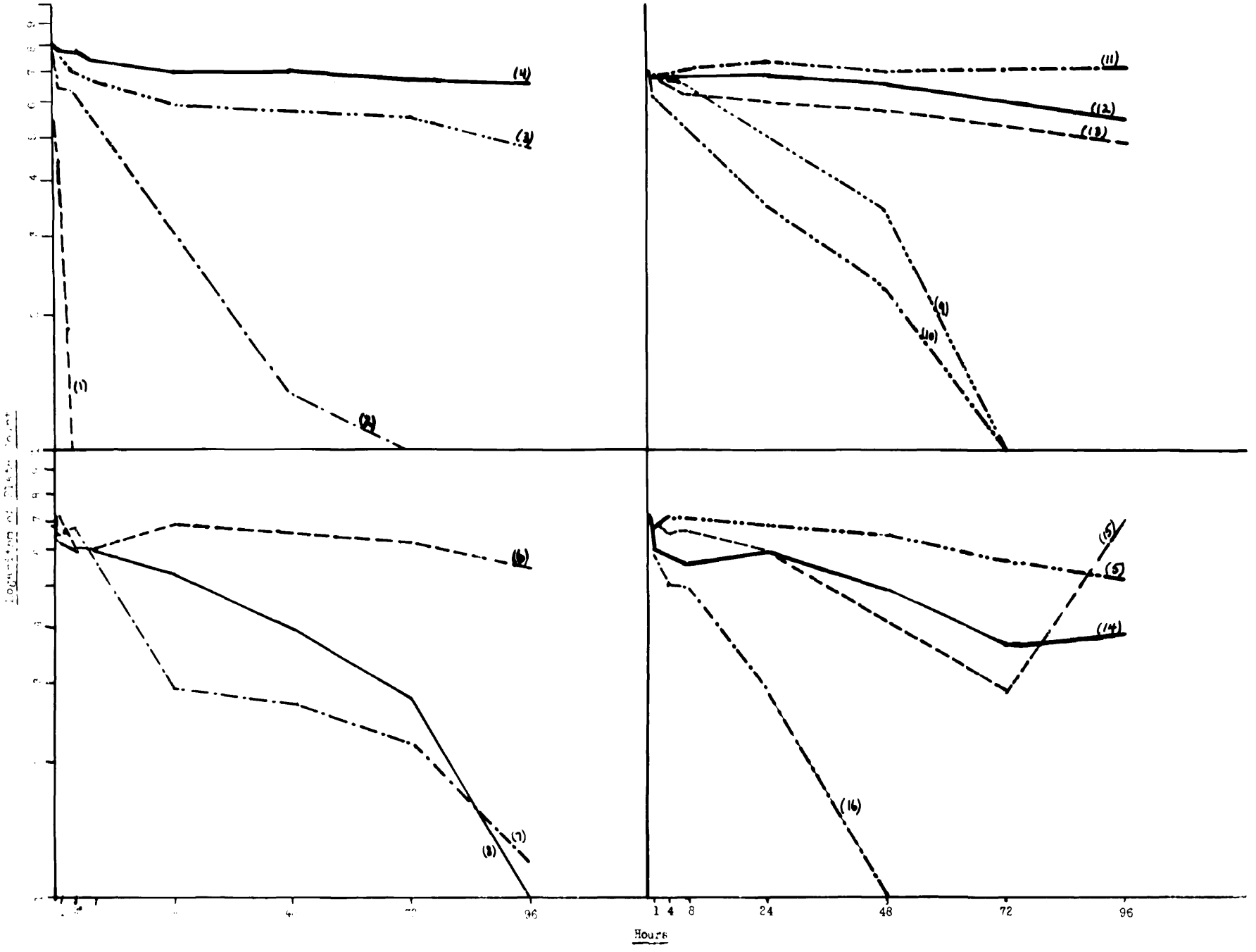

FIGURE 19.

Effect of Test Solutions upon S.sonnei(11).

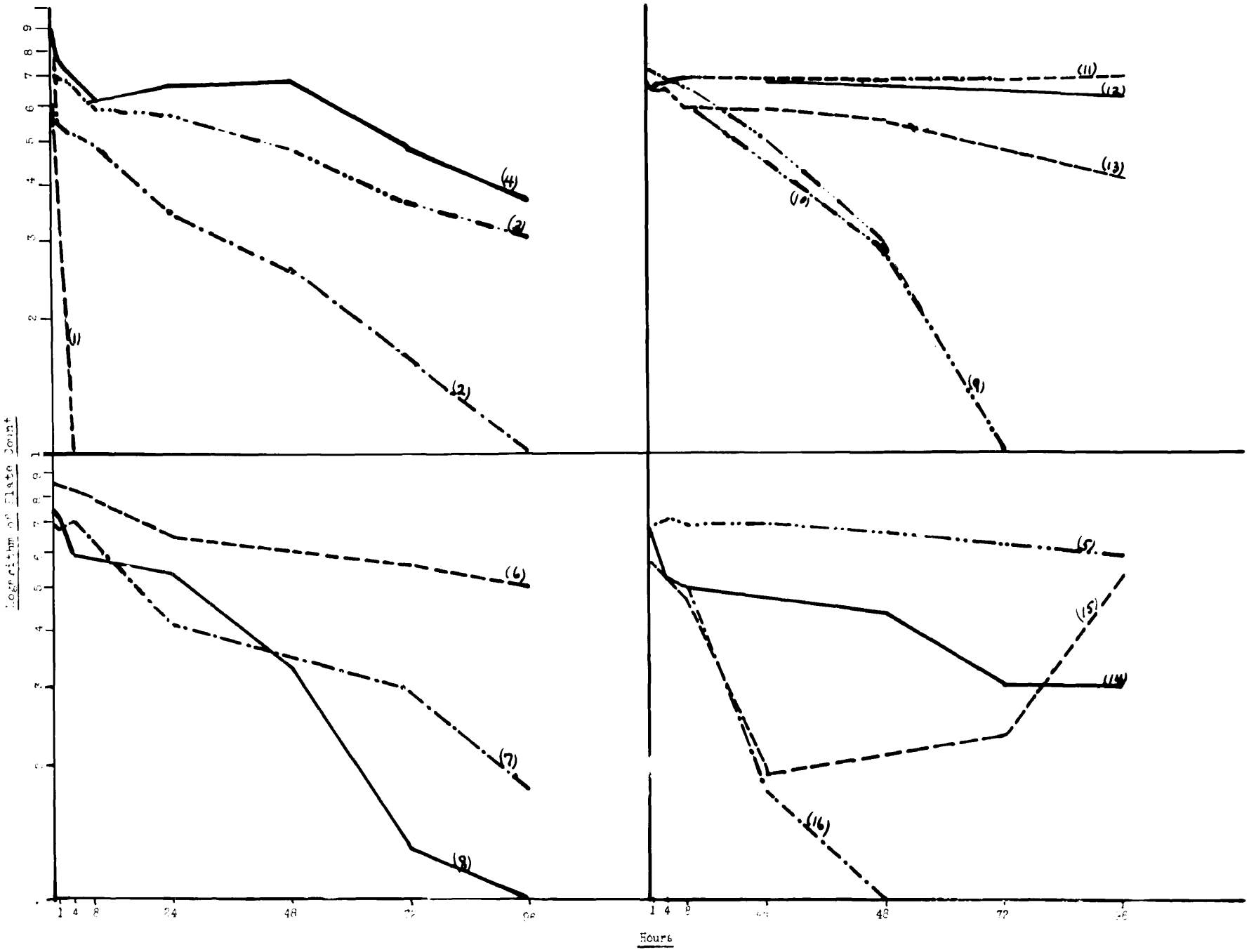

FIGURE 20.

Effect of Test Solutions upon S.sonnei(191).

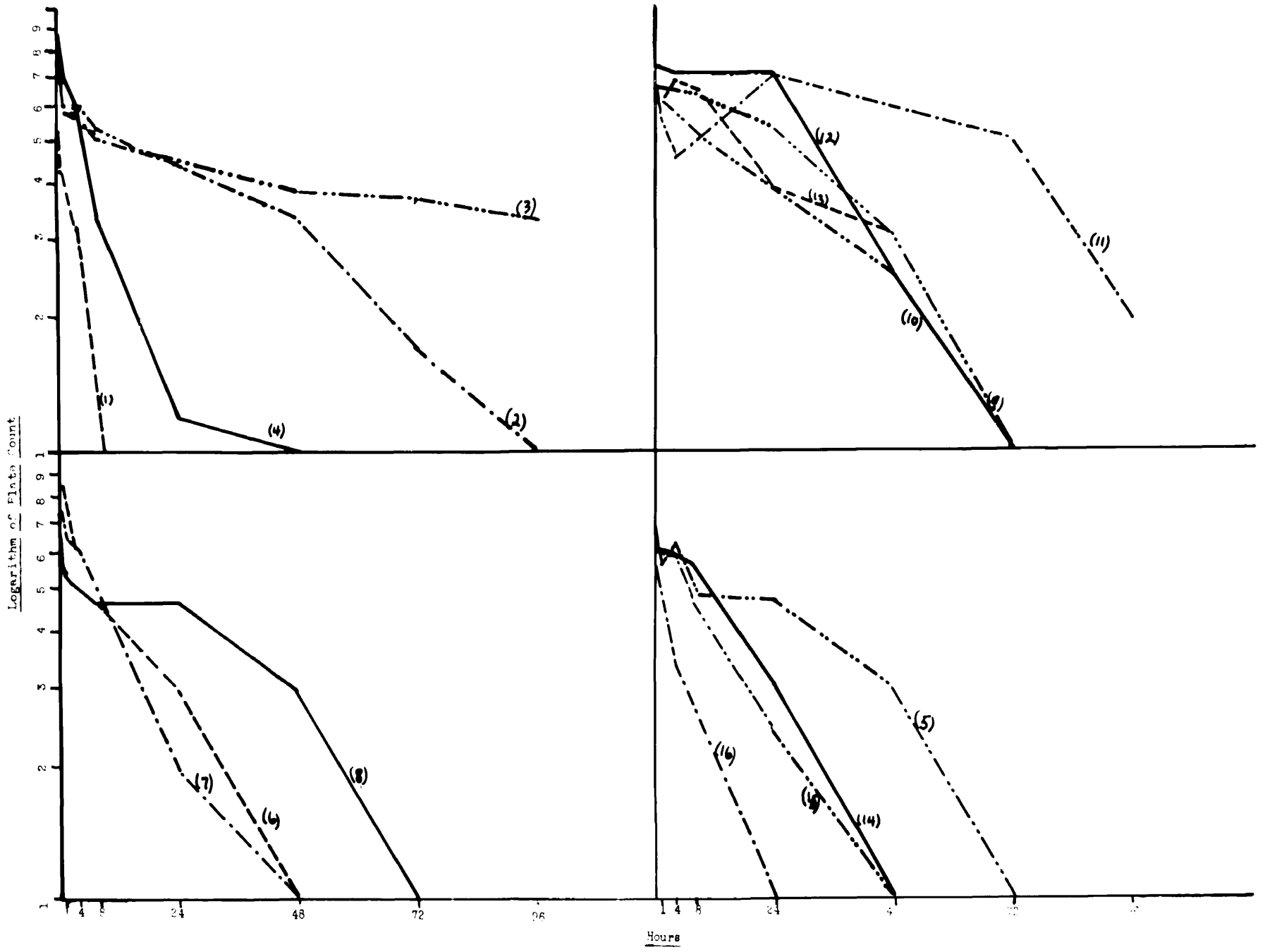

FIGURE 21.

Effect of Test Solutions upon S.sonnei(SonUC).

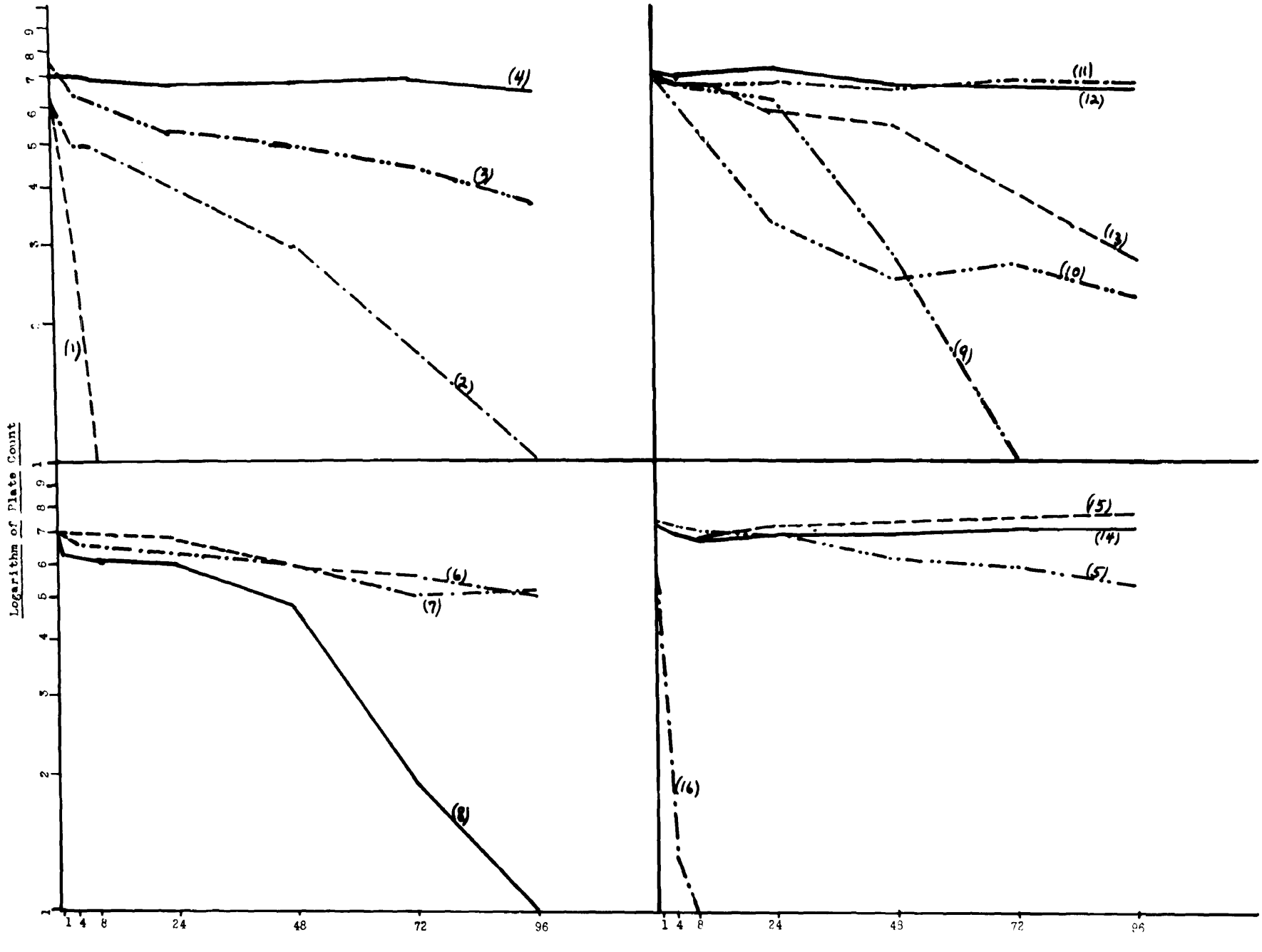

FIGURE 22.

Effect of Test Solutions upon E.coli.

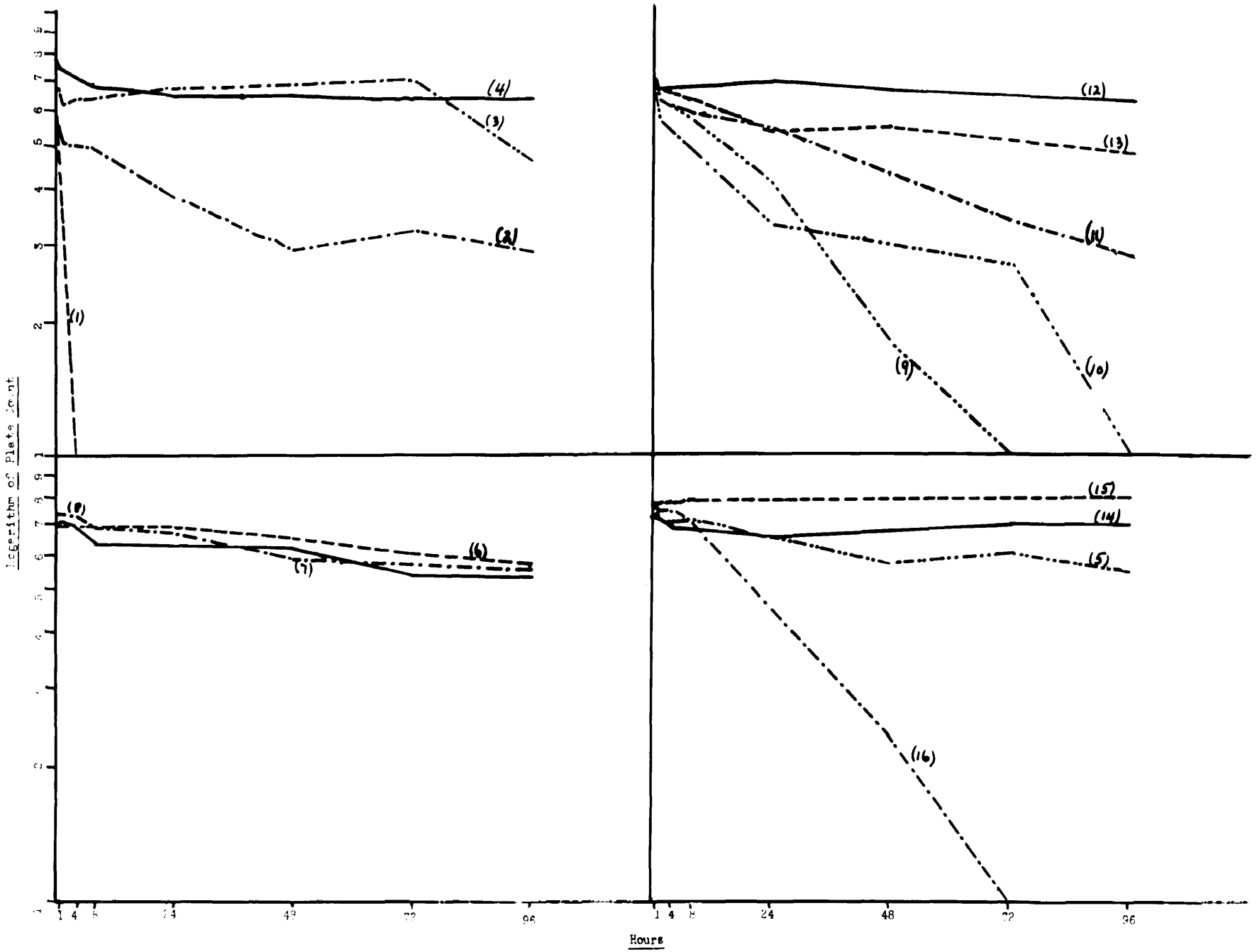

FIGURE 23.

Effect of Solutions 11 and 15 upon Organisms in Simulated Specimens.

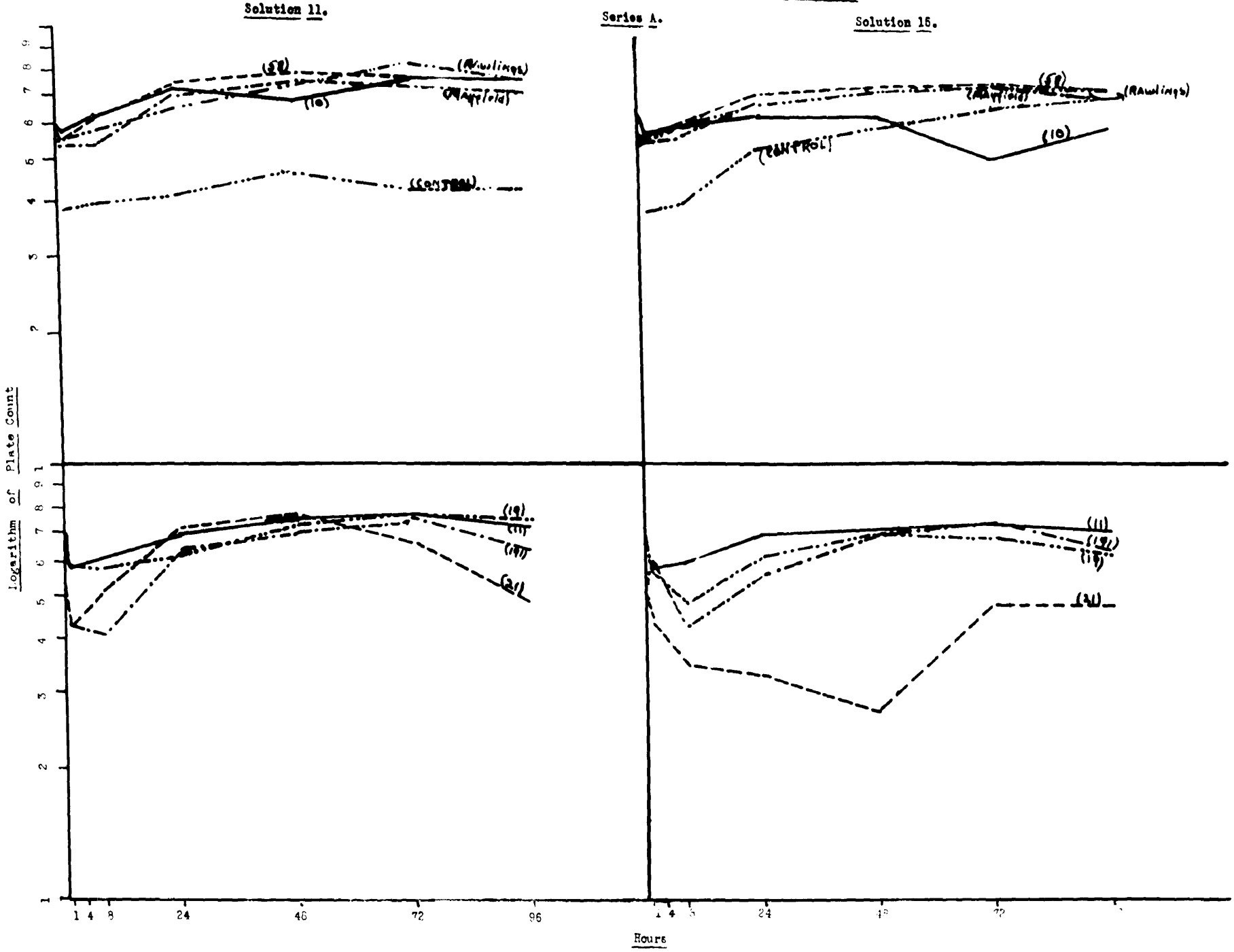

FIGURE 24.

Effect of Solutions 11 and 15 upon Organisms in Simulated Specimens.

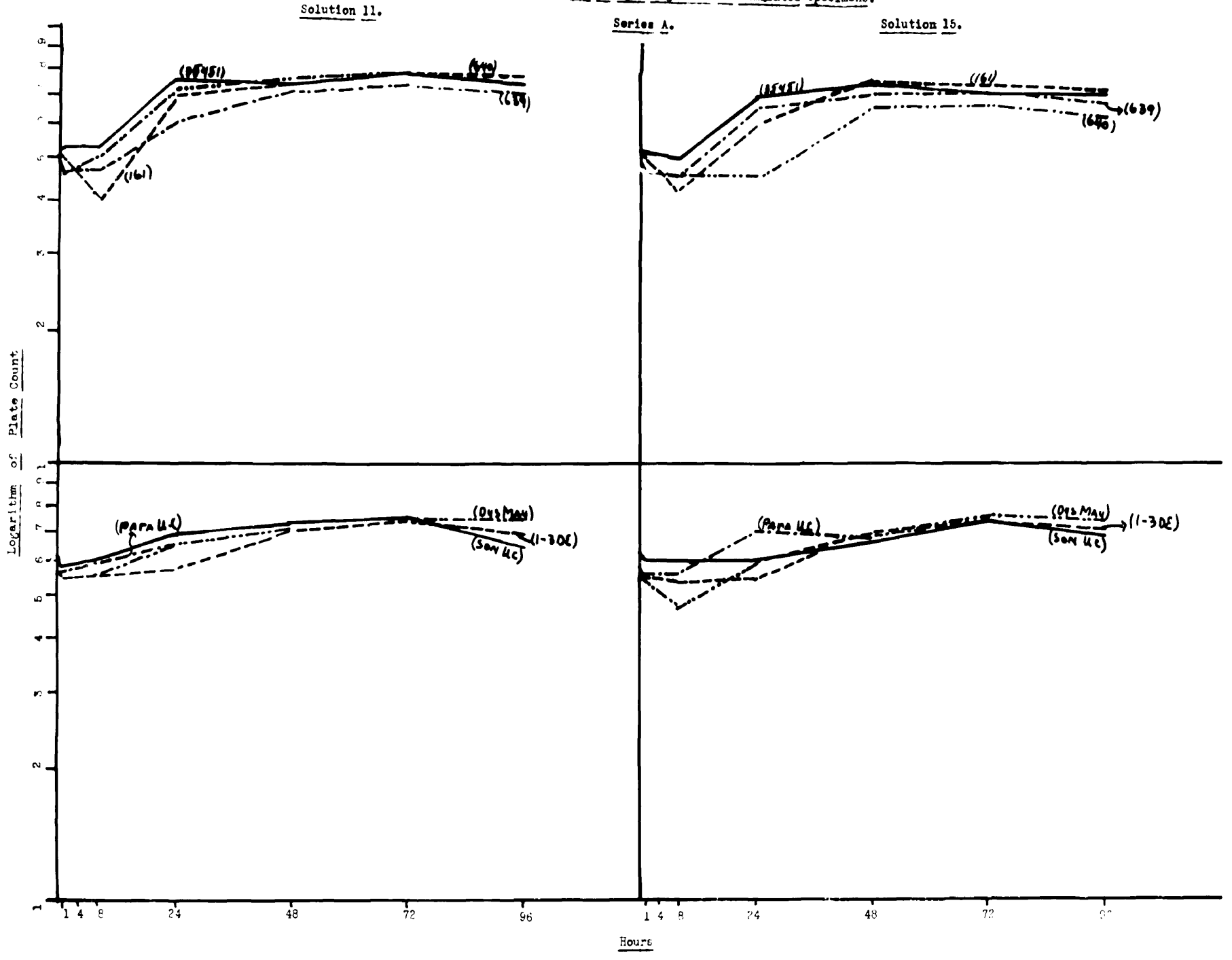

FIGURE 25.

Effect of Solutions 11 and 15 upon Organisms in Simulated Specimens.

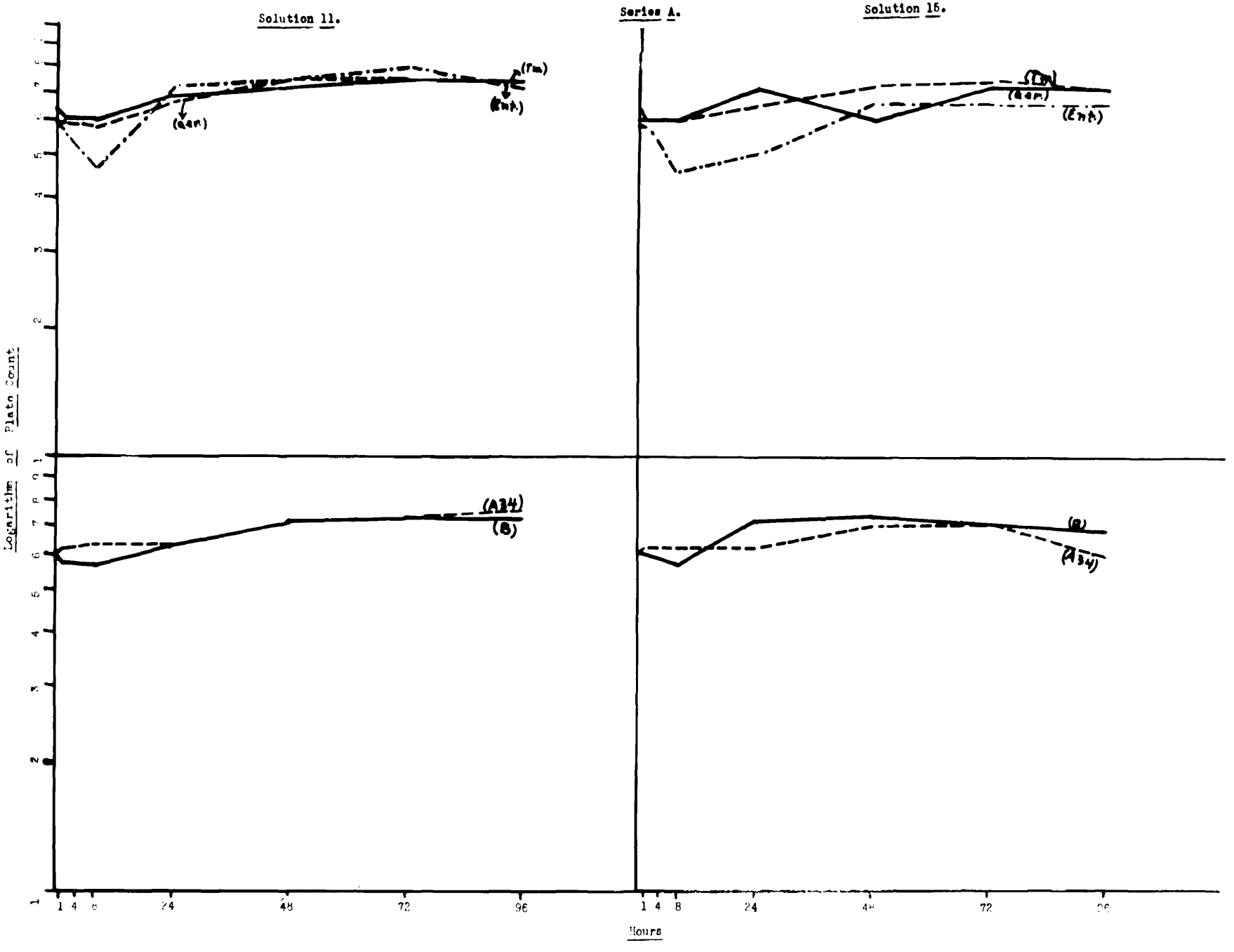

FIGURE 26.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series B.

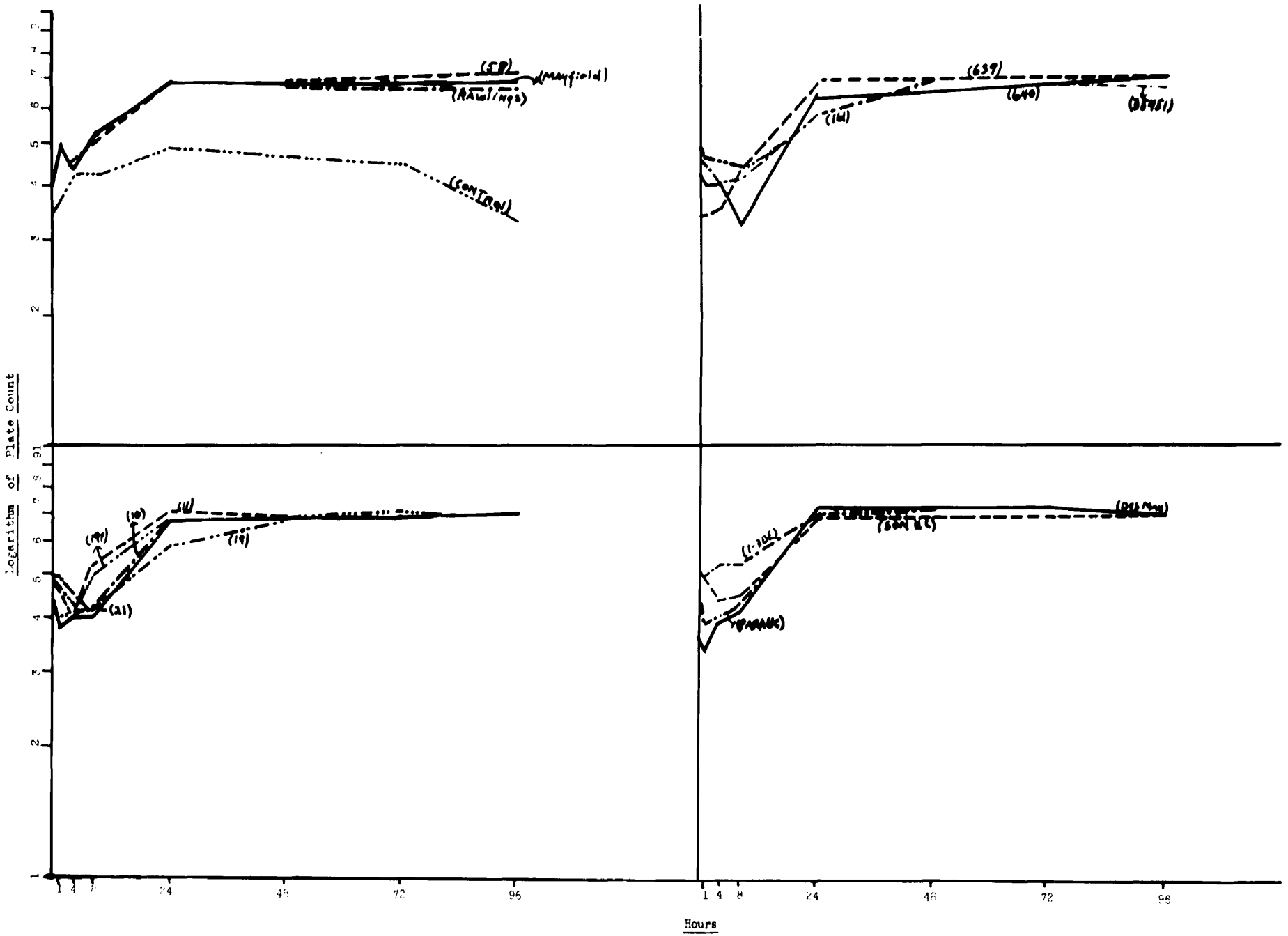

FIGURE 27.

Effect of Solution 11 upon Organisms in Similated Specimens.

Series B.

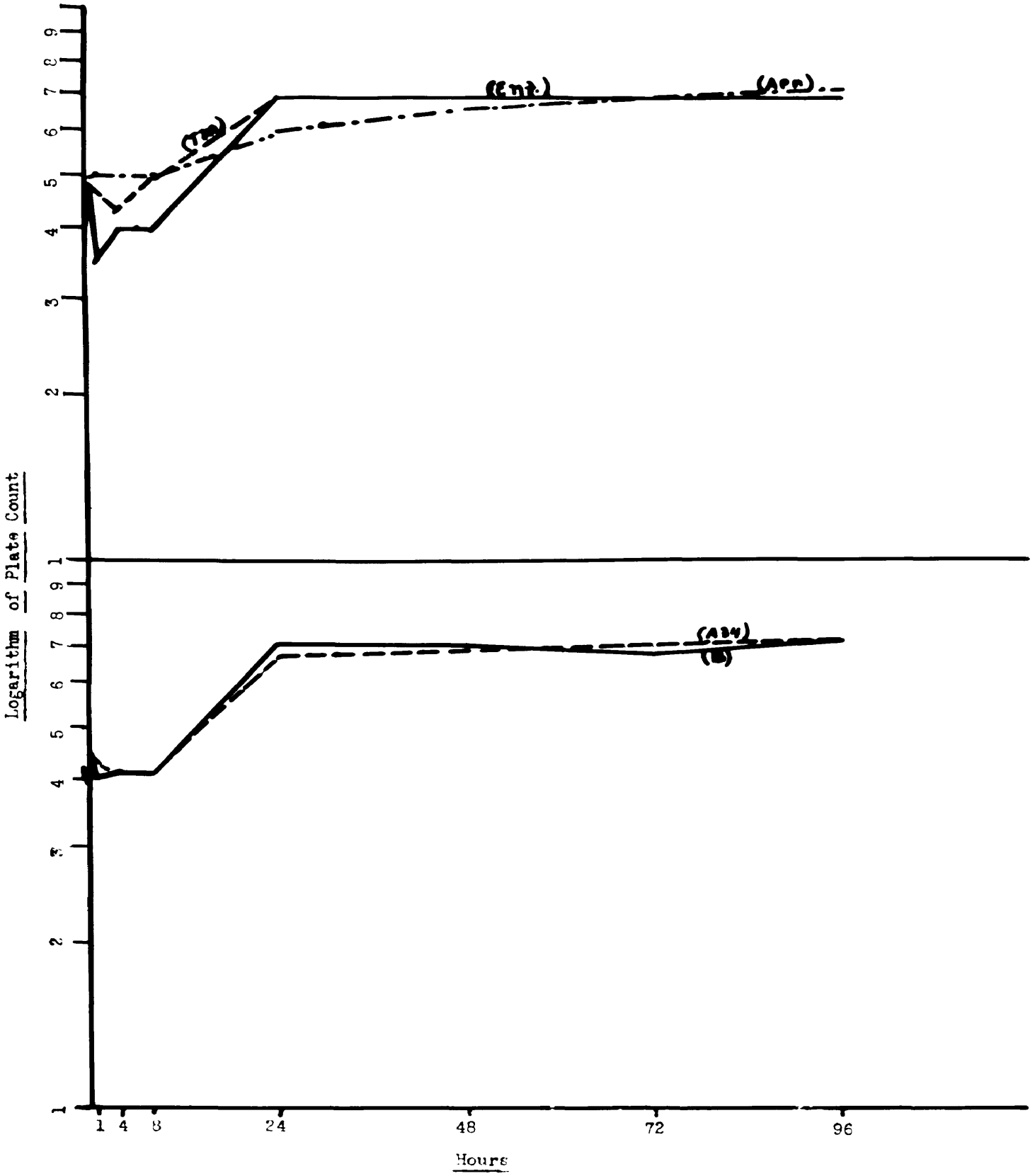

FIGURE 28.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series C.

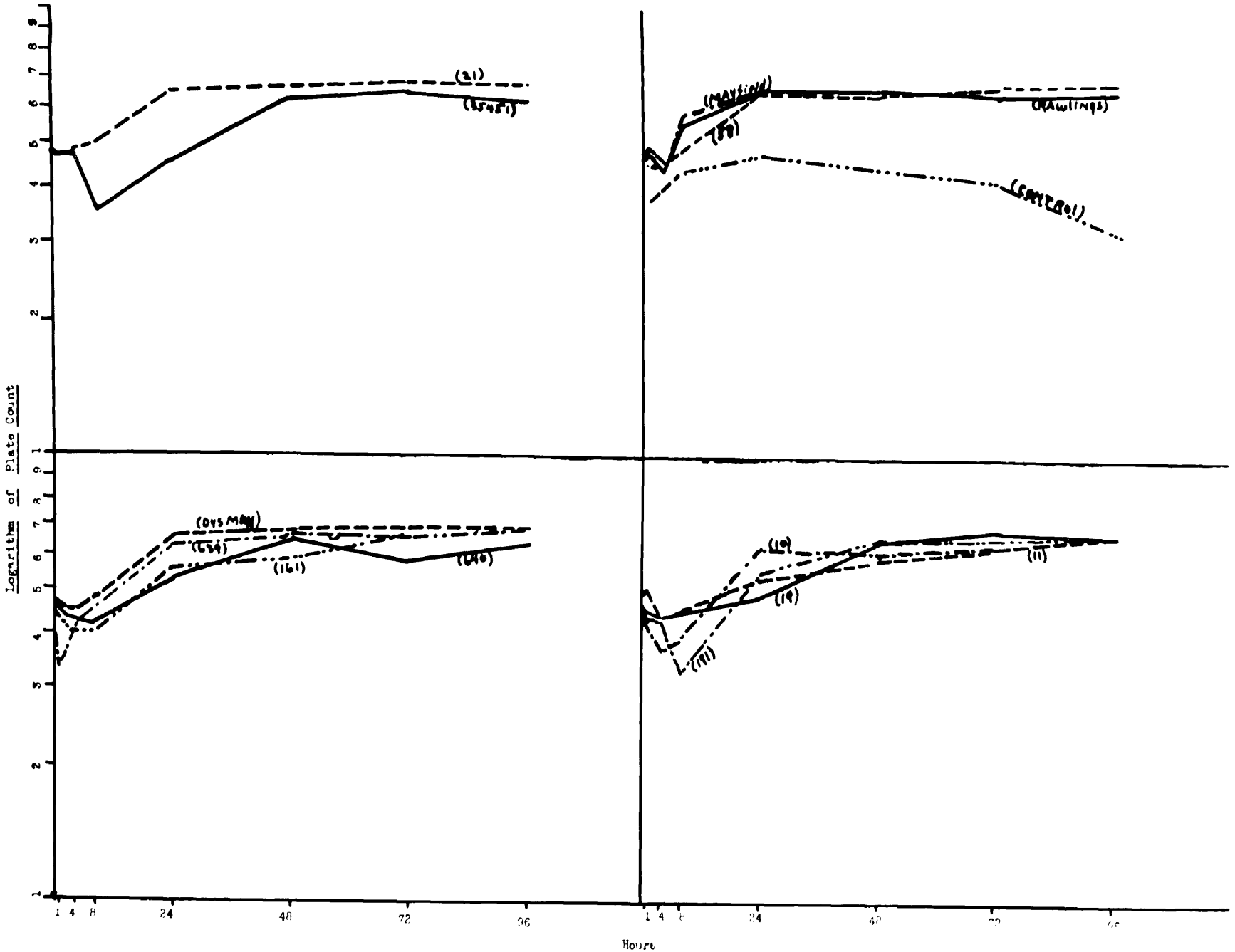

FIGURE 29.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series C.

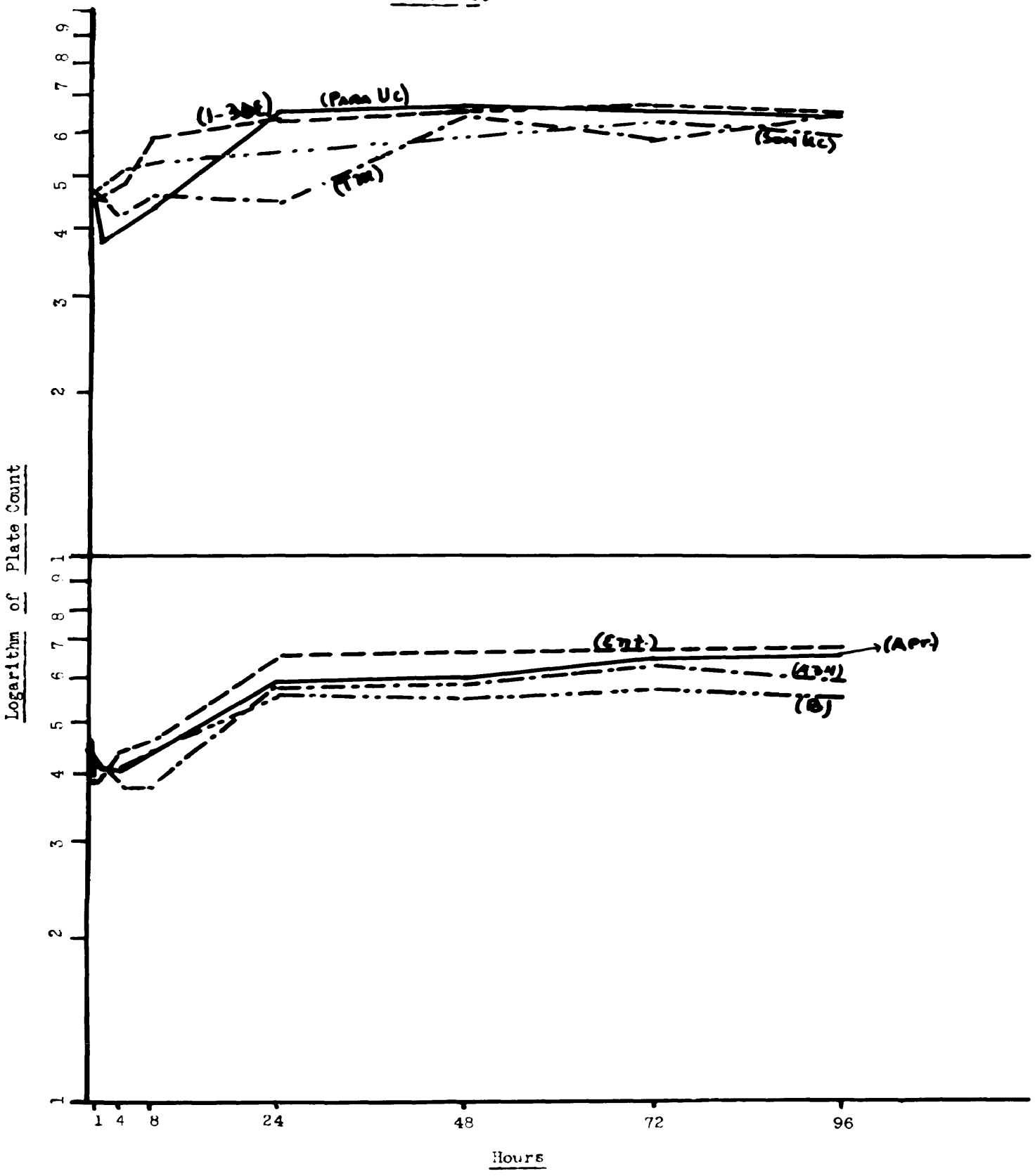

FIGURE 30.

Effect of Solution 11 upon Organisms in Simulated Specimens.

Series D.