CORRELATION BETWEEN SUSCEPTIBILITY TO NEOARSPHENAMINE AND SULFATHIAZOLE IN VITRO AND CERTAIN OTHER CHARACTERISTICS OF STREPTOCOCCI FROM SUBACUTE ENDOCARDITIS

by

JULIA JOSKI

A THESIS

Presented to the Department of Bacteriology
and the Graduate Division of the University of Oregon
in partial fulfillment
of the requirements for the degree of
Master of Science

June 1941

APPROVED: [redacted]

(Advisor for the Thesis)

[redacted]

(For the Committee)

May 15/1941

In Acknowledgement and Appreciation

of the Guidance, Assistance, and Instruction by

Dr. H. J. Sears

Department of Bacteriology

and

Dr. E. E. Osgood

Department of Experimental Medicine

of the

University of Oregon Medical School

TABLE OF CONTENTS

	Page
CLINICAL AND BACTERIOLOGICAL DATA ON THE STRAINS STUDIED	5
THE CLASSIFICATION OF THE STREPTOCOCCI BY THEIR PHYSIOLOGICAL REACTIONS	7
SEROLOGICAL RELATIONSHIPS	9
DRUG SENSITIVITY IN VITRO OF THE STREPTOCOCCI OF SUBACUTE ENDOCARDITIS	12
Discussion of Results with Sulfathiazole	
Discussion of Results with Neoarsphenamine	
SUMMARY	19
CONCLUSIONS	21
BIBLIOGRAPHY	22
APPENDIX	24

CORRELATION BETWEEN SUSCEPTIBILITY TO NEOARSPHENAMINE AND SULFATHIAZOLE IN VITRO AND CERTAIN OTHER CHARACTERISTICS OF STREPTOCOCCI FROM SUBACUTE ENDOCARDITS.

by

JULIA JOSKI, B. S.

Recent developments in drug therapy in reference to subacute endocarditis have shown that the non-hemolytic streptococci vary in their response to certain drugs. These findings, therefore, seemed to indicate a reinvestigation of the problem to see if there is a correlation between the drug sensitivity of the different strains from pathogenic sources and their cultural and serological reactions.

This investigation deals first with the metabolic and biochemical reactions of the streptococci isolated from blood cultures of patients with subacute bacterial endocarditis; second, it deals with the serological relations between the individual strains and those that differed in their response to neoarsphenamine and sulfathiazole; third, it deals with the in vitro reactions of these streptococci to neoarsphenamine and to sulfathiazole in simple bacteriological media and the relationship which these results may have to those of previous research in bone marrow cultures.

Review of the literature indicates that many attempts at classifying the non-hemolytic streptococci from pathogenic and from saprophytic sources have been made, but that no adequate means of differentiation of these heterogeneous organisms has been found. Horder (1908) reported the

first intensive study of the micro-organisms associated with subacute endocarditis. Twenty-two of the 35 strains isolated from blood cultures of patients corresponded to Str. salvarius and Str. fecalis in their cultural reactions to Gordon's tests. He provided absolute proof of this by taking example of Str. salvarius from a normal person's throat and Str. fecalis from a normal feces and producing with each, by intravenous injection into rabbits, the classical valvular lesions of subacute endocarditis. Andrewes and Horder (1906) found no characteristics by which the streptococci from malignant endocarditis could be differentiated from Str. salvarius, which includes the defunct Str. anginosus species, and Str. fecalis from saprophytic sources. The Str. salvarius of Andrewes and Horder (1906) has been accepted by Safford, Sherman, and Hodge (1937) as valid.

Holman (1916) based his classification of streptococci on their respective reactions on blood, lactose, mannitol, and salicin. Gordon, and Andrewes and Horder, though, reported that reactions with salicin are liable to vary with the same strain. Blake (1917) based his classification on the fermentation of lactose and mannitol. His six strain of endocarditis streptococci all fermented lactose and sucrose, but not mannitol. The fermentation of salicin, raffinose, and inulin varied with the different strains.

Kinsella and Swift (1917) reported on 14 strains from subacute endocarditis, but the number of tests run with these strains was insufficient to give this paper importance today. Their work showed that there was no correlation between the fermentation and agglutination tests. Hopkins and Lang (1914) reported a classification of pathogenic streptococci by fermentative reactions. Their series included 14 strains from

subacute endocarditis. These strains did not show similarity in their reactions to tests, and their classification on cultural methods proved to be impractical.

Moran (1938) investigated 20 strains from subacute endocarditis. Her classification of these organisms was based on Sherman's scheme. Of these 20 strains, 12 corresponded to Str. salvarius, 3 to Str. fecalis, 2 to Str. zymogenes, and 3 differed from any other established species by producing an alkaline reaction in milk.

Serological methods, too, have not proved useful for the classification of these organisms. Lancefield (1925) found that the specific carbohydrate appears to be type specific in the Str. viridans instead of group or species specific as in the case of the hemolytic forms. She also found that the nucleoproteins of the non-hemolytic streptococci were serologically similar.

Krumwiede and Valentine (1916) made a study of the agglutination and cultural relationship of the streptococci from 15 cases of subacute endocarditis and from a series of strains isolated from tonsils of healthy individuals. Their conclusions were that the agglutination results are in accord with the enormous multiplicity of types which can be differentiated by the determination of fermentative characteristics. On the other hand, there was no correlation between interagglutinative and fermentative reactions, even where the former were very marked. Most of the strains did not agglutinate with immune sera of other strains.

Certain strains from saprophytic and from pathogenic sources have been noted to be agglutinated by more than one serum and yet the homologous strains for the serums did not interagglutinate. No classification seems apparent on the basis of agglutination reactions.

Norton (1923) studying the serological relationships in the viridans group from saprophytic sources concluded that there is no correlation between cultural characters and biological reactions or between sources and agglutinin absorbing powers. Clawson (1920) immunized five rabbits with strains of Str. viridans. In a series of the non-hemolytic streptococci, 89 per cent failed to agglutinate with the above serums. All of these strains were from non-pathogenic sources.

Thus we find, in the field of the non-hemolytic streptococci, much intensive investigation in an attempt to find some form of classification or some form of correlation as to pathogenicity of this heterogeneous group of organisms.

Osgood (1940) found that sulfathiazole in the concentration of 1:10,000 is ineffective against some strains but leads to sterility with other strains in his bone marrow cultures; that neoarsphenamine in the concentration of 1:150,000 is effective against many strains and is less effective than sulfathiazole against some strains. He also found that clinically patients with subacute endocarditis, whose infective organisms responded to neoarsphenamine in bone marrow cultures, showed similar response to the drug therapy. Osgood has used tests in the bone marrow culture media successfully for predicting the in vivo susceptibility of strains of streptococci to neoarsphenamine and sulfathiazole. The strains differentiated into three group, according to their sensitiveness to these drugs.

CLINICAL AND BACTERIOLOGICAL DATA ON THE STRAINS STUDIED

The fifteen strains of non-hemolytic streptococci were obtained from blood cultures of patients with subacute bacterial endocarditis. As far as could be determined, the organisms were isolated before the patients were started on neoarsphenamine and sulfathiazole therapy. In two instances, the original strains were lost and subsequent cultures were isolated from the blood after the patients had been on the drug therapy for some time. These latter strains are referred to in this investigation as Kimberly and Bland. The original Kimberly strain was neoarsphenamine sensitive but sulfathiazole resistant, while the first Bland strain was sensitive to both of the drugs in bone marrow cultures. The two strains of the second isolations were found to be no longer sensitive to neoarsphenamine.

Soon after Bland and Kimberly came to our attention and their infective organisms were found to be sensitive to neoarsphenamine, therapy with this drug was begun. The patients showed a very favorable response to this treatment until, due to inadequate dosages, an allergic reaction to the drug developed and the drug had to be discontinued with each. Both the patients were then given sulfathiazole, but it was not tolerated very well. The organisms isolated during this period no longer showed sensitivity to neoarsphenamine in bone marrow cultures, although neither of the strains varied in their reactions to sulfathiazole. As we did not believe that the physiological characteristics of these two strains would necessarily be altered, they were tested and compared on the basis of their original reactions in bone marrow cultures.

Table I summarizes the preliminary data on all the strains. In a number of cases, the effect of the drug therapy was not known because these drugs had not been tried. The initial inoculation of the bone marrow cultures was between 50 and 400 organisms per cc. Larger inoculations, of approximately 1000 per cc., in the experiments with the Hillis and Williams strains in the presence of neoarsphenamine did not show any limitation of growth by the drug that the smaller initial numbers revealed in 24 hours. It must be mentioned that Hillis was a strain isolated from a case of allergy and not from subacute endocarditis. As its reactions in the bone marrow cultures compared with those streptococci from the more pathogenic sources, it seemed desireable to determine if this strain could be differentiated from the others on the basis of its physiological or serological characteristics.

TABLE I

CLINICAL AND BACTERIOLOGICAL DATA ON THE STRAINS STUDIED

Strain	Response of Patient to Drug Therapy.	Organism Isolated.	Drug Susceptibility of Organism in Bone Marrow Cultures.
Fitzmaurice	Neoarsphenamine ≠ Sulfathiazole -	Str. salvarius	Neoarsphenamine ≠- Sulfathiazole -
Kimberly	Neoarsphenamine ≠ Sulfathiazole -	(Strain lost)*	Neoarsphenamine ≠ Sulfathiazole -
"	Neoarsphenamine -	Str. fecalis**	Neoarsphenamine - Sulfathiazole -
Bland	Neoarsphenamine ≠ Sulfathiazole -	(Strain lost)*	Neoarsphenamine ≠ Sulfathiazole ≠
"	Neoarsphenamine - Sulfathiazole -	Str. salvarius**	Neoarsphenamine ≠- Sulfathiazole ≠
Peterson	Neoarsphenamine ? Sulfathiazole ?	Str. salvarius	Neoarsphenamine ≠- Sulfathiazole ≠
Wilson	Neoarsphenamine ? Sulfathiazole -	Str. salvarius	Neoarsphenamine ≠- Sulfathiazole ≠
Pierce	Neoarsphenamine ? Sulfathiazole ?	Str. salvarius	Neoarsphenamine ≠ Sulfathiazole ≠
Hillis		Str. salvarius	Neoarsphenamine ≠ Sulfathiazole ≠
Williams	Neoarsphenamine ? Sulfathiazole ?	Str. salvarius	Neoarsphenamine ≠;- Sulfathiazole ≠
Rothchild	Neoarsphenamine - Sulfathiazole -	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠

* The strain was isolated before drug therapy but was lost after initial studies in the bone marrow cultures.

** The strain was isolated after the drug therapy.

? indicates the drug was not tried, or this information was not available.

≠ indicates the organism was susceptible to the drug; ≠- indicates the organism was partially sensitive to the drug.

TABLE I (CONTINUED)

Strain	Response of Patient to Drug Therapy.	Organism Isolated.	Drug Susceptibility of Organism in Bone Marrow Cultures.
Brill	Neoarsphenamine - Sulfathiazole -	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠
Ingle	Neoarsphenamine - Sulfathiazole -	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠
Southwell	Neoarsphenamine ? Sulfathiazole ?	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠
Magolino	Neoarsphenamine ? Sulfathiazole ?	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠
Thomson	Neoarsphenamine ? Sulfathiazole ?	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠
Halpert	Neoarsphenamine ? Sulfathiazole ?	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠
Matlow	Neoarsphenamine ≠ Sulfathiazole ?	Str. salvarius	Neoarsphenamine - Sulfathiazole ≠

CLASSIFICATION OF THE STREPTOCOCCI BY THEIR PHYSIOLOGICAL REACTIONS

It is generally accepted that the classification of the non-hemolytic group must rest on physiological characteristics. In this investigation, a large number of tests were run routinely in an anticipation that the comparison of reactions might reveal certain distinguishing characteristics which would correlate with the groupings of the organisms obtained by the study of the drugs in the bone marrow cultures. On the whole, the classification is based on Sherman's scheme (1937).

All tests were made in duplicate, using young growing broth cultures, and employing established bacteriological techniques. Wherever an occasional difference in reaction was noted in the two examinations, the test was repeated several times until consistant results were obtained.

The fermentation tests were made in sugar-free serum broth with 1 per cent of the test substance added. The tubes were incubated at 37°C for 7 days. Daily observations were made during the period of incubation. Wherever the tubes failed to show acid, they were tested for growth. The pH after four days of growth in one per cent glucose infusion broth was determined in all but three instances with the glass electrode.

Table II shows that 14 of the 16 strains, on the basis of their physiological reactions, were identical with Str. salvarius. Additional characteristics, as seen in Tables III and IV, revealed that the Fitzmaurice and the Pierce strains were atypical in that the former fer-

mented mannitol, hydrolyzed starch, and gave no alpha hemolysis on blood agar, and the latter fermented xylose and grew in the presence of 4 per cent salt. In as much as these strains did not vary in the basic reactions (Table II), they were classified as Str. salvarius. Two of the 16 strains proved to be enterococci.

The Kimberly strain, as seen in Table II, was a typical Str. fecalis, while the Matlow strain resembled this species very closely in some of its reactions and yet it differed in its heat tolerance, its methylene blue tolerance, and in its failure to give any acid reaction in milk. The Matlow strain did ferment lactose and it gave a low pH in 1 per cent glucose infusion broth. Moran (1938) reported 3 strains that fermented lactose and did not give acid in milk. But, her strains did not compare to our strain in the other characteristics.

Tables III and IV give the detailed results of all the strains tested. The salvarius strains, as a whole, showed a relationship in most of the chief characteristics and showed a wide variation in the less important. No subgroups were revealed which might aid in the recognition of the different drug sensitive strains.

TABLE II

DIVISIONS OF THE STREPTOCOCCI ACCORDING TO SHERMAN

Strains	Hemolysis	Growth at		Growth in Presence			Survival at 60° C per 30 minutes.	Division
		10° C	45° C	6.5 % NaCl	pH 9.6	0.1 % Methylene Blue		
Fitzmaurice	none (gamma)	-	+	-	-	-	-	Strep. salvarius
Kimberly	alpha	+	+	+	+	+	+	Strep. fecalis
Bland	alpha	-	-	-	-	-	-	Strep. salvarius
Peterson	alpha	-	-	-	-	-	-	" "
Wilson	alpha	-	-	-	-	-	-	" "
Pierce	alpha	-	-	-	-	-	-	" "
Hillis	alpha	-	-	-	-	-	-	" "
Williams	alpha	-	-	-	-	-	-	" "
Rothchild	alpha	-	-	-	-	-	-	" "
Brill	alpha	-	-	-	-	-	-	" "
Ingle	alpha	-	-	-	-	-	-	" "
Southwell	alpha	-	-	-	-	-	-	" "
Mazolino	alpha	-	-	-	-	-	-	" "
Thomson	alpha	-	-	-	-	-	-	" "
Halpert	alpha	-	-	-	-	-	-	" "
Matlow	alpha	+	-	+	+	-	-	Strep. fecalis type

TABLE III

ADDITIONAL CHARACTERISTICS OF THE STREPTOCOCCI

STRAINS	Bile soluble	Growth on agar		Growth in presence					Survival at, 30 minutes.					Growth on gelatine	Dextrin split	Starch hydrolysis	Esculin split
		10% Bile-blood	40% Bile-blood	2% NaCl	4% NaCl	6.5% NaCl	0.01% M.B.	0.1% M.B.	50° C	55° C	60° C	65° C	70° C				
Fitzmaurice	-	≠	≠	≠	-	-	-	-	≠	≠	-	-	-	≠	≠	≠	≠
Kimberly	-	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	≠	≠	-	≠
Bland	-	≠	≠	≠	-	-	≠	-	≠	≠	-	-	-	≠-	≠	-	≠
Peterson	-	-?	-	-?	-	-	≠	-	≠	≠	-	-	-	-	≠	-	≠
Wilson	-	-	-	≠	-	-	-	-	≠	≠	-	-	-	-	≠	-	-
Pierce	-			≠	≠	-	≠	-	≠	≠	-	-	-	-	≠	-	-
Hillis	-	≠	≠	≠	-	-	-	-	≠	≠	-	-	-	-	≠	-	≠
Williams	-	≠	≠	≠	-	-	≠	-	≠	≠	-	-	-	≠-	≠	-	≠
Rothchild	-	≠	≠	≠	-	-	-	-	≠	≠	-	-	-	≠-	≠	-	-
Brill	-	≠	-	≠	-	-	-	-	≠	≠	-	-	-	≠-	≠	-	-
Ingle	-	-	-	-?	-	-	-	-	≠	≠	-	-	-	-	≠	-	-
Southwell	-	-	-	≠	-	-	-	-	≠-	≠	-	-	-	-	≠	-	-
Mazolino	-	≠	-	≠	-	-	-	-	≠	≠	-	-	-	≠-	≠	-	-
Thomson	-	≠	-	-	-	-	-	-	≠	-	-	-	-	-	≠	-	-
Halpert	-			-	-	-	-	-	≠	≠	-	-	-	-		-	-
Matlow	-			≠	≠	≠	-	-	≠	≠	-	-	-	≠		≠	≠

TABLE IV

ADDITIONAL CHARACTERISTICS OF THE STREPTOCOCCI

STRAINS	Litmus Milk -- acid.	Final pH in 1% Glucose Infusion Broth.	Acid Produced From												
			Lactose	Sucrose	Maltose	Raffinose	Trehalose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin	Arabinose	Xylose
Fitzmaurice	+	4.38	+	+	+	+	+	+	-	+	-	+	-	-
Kimberly	+	4.24	+	+	+	-	+	-	+	+	+	+	-	-
Bland	+	4.61	+	+	+	+	+	+	-	-	-	+	-	-
Peterson	+	4.62	+	+	+	+	+	+	-	-	-	+	-	-
Wilson	+	4.72	+	+	+	+	-	-	-	-	-	-	-	-
Pierce	+	4.2-4.4	+	+	+	-	+	+	-	-	-	+	-	+
Hillis	+	4.41	+	+	+	+	-	-	-	-	-	+	-	-
Williams	+	4.54	+	+	+	-	+	+	-	-	-	+	-	-
Rothchild	+	4.41	+	+	+	-	+	-	-	-	-	+	-	-
Brill	+	4.45	+	+	+	-	-	-	-	-	-	-	-	-
Ingle	+	4.33	+	+	+	-	-	-	-	-	-	-	-	-
Southwell	+	4.61	+	+	+	+-	-	-	-	-	-	-	-	-
Mazoline	+	4.82	+	+	+	-	-	-	-	-	-	-	-	-
Thomson	+	5.20	+	+	+	-	-	-	-	-	-	-	-	-
Halpert	+	4.2-4.4	+	+	+	-	+	+	-	-	-	-	-	-
Matlow	-	4.2-4.4	+	+	+	-	+	-	+	+	-?	+	-	-

SEROLOGICAL RELATIONSHIPS

Serological behavior of the strains was studied by means of sera from rabbits immunized against living bacteria. This survey is aimed to determine if there is any antigenic similarity among the strains belonging to the same drug sensitive group.

Three rabbits were immunized with 24 hour broth cultures of live organisms, according to the technique used by Lancefield (1928). The three strains selected for the immunization represented strains having different sensitivity to the drugs in bone marrow cultures. The Kimberly strain represented those that were resistant to sulfathiazole, the Bland strain those that were sensitive to both neoarsphenamine and sulfathiazole, and the Brill strain the group that was susceptibile to sulfathiazole alone.

The agglutination tests were made using the method described by Lancefield (1925). As the streptococci did not give heavy growth in 24 hour broth cultures, the antigens were not deeply turbid. By using a hand lens and carefully comparing the agglutination tubes to the controls, agglutinations could be easily read even where the organisms tended to settle out spontaneously on standing overnight in the ice box.

Normal content of agglutinins in each of the rabbit sera was determined with each strain. These values were deducted and the results of the agglutination tests summarized in Table VIII. Tables V, VI, and VII give the detailed data of the strains with each of the sera.

Serological behavior of the strains did not reveal any consist-

ent antigenic similarity among the organisms. The Bland and the Peterson strains were very much alike in their drug sensitivity in the bone marrow cultures, in their cultural and biochemical reactions, and in the agglutinations with the Bland immune serum (Table VI). Furthermore, these two strains gave identical reactions with the Kimberly immune serum. But, with the Brill immune serum their reactions differed. The Bland strain gave partial agglutination with this serum in the lower dilutions, while the Peterson strain gave as high a titer as the homologous strain did. The Peterson strain, just as the Brill strain, gave definite agglutinations up to 1:40 with the normal rabbit sera while the Bland strain did not show any reactions.

The Thomson strain, which was similar to the Brill strain in most characteristics, showed a close resemblance serologically, too. But, other strains, as the Mazolino and the Southwell, which fell in the same group, did not agglutinate with any of the sera.

Whereas the Str. fecalis strain of Kimberly showed no serological relations with either of the two strains of Str. salvarius, most of the salvarius strains that agglutinated with the other two immune sera gave partial or slight agglutination with the Kimberly immune serum. The Matlow strain, on the other hand, culturally resembled a Str. fecalis but it did not show any antigenic similarity with the Kimberly strain.

On the whole, the data seem to us to justify the conclusion that these pathogenic streptococci constitute a heterogeneous group, and that the groupings revealed in the studies of the drugs are not the same as those established by serological methods. Krumwiede and Valentine (1916) interpreted similar serological findings by stating that many of

the strains tended to possess common agglutinin-binding content but did not necessarily show a common agglutinogenic content.

TABLE V

AGGLUTINATIONS WITH BRILL STREPTOCOCCUS IMMUNE RABBIT SERUM

STRAINS	1:10	1:20	1:40	1:80	1:160	1:320	1: 640	1:1,280	1:2,560	1:5,120
Fitzmaurice	* -	-	-	-	-	-	-	-	-	-
Kimberly	* -	-	-	-	-	-	-	-	-	-
Bland	* -	++	+	+- L	-	-	-	-	-	-
Peterson	* +++	++	+- L	+++	+++	+++	++	+-	+	+- L
Pierce	* -	-	-	-	-	-	-	-	-	-
Hillis	* -	-	-	-	-	-	-	-	-	-
Williams	* -	++	+	+- L	-	-	-	-	-	-
Rothchild	* -	-	-	-	-	-	-	-	-	-
Brill	* +++	++	+++	++++	+++	+++	+++	++	+	+- L
Ingle	* -	-	-	-	-	-	-	-	-	-
Southwell	* -	-	-	-	-	-	-	-	-	-
Mazolino	* -	-	-	-	-	-	-	-	-	-
Thomson	* ++	+++	+- L	+++	+++	++	++	+	+-L	-
Halpert	* -	-	-	-	-	-	-	-	-	-
Watlow	* -	-	-	-	-	-	-	-	-	-

* Indicates agglutination with normal serum.

+- L indicates slight agglutination seen with a hand lens.

TABLE VI

AGGLUTINATIONS WITH BLAND STREPTOCOCCUS IMMUNE RABBIT SERUM

STRAINS	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
Fitzmaurice	* ?	-	-	-	-	-	-	-	-	-
		± L								
Kimberly	* -	-	-	-	-	-	-	-	-	-
		-								
Bland	* -	-	####	####	####	####	####	###	###	##	±-L
Peterson	*###	##	###	###	###	###	##	##	±	±-L	-
Pierce	* -	-	-	-	-	-	-	-	-	-	-
		-								
Hillis	* -	-	##	±	-	-	-	-	-	-	-
		±								
Williams	* -	-	±	±	± L	-	-	-	-	-	-
Rothchild	* -	-	? L	-	-	-	-	-	-	-	-
		-								
Brill	* ##	±	###	###	##	±	±-L	-	-	-	-
Ingle	* -	-	±	±-L	-	-	-	-	-	-	-
		-								
Southwell	* -	-	-	-	-	-	-	-	-	-	-
		-								
Mazolino	* -	-	-	-	-	-	-	-	-	-	-
		-								
Thomson	*###	##	###	###	±	±-L	-	-	-	-	-
		±								
Halpert	* -	-	-	-	-	-	-	-	-	-	-
		-								
Matlow	* -	-	-	-	-	-	-	-	-	-	-
		-								

* Indicates agglutination with normal serum.
± L indicates agglutination slight, read with a hand lens.

TABLE VII

AGGLUTINATIONS WITH KIMBERLY STREPTOCOCCUS IMMUNE RABBIT SERUM

STRAINS	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
Fitzmaurice	* -	-	-	-	-	-	-	-	-	-
Kimberly	* -	++++	++++	++++	++++	+++	+++	++	+	+-L
Bland	* -	+++	+++	++	+	+-L	-	-	-	-
Peterson	*+++	+++	++	++	+	+-L	-	-	-	-
Pierce	* -	-	-	-	-	-	-	-	-	-
Willis	* -	++	+	+-L	-	-	-	-	-	-
Williams	* -	+	+-L	-	-	-	-	-	-	-
Rothchild	* +	+-L	++	-	-	-	-	-	-	-
Brill	*+++	+++	++	+	+-L	-	-	-	-	-
Ingle	* -	-	-	-	-	-	-	-	-	-
Southwell	* -	+-L	-	-	-	-	-	-	-	-
Marolino	* -	-	-	-	-	-	-	-	-	-
Thomson	*++++	+++	++	+	+-L	-	-	-	-	-
Halport	* -	-	-	-	-	-	-	-	-	-
Matlow	* -	-	-	-	-	-	-	-	-	-

* Indicates agglutination with normal serum.

+- L indicates slight agglutination, read with a hand lens.

TABLE VIII

SUMMARY OF THE AGGLUTINATION RESULTS WITH THREE IMMUNE RABBIT SERA

	Brill Rabbit Serum		Bland Rabbit Serum		Kimberly Rabbit Serum	
	Normal	Immune	Normal	Immune	Normal	Immune
Fitzmaurice	0	0	0	0	0	0
Kimberly	0	0	0	0	0	1:2,560
Bland	0	1:40	0	1:2,560	0	1:160
Peterson	1:20	1:2,560	1:20	1:1,280	1:40	1:160
Pierce	0	0	0	0	0	0
Hillis	0	0	0	1:40	0	1:40
Williams	0	1:40	0	1:40	0	1:20
Rothschild	0	0	0	0	1:10	1:20
Brill	1:40	1:2,560	1:20	1:160	1:80	0
Ingle	0	0	0	1:20	0	0
Southwell	0	0	0	0	0	0
Mazolino	0	0	0	0	0	0
Thomson	1:20	1:1,280	1:20	1:80	1:80	0
Halpert	0	0	0	0	0	0
Matlow	0	0	0	0	0	0

DRUG SENSITIVITY IN VITRO OF THE STREPTOCOCCI OF SUBACUTE ENDOCARDITIS.

It has been generally assumed that these drugs in the low concentrations in which they can be used clinically have a bacteriostatic action in which multiplication slows down or is interrupted. The best evidence for determining the actual effect of these drugs is derived from the study of the growth curves of the organisms in a medium containing a low concentration of the drugs as compared to the same organisms in the same medium without the drugs.

Procedure

The following studies were made with beef infusion broth at pH 7.4 in which our strains grew readily. This medium was used in 500 cc. flasks with 250 cc. quantities. Each strain was inoculated into 3 flasks. To one was added 0.0056 mg. neoarsphenamine per cc., to another 0.1 mg. sulfathiazole per cc., and the third contained no drug. These were incubated at 37°C and at stated intervals, appropriate samples were withdrawn and pour plates for colony counts were made. From the tables giving the total colony counts, graphs were constructed. In the graphs, the logarithms of the number of bacteria per cubic centimeter were plotted as the ordinates and the time in hours as the abscissa.

Discussion of Results with Sulfathiazole

Sulfathiazole showed the most marked bacteriostasis. Except in the two instances with strains resistant to this drug, quantities greater than 0.3 or 0.2 cc. of the undiluted cultures with sulfathiazole could not be plated because of the inhibitive action of the drug in the plates. Plates with 1.0 cc. and 0.5 cc. of culture would show no colonies, while 0.2 and 0.1 cc platings showed good growth of colonies. Therefore, the colony counts from the sulfathiazole cultures were based on 0.2 cc. and 0.1 cc. platings. Consequently, with some strains, as the Bland, the Rothchild, the Mazolino, and the Halpert, represented in Series I figures 3, 8, 12, and 13, irregularities in the curves are due to inaccuracies in the total colony counts which had to be calculated on the findings of one or two colonies per 0.1 or 0.2 cc. In the early phases of growth, the colony counts of the control and the neoarsphenamine cultures were based on 1.0 and 0.5 cc. platings, respectively.

The Effect of Sulfathiazole on the Growth Curves. Figures 1 and 2 in graph Series I show that sulfathiazole had no effect upon the growth curves of the Kimberly and Fitzmaurice strains. The bacterial counts of the sulfathiazole and the control experiments were almost identical. The four strains represented in figures 4, 5, 7, and 10, illustrate a delayed bacteriostatic action of the drug. Figures 4 and 10 definitely show that the multiplication of the organisms in the 4 and 6 hours, respectively, was uninhibited. After this early rapid increase, a gradual slowing took place until reproduction ultimately ceased or came to a stationary phase, and the total number of cells began to decline. This decline was quite noticible in figure 4 in 30 hours. This experiment was repeated,

with the bacterial counts made every two hours for 30 hours. The growth curves represented in graph Series II, figure 2, give the more complete picture of this bacteriostatic action of sulfathiazole.

Some strains were more sensitive to this drug than the above. Three strains, illustrated in graph Series I, figures 6, 9, and 14, showed such a rapid decline of the number of organism that none could be found by plating after one hour of incubation. This fact does not necessarily indicate that the cultures became sterile, because in several later experiments it was shown that such apparently negative cultures did not prove to be sterile even after 30 hours of incubation. It is possible that the action of the drug affected the reproduction of the organisms without killing them.

Figures 3, 8, 11, 12, and 13 in Series I showed the presence, but no multiplication, of the organisms in the early hours of incubation. Plate counts revealed almost the same number of cells as in the initial inoculation. Later, no colonies were obtained in the pour plates.

The growth curves of the bacteria in the presence of sulfathiazole vary with the different strains. Some strains seem to be more sensitive than others. The sulfathiazole affects the reproductory system of the bacteria in such a manner as to produce, in the most strains, a complete stasis of growth, while in the presence of this drug.

Sulfathiazole in Hartley's Broth Cultures. In some instances, the type of medium, in which the in vitro studies of a drug are made, may influence the reactions. It seemed, therefore, desireable to find if sulfathiazole active in beef infusion broth would exert a similar effect on the drug sensitive strains in another medium, as in that of Hartley's broth. Substituting Hartley's broth for the other medium, identical ex-

periments were set up with three strains. The growth curves, represented in the graphs of Series III, compared with those obtained in infusion broth. The drug was bacteriostatic irrespective of the medium used.

To determine whether these three cultures that gave negative plates after four hours actually became sterile, 1.0 cc. of the culture, after 30 hours of incubation, was removed from each sulfathiazole flask, and inoculated into flasks containing 100 cc. of infusion broth. The latter were then incubated at 37°C and daily observations were made. No gross evidence of growth was observed after 48 hours of incubation, but when the cultures were examined on the fourth day, they were clouded with growth. The sulfathiazole apparently had not sterilized the cultures of the bacteria, but had affected the cells to such an extent that they were temporarily dormant in a sulfathiazole-free medium.

Bacteriostatic Effect of Lower Concentrations of Sulfathiazole. Sulfathiazole in the concentration of 0.1 mg. per cc. exerted a definite bacteriostasis of most of the strains. Two lower concentrations of this drug were tried with the Pierce strain. The growth curves in the Series IV show that there is practically no difference in or loss of the bacteriostatic effect when the concentration is decreased one-half but a distinct reduction of effectiveness is noted with a decrease of ten times.

The Effect of Sulfathiazole on a Large Inoculum of Organisms. There is evidence that the action of the drug may vary according to the size of inoculations. Work on the hemolytic streptococci in the presence of sulfanilamide has shown that if the initial number of organisms inoculated is large, the cultures grow out to large numbers in spite of the presence of the effective drug (Osgood, 1938). As all our experiments were set up with relatively small inoculations, some with a

larger number of organisms were tried.

The Brill strain with an initial inoculation of 440 and of 1700 organisms per cubic centimeter showed no colonies when the cultures were plated six hours after the incubation. The sulfathiazole flasks showed no growth at the end of five days, when they were last observed, while the controls became turbid within 24 hours and remained so. The experiment was repeated with an initial inoculation of over 200,000 organisms per cubic centimeter, or 0.5 cc. of a 20 hour infusion broth culture in 250 cc. of broth. In 24 hours, the plate counts of the sulfathiazole culture showed 640 organisms per cubic centimeter. The medium was clear, but in 48 hours, it became turbid and the bacterial count was then over 1,000,000. It was not determined whether the bacteria that grew in the presence of the drug were actually resistant to the drug.

Cultures of the Bland strain, with an inoculum of 1600 and of 6400, gave negative plates at the end of six hours of incubation.

It may be concluded that if the number of organisms is large enough that, even with the sulfathiazole sensitive strains, the drug may not stop multiplication completely. But, the bacteriostatic action is powerful enough to definitely limit the growth of the susceptible bacteria in 24 hours. Thus, in 24 hours, the drug sensitive strains may be differentiated from the resistant ones. The best information of the in vitro action of sulfathiazole is obtained using a small inoculum.

Discussion of Results with Neoarsphenamine

Neoarsphenamine did not have a significant inhibitive action on the strains in the beef infusion broth cultures. Some slowing of the multiplication in the first four or eight hours, respectively, was noticed with some strains, but this effect was not consistant with the drug sensitivity of these strains in the bone marrow cultures. The drug had no bacteriostatic action on the growth of the organisms in the pour plates made.

The Effect of Neoarsphenamine on the Growth Curves. Figure 1 of the graph Series II shows that this drug had some toxic effect on the growth of the organism in the first eight hours. The bacterial counts in that period revealed a lengthened lag phase not comparable to that of the drug-free culture. On the other hand, the strain represented by the growth curves of figure 2 of the same graphic series did not respond to neoarsphenamine. Osgood's results gave opposite conclusions in that he found the former strain to be neoarsphenamine resistant and the latter neoarsphenamine sensitive.

The Bactericidal Effect of Increased Concentrations of the Drug. As neoarsphenamine at the concentration of 0.0056 mg. per cc. very slightly affected the growth of the organisms, experiments with higher concentrations of the same were repeated with the Brill and the Peterson strains.

Each strain was inoculated into three flasks of 250 cc. of broth. The first one contained no drug; the second had of concentration of ten times, and the third, twenty times that of the neoarsphenamine in all the previous experiments. The cultures were incubated and tested in the usual

manner. In addition, to determine whether the cultures with the high concentrations of the drug became actually sterile after 30 hours of incubation, 1.0 cc. was removed from each of the drug cultures and inoculated into flasks with 100 cc. of fresh infusion broth. Graphs were plotted from the bacterial counts.

The growth curves in Series IV show that 0.06 mg. per cc., of neoarsphenamine, had a bacteriostatic effect on the Peterson strain and a bactericidal effect on the Brill strain. The culture with the latter strain proved to be sterile. At the concentration of 0.12 mg. per cc., the organisms of both strains were killed. The bacterial counts in the latter cultures showed a gradual decline until none could be obtained by plating the 30 hour cultures, and no growth could be detected when these cultures were inoculated into a drug-free medium. Therefore, this decline and the absence of bacteria in 30 hours seems to indicate Neoarsphenamine in high concentrations is bactericidal rather than bacteriostatic aswas the case with sulfathiazole.

The lack of correlation between the drug sensitiveness of the strains in the medium employed in our studies and that obtained by studies in the bone marrow cultures indicated that simple bacteriological media, as the beef infusion broth, cannot be used to evaluate the neoarsphenamine sensitiveness of the streptococci.

SERIES I

Growth Curves of Streptococci in the Presence of Neoarsphenamine and Sulfathiazole in Infusion Broth.

TOTAL COUNTS AT GIVEN INTERVALS OF STREPTOCOCCI IN INFUSION BROTH (CONTROL) IN PRESENCE OF NEOARSPHENAMINE AND OF SULFATHIAZOLS.

1. Fitzmaurice Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.0056 mg./cc.	Sulfathiazole 0.1 mg./cc.
0	32	32	32
1	80	50	114
2	360	100	330
3	950	300	690
4	2,000	970	2,100
6	30,000	14,000	14,000
8	148,000	89,000	106,000
25	235,000,000	134,000,000	331,000,000
30	270,000,000	91,000,000	313,000,000

2. Kimberly Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.0056 mg./cc.	Sulfathiazole 0.1 mg./cc.
0	70	70	70
1	100	65	108
2	490	216	480
3	2,140	1,000	1,600
4	8,650	3,200	6,400
6	310,000	81,000	200,000
8	2,000,000	1,130,000	1,500,000

(Continued on following page.)

2. Kimberly Strain (Continued)

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
25	530,000,000	632,000,000	537,000,000
30	670,000,000	750,000,000	570,000,000
49	700,000,000	658,000,000	500,000,000

3. Bland Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	8	8	8
1	14	3	2
2	11	15	3
3	22	16	0
4	16	16	0
6	30	19	0
8	60	48	0
22	38,000	31,000	0
24	62,000	50,000	0
30	750,000	/50,000	0
50		195,000	0

4. Peterson Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	18	18	18
1	18	18	15
2	46	40	60
3	90	76	80
4	275	170	210
6	1,700	1,200	550
8	8,800	6,000	1,370
25	156,000,000	56,000,000	260
30	178,000,000	88,000,000	70

5. Williams Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	36	36	36
1	36	28	0
2	45	39	0
3	148	42	0
4	360	84	0
6	3,500	540	0
8	8,000	1,500	0
25	150,000,000	66,000,000	0
30	140,000,000	110,000,000	0

6. Hillis Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.0056 mg./cc.	Sulfathiazole 0.1 mg./cc.
0	3	3	3
1	3	5	5
2	12	5	0
3	57	4	0
4	137	18	5
6	1,800	149	50
8	14,000	1,340	150
25	16,000,000	about same as control	0?
30	42,000,000	" " " "	

7. Pierce Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.0056 mg./cc.	Sulfathiazole 0.1 mg./cc.
0	78	78	78
3	84	90	0?
6	525	220	220
24	425,000,000	1,200,000	120

8. Rothchild Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.0056 mg./cc.	Sulfathiazole 0.1 mg./cc.
0	38	38	38
1	55	40	0
2	216	122	
3	815	420	0
4	4,950	2,000	3?
6	100,000	45,000	20?
24		108,000,000	0
30	12,500,000	86,500,000	0

9. Brill Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.0056 mg./cc.	Sulfathiazole 0.1 mg./cc.
0	36	36	36
1		30	1?
2	80	40	1?
3	240	115	0
4	824	390	0
6	8,800	4,000	0
8	/12,000	/5,000	0
22	3,000,000		0
24	125,000,000		
26		154,000,000	0
30	166,000,000		
50	94,000,000		

10. Thomson Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	28	28	28
1	26	23	28
2	60	46	64
3	112	66	147
4	267	124	330
6	1,300	296	1,000
8	5,500	1,300	1,600
24	130,000,000	131,000,000	1,300
30	152,000,000	183,000,000	1,200
49	146,000,000	249,000,000	880

12. Mazoline Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	28	28	28
1	44	34	45
2	100	57	20
3	650	146	40
4	4,600	750	60
6	97,000	24,000	0
24	62,000,000	44,200,000	0
30	61,000,000	81,000,000	0

13. Ingle Strain

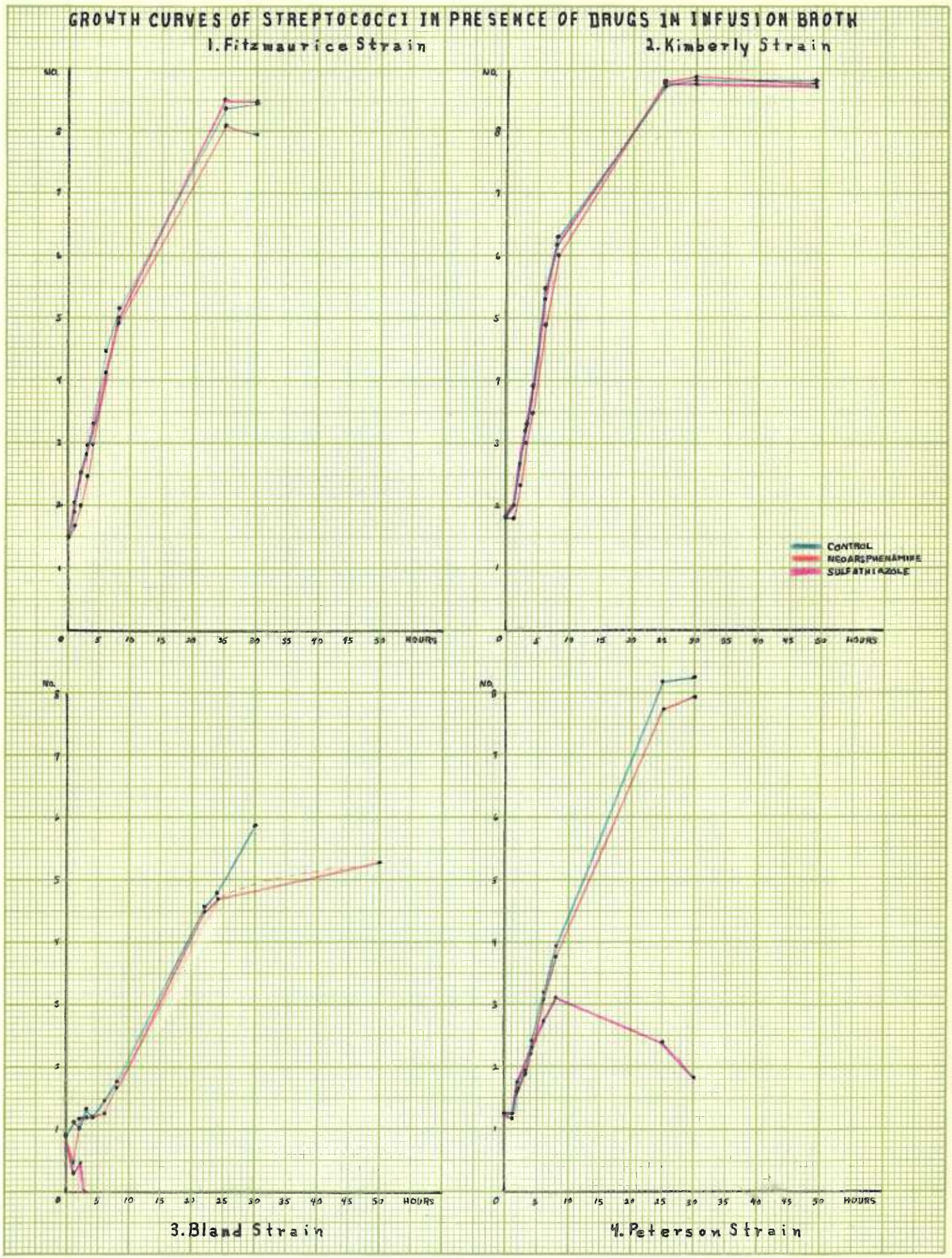
Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	18	18	18
1	18	17	10
2	11	10	13
3	15	11	0
4	30	20	0
5	40		0?
6	120	40	0
8	370		
10	900	160	
24	330,000	100,000	0
30	2,150,000	375,000	0
48	2,500,000	500,000	0

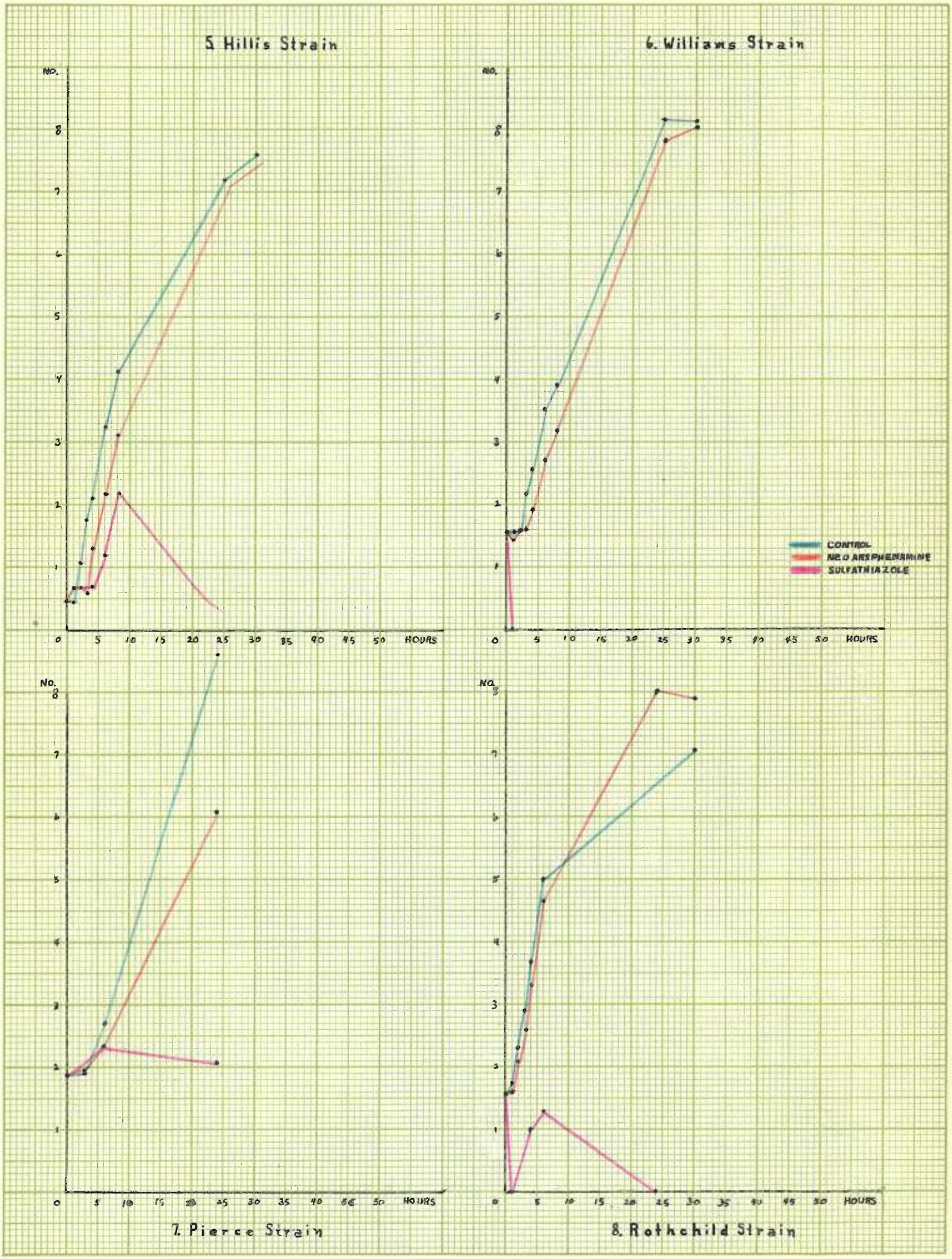
14. Southwell Strain

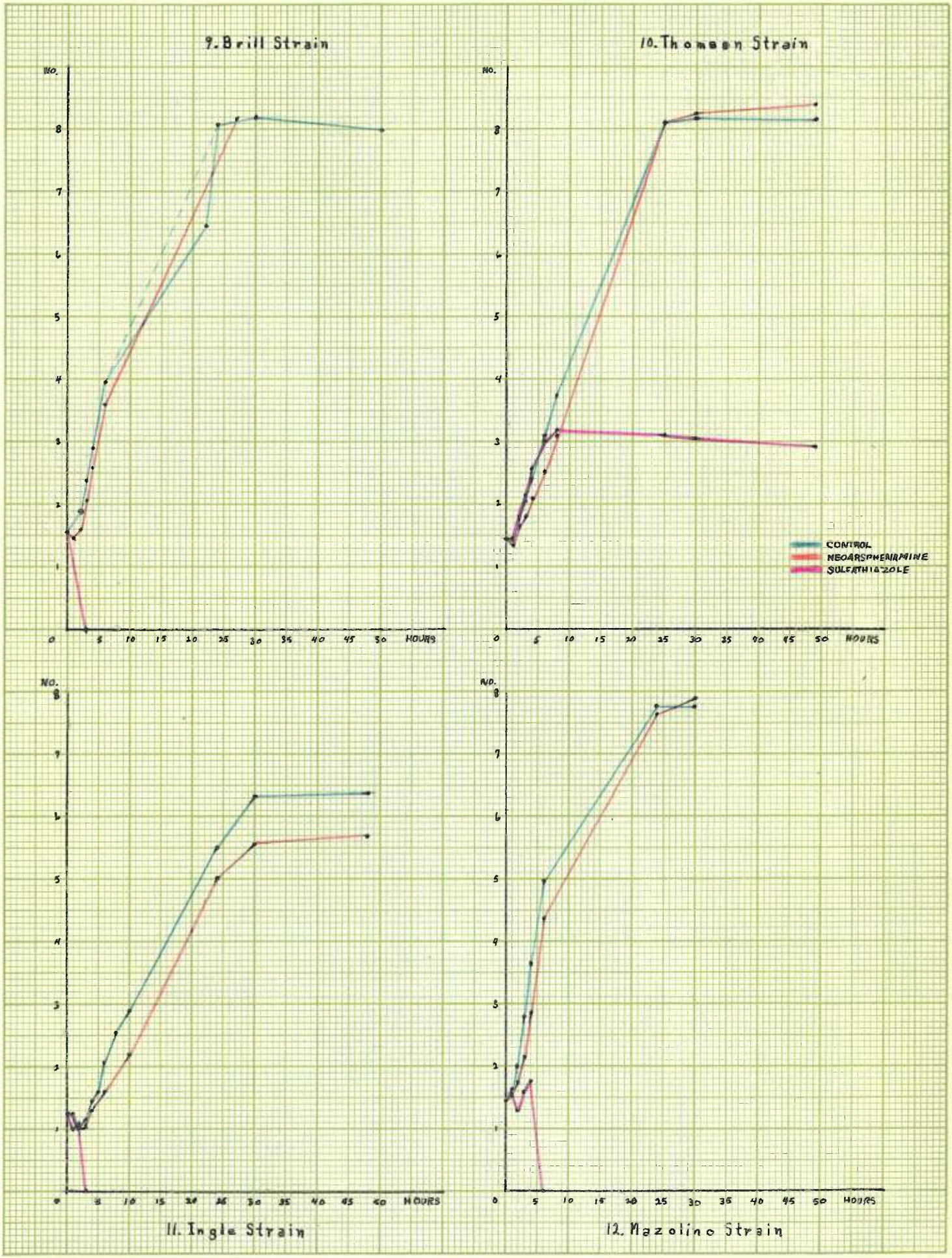
Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	53	53	53
1	70	48	0
2	125	70	0
3	634	100	0
4	2,500	160	0
6	55,000	700	0
8	700,000	5,400	0
24	91,000,000		
30	286,000,000	128,000,000	0

15. Halpert Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0		80	80
1		32	120
2		130	70
3		370	30
4		900	40
6		1,880	9
8		2,240	0?
30	/100,000,000	180,000,000	0

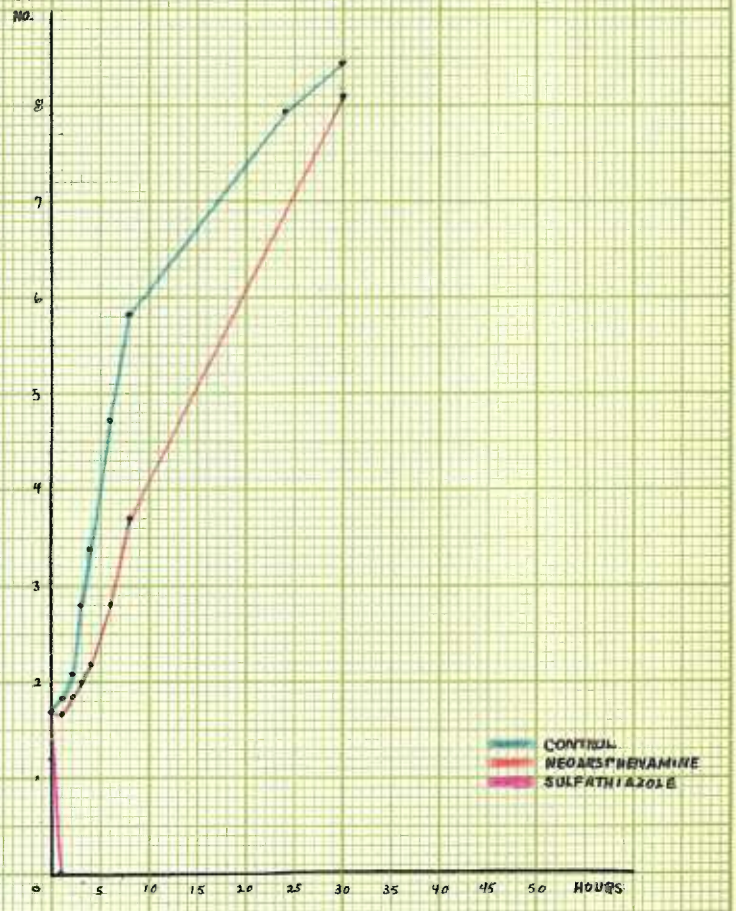

GROWTH CURVES OF STREPTOCOCCI IN PRESENCE OF DRUGS IN INFUSION BROTH
1. Fitzmaurice Strain
2. Kimberly Strain
3. Bland Strain
4. Peterson Strain
NO.
HOURS
CONTROL
NEOARSPHENAMINE
SULFATHIAZOLE


5. Hillis Strain
6. Williams Strain
7. Pierce Strain
8. Rothchild Strain
NO.
HOURS
CONTROL
NEOARSPHENAMINE
SULFATHIAZOLE


9. Brill Strain
10. Thomsen Strain
11. Ingle Strain
12. Mazolino Strain
NO.
HOURS
CONTROL
NEOARSPHENAMINE
SULFATHIAZOLE

13. Halpert Strain

14. Southwell Strain



SERIES II

Growth Curves of Streptococci over a Period of Thirty Hours.

TOTAL COUNTS OF STREPTOCOCCI OVER THIRTY HOURS

1. Brill Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	60	60	60
2	97	76	0
4		460	
6	173,000	4,600	0
8	2,000,000	56,000	0
10	30,000,000	1,700,000	0
12	185,000,000	77,500,000	0
14	332,000,000	246,000,000	0
16	342,000,000	374,000,000	0
18		394,000,000	0
20	412,000,000	285,000,000	0
22	388,000,000	360,000,000	0
24		391,000,000	0
26	386,000,000	284,000,000	0
28	425,000,000	330,000,000	0
30	350,000,000	318,000,000	0

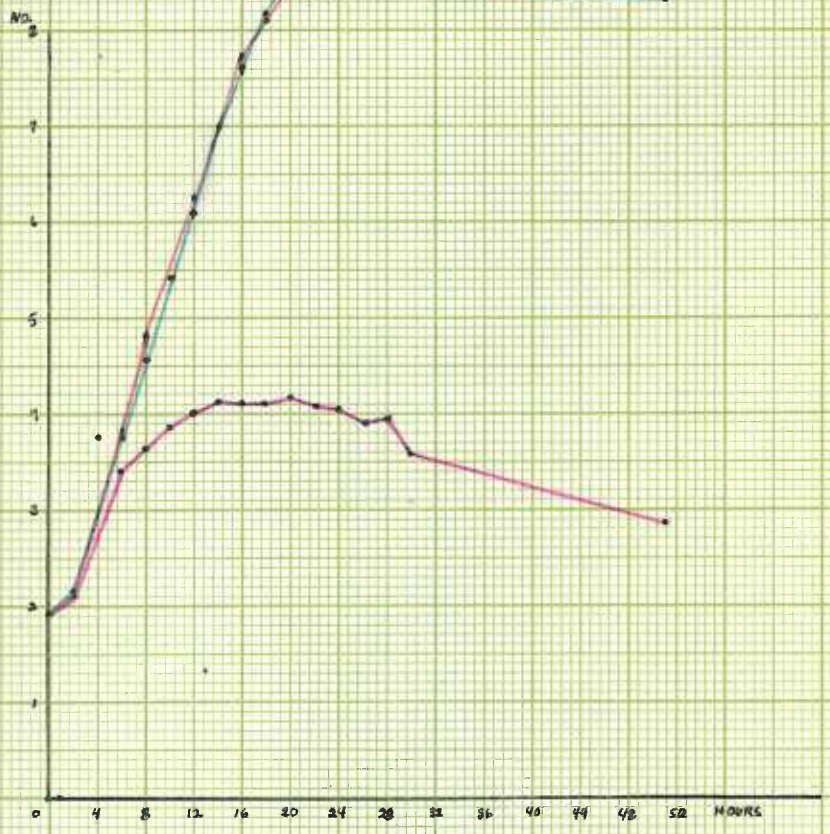
TOTAL COUNTS OF STREPTOCOCCI OVER THIRTY HOURS

2. Peterson Strain

Hours	I Control	II Neoarsphenamine 0.0056 mg./cc.	III Sulfathiazole 0.1 mg./cc.
0	86	86	86
2	150	140	126
6	5,700	6,900	2,560
8	38,000	68,000	4,600
10	275,000	265,000	7,600
12	1,900,000	1,280,000	12,000
14	12,000,000	10,000,000	15,600
16	44,000,000	58,000,000	13,000
18	157,000,000	136,500,000	13,000
20	395,000,000	329,000,000	15,000
22	400,000,000	260,000,000	12,000
24	431,000,000	282,000,000	11,100
26	401,000,000	344,000,000	8,200
28	368,000,000	325,000,000	9,100
30	344,000,000	325,000,000	3,900
51	211,000,000		770

GROWTH CURVES of STREPTOCOCCI over THIRTY HOURS

1. Brill Strain



2. Peterson

SERIES III

Growth Curves Comparing the Growth in the

Presence of Sulfathazole in

in Infusion Broth and

Hartley's Broth.

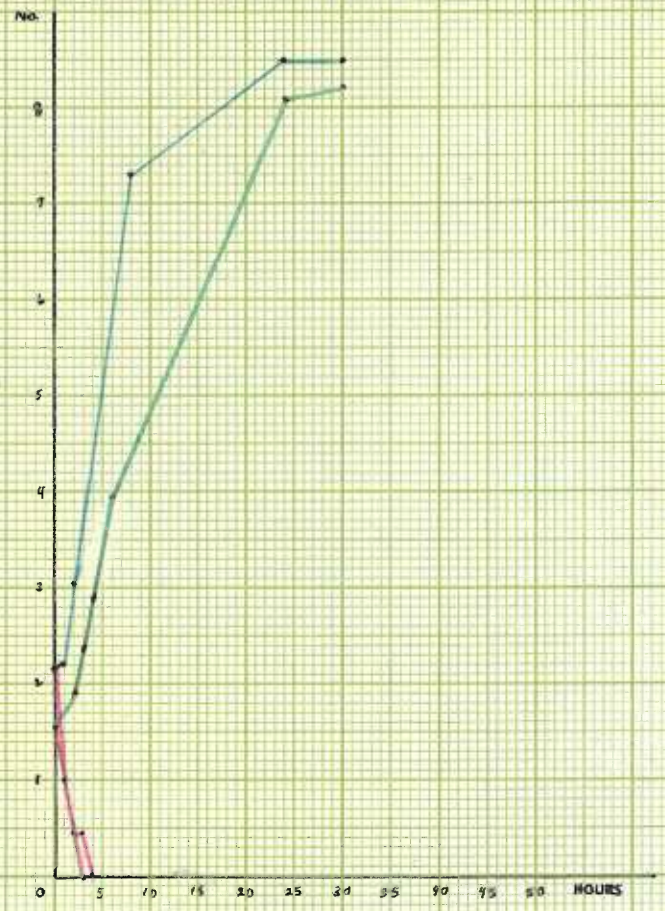
TOTAL COUNTS OF THREE STRAINS OF STREPTOCOCCI IN HARTLEY'S BROTH (CONTROL) IN PRESENCE OF SULFATHIAZOLE.

	1. Bland Strain		2. Brill Strain	
	I	II	I	II
Hours	Control	Sulfathiazole 0.1 mg./cc.	Control	Sulfathiazole 0.1 mg./cc.
0	205	205	150	150
1		0	170	10
2	350	0	1,100	3
3		0		3
4½	2,700	0		0
6	46,000	0		0?
8	700,000	0	21,000,000	0
24	332,000,000	0	325,000,000	0
30	338,000,000	0	325,000,000	0

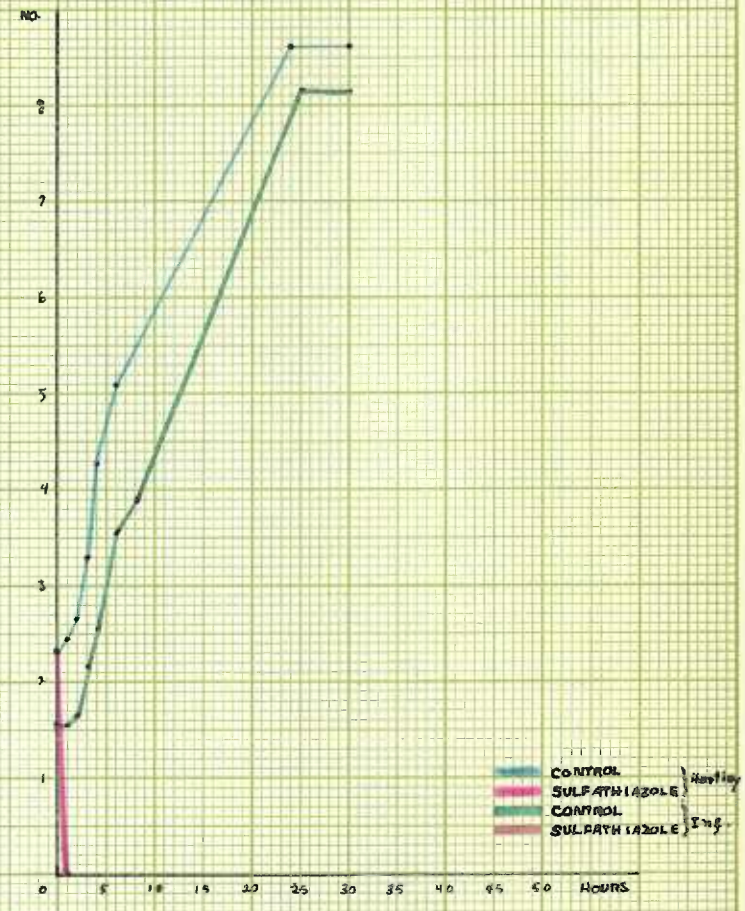
	3. Williams Strain	
	I	II
Hours	Control	Sulfathiazole 0.1 mg./cc.
0	220	220
1	290	0
2	470	0
3		0
4½	2,200	0
6	19,000	0
8	133,000	0
24	430,000,000	0
30	430,000,000	0

Comparison of Growth in Infusion Broth To-In Hartley's Broth

1. Brill Strain



2. Williams Strain



3. Bland Strain

SERIES IV

Growth Curves of Streptococci in

Various Concentrations of

Drugs.

TOTAL COUNTS ON TWO STRAINS OF STREPTOCOCCI IN PRESENCE OF VARIED CONCENTRATIONS OF NEOARSPHENAMINE.

1. Peterson Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.06 mg./cc.	Neoarsphenamine 0.12 mg./cc.
0	75	75	75
2½	333	73	60
5	2,800	150	70
7½	50,000	100	40
30	170,000,000	/100,000	0

2. Brill Strain

	I	II	III
Hours	Control	Neoarsphenamine 0.06 mg./cc.	Neoarsphenamine 0.12 mg./cc.
0	105	105	105
2½	416	75	
5	57,000	70	15
7½	500,000	6	4
30	270,000,000	0	0

TOTAL COUNTS ON ONE STRAIN IN VARIOUS CONCENTRAIONS OF SULFATHIAZOLE.

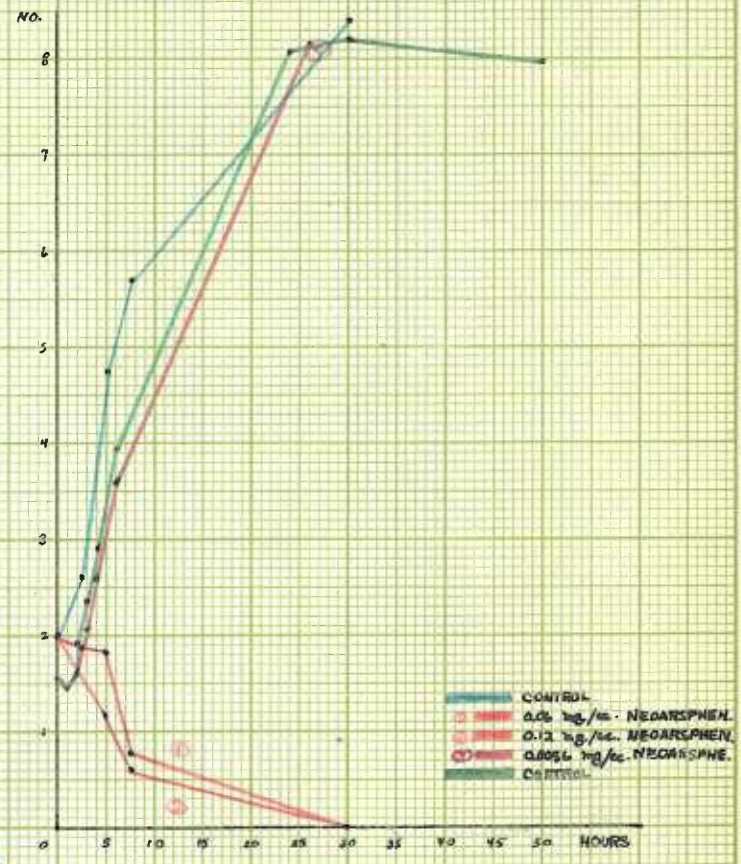
Pierce Strain

	I	II	III	IV
Hours	Control	Sulfathiazole 0.1 mg./cc.	Sulfathiazole 0.05 mg./cc.	Sulfathiazole 0.01 mg./cc.
0	78	78	78	78
3	84			
6	570	220	220	270
30	430,000,000	120	110	$\angle$10,000

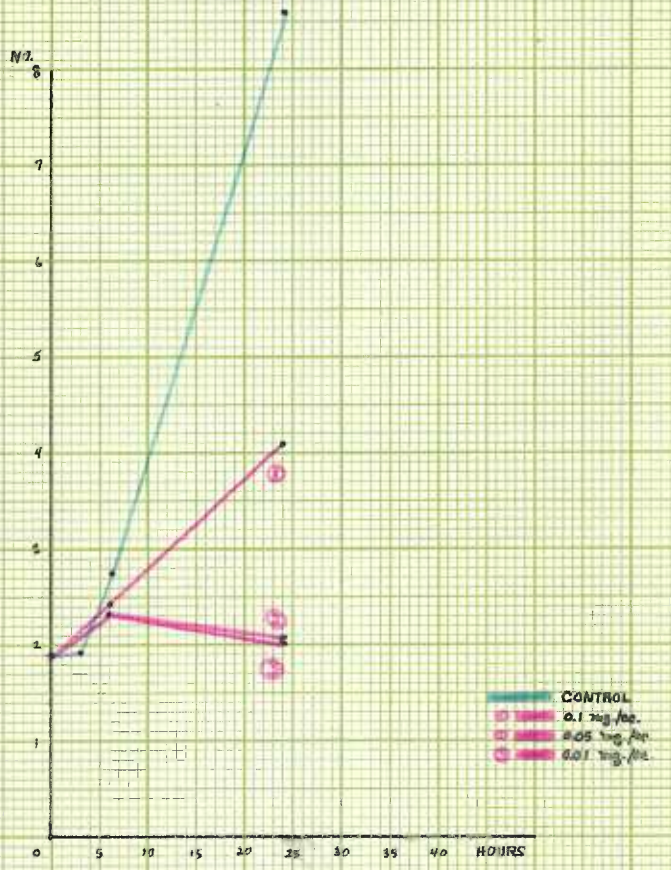
Growth Curves
Neoarsphenamine
Varied

1. Peterson Strain

2. Brill Strain



1. Pierce Strain



With Sulfathiazole Varied

SUMMARY

In this paper is reported a study of fifteen strains of streptococci isolated from the blood of the same number of cases of subacute endocarditis. Classified on the basis of those characters regarded by Sherman as of major classificatory value, these strains fell into two groups showing, with minor variations, the characteristics of Sherman's species Str. salvarius and Str. fecalis. Thirteen of the strains belonged to the former species, two to the latter.

It was found that representatives of each of Osgood's three groups based on sensitiveness to the drugs neoarsphenamine and sulfathiazole were included in each species. No single character or group of characters that we studied seemed to correlate with the drug sensitiveness.

Agglutination tests carried out with sera specific for a member of each of Osgood's groups failed also to classify the strains in the same manner.

The drug sensitivity in vitro was found by Osgood to correlate very satisfactorily with the clinical results in drug treatment. His method, involving the use of growing bone marrow cultures, is, of course, too difficult and complex to use in many laboratories. An attempt was made to determine the drug sensitiveness in simple beef infusion broth cultures. The results correlated with those of Osgood in all cases when sulfathiazole was used, but failed with these authors' results when neoarsphenamine was employed.

In these studies, a sufficient number of bacterial counts were made to construct growth curves for the organisms being investigated in the meat infusion broth and in the same medium containing the above drugs. These curves show graphically some interesting characteristics of the effects of the drugs. In the case of sulfathiazole, particularly, it was demonstrated that when the initial inoculation was small, multiplication ceased within a few hours, whereas when the initial inoculation was large, an early cessation was followed by the appearance of a revival of the culture which led to a moderate clouding of the medium in forty-eight hours.

A brief discussion of the literature pertaining to the classification of the streptococci from subacute endocarditis is included in the paper.

CONCLUSIONS

1. The streptococci isolated from the blood of fifteen cases of subacute endocarditis were classified on the basis of their physiological characteristics as Str. salvarius and Str. fecalis.

2. Neither the cultural nor the serological chracters of these strains correlated with Osgood's three groups based on the sensitiveness to the drugs neoarsphenamine and sulfathiazole.

3. In vitro studies of these two drugs in simple bacteriological media correlated with those in the bone marrow cultures of Osgood in all cases when sulfathiazole was used, but failed when neoarsphenamine was employed.

BIBLIOGRAPHY

Andrewes, F. W., and Horder, T. J., "A Study of Streptococci Pathogenic for Man," Lancet, II,(1906), 708-713;775-782; 852-855.

Blake, F. G., "The Classification of Streptococci," J. Med. Res., XXXVI, (1917), 99-133.

Clawson, B. J., "Varieties of Streptococci with Special Reference to Constancy," J. Inf. Dis., XXVI, (1920), 93-116.

Gordon, M. H., "The Differentiation of Streptococci," J. Path. and Bact., XV, (1910), 523.

Holman, W. L., "The Classification of Streptococci," J. Med. Res., XXXIV, (1916), 377-443.

Hopkins, J. G., and Lang, A., "Classification of Pathogenic Streptococci by Fermentation Reactions," J. Inf. Dis., XV, (1914), 63-84.

Horder, T. J., "Report on a Study of the Micro-organisms Associated with Rheumatic Fever and Malignant Endocarditis," Sessional Papers of the House of Lords, CXXXIV, (1908), 270-310.

Kinsella, R. A., and Swift, H. F., "A Classification of Non-Hemolytic Streptococci," J. Exp. Med., XXV, (1917), 877-896.

Krumwiede, C., Jr., and Valentine, E. L., "A Study of the Agglutination and Cultural Relationship of Members of the So-Called Streptococcus Viridans Group," J. Inf. Dis., XIX, (1916), 760-766.

Lancefield, Rebecca C., "The Immunological Relationships of Streptococcus Viridans and Certain of Its Chemical Fractions," J. Exp. Med., XLVII, (1925), 377-395.

Moran, Helen, "Classification of Streptococci from Cases of Endocarditis," Proc. Soc. Exper. Biol. and Med., XXXVIII, (1938), 805-808.

Norton, John, "Serological Relationships in Streptococcus Viridans Group," J. Inf. Dis., XXXII, (1923), 37-40.

Osgood, E. E., Unpublished data, 1940.

Osgood, E. E., "Culture of Human Marrow. Studies on the Mode of Action of Sulfanilamide," J. A. M. A., CX, (1938), 349-356.

Osgood, E. E., "Culture of Human Marrow. Studies of the Relative Effectiveness of Neoarsphenamine, Mapharsen, Sulfathiazole, Sulfanilamide, Sulfapyridine, and Sulfamethylthiazole on Infections with Streptococcus Viridans (Alpha Hemolytic Streptococcus)," Am. J. Med. Sc., CC, (1940), 596-603.

Sherman, J. M., "Streptococci," Bact. Rev., I, (1937), 3-97.

Safford, C. E., Sherman, J. M., and Hodge, H. M., "Streptococcus salvarius," J. Bact., XXX, (1937), 263-274.

APPENDIX

Discription of Procedures Employed in the Text

A. Cultural and Biochemical Tests.

Every experiment was begun with a 20-24 hour beef infusion broth culture.

1. Sugar fermentation. Sugar-free serum broth with 1 per cent of the test substance added was used as the basic medium for all the fermentation experiments. The broth consisted of 2 per cent neo-peptone in sheep serum diluted one part of serum to three parts of water. The pH was adjusted to 7.4. The sugar was made up in a 20 per cent water solution and autoclaved separately under ten pounds of pressure. The broth was later added to the sugar solution and tubed aseptically. The tubes were incubated for 24 hours before inoculating the cultures. All tests were made in duplicate, and wherever the results differed, the tests were repeated until consistant reactions were obtained. Tubes that failed to show acid were checked for growth.

2. Salt tolerance. Sodium chloride was weighed out in 2, 4, and 6.5 gram portions and 5 cc. of water was added to each, and autoclaved at ten pounds pressure. Later, 95 cc. of 1 per cent glucose infusion broth was added to the different portions of salt. After the salt was dissolved and well mixed, the resulting medium was tubed aseptically and incubated 24 hours. When the cultures were inoculated into these tubes with the different concentrations of salt, they were incubated at 37°C and daily observations over a period of four days were made.

3. Methylene blue tolerance. Ten and 100 milligram portions of methylene blue in 5 cc. of water were autoclaved at ten pounds pressure. Ninety-five cubic centimeter of sterile milk were added to each ammount, mixed well, and tubed asceptically. The tubes were incubated over night and inoculated the next day. Daily observations for growth in the form of reduction of the dye were made over the period of one week.

4. Litmus milk. For the determination of the reactions of the streptococci in milk, skimmed milk with bromo creosol purple as indicator was used.

5. Heat tolerance. Tubes with 1 per cent glucose infusion broth were warmed and inoculated with approximately 0.2 cc. of a 24 hour broth culture. These were then immersed in the water bath set at the test temperature. The tubes were constantly agitated while in the water bath inorder that the temperature would be maintained constant throughout. At the end of thirty minutes, the tubes were removed from the bath and immersed in cold water. After this rapid cooling, they were then placed in the incubator at 37°C. The final examinations for the growth of the organisms that may not have been killed by the temperature were made at the end of four days of incubation.

6. Esculin splitting. The media used in this test is the modified form of that employed by Harrison and Van der Leek (Sherman 1937). It consisted of 1.5 per cent infusion agar with 0.1 per cent of esculin and 0.05 per cent ferric chloride added. This medium was tubed and autoclaved at ten pounds. The cultures were inoculated by streaking and stabing the slants. Positive tubes showed a striking blacking of the media.

7. Starch hydrolysis. The medium was made up of 0.15 per cent starch and 1.5 per cent infusion agar. It was tubed and autoclaved. The cultures were inoculated by streaking and stabbing the slants. After 24 hours of incubation, 0.3 cc. of Lugol's iodine was poured over each slant. The negative tests gave a blue color when the iodine was added, while the positive ones showed a complete absence of this color.

8. Final hydrogen-ion concentration. The cultures were grown for four days in 1 per cent glucose infusion broth. At the end of this time, the pH of the tubes was determined with a glass electrode.

9. Growth at pH 9.6. Exactly 8 cc. of infusion broth was pipetted aseptically into tubes. The amount of normal sodium hydroxide required to give a pH of 9.6 was determined with the glass electrode, and this amount was then added to each tube. The tubes were incubated over night and the next day 0.2 cc. of a 24 hour broth culture was inoculated, and again incubated at 37°C. The tubes were observed for four days before they were finally discarded.

10. Bile tolerance. Six fresh beef gall bladders were obtained and the bile was drained into a sterile flask. The flask was heated to the boiling point and held there for a few minutes. The gall was then filtered through sterile cotton and placed in the ice box. On the three successive days, the bile was sterilized in the Arnold's sterilizer. For the medium containing 10 per cent bile, 3 per cent infusion agar was made up and to every 80 cc. of melted agar, 10 cc. of warmed bile and 10 cc. of defibrinated horse blood was added. The medium was mixed well and poured into petri dishes. When the plates were hardened, they were streaked and incubated. The streptococci that tolerated this concentration of bile gave some discoloration of the medium around the colonies.

Forty per cent bile medium was made up of 40 cc. of bile and 10 cc. of defibrinated horse blood to every 50 cc. of 4 per cent infusion agar. Plates were made of the medium and when these were hardened, the cultures were streaked.

11. Growth at 45°C and 50°C. Two water baths were adjusted to the given temperatures. Glucose infusion broth tubes were inoculated with 0.2-0.3 cc. of 24 hour broth cultures and immersed in the water baths for 48 hours.

All the tests used in the determination of the physiological reactions of the streptococci studied in this investigation are those reviewed by Sherman (1937).

B. Agglutination Tests.

The agglutination tests were made with broth cultures and immune rabbit sera according to the method of Lancefield (1925). When the broth cultures were removed from the incubator, they were well shaken to resuspend the sediment. The tubes were then allowed to stand one to two hours in order that the larger particles could settle out. The supernatant cultures were used as antigens. Consequently, most of the antigens were not deeply turbid. By using a hand lens and carefully comparing the agglutination tubes and the controls, readings could be easily made even in most of the tubes where the organisms tended to settle spontaneously on standing overnight in the ice box. On shaking, the tubes that were positive showed agglutination or precipitation not like that of the negative tubes and of the antigen controls.

The serum dilutions were made with equal quantities of broth and serum. Agglutination tests were made with 0.5 cc. of the serum

dilution and 0.5 cc. of a 24 hour broth culture. The tubes were well shaken and incubated in the water bath for two hours at 56°C. Readings were made at the end of this incubation, and the tubes were then put in the ice box for 18 hours and read again at that time. On the whole, the agglutinations in the higher dilutions did not appear until the tubes had stood over night in the ice box.

C. Procedures Used in the In Vitro Studies of Drugs on the Streptococci.

The cultures were grown in 250 cc. quantities of beef infusion broth in 500 cc. Erlenmeyer flasks. This large volume of media was used in order that the frequent removal of samples would not alter, appreciably, the total volume of the culture.

At each testing, the flasks were removed from the incubator, shook well, and 5 cc. of the culture was taken out with a pipette. The flasks were then returned to the incubator and the test portions were put into 50 cc. vaccine vials. The vials were shaken in a mechanical shaker for approximately one minute, and measured volumes from these cultures were plated. Tuberculin syringes were used for measuring the plated volumes.

Initial inoculation of cultures. A 20-24 hour infusion broth culture was used to inoculate every experiment. To obtain a small inoculum, the culture was diluted in the following manner: 0.1 cc. of the 24 hour broth culture was diluted in 50 cc. of saline. The saline vial vial was capped and shaken in a mechanical shaker; then 1.0 cc. was removed from this and diluted to 50 cc. of saline and again shaken in a shaker. From this last dilution, 1.5, 2.0, or 3.0 cc. (depending on the turbidity of the initial broth culture) was inoculated into each

flask of 250 cc. broth. The flasks were prewarmed and those designated for the in vitro study of a drug, contained the same at the time of the inoculation. On inoculating, the flasks were well shaken and 5 cc. was removed from the one containing no drug, for the initial plate counts. The flasks were incubated at 37°C. At given intervals of time, the cultures were removed from the incubator, shook well, and 5 cc. samples were pipetted for plating. The pour plates were made with infusion blood agar.

Method of plating the cultures. The initial inoculation of organisms was small enough that 1.0 or 0.5 cc. and 0.2 or 0.1 cc. amounts could be plated of the undiluted cultures in the first four hours of incubations. Two plates were made from each sample of culture. At 6 and 8 hours, the multiplication was usually so extensive as to require the dilution of the cultures before plating them. At this time, 0.1 cc. of the culture was diluted with 50 cc. of saline. Four pour plates were usually made by plating 0.2 and 0.1 cc. of the undiluted culture and 0.5 and 0.1 cc. of the dilution of 1:500. In the period between 10 and 50 hours, greater dilutions were necessary. At that time, 0.1 cc. of the culture was diluted with 50 cc. of saline, and 0.5, 0.3, or 0.2 cc. of the latter in another 50 cc. of saline. Four plates of the last dilution were made with 1.0 and 0.2 cc., 0.5 and 0.1 cc., or 0.2 and 0.1 cc., depending on the extent of the turbidity of the cultures.

The counting of colonies. Wherever it was possible, all the colonies on a plate were counted, after the plates had been incubated for 24 or 48 hours. As sulfathiazole was bacteriostatic in the plates when 1.0 or 0.5 cc. of that culture was plated (except in the cases of

the two sulfathiazole strains) no colonies could be found. Therefore, wherever the undiluted culture with sulfathiazole was plated, the colony counts are based on 0.2 and 0.1 cc. Plates with less than 20 colonies carried a greater error than those with larger numbers. The percentage error of 1 cc. tuberculin syringes was found to be between 5 and 10 per cent and sometimes as high as 20 per cent. This fact and the fact that streptococci tend to stay in chain which may or may not be broken up required at least two pour plate readings to be averaged in order that reliable counts could be obtained. In the instances where two readings were unavailable, the bacterial counts were based on the one . Plates with as many as 1000 colonies have been carefully counted and found to check with those of the higher dilutions.

The dilution of neoarsphenamine. All the experiments in this series, with the exception of one, in which neoarsphenamine was used, contained 0.0056 mg. of this drug per cc. of media. The drug was made up each time by dissolving 0.15 gm. ampule in sterile water.

The dilution of sulfathiazole. The sulfathiazole cultures contained 0.1 mg. of this drug per cc. A 4 per cent solution was made up in saline and 6.25 cc. of this was added to a flask of 250 cc. of broth. As sulfathiazole is not very soluble, the solution was first well shaken and the desired amount was removed with a ten cc. syringe having a 19 guage needle.

Typed by:

JULIA JOSKI