



A SHORT STUDY OF THE STREPTOCOCCI

by

Mary Elizabeth Guernsey

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INTRODUCTION

The study of streptococci is interesting and of great general importance, because of their widespread occurrence in the diseases of today. They are an important factor in such serious maladies as scarlet fever, septicaemia, pyemia, focal infections, puerperal fever, erysipelas, endocarditis, osteomyelitis, mastoiditis, meningitis, and others. They also cause complications as a secondary infection in pulmonary tuberculosis, septic diphtheria, measles, smallpox, pneumonia, etc. Some strains of streptococci are not specific in their production of disease. For example, *Streptococci pyogenes* can be isolated from cases of mastoid, pyorrhea, gangrene of the leg, peritonitis and septicaemia.

Besides the great invasive power and high degree of virulence of these organisms, another factor which increases their importance is the lack of production of immunity. Severe general infections usually have a fatal termination; however, in minor local infections there is a certain recovery but the infection may recur and progress to a greater extent than originally, since no great degree of immunity is produced. Anti-streptococcus serum has been of benefit in the treatment of local cases, but has met with little success in severe cases. In experiments with domestic animals where serum treatment has benefited, the serum was administered before infection or immediately after. If given several hours later, it showed no curative effect.

An attempt has been made to isolate a variety of strains of streptococci and to study them under the following headings:

1. Suitable media.

2. Classification - morphology
- biological characters
3. Serological characters.
4. Fluorescence.

Pathogenic streptococci when growing in the body have a high degree of virulence, but their growth in the laboratory on prepared media is uncertain. Great attention has to be paid to their requirements in order to keep them living and multiplying. The addition of some animal fluid, such as serum, or ascitic transudates, aid greatly in their growth and maintenance. The addition of glucose also facilitates growth but hastens

ction of acid.

best in moderately alkaline media - Ph 7.4 - 7.6.

cultivation and study of these various types, several kinds used.

. infused broth.

grs. salt.

10 grs. peptone. Sterilization - 15 lbs. for 20 mins.

BEEF INFUSION AGAR.

100 cc. infusion broth (double strength).

100 cc. distilled water.

1.7 agar. Sterilization - 15 lbs. for 20 mins.

GELATIN

100 cc. infusion broth

0.5% salt.

1.0% peptone

12 grs. gelatin. Sterilization 15 lbs. for 20 mins.

LITMUS MILK.

Skim milk.

Litmus to color.

Sterilization 100°C. for 30 mins. on

3 successive days.

HISS' SERUM WATER

100 cc. blood serum.

300 cc. water.

1% sugar.

Indicator (Brom thymol blue). Sterilization 100°C. for 30 mins. on

3 successive days.

LOEFFLER'S BLOOD SERUM.

300 cc. blood serum.

100 cc. infusion broth.

1% glucose

0.5% glycerin.

Sterilization 15 lbs. for 20 mins.

CALCIUM CARBONATE BROTH

100 cc. infusion broth.

1% glucose.

1% calcium carbonate (sterilized). Sterilization 15 lbs. for 20 mins.

CASEIN AGAR.

Beef infusions broth.

1% peptone.

1% gelatin.

0.5% casein.

0.65% glucose.

0.4% sodium acid phosphate.

0.3% sodium citrate.

0.75% agar.

Sterilization 15 lbs. for 20 mins.

BLOOD AGAR PLATES.

Fresh blood was added to melted agar in test tubes and this was poured into sterile Petri dishes, then incubated for 24 hours.

AGAR.

This medium, together with Loeffler's serum, was the material used for growth and maintenance of the cultures. The cultures were transferred about every 6 days and kept at room temperature.

The growth on agar was very satisfactory in most cases, only a few strains growing poorly on it. The growth was moderate filiform, in fine separate colonies, transparent. In some cases, the growth seemed to become slimy and the colonies to enlarge; this was noticeable when the culture was transferred from serum water media. As the cultures grew older, the growth appeared the same, but was much slower and a typical growth did not appear until nearly 48 hours.

Cultures were isolated by streaking on agar slants, as plating was found to be unsuccessful. The colonies were picked and sown in infusion broth.

The appearance of the streptococci in agar is not a criterion of their morphology. A short-chained streptococcus resembles staphylococci on agar, and even with the long-chained type it is difficult to distinguish.

The colonies on agar slants are round, small and translucent. The edges are regular, the colonies being raised slightly from the media.

SERUM SLANTS.

For stock cultures, the strains were transferred about once a week, although this was not necessary as the culture were found living after two or three weeks.

All the streptococci grew well on this medium, a moderate growth being produced with small transparent colonies along the line of inoculation.

The growth in the water of condensation at the bottom rendered it either flocculent or turbid depending on the morphology of the particular streptococci. A few cultures gave a rather scanty growth on the slant but grew vigorously in the water of condensation.

The streptococci lived longer on this medium than on the agar, probably due to the fact that there is a natural food substance contained in it - and also that the water of condensation keeps the growth moist. On agar, the colonies dried up and became leathery.

The formation of chains on the serum slant was not particularly good, but in the water of condensation, was excellent. However, great irregularities were apparent in an old culture when grown in serum water of condensation.

BROTH.

The inoculations into the serum water for fermentation tests were made from broth, thus insuring an equal quantity of inoculum in each tube.

The growth in broth was found to be more abundant in some cultures more than in others. The type of growth either produced a heavy turbidity with fine sediment at the bottom, or else granular and flocculent with little turbidity.

Von Lengelsheim made a rough classification using these facts as basis. The long-chained streptococci which produced a flocculency in broth he claimed were pathogenic while the short-chained types, which rendered the broth turbid, were saprophytic with little pathogenic power.

I have found this to be partially true but no sharp distinction can be drawn between the two as the type of growth in broth may vary from time to time.

GELATIN.

The gelatin was used to test for liquefaction. The growth was slow and on older cultures doesn't appear for several days. There was no surface growth, minute isolated colonies being formed along the line of inoculation only. No streptococci that were studied liquefied gelatin.

The gelatin has not a poisonous effect on the streptococci as cultures inoculated on January 18th showed growth when resown March 15th.

LITMUS MILK.

Acid was produced in all strains, the rapidity and degree of the acid formation varying with the different strains. Some coagulated it very quickly, others more slowly, while others did not clot the milk, but only produced an acidity which coagulated on the application of heat.

The formation of chains and morphology was not characteristic. Short chains formed diplococci, while long chains showed irregularities.

CASEIN AGAR.

This medium was recommended for use as stock cultures of streptococci.

The medium, being semi-solid was inoculated by stabbing. The growth was not confined to the line of inoculation but extended out into the medium. The growth was abundant and the chain formation excellent. In fact, the growth seemed to be too vigorous, as the cultures died even with frequent transference. The salts in the medium were evidently not in sufficient quantity to neutralize the acid formed from the glucose by the organisms.

Although the chain formation was good, irregularities in size and staining were apparent in new cultures after growing in the medium for a few days.

SERUM WATERS (HISS)

The fermentation tests were carried out with this media. Blake used only ordinary infusion broth with the addition of 2% peptone and 1% sugar claiming that the addition of serum was unnecessary. However, the addition of the serum facilitates the growth of the organism and causes the formation of good chains.

The sugars were incubated for 24 hours at 37°C. and then read - and were read each day for four days. Acid was generally produced in the first 24 hours, probably due to the addition of the serum which aided growth.

CALCIUM CARBONATE BROTH.

This broth was used for obtaining mass cultures of streptococci for agglutination purposes. The calcium carbonate present neutralizes the acid formed from the glucose and the culture continues to live and multiply. The chains are not of great length but tend to become shortened and do not form a network as they do in serum.

BLOOD AGAR PLATES.

The cultures were streaked on the surface of the agar and incubated for 24 hours. The formation of hemolysin was apparent then, but the presence of methemoglobin was not seen for 48 hours and longer.

A narrow band of hemolysis was seen around the green streak. This was misleading, but according to Mendebaum and Le Blanc this is not a true hemolysis.

The colonies on the agar were small and isolated, varying in size. The band of hemolysis was either broad or narrow.

CLASSIFICATION

Introduction

The first streptococcus was discovered by Koch in septic processes of tissues, and by Ogston in pus from acute abscesses, but it was not obtained in pure culture until Fehleisen isolated it from a case of erysipelas. Rosenbach, Krause and Passet also isolated the organism and called it *Streptococcus pyogenes*.

Morphology

Streptococci occur in chains and occasionally in pairs. The size of the individual coccus ranges from 0.5 to 1.5 micron in diameter.

Since they divide perpendicular to the axis of the chain, the single coccus often appears flattened at the contiguous surfaces. In many cases, one coccus in a chain may appear longer than its fellows. This is due to the fact that it is dividing and has not yet separated into the two resulting cocci.

The streptococci stained well with anilin dyes and were not decolorized, but retained the Gram's stain. When stained by the copper sulphate method, no capsules were seen.

In old cultures, especially in media containing serum, the chains showed irregularity in morphology. The presence of swollen cocci in a normal chain was often seen. These irregular forms were studied by Heuppe and named "arthrospores" by him.

Old cultures soon lose their power of staining regularly. With the Gram's stain, some cocci in a chain stained quite darkly, while other appeared to be distinctly Gram negative. One might have thought the cultures were mixed, if these irregularities did not occur at intervals in an otherwise

normal chain.

The arthrospores seemed to occur mostly in the hemolytic strains and not often in the non-hemolytic ones.

CLASSIFICATION.

As was stated above Von Lingelsheim endeavored to classify the streptococci according to their morphology and action in broth. He designated the short chained type as *Streptococcus brevis* and the long-chained type as *Streptococcus longus*.

It was Marmorek in 1895, who first saw the phenomenon of hemolysis, but it was Schattmuller in 1903, who applied it in the classification of streptococci.

This was an important step in the right direction and today the classifications are based on this phenomenon of hemolysis.

Schottmuller firmly believed that the hemolytic character of the organisms is unchangeable and cannot be lowered by loss of virulence. Rosenow claimed the hemolytic power is not constant and can be changed from hemolytic to non-hemolytic and vice versa.

Gordon (1903) was the first to attempt a classification based on fermentation reactions. Andrewes and Horder later published their classification very much like Gordon's but with the addition of several factors. They distinguished between *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus anginosus*, *Streptococcus salivarius* and *Streptococcus fecalis*, and *Streptococcus equinus*.

Smith and Brown in 1915 published their classification based on hemolytic and fermentative powers both. They divided the Streptococci into

two groups:

- (1) Type Alpha - viridans - producing green pigment.
- (2) Type Beta - producing hemolysis.

In Blake's classification he only recognizes two groups:

- (1) Streptococcus hemolysans - zone of hemolysis.
- (2) Streptococcus viridans - formation of methemoglobin, or
no alteration.

He then divides Streptococcus viridans into three groups, by their action on mannite and lactose.

Halman bases his classification on the action on blood agar and on three sugars - lactose, mannite and salicin. He divides both hemolytic and non-hemolytic up into eight groups.

I have classified the types of streptococci under study according to Halman's classification. It is the most definite and provides for nearly all types.

Andrewes and Horder's classification while being of importance is not definite enough as there is great variation in their fermentation reactions.

The sugars used for the fermentation tests were dextrose, lactose, mannite, salicin, sucrose and raffinose. Dextrose was not put in the following table as all the strains fermented it and so it is of no value. Sucrose was not fermented by some, but this is of no significance. As human throat strains are said to ferment raffinose and milk strains are not, this sugar was added to confirm this. All were negative to inulin.

Streptococcus infrequens.

#10 and #16 - both showed good chain formation in all except agar. There was no definite fermentation of raffinose in either case but all the sugars were fermented. The growth on agar was typical, being fine and transparent.

Streptococcus hemolyticus I.

#14 and #35 - showed chains of medium length. Arthrospores and irregularities in staining were seen. #14 on agar, after a time became white and slimy, while #35 formed large white colonies. Salicin was not fermented.

Streptococcus pyogenes.

#21 from mastoid, #25 from foot infection and #45 from scarlet fever patient all showed no fermentation in mannite. Scarlet fever streptococci should come under *Streptococcus anginosus* and be positive to raffinose. This strain seems to be a typical. #21 produced a white slimy growth on agar while #25 and #45 gave the usual growth. #46 formed short chains, even in serum water. Irregularities were seen in 24 hour cultures of #21 and #25.

Streptococcus anginosus.

This group contains the most number of strains isolated, not fermenting mannite or salicin.

Three strains from mastoid.

Two strains from throat.

One strain from wound.

One strain from spinal fluid.

The three mastoid strains all formed moderately good chains; the ones from throat cultures being shorter. Both #15 and #19 from wound and spinal fluid formed long chains and were large cocci. Halman found most of his strains in the *Streptococcus anginosus* group came from nose and throat and fermented raffinose, but only the majority of streptococci in this group do

not ferment raffinose. This seems to be contrary to the general opinion.

Irregularities in size were found in all the types in this group. The cocci in one chain ranged from 1 to 3 microns in diameter.

#15, #18, #19, #20, and #26B all produced a slimy white growth on agar. With the exception of #26B all formed chains of good length. #36 clotted milk in 2 days and #19 in 3 weeks. The rest of the cultures only clotted after heating. #18 in dextrose showed very plainly the squeezing together of the cocci which is found in hemolytic forms.

Streptococcus subacidis.

Only one strain was found in this group. #36 which was isolated from an ear. The chains were of medium length and the growth on agar typical. Very little acid was produced in milk.

NON-HEMOLYTIC

Streptococcus fecalis.

Both #26A and #32 isolated from throats have short chains, #32 being almost a diplococci. #26A produced a slimy growth on agar, but #32 would hardly grow on it, the colonies being small and scarce. Both clotted milk in two days. #32 was faster in its fermentation powers and reacted on all the sugars, while #26A did not ferment sucrose or raffinose.

Streptococcus mitis.

#23 - mastitis - the only member of this group.

Holman says that the true mastitis is probably hemolytic but the two cultures obtained from cases of mastitis showed no hemolysis. Jones found strains which fermented salicin, lactose and dextrose and others which acted the same with the exception of salicin. The agar growth was white and slimy, the streptococci were large with long chains and seemed to be elongated.

Date Rec'd	No.	Morphology Chains	Hemolysis	Lactose	Mannite	Salicin	Sucrose	Raffinose	Isolated From	Classified As:
Nov. 28	10	Long	**	*	*	*	*	*	Pleural Fluid	Streptococcus infrequens
Jan. 5	16	Long	**	*	*	*	*	-	Mastoid	Streptococcus infrequens
Jan. 5	14	Medium	**	*	*	-	*	-	Pleural Fluid	Streptococcus hemolyticus I
Mar. 12	35	Medium	***	*	*	-	*	-	Blood Culture	Streptococcus hemolyticus I
Jan. 5	21	Long	**	**	-	*	*	*	Mastoid	Streptococcus pyogenes
Feb. 12	25	Long	**	*	-	*	*	*	Foot Infection	Streptococcus pyogenes
Mar. 30	46	Short	*	*	-	**	**	-	Scarlet Fever	Streptococcus pyogenes
Jan. 5	15	Long	***	*	-	-	*	-	Wound	Streptococcus anginosus
Jan. 5	17	Medium	*	*	-	-	-	-	Mastoid	Streptococcus anginosus
Jan. 5	18	Long	*	*	-	-	*	-	Mastoid	Streptococcus anginosus
Jan. 5	19	Long	***	*	-	-	*	-	Spinal Fluid	Streptococcus anginosus
Jan. 5	20	Long	**	**	-	-	*	-	Mastoid	Streptococcus anginosus
Feb. 20	26B	Short	**	**	-	-	*	*	Throat	Streptococcus anginosus
Mar. 20	37	Medium	**	*	-	-	*	*	Throat	Streptococcus anginosus
Mar. 16	36	Short	**	-	-	-	*	*	Ear	Streptococcus subacidus
Feb. 20	26A	Short	-	*	**	*	-	-	Throat	Streptococcus fecalis
Feb. 28	32	Short	-	**	*	**	**	**	Throat	Streptococcus fecalis
Feb. 2	23	Long	-	**	-	**	**	*	Mastitis	Streptococcus mitis
Feb. 27	30	Short	-	**	-	-	**	*	Blood Culture	Streptococcus salivarius
Mar. 12	34	Long	-	**	-	-	**	-	Mastitis	Streptococcus salivarius

Hemolysis

*** - broad zone
 ** - medium zone
 * - narrow, but true

Sugars

** - very acid
 * - acid
 - - indefinite
 - - no acid

It looked very much like a mixed culture as there were irregularities. Milk was coagulated in two days forming a uniform pink clot.

Streptococcus salivarius.

#30 from throat culture showed very short chains, with the individual cocci being elongated and rod-like. The growth on agar was very scarce and only short chains were formed in the serum waters.

#43 from a case of mastitis did not ferment mannite, salicin and raffinose. The growth and morphology resembled #23 to a great extent. The colonies were large and white. Milk was clotted in 48 hours.

A non-hemolytic streptococcus was isolated from a scarlet fever throat swab but the growth was scanty and it soon died.

The streptococci forming the long chains were found mostly in the hemolytic group - while the short chained ones were found in the non-hemolytic group.

Hemolytic streptococci have high invasive powers and active virulence.

The type of infection caused by these are either local or general reactions. The non-hemolytic forms have been found to be the common cause of chronic streptococcus infections. Death results only after a prolonged course.

PATHOGENICITY.

White mice were used in the tests for pathogenicity, they being very susceptible to streptococci.

0.5 cc. of cultures #19, #30, #32 and #34 was inoculated intraperitoneally into mice but no deleterious effects were noted.

#25 was inoculated intravenously and a swelling formed at the point of inoculation and later a scab formed but the mouse did not die.

#21 was inoculated into a mouse intraperitoneally and four days later it died. The autopsy showed adhesions at the point of inoculation and pus in the peritoneal cavity. Cultures taken showed streptococci predominating.

#35 was inoculated intraperitoneally and the mouse died one day later. The autopsy showed adhesions of the peritoneum. Streptococci were found in the body fluid.

#46 was inoculated intraperitoneally and the mouse died one day later. Cultures taken from subcutaneous tissue, peritoneal cavity and heart showed the presence of streptococci in great numbers.

As all the cultures had been growing on media for some time the virulence had been lessened considerably. Of the three cultures that killed, all were hemolytic and were isolated from serious infections.

#25 and #34 were not very virulent to begin with and #19 possibly did not effect anywhere but the spinal cord so did not cause death.

SEROLOGICAL CHARACTERS

Any attempts to classify streptococci by means of the agglutination reaction meets with great difficulty. Both Reudiger and Davis found cross agglutination occurring between different strains of hemolytic streptococci, but not between hemolytic and viridans strains. Smith and Brown also found no cross agglutination between their Alpha and Beta types. As a rule the highest agglutination is between the one organism and its immune serum - although cases have been seen where the highest agglutination is with another streptococci. Eagles found in his experiments with streptococci from scarlet fever that scarlet fever formed a clear cut group by itself and that the other strains only gave agglutination in the low dilution, while the scarlet fever organism gave agglutination up to the 1:2560 dilution.

Douglas, Fleming, and Colebrook have shown that hemolytic streptococci from war wounds fall into one group and Tunncliffe in her agglutination experiment found that there was some relationship between the streptococci from erysipelas.

The agglutination tests were carried out with immune sera from scarlet fever patients.

- A. Serum from scarlet fever patient of four weeks.
- B. Serum from Scarlet fever patient of three weeks.

The serum was obtained by centrifuging and preservative with 0.1 cc. of 5% phenol in saline to every 1 cc. of serum was added. This preservative at first formed a precipitate, which soon disappeared and after filtering the serum was as clear as originally.

The antigens were made of numbers 14, 15, 16, 17, 18, 19, 20, 21, 23, 26, 34 and 46 and were prepared by growing the cultures on agar slants, then washing them off with 0.85% saline solution. They were then standardized to 0.5% turbidity and sterilized with 0.5% formalin. Microscopic tests were tried in dilutions of 1/20 and 1/80, incubating for 5 minutes at 37°C. This method was not satisfactory as the streptococci were too clumped in the control and could not be broken up. It was impossible to tell whether agglutination had occurred or not. The microscopic tests were not successful, the results being of no significance and so are not given here.

Just at present a new media on which to grow the antigens is being tried. This is the calcium carbonate broth and prevents the clumping of the organisms--which is so deceiving in the antigens made from agar washings.

IMMUNITY

As stated above the absence of any real immunity from streptococcal infection is of great importance.

Marmorek in 1895 was the first worker to attempt to produce a serum for passive immunization of human beings. He believed that the antistreptococcal serum produced by one strain would insure protection against any other type of streptococci infection and consequently made his serum with only one type of streptococci.

Aronson at first adopted Marmorek's idea, but later came to the conclusion that there were many different types of streptococci and that different strains should be used for immunizing purposes. Tavel, Demp, Menger and Maser also saw the importance of using a polyvalent serum. Maser claimed at the time that horse serum immunized against the streptococci found in a scarlet fever case showed marked curative effect when injected into a scarlet fever patient.

However, although local infections seem to benefit, to some degree by treatment with immune sera, the more severe general infections do not seem to respond. "In the treatment of acute streptococcal infection the failure of vaccine therapy is notorious, while in chronic streptococcal endocarditis response to therapeutic inoculation does not occur".

Besredka has made considerable advance by his work on specific dressings. He grows the organism responsible for the infection in broth and uses dressings soaked in the filtrate, applied to the skin as a general vaccine or directly to the affected part.

ECTOANTIGEN

Following Wright's discovery that suspensions of dead bacteria were capable of protecting against bacterial infection, vaccines have been used for this purpose almost exclusively.

A great many good antigens have been prepared but the original sus-

pension seems to be the closest to the ideal.

A good antigen should be in aqueous solution, free from protein, no toxic properties, but high in immunizing power. The various types of antigen have been -

- (1) bacterial suspensions grown on solid media.
- (2) whole broth culture of the organism.
- (3) filtrate of broth culture.
- (4) extract of bacterial cells.

Work was first done in 1921 by Ferry and continued by Ferry and Fisher, in a new type of antigen approaching the ideal. Experiments were first performed on *B. typhosus*, *B. coli* and *Pneumococcus*, all of endotoxic nature. They found that the filtrates of these organisms had higher antigenic powers than they had imagined. They found the shorter the incubation time (18 - 24 hours) the higher was the antigenic property of the filtrate and filtrates from cultures 4 - 5 days old had low power. They came to the conclusion that antigenic substance was not of extracellular type because of length of incubation. They also found that the antivenic substance was not of the toxic variety because it would be stronger after 4 - 5 days incubation.

They were of the opinion that the antigenic substance must be loosely bound up with bacterial cells and can be washed off from bacterial surfaces, or ectoplasm, rather than from endoplasm. This new type of antigen they termed "ectoantigen".

They then tested the washings from agar growth, passing them through Sharple's centrifuge. They found these washings had high antigenic properties, even more so than the broth filtrate. The washed organisms showed low in antigenic power.

A very important fact about this ectoantigen is that the antigenic substance is washed off and the toxic substances left behind.

Tests were made to show that washings were nontoxic while the washed bacteria were as toxic as ever. This is contrary to the general opinion which is that antigenic substances are related to toxins while this serves to show that they are entirely different substances.

Experiments were performed on streptococci to see if the results coincided. As streptococci seem to have a patent diffusible toxin, and yet seem also to contain an endotoxin the results would be interesting.

For these experiments, cultures of streptococci were inoculated into broth and on agar slants. After 24 hours inoculation, the agar cultures were washed with saline and immediately centrifuged. The broth culture was put through a Sharple's centrifuge.

The tests were performed on rabbits, 1 dose of 0.5 cc. subcutaneously, 2 doses of 1 cc. each and 2 doses of 2 cc. each given intravenously, three days apart. Five days later the animals were bled to death. Agglutination and complement fixation tests were performed. For agglutination purposes, the suspensions grown in broth were centrifuged and diluted with 0.85% saline and sterilized with 0.5% formalin. The technique for the complement fixation test was the same as Kolmer uses in his text book, - Infection, Immunity and Specific Therapy.

	<u>Agglutination</u>	<u>Complement Fixation.</u>
The broth culture	1-500	1-10
The broth centrifugate	1-600	1-0
The agar suspension	1-800	1-60
The agar washings	1-800	1-50

Of the broth culture, the centrifugate showed higher antibody response while the agar washings just equal the agar suspension.

Organism	Fluorescence	Hemolysis	Morphology	Isolated from:
Diphtheria	***		Rod	Throat
Pneumococcus (1)	***		Coccus	Lung
Pneumococcus (2)	***		Coccus	Lung
Streptococcus #21	**	**	Medium Chains	Mastoid
Streptococcus #26B	**	**	Short Chains	Throat
Streptococcus #36	**	**	Short Chains	Ear
Streptococcus #15	*	**	Long Chains	Wound
Streptococcus #17	*	*	Medium Chains	Mastoid
Streptococcus #18	*	*	Long Chains	Mastoid
Streptococcus #19	*	***	Long Chains	Spinal Fluid
Streptococcus #25	*	**	Long Chains	Foot Infection
Streptococcus #30	*	-	Short Chains	Blood Culture
Streptococcus #32	*	-	Very Short	Throat
Streptococcus #35	*	*	Medium Chains	Blood Culture
Streptococcus #23	* -	-	Long Chains	Mastitis
Streptococcus #34	* -	-	Long Chains	Mastitis
Streptococcus #37	* -	**	Medium Chains	Throat
Streptococcus #46	* -	*	Short Chains	Scarlet Fever
Streptococcus #26A	-	-	Short Chains	Throat
Staphylococcus aureus	-	*	Bunches	Non-virulent
Staphylococcus albus	-	*	Bunches	?

*** - very good
 ** - good
 * - fair
 * - faint
 - - no fluorescence.
 -

FLUORESCENCE.

The effect of ultra violet light on the streptococci was suggested as a means of differentiating between the various strains. It was thought that the different types would give different degrees of fluorescence or different colors. This was found to be partially true..

The cultures were grown on agar slants for 24 hours, then washed off with distilled water. The suspensions were then transferred to clean test tubes and diluted to the same degree of turbidity as far as possible. It was found later that a difference in turbidity made no difference in the degree of fluorescence; one with an almost imperceptible turbidity fluoresced more than one with a heavy one, depending on the culture entirely.

Cultures # 15, 17, 18, 19, 21, 24, 25, 26A, 26B, 30, 32, 34, 35, 36, 37, 46 were tested, also one culture of *Staphylococcus albus*, *Staphylococcus aureus*, *B. diphtheriae*, and two cultures of *Pneumococci*.

As shown by the accompanying table, the pneumococci and diphtheria cultures showed good fluorescence; all were known to be virulent. The staphylococci, which were hemolytic, showed no fluorescence, but the cultures used were not virulent.

There was no relationship between the fluorescence and the same types of streptococci; for example, #21 which fluoresced well is classified as *Streptococcus pyogenes*, while #46, also *Streptococcus pyogenes*, gave very poor fluorescence. Both were pathogenic for mice.

The types that fluoresced the greatest were all hemolytic, while three out of the five non-hemolytic ones gave very little fluorescence or none at all.

Of the four strains that showed little fluorescence two were from cases of mastitis and the other two were isolated from the throat swabs, one being

a scarlet fever throat. #26A, which did not fluoresce, was also from a throat swab. However, this is really of little significance as #26B and #32 both from throat cultures gave good fluorescence.

Suspensions were made of cultures #19, 21, 35, grown for one week on agar. These were compared with 24 hour cultures of the same organisms. No difference in fluorescence could be detected between the two. The suspensions were also left standing for 72 hours and then compared with fresh cultures. No loss in fluorescence was apparent.

When the organisms were exposed to the ultra violet light for a while, the fluorescence seemed to increase. The germicidal action of ultra violet rays is well known and whether the light killed the organisms and the toxin was liberated is yet to be proven.

24 hours suspensions of #17, 19, 46 were made. These were then divided into three parts:

- (a) Original suspension.
- (b) Put through Berkefeldt filter.
- (c) Put through asbestos filter.

Comparison of the three showed that the filtered materials (b) and (c) were fainter than the original suspension (a) and that (c) was fainter than (b). No difference was seen between (a), (b) and (c) of #46, the original suspension being weak to start with.

These experiments may go to prove that the material causing the fluorescence is liberated from the bacterial cell and is present in the filtrate.

Tests are now being performed to compare the fluorescence of the bacterial suspension, the filtrate and the washed organisms; also to see if there is any difference between the living and dead organisms.

SUMMARY AND CONCLUSION

Great difficulty has been encountered throughout the work by the failure of the streptococci to grow when desired.

In the fermentation tests, little correlation was found between the source and the type, but there was a relationship between the length of the chains and the hemolytic power.

The strains from mastoid all showed fairly long chains, while those from the throat produced short ones.

#46 should have been in the *Streptococci anginosus* group instead of in *Streptococci pyogenes*.

The failure of the agglutination tests may possibly be due to the fact that scarlet fever streptococci are specific in their agglutination reactions and the immune scarlet fever sera refused to agglutinate with the other streptococci to any extent.

Great possibilities can be seen in the experiments with the "ectoantigen" and further work may be done on this.

The study of the fluorescence of streptococci was undertaken as a new phase. Interesting results were obtained and further experiments should be performed on the relationship between toxins and fluorescence. It would seem that the toxins have a slight fluorescence as the filtrates from the Berfeldt and asbestos filter showed.

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