An Investigation into the Specific Etiology of Scarlet Fever.

being a

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by

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Summary of Contents.

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The infection of Scarlet Fever has been known for a considerable time to be associated with a specific entity or germ which was conveyed in the vapors of persons suffering from the disease, was liable to be carried by fomites, and by articles of food and drink.

Although the persistence and virulence of this infection, under varying circumstances and conditions, has from time to time been studied with great detail, a certain amount of doubt hangs round the specific Etiology of the disease.

Various forms of organism have been described, and of recent years much has been written regarding the specific characters of certain Streptococci.

Scarlet Fever probably existed

in the time of Hippocrates. The Plague of Athens is supposed to have been this disease but the observations of Hirsch have led him to believe that the first definite record of the disease was in the year 1543.

Only of recent years, however, with improved methods of investigation, has the investigation of the Specific cause of the disease ceased to be a matter for of conjecture. The nature of the early microorganisms may, now, be considered only as a matter of historical interest.

In 1872 Mac Kendrick described a micrococcus, and in 1883 Pincus, a bacillus, neither of which have been considered distinctive.

In 1883 Crook[1] found a bacillus in the fluid exuding from the septic cellulitis of the neck, present occasionally in severe septic cases, and also in the Nasal discharge. It was

(1) Lancet 1883 Vol I Page 857.

composed of long segmented leptothrix-like filaments. These undoubtedly came from some outside source.

In 1887, Edington and Jameson[1] found an organism somewhat resembling the above, in the desquamating scales of epidermis in Scarlet Fever cases after the third week, also in the blood of Scarlet Fever before the third day of disease. The method of obtaining it from the blood was by pricking the finger. The nature of this organism has been critically enquired into by Klein,[2] and its specific nature disproved

In 1870 Dr. M. Taylor[2], while observing the course of an epidemic at Penrith found, that in addition to the previously believed methods of conveyance of infection, the materies morbi of the disease, was capable of living and multiplying in articles of food, in this instance milk, which, since that date, has so frequently been shown to be a breeding

(1) Brit. Med. Journal June 11, 1887

(2) Stevenson & Murphy. Hygiene & Pub. Health Vol II.

source of dissemination of the disease.

In 1881 (1), Ernest Hart, found that there were on record up to that date fifteen epidemics, which had been thoroughly investigated, and where the source of infection had been traced to milk. Till this date the specific cause which was carried by, and multiplied in milk, was believed in every instance to have been derived from a human source.

In lieu of subsequent investigations however, it appears highly probable, that infection may come originally, from other than a human source.

Investigations in connection with the historic Epidemic in London in 1881 showed that the cause of infection was conveyed by milk ~~and~~ when all contamination from a human source was excluded.

It was found that a cow used for milking purposes in a Surrey Farm, and from which a portion of the milk supplied was obtained, suffered from

(1) Sir Wm. Murphy Hyg. & Pub. Health Vol II & BMJ. 1881 Vol I.

a peculiar disease which in some respects resembled Scarlet Fever. The relation of milk infection and disease in the cow remained doubtful till a striking example was given by the Hendon Cow Epidemic in North London in 1885.(1).

The disease in every case was traced to the milk supplied by a certain farm, where the cows were found to be suffering from a disease of the udder & skin associated with certain visceral conditions.

Will. The investigation of this epidemic are associated the earliest observations on the streptococcus which is believed to be so closely related to the disease.

The milk from the farm at Hendon which caused the Scarlet Fever Epidemic by its infectiveness, was found to be distributed in certain sections, and the ability to convey infection had relation to particular cows. The more scientific truth in this epidemic was given to Dr Klein,(2) who carried out the investigations for the Local Govt. Board.

(1) Outbreaks of Scarlet Fever Hanley & Hendon. Report of Med. Officer L.G.B. 1886. appendix & ditto.
Klein. Reports Milk Scarlet Fever 1885-86 1886-87
Report Med. Officer L.G.Board 1897-98
(2) Infectious Diseases of Man & Lower Animals. Trans Epidem. Soc. Vol. N.S.

The cows referred to, suffered from ulcers on the teats and udders, with sores, scanty hairless and loss of hair, associated with certain conditions of the internal organs.

Inoculation of calves with the lymph from some of these ulcers produced the identical disease. A micrococcus was obtained from the lymph, which was found to have distinctive modes of growth under cultivation. Inoculation of calves with a pure growth of this coccus produced in them a disease similar to the Hendon Cow Disease and Scarlet Fever.

A micrococcus was found in the blood and circulation of persons suffering from Scarlet Fever, identical in cultural & microscopic characters with that obtained from the Hendon cows (1)

Having ascertained the identity of the micrococcus in morphological, and cultural respects strains of each were killed on animals and the results compared. Mice inoculated were found to become affected in the same manner

Death took place in mice on the 7th day and a micrococcus obtained from the

(1) Etiology of Scarlet Fever Proceedings of the Royal Society Vol 42

blood and heart-, has the same characters as that recovered from the Hudson cows, and from cases of Scarlet Fever.

Lastly the micrococcus recovered from the blood of these mice was inoculated into calves, and a disease was produced, the same as that produced by inoculation of the cocci from the Hudson cows; and from the heart and blood of these calves the same micrococcus was recovered by cultivation.

The relationship of Klein's micrococcus (which he called the Streptococc. Scarlatinae) to Scarlet Fever, and to the peculiar disease found in cows led him to believe it to be the Specific cause of scarlet Fever.

Serious Objections to these views have been taken (1)

It has been urged that the Organism recovered by Klein, from these sources was nothing more or less than the Streptococcus pyogenes.

Cultures in various media and inoculation

(1) see. Trans. of Epidemiological Society Vol ii New Series.

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in animals, Klein urged, showed destruction.

The "Streptococcus pyogenes" produces in mice and rabbits local suppurations, which may lead to metastatic abscesses in the viscera; the Streptococcus of Scarlet-fever produces in a small percentage of rabbits and a large percentage of mice in the course of one or two weeks, a distinct visceral disease leading to death.

It has further been observed, that Hendon Cow disease, & that disease, produced in calves with the cocci from Scarlet-fever are neither of them the same disease as human Scarlet-fever.

It has never been proved that this organism recovered from Hendon Cow disease or from the human subject in cases of Scarlet-fever, can after being passed through several animals reproduce the disease in man, or, that this organism recovered from man in cases of Scarlet-fever, is the cause of the Symptoms of Scarlet-fever. That it is frequently if not invariably found in Scarlet-

Fever is well known but there the matter rests.

The relation of this Streptococcus to Scarlet Fever has subsequently been more thoroughly studied.

The fact that there is in a large proportion of Scarlet Fever cases a distinctly septic element, that septic processes are so frequently associated with streptococcus pyogenes, and that streptococcus is an organism which resembles closely the so-called Streptococcus of Scarlet Fever has rendered the investigation a more than ordinarily complex one. Thus we find Streptococcus pyogenes in ulcerated throat, suppurative arthritis, abscesses, & other processes which are so frequently associated with the more severe type of Scarlet Fever.

The characters by which the Streptococcus of Klein were differentiated are cultural and pathogenic —

In broth the fluid remained clear, while the growth formed a coherent conglomerated mass at bottom of tube. Milk was clotted in 4 days, agar colonies studded to develop nodules. Intravenous into mice these die in a week when, in rabbits only redness at site of inoculation is produced & death does not result. In all media the coherency of the growth separates it was marked & distinguished this from other forms of Streptococci.

Most observers agree that Streptococcus Pyogenes plays a very important part in Scarlet Fever

(1) Babes considers that the whole infection in Scarlet Fever is caused by a modified Streptococcus infection

Raskin found Streptococcus pyogenes in severe cases but cried not demonstrate it in a single mild case. Hence he considered that Strept. Pyogenes was only connected with the secondary infections

(2) Lenhartz found the organism in here

Ref (1) Bacter. Untersuch. über Septische Prozesse des Kindesalters Leipzig 1889

(2) Beitrag zur Kenntnis der Secund infection bei Scharlach. 1888

Culture in the blood in case of Scarlet Fever associated with Suppurative affections of throat.

(1) Blaxall in 1894 investigated the bacteriology of Diphtheria occurring in Scarlet Fever and found that (1) the organism most frequently in the cause of this complication was the Streptococcus pyogenes. (2) That bacilli present are due to a secondary infection. (3) next in frequency are the Staphylococci. (4) and that the diplococcus of Pneumonia does not play such an important in this as in Diphtheria due to other causes, than Scarlet Fever.

In (2) 1899, Class of Chicago described an organism which he believed to be the cause of the disease. It was a Coccus which exhibited peculiar adhesive properties due to an intercellular substance. The cells "Cocci form short chains + diplococci and, have been recovered from the blood in Scarlet Fever. His pleomorphic in character, + he maintains that it is the same organism as that described by (3) Baginsky and Sommerfeld in 1900.

(1) Bacteriol. Investigation of Suppt. Ear Discharge in Scarlet-fever. Brit. med. Journ. 21/7/94.

(2) Class. Chicago med. Recorder May 1899 + Lancet Sept 29, 1900

(3) Baginsky & Sommerfeld Berlin klin woch 29/7/1900

The latter investigated the pharynx and mucus in cases of Scarlet Fever. A Streptococcus was isolated, which exhibited great length of chains, retained Gram's stain. It answered the general description of Streptococci in general, but did not seem to have any distinctive characteristics

They Contended that the presence of this organism in the cadaver in rapidly fatal forms shows the close relation between it and the actual cause of the disease.

It was uniformly found present in all cases of the disease, and they argued that all the clinical manifestations of the disease could be produced by invasion of this organism.

(1) In 1891 Kurth made a valuable contribution regarding the Streptococcus present in cases of Scarlet Fever and which he named the Strept. Conglomeratus. He recognized the exceptional conglomerating powers of the Streptococcus in broth. From the general description it appears to be identical with the Streptococcus described by Klein.

(1) Arbeiten aus dem Kaiserlichen gesundheit-samte Band VII 1891

The most recent observations on the Streptococcus believed to be the specific cause of Scarlet Fever have been made by W. Mervyn H. Gordon of St. Bartholomew's Hospital, a large part of his original material having been obtained from the Isolation Hospitals [Brook Hospital, Met. Asylum Board] of the Board.

He has conducted a most elaborate investigation into the morphology & pathogenicity of the Organism [Klein's] obtained from the throat & nasal organs during the disease, from various complications and also from the cadaver. It was invariably found by him in the tonsillar secretion of early cases of scarlet-fever. It exhibited a remarkable pleomorphism in its individual cocci. This under the microscope at once differentiated it from Streptococcus pyogenes, but this feature was found to vary considerably in

(1) Gordon. Reports of Med. Officer Loc. Govt. Board 1898-99 1899-00 1900-01.

and B.M.J. May 16, 1902.

different culture media, and was more marked on solid media than in liquid ones. This "bacillus formation" was more often masked by passing the organism thro' mice. While the virulence was increased. In some instance it was found that the "bacillus formation" was increased. From the Scarlet. fever cadaver a streptococcus in pure culture was obtained which only in a limited number of cases could be distributed with the Scarlatinal streptococcus (Streptococcus conglomeratus) in its usual typical form, and was indistinguishable from Streptococc. Pyogenes. By lengthened culture on different media however, the organism assumed some of the typical characters of the Streptococc. conglomeratus. These organisms then showed more clearly differentiating characters from the Streptococcus pyogenes than did the unmodified Streptococcus conglomeratus modified by passage through a mouse.

Thus the organism recovered from Scarlet Fever after death, resembled that

recovered after causing death in a mouse.

The organism as it increased in virulence seemed to approximate to the Streptococcus hyogenes.

Investigation of the tissues of the cadaver showed that the invasion of organisms was through the buccal of the tonsils, whence it spread by the lymph glands to the blood.

As a contrast to the organism recovered from the tonsil during life, that on the surface of the tonsil after death exhibited the loss of cultural peculiarity (bacillus formation) which was associated with increased virulence.

In the later reports of London, a more thorough investigation of the characters of S. conglomerata has been made, and a quantitative determination of the organisms on the surface of the tonsil.

A diphtheroid organism was found on the surface of the tonsil associated with streptococcus hyogenes, the latter was less frequently present in milder cases of the disease.

The Streptococcus conglomeratus exhibited the following distinctive characters:-

(1) Conglomeration in broth and marked coherency of the chains under the microscope

(2) "Lace work" in agar condensation fluid the coherency of chains, in spreading out the growth give an appearance of lace pattern

(3) "bacillus formation", the individual cocci tending towards the forms of rods, spindles & cones.

(4) Litmus Milk clotted firmly in 3 days and a marked acid reaction given.

The organism may be fixed in its characters or alter on passage thro' mice, or on cultivation.

The Streptococcus obtained from the cadaver Dr. Gordon has subsequently been unable to distinguish with the above, and believes that it is an organism which plays a subsidiary part (Streptococcus progenes) altho' ultimately causing death.

The Question to settle is whether the Streptococcus progenes, and the S. conglomeratus

both present. In the Surface of the Tonsil are one and the same organism, or whether the S. pyogenes plays a varying role: in mild cases absent, while in fatal cases predominating and causing death by septicaemia.

This view seems to me to be the most rational one and explains the clinical manifestations of the disease.

The conglomerates Streptococcus would appear to be the active cause (Specific) in the first instance, giving rise to the classical Symptoms, and by its local effects allowing the invasion into the System of the Streptococcus pyogenes. In severe or septic cases the latter assumes a more and more important rôle as the disease goes on, and would appear to become inimical to the life of the conglomerates Streptococcus, so that in fatal cases the latter is absent.

In my own cases of ten fatal cases I was fortunate able to find the S. conglomeratus

The Streptococcus conglomeratus was found in mice to produce, in addition to the local conditions at the seat of inoculation, by its toxine a general disease, while the organism itself was restricted to the site of inoculation.

In the toxic variety of scarlet fever which is so extremely fatal, the septic symptoms are absent, while the affection of the throat may be comparatively slight. Death would appear to be caused by the virulence of the toxic infection of the system.

It is an interesting fact that the Streptococcus conglomeratus has not been found in the blood of these cases, the severe & fatal symptoms being caused by dissemination thro' the system of a highly virulent toxine produced at the seat of invasion, the throat, the organism itself being restricted to this site.

In the septic type of the disease the clinical picture generally changes

a few days after the onset of the disease
the temperature chart, the facies &
symptoms become those of a severe
septic invasion of the system
Abscess, Septic arthritis & cellulitis
& phlebitis are all accounted for by
the presence of Streptococcus hyogenes.
On the other hand the complications
caused by the specific virus of the
disease seemed often to be
late adenitis, and acute Nephritis
which appear to bear no relation
to the severity of the original "septic
feature" of the disease.
The infection of Scarlet Fever d.
may be urged remains for a considerable
time in septic cases. This may
be due to the specific germ which
may still remain all ached to clothes, &c
while the active agent in the
blood & tissues is the Streptococcus
hyogenes.

From the unquestionable experiments of Stickler (1) it seems certain that in the early or eruptive stage of the disease the mucous of the fauces contains the contagium of the disease

During the last year, having a large amount of clinical material at my disposal I have tried to find how far I could demonstrate for my own instruction, the conclusions of Roux.

Interest in the matter was increased, from a desire to find a bacteriological clinical test as in the case of Diphtheria. In a large number of cases, a positive diagnosis of Scarlet Fever cannot be made & in many instances where a positive diagnosis is made with great care by an expert. In the disease, too frequently the onset of the classical symptoms a few days later

Ref:-
(1) Stickler New York Med. Record
Sept 9, 1899 p. 363.

shows the conclusion to be fallacious.

In twenty cases of mild Scarlet Fever, the majority in the early stage of the disease, I have made a bacteriological examination of the tonsillar secretion for the Streptococcus Conglomerate, at the same time being devoted to the presence of the Streptococcus progress ~~of the disease~~

Further I have made a control examination of twenty normal throats not exposed to the infection of Scarlet Fever

Finally I have obtained from D. Sype of Lyon's Institut Bacteriological Laboratory two strains of Streptococc Pyogenes from an entirely different source which I have examined with a view to comparison.

I regret that owing to the illness of Dr. Sype I have been unable to carry out with the strains I have isolated pathogenic experiments on mice at the date of writing this preliminary Note

Technique of collection of material and description of preliminary and subsequent cultivations :—

In every instance in the examination of Scarlatinal throats the muco-purulent secretion on the surface of the inflamed tissue was selected for bacteriological examination.

A measured platinum hook was used for this purpose. A drop of water picked up in the hook was found (on being drawn into the capillary thread of a Hawksley's Pipette) to measure .0025 Cubic centimetre.

$.0025\ cc = \frac{1}{400}$ Cubic centimetre

In London's investigations a hook containing $\frac{1}{500}$ cubic centimetre was used; two loopfuls in each instance being obtained, by being distributed in 2 cubic centimetres of sterile normal salt solution. To Under my

observations somewhat parallel, for purposes of comparison, I took 4 loopfuls of tonsillar secretion and diluted it in 5 cubic centimetres of sterile normal salt solution.

Therefore in 1 cubic centimetre of my diluted tonsillar secretion, as in his, there was contained $\frac{1}{500}$ cubic centimetre of original tonsillar secretion

Two loopfuls of this dilution (my loop measuring $\frac{1}{400}$ cc) represent $\frac{1}{200}$ of $\frac{1}{500}$ cc

or $\frac{1}{100,000}$ c.c. of tonsillar secretion

1 loopful = $\frac{1}{200,000}$ cc tonsillar secretion

4 loopfuls = $\frac{1}{50,000}$ cc of tonsillar secretion

and so on. Hence in each

case examined a rough approximation to the number of organisms present can be made.

If a culture be made in a medium with two loopfuls of this dilutions, if any colonies develop, organisms must at least have been present to the extent of one hundred thousand per cubic centimetre.

In the first instance in the cases examined, two loopfuls of dilution were used, and spread over the surface of a blood-serum slope-cultivation tube, and also agar slope. These were incubated at 37°C for 48 hours, and then the colonies examined. If a too proliferous growth of colonies was obtained, a

lesser quantity of dilution was used on the same media, and vice versa.

In the majority of instances colonies of streptococci, staphylococci, etc grew well on both media, in a few cases growths of streptococci were got on one or other media only.

The media used were prepared at Guy's Hospital Bacteriological Laboratory, and cultures made under the supervision of Dr. Eyre, Lecturer on Bacteriology at Guy's Hospital.

Before removal of the secretion, one or two mouthfuls of water were swallowed by patient to remove any loose particles of food, and any extraneous matter. In no instance had gargles or antiseptic solutions been used prior to the removal of secretion.

When the tubes were incubated for 48 hours, various colonies which

resembled Staphylococci growths were Subcultured on Blood-Serum slopes, and on agar & incubated at 37°C for 48 hours, and growths which resembled the Staphylococcus conglomeratus in cultural and microscopical features were eventually selected in each instance

In all the cases selected the above methods have been followed, and subsequently a more thorough examination as to their macroscopic & microscopic appearances, by growth on the following media:—

a Blood Serum 1 day at 37°C
b Blood Serum condensation fluid
c Agar slopes
d Agar condensation fluid
e. Gelatine slopes and plate Cultures
f. Impression preparations from gelatine colonies.
g. Litmus Milk.
h Broth

A culture examination of the throats in each media was made, & staining by the following methods.

(1) Hanging Drop Preparations (mustained)
(2) Löfflers Methylene Blue.
(3) Grams Method.
(4) Dilute Carbol-Fuchsine.

Each strain was examined as regards capsular staining, by Richard Muirs Method.

In the series of Normal throats examined as regards the presence of streptococci in the tonsil, in many instances it was found impossible to obtain sufficient mucus to fill the measured loop; Cultures were therefore made with a straight needle from the tonsillar crypts or the surface of the tonsil directly

on to the surface of blood serum and agar slopes and spread thoroughly all over the surface. The subsequent examination was the same as in the case of the Scarlatinal throats.

Series of 20 cases Scarlatinal Throats examined

Case 1 [Lab. 2]

J.R. male age 18

5th day of disease

well marked typical case – rash fading on chest, present on abdomen and limbs. Temp. 92°F — 102°F. Tongue coated – papillae showing through. Tonsils swollen and covered with slimy muco-purulent secretion.

Four loopfuls of secretion were taken from the surface of the tonsil and diluted in 5 cc of Normal Sterile Salt. Sol^{n}.

Two loops of dilution were spread on the surface of blood serum slope

tube, and similar quantity on agar slope. Both tubes were incubated at 37°C for 48 hours.

After this period there were found on the surface of blood serum from 40-50 colonies which were of three varieties

(1) Yellowish white large colonies with colour deeper in centre than at edge, circular and tending to fuse with other colonies in places.

These on being subcultured and examined were found to be composed of staphylococcus pyogenes aureus.

(2) Smaller and more numerous colonies which were of two kinds

(a) discrete, very slightly elevated greyish semitransparent colonies,

(b) discrete colonies, about the same size as last, but more raised and with well defined edges.

Several sub-cultures were made

from "2.a" and "2.b" both of which were colonies of streptococci, and those of (b) were identified by the following characters as being those most nearly resembling the descriptions of the 'specific' streptococcus.

(1) The presence of bacillary forms, in many of the chains, which were undoubtedly well marked.

(2) The conglomerative or "mummular" tendency in broth, which remained clear. This character was further observed in hanging drop preparations

The colonies of "2a" examined were composed of chains, and a thorough search of colonies on blood serum failed to reveal the bacillary formation.

A characteristic growth of "2b." was then obtained in the following media and more thoroughly

observed as regards its mode of growth and staining reactions.

I Liquid Media

(a) Broth 48 hours :- The fluid is clear: yellowish while granular deposit at bottom which preserve their coherency on gently shaking the fluid, (somewhat resembling mucous sputum in this respect). This mass formation is very marked, each granule or mass rising in the fluid when shaken in toto, and then sinking down to bottom of tube again, to form a continuous layer of sediment covering bottom of tube; one or two detached masses adhere to sides of tube but the ~~great mass of the~~ fluid is clear.

Specimens were made for microscopic exam.n, films & hanging drop preparations - in the latter some maceration was required before the individual elements could be satisfactorily examined. The masses were formed of short chains of cocci 2 - 7 cocci in each, but no bacillary formation could be made out.

and no further distinctive characters could be made out in stained specimens.

(b) Blood-serum [24 hours] Condensation fluid. Slightly turbid. On microscopic examination numerous long chain (10 - 30 cocci sometimes) with here and there a chain with bacillary forms fairly well marked. This character was observed in a film stained by Gram's Method. The cocci retained the stain very strongly, and bacillary forms of long & short length could be seen in almost every chain examined carefully.

(c) On agar Condensation fluid. The chains are "arranged like pearls" but not so long, are more curled, & show bacillary forms 'clubs' These appear to be less numerous.

2 Solid Media :-

(a) Blood Serum Colonies [24 hours at 37°C]

very copious growth of well marked colonies, which are well defined, slightly raised, circular, and of a greyish colour - Microscopically

(1) This was not observed in fresh specimens, but in carefully prepared films (stained)

These colonies are found to be composed of medium sized chains (8 - 15 cocci), which show slight sinuosity, and irregularity in the size of the cocci. Many of the cocci are oval or wedge-shaped with occasional forms which by themselves would be described as bacilli. There is no agglomeration tendency, in preparations, made from these colonies. There are some wedge-shaped and lozenge shaped cocci

There are numerous single-cocci and occasional pairs of cocci

The growth as a whole could not be mistaken (microscopically) for a growth of bacilli, for while there are numerous bacillary forms, & forms approximating to that type, the coccus forms are in great preponderance.

(b) <u>Agar</u>. :- The colonies are smaller, less numerous, greyish and more transparent than on blood-serum. They are circular or oval, and are less defined than on blood-serum.

microscopically, bacillary forms are numerous, and on the whole the preparations resemble those from blood-serum.

(c) Gelatine (1 week at 20°C)

The growth on this medium is very slow. In about 1 week's time the colonies which are discrete can be examined with the help of a magnifying glass.

(1) Streak preparation – Minute semitransparent colonies. which do not liquefy the medium.

(2) Stab culture – discrete colonies microscopically the chains are found to be very short (two three forms) and single cocci preponderate. Spindle & bacillary forms are numerous

(3) Gelatine Impression preparations were made from plates. The bacillary forms are so numerous as to lead one to believe that the bacillary formations must be more marked

on the surface of the actively growing colonies. The impression in this instance was made from a plate growth on the 10th day. (incub. at 20°.C.)

(d) Litmus Milk. In 48 hours coagulation of the milk was well marked, and an acid reaction was given.

Case 2. [Lab Q]

A.N. female. age 12.

3rd day of disease

Rash very typical. A moderately severe attack. Tonsils congested with some white smeary deposit. Tongue coated. Temp. range from 100 – 102°F. Submaxillary glands palpable & tender.

Four loopfuls of secretion from throat diluted in 5 cc normal saline salt solution. Two loopfuls of dilution spread on blood-serum cultural slope, and one loopful on agar.

Both tubes were incubated at 37°C for 48 hours. On blood-serum a very numerous growth of colonies was obtained on agar less numerous growth.
From the latter several colonies were examined which were in every instance streptococci. In this instance the colonies much resembled one another in macroscopic appearances, but on careful microscopic examination, the certain colonies were found to be composed of a smaller streptococcus than in the others with more massing of the chains together, but no bacillary forms could be found in stained preparations, from the original cultures.

Subcultures were made on the various media as in Case I, the results of which led me to believe that this streptococcus had specific

characteristics sufficient to distinguish it from the other forms of staphylococci.

In broth after incubation for 48 hours the growth formed a sediment, and the conglomerative character was more marked than in "case I." The supernatant fluid was clear. On microscopic examination the conglomerations on first examination somewhat resembled masses of staphylococci, but under the higher powers of the microscope, the growth was found to be composed of medium length chains of streptococci with an occasional oval coccus in each chain. This feature was not however so marked as in Case I.

In serum and sweetbread fluid the chains were longer, and bacillary forms more numerous, in agar and sweetbread fluid "lace work" was well marked.

On blood-serum the colonies were

Small but well defined, on gelatine the growth was very slow (14 days) On gelatine no bacillary forms were found, but on blood-serum and agar these altho' not numerous, were distinctive.

Urinus muck was clotted and an acid reaction was given.

Case 3. (Lab M.)

G.M. female age 5.

3rd day of disease

Very mild case of the disease. Rash fairly well marked, and throat, & tongue characteristic. Maximum temperature was 100.2°C on 2nd day, i.e. day of admission to hospital - Normal temp. on 3rd day. Throat shows general injection of fauces, but tonsils are not swollen.

Four loopfuls were originally diluted in 5 cc normal Saal. Solution and subsequently 4 loopfuls in 2½ c.c.

of salt solution. In the first instance there was no growth, but on the second dilution being used, two loopfuls being spread over blood-serum slope, eight to twelve colonies were obtained after 48 hours incubation at 37°C. Several of these were examined, and in every instance a streptococcus was found with well marked bacillary forms, in varying degree.

A greater quantity of dilution (6 loops) was spread over blood-serum and incubated for 48 hours. In this instance also all the colonies were of one type, well marked, slightly raised, more numerous [40-50] and composed of a streptococcus manifesting characteristics which led me to consider it as the Specific streptococcus.

It did not clot Litmus Milk, and the reaction was only faintly acid the coagulative faculty was less

marked than in Case I or Case II, and there was no "Case work" in agar condensation fluid. The bacillary forms were however well marked in the various media, and the chains appeared larger, and the growth more proliferous in the solid & liquid media, than in the cases already described.

Case 4 [Lab P] Relapse Case

V. B. female, age 12 years

2nd day of disease. Moderately severe attack of Scarlet Fever. This case was supposed to be a relapse on the 14th day of disease. Patient was convalescent, desquamation had not commenced when on the day stated (14th) symptoms of vomiting & pyrexia supervened followed about 30 hours later by well marked rash. Tonsils were much enlarged & congested, with copious secretion of muco-pus. Tongue was thickly coated, & submaxillary glands became much enlarged and tender

Temperature ranged from 102.5 to 103.8°F and pyrexia lasted for 5 days.
Two loopfuls of tonsillar secretion were diluted in 5 cc. Normal Salt Solution, two loopfuls of dilution being spread over Blood Serum Tube and Agar Slope.
A very copious growth was obtained on both media on removal from incubator at 37° 48 hours later. The majority of the colonies were found to be composed of Streptococcus pyogenes, several of Staphylococcus pyogenes aureus & albus and a considerable number of Streptococcus viridans. Identified as the Streptococcus of Scarlet Fever
The latter showed bacillary forms, which were very well marked in gelatine impression preparations, Conglomeration was well marked, "lace work" arrangement of chains in agar consistency fluids, and clotting of Litmus milk
In this instance little difficulty was experienced in identifying the Streptococcus

Case 5 [Lab E]

W.F. male age 3 years.

Third day of disease

well marked rash. Temp 102°F.

Fauces and tonsils infected. Tongue

coated white creamy. Fingers clean. Papillae

enlarged.

Two loopfuls of tonsillar secretion were

diluted in 5 cc of Salt Solution, and

two loopfuls of dilution spread on

blood serum ~~agar~~ slopes.

The majority of the colonies examined

were found to be composed of a

Streptococcus with bacillary forms.

On further examination it was found

to exhibit conglomerative characteristics

in both growth, "lace work" arrangement

was well shown, ~~[illegible]~~ acid reaction

in Litmus milk which was firmly

clotted in three days.

Some of the colonies which did

not show any bacillary formation on

being subcultured were found in some

reacts to resemble the suffused streptococcus, viz conglomerative finding in broth, fluid remaining clear, while no acid reaction in litmus milk was obtained.

Case 6 [Lab. K]

DB male - age 7 years
Fourth day of disease - Mild case.
Rash characteristic. Tongue clean
Palatine enlarged. Throat slightly
injected. Temp 99.8° on 4th day.

Four loopfuls of secretion off the
surface of the tonsils were diluted
in 2½ cc of sterile normal salt solution.
and two loopfuls were spread over
blood serum slope, & four loopfuls
over agar slope. No growth
was obtained on agar after incubation
at 37°. for 48 hours, — on blood serum
there was a growth of 6 or 8 colonies
all of which were examined in

hanging drop preparations & cultures
uniformly of the same nature and were
identified as the Streptococcus
Bacillary formation in all degrees, (Streptococcus
coals & distinct bacilli) were observed
on subcultures on blood-serum
Conglomeration & acid reaction resulting
& bilious milk were observed.
In this, a comparatively mild case,
pure cultures of this organism were
obtained.

Case 7 [Lab R]

E.W. female age 13½ years.
Second day of disease
Typical attack of Scarlet Fever of a mild
type. Rash well marked on chest
and abdomen. Tongue slightly furred -
papillæ. Enlarged - Temp 100° F
Tonsils enlarged with spots of soft deposit
on left side.
Four loopfuls of soft exudation taken
and diluted in 5 cc Dest. Solution.

Two loopfuls of dilution were spread on blood-serum agar slopes. Several colonies developed after incubation for 48 hours at 37° on agar, on blood serum there was a much more copious growth. There were two types of colonies which were composed of a bacillus (rather slender) and two varieties of streptococci, one form grew in long chains & resembled in character a streptococcus of the "Longus" group, the majority of the colonies were composed of streptococci with very marked bacillary forms, the bacillary forms being more numerous, than cocci. A strongly acid reaction in litmus milk & a firm clot in two days was obtained by this strain. Conglomeration was fairly well marked in both. The chains in fluid media were short.

Note —
In the following five cases the streptococci were examined as in the previous cases, but the characters were ~~not so~~ less typical and I was unable to identify so easily the streptococcus as in the previous instances.

Case 8. [Lab A.]

V. B. female. age. 12 years.
Fourth day of disease. Admitted on 3rd day of disease with patchy rash on chest and limbs. Tongue furred throat slightly injected. Temp 99°F.
Four loopfuls of secretion from surface of tonsil diluted with 2½ cc. Saline and Blood serum inoculated by streaking 2 loopfuls of dilution over surface of slope tube.
Two strains of streptococcus were examined in pure culture, as usual. on incubation for two days. Neither streptococcus showed bacillary forms

conglomeration in both was only slightly marked in both instances, — so far they resembled one another — One strain gave a marked acid reaction in Lakmus Milk and a firm clot was formed in two days, the other streptococcus only gave a feebly acid reaction and no clot was formed.

Case 9 [Lab B]

B.H. Female age 3 years.

Third Day Case.

Rash patchy on limbs. Fauces congested Tonsils enlarged Temp 99°—100.5° F on third day. Tongue furred - papillae enlarged.

Two loopfuls of tonsillar exudate were taken and diluted in 5 cc Salt. Solution and with two loopfuls Serum slope was inoculated, and incubated for two days.

A Staphylococcus and streptococcus were isolated, the latter formed medium sized chains, without any tendencies

of bacillary forms, and milk was not clotted. It was eventually identified as being of the Streptococcus Pyogenes class.

Case 10 (Lab C)

4th day of disease

H.F. female age 12.

Well marked rash - throat generally congested. Tongue characteristic

Four loopfuls of secretion were taken in the usual way & dil. with 5 cc Salt Solution - Two loopfuls of secretion dil. were distributed in blood-serum agar tubes.

Two varieties of streptococcus were obtained. The first type culturally & microscopically in all characters resembles Streptococc. pyog. The second form also exhibiting bacillary forms, & more often conglomeration readily did not clot.

milk and no acid reaction was obtained.

Case 11 [Lab D]

H.B. male aet. 7.

Sixth day of disease. Rash fading and throat symptoms subsiding desquamation commenced on face.

$\frac{1}{200,000}$ cc of tuberculin secretion inoculated in blood serum negative.

A pure growth of staphylococcic colonies were obtained which resembled the streptococcus in the cultural characteristics except in the clotting of milk (which was absent). Acid reaction in milk was present after three days.

Case 12. [Lab I] 4th day Case

H.B.M. age 7. male.

A mild attack - (without septic symptoms)

Rash faintly marked. Tongue clean papillae enlarged. Throat infected submaxillary glds tender. Max. T. 102°.

1/100,000 of Tonsillar secretion used in inoculating Surface of agar & blood serum Tubes. A bacillus growth, several colonies of staphylococci & numerous colonies of streptococcus which on further examination was found to have only slight adherent conglomerative tendency. Bacillary forms were doubtful - Some of the cocci might be described as oval. Neisser's culture gave acid reaction and a pure cult. in three days.

Case 13 [Lab H]

A.F. male age 5 years
Fourth day of disease. Previously suffered from chronic tonsillitis. Tonsils much enlarged and congested. Tongue furred. Rash characteristic.
1/100,000 of tonsillar secretion used. Blood serum & agar Tubes inoculated

Colonies of staphylococci & streptococci examined. The latter were further examined from both original Blood-Serum & agar colonies. The streptococcus in every instance was found in cultural & microscopic exam: to correspond to description of streptococcus pyogenes.

Case 19. [Lab I.]

S F. male age 5.
Second day of disease. Rash and throat symptoms characteristic. General symptoms slight.
1/200,000 of tuberculin secretion in blood serum.
A culture growth of small colonies which were of streptococci which showed none of the characteristic features of strept. conglomeratus, but gave the cultural characters of strepto-pyogenes.

Case 14 [Lab J] 14th day of Disease

W.H. male age 14½ years.
Moderately severe attack – Rash very well marked. Corpuscle Rhinorhoea
1/50,000 of Tonsillar secretion.
Two forms of Streptococci were isolated. Streptococc. Pyogenes and Streptococcus Longus. None of the colonies examined resembled Streptococcus conglomeratus.
There was present in the original growth from selection a motile bacillus probably bac. coli communis.

Case 15. [Lab O]

M N. female age 3 11/12 years.
3rd day of disease
No complications but characteristic rash. Throat very slightly injected. Tongue clean.
1/100,000 of tonsillar secretion.
Streptococcus pyogenes + Staph. Pyog. Aureus.

Case 17 [Lab X]

P. T. male age 14 years.

Sixth day of disease
well marked rash. Throat Symptoms
severe with copious faucial secretion
ulcerative patches. Submaxillary glands
enlarged. Temp. ranged from 100.5°
to 103.2° C. and was of remittent type
1/200,000 cc of nasal secretion used
for making cultures on Blood Serum
and agar tubes.
Streptococcus Pyogenes obtained
copious growth — also Streptococcus
longus. There were in addition
various bacilli & Staphylococci

Case 18 [Lab Y]

E P. male age. 1/100000 cc of
Tonsillar secretion. Rash well
marked. Throat Symptoms moderately Severe.

Streptococcus Pyogenes and Staphylococci isolated.

Case 19. [Lab G.]

K R. male age 17.

7th day of disease. Throat. alimt. quite clean. Tongue clean. Palatine glands enlarged. Rash faint. on chest. Temp 100.5° on 2nd day.

1/50,000 of tonsillar secretion.

No streptococci were isolated.

Case 20 [Lab N] Fourth day of Disease

H W. Female age 4. Years

1/100,000 tonsillar secretion

Throat much congested. Rash typical. Max. Temp 103°. on 4th day.

In this case no streptococci were obtained, but an almost pure culture of Klebs Löffler Bacilli

The chief manifested here of the clinical symptoms of Diphtheria and the mode an uninterrupted recovery.

Table showing Results of Bacteriological Examination of Tonsil in Scarlet Fever as regards Streptococci

Case	Day of Disease	Amount Secretion	Organism with Characters of S. Conglom.	Other Streptococci
I	5	1/100 000 cc	+	+ Strept. Pyog.
II	3	1/100000 cc	+	+ Strep Pyog.
III	3	1/50,000 cc	+	−
IV	2	1/100000 cc	+	+ Strep pyog
V	3	1/100000 cc	+	+ Strep pyog
VI	4	1/50,000 cc	+ (?)	−
VII	2	1/100000 cc	+ (?)	+ Str long.
VIII	4	1/50,000	+ (?)	+ Strep pyog
IX	3	1/100 000 cc	−	+ Strep pyog.
X	4	1/100000	+ (?)	+ Strept pyog
XI	6	1/200,000	+ (?)	−
XII	4	1/100000 cc	+ (?)	−
XIII	4	1/100000 cc	−	+ Strep pyog
XIV	2	1/200000 cc	−	+ Strept pyog
XV	14	1/50,000	−	+ Strep pyog Strep Conglo
XVI	3	1/100000	−	+ Strept. pyog
XVII	6	1/200000	−	+ Strep pyog Strept Conglo
XVIII	5	1/100000	−	+ Strep. pyog
XIX	7	1/50,000	−	−
XX	4	1/100000	−	+ − [Klebs Löffler Bacilli]

Analysis of Specific Characters of Shiga Conj. isolated

Case	Bacillus Formation	Conglom.n = pressw.	Lac. work in agar Plate	Litmus Milk Coagulation	Acid Reac. in Litmus Milk.
I	+	+	+	+	+
II	+	+	+	+	+
III	+	+	+	−	+
IV	+	+	+	+	+
V	+	+	+	+	+
VI	+	+	+	+	+
VII	+	+	+	+	+
VIII	+	+ slight	+	+	+
IX	−	−	−	−	−
X	+	+	+	−	−
XI	+	+	+	−	−
XII	+	−	−	+	+
XIII	−	−	−	−	−
XIV	−	−	−	−	−
XV	−	−	−	−	−
XVI	−	−	−	−	−
XVII	−	−	−	−	−
XVIII	−	−	−	−	−
XIX	−	−	−	−	−
XX	−	−	−	−	−

Table of Bacteriological Exam.ⁿ of Tonsil in 20 normal Throats regarding the presence of Streptococci.

No	Streptococcus with character resembling Strept. Conglom.	Strept. Pyog	Other Streptococci
I	–	–	+ Strept longus
II	–	–	–
III	–	+	+ Strept. longus.
IV	–	+	–
V	–	–	–
VI	–	–	–
VII	–	+	+ Strept. long
VIII	–	–	–
IX	–	–	+ Strept. long
X	–	–	–
XI	–	+	+ Strept long
XII	–	–	–
XIII	–	+	–
XIV	–	–	+ Strep long
XV	–	+	+ Strept. long
XVI	–	–	–
XVII	–	–	–
XVIII	–	–	–
XIX	–	+	+ Strept long
XX	–	+	+ Strept. long

A special examination of two strains of typical Streptococcus Pyogenes was made special attention being given to those special features which are characteristic of the Streptococcus conglomeratus

The following characters were observed :-

Broth - The fluid after incubation for 48 hours is slightly turbid, and a distinct sediment forms at the bottom of the tube which does not exhibit the same characteristic characters as in the case of S. conglomeratus. The growth is more readily spread out as a film preparation and microscopically it is found to be composed of massed small curled cocci chains, not forming any distinct grouping.

In agar condensation fluid a certain amount of pattern grouping can sometimes be obtained if the film is carefully prepared.

In Lehmis Mlek incubated for 3 days at 37°
I uniformly got a faint acid reaction
Clotting did not occur.

In agar and gelatine plates I was
unable to make out any distinguishing
characters by the naked eye, which
would separate one class of colony
from the other. Various impression
preparations of Streptococcus pyogenes
were made but in none of these
were bacillary forms discovered

The growth of Streptococcus
conglomeratus on gelatine I found very
much slower than in the case of
Strept. Pyogenes. Examined under
the lens a streak growth of
S. Conglomeratus is invisible to the
naked eye, and a negative
conclusion cannot be arrived at
in the case of a growth on this
medium for several weeks. The
growth of S. pyogenes on this
medium is much more profuse

than that of S. conglomeratus, and the colls is a more delicate organism, and dies more readily. I found it necessary to have several fluid growths of each strain as tubes frequently ceased to give any growth without apparent cause.

Summary of Results and Conclusions:—

(1). In twenty cases of mild Scarlet fever examined the organism with the distinctive characters of Streptococcus conglomeratus (or scarlatinae) was isolated in eleven instances.

(2) In four of these cases Streptococcus conglomeratus was the only streptococcus found present in the secretion.

(3) In seven of the instances where streptococcus conglomeratus was found, streptococcus pyogenes was also found associated with that organism.

(4) In seven cases streptococcus pyogenes was the only form of

Staphylococcus discovered in the Secretion.

(5) In only one instance (a very mild case) was no Staphylococcus discovered.

(6) In one instance the Klebs Löffler Bacillus was discovered in a case which at the time and subsequently manifested none of the Symptoms of diphtheria.

(7). Case 3 & Case 4 (it is interesting to note) were the same patient. In the post-attack (Case 4) cultures were made on the 4th day of disease and the characters of the Staphylococci obtained were anomalous, although slight bacillary formation was noted. On the 14th day patient had a relapse more severe in nature than the initial attack, and cultures were made from the secretion on the 2nd day of disease. The Staphylococcus was isolated showing all the distinctive features of S. conglomeratus.

(8) Variation in the cultural characters of Streptococcus conglomeratus were found, 'bacillary formation' may be be slight or well marked, conglomeration varies somewhat in degree, clotting of milk may be absent, while all the other characteristic features are present.

(9) In no case of Streptococcus Conglomeratus examined was the bacillary formation so marked as to suggest the presence of the Klebs Löeffler Bacillus.

The presence of Klebs Löeffler Bacilli in one instance noted was probably accidental, & probably due to conveyance of infection in some way from a Diphtheria ward in the same hospital.

In the absence of proof of the specific nature of the S. Conglomeratus by inoculating the human subject it is impossible to say absolutely that it is the specific cause of the disease although this seems highly probable.

its presence in Scarlet Fever, and absence in the normal throat, [redacted] even in the absence of proof of its absolute specificity), is of clinical value in the diagnosis of the disease

In 20 Cases of Normal throats examined, in no instance was a streptococcus found manifesting the distinctive characters of the streptococcus which would appear to be specially associated with Scarlet Fever.

From the results above noted it would appear to be a good positive sign in the diagnosis of the disease, while a less trustworthy negative sign.

The pathogenesis of the organism in mice is an important feature of the organism and I regret that at the time of writing I am not able to append results obtained in this direction to these preliminary notes.

All shades of gradation of the characters of Streptococc. glomeratus, into those of Streptococcus hyog. may be found, and by culture and inoculation of animals its characters may be so changed as almost to resemble the Strept. hyogenes

On the other hand cultivation and inoculation of the Streptococc hyogenes has failed to make it resemble Strib. coccus conglomeratus in the main features of that organism.

Both organisms may be found in the fauces in cases of Scarlet. Fever.

The nature of infections by the Streptococcus hyogenes are well known, and many of the symptoms of Scarlet. Fever resemble infection by this organism. On the other hand certain features of Scarlet. Fever cannot be explained by this

infection of Streptococcus pyogenes

No other germ has been described or has been found in the fauces, which is only found in Scarlet Fever.

Hence in the present state of our knowledge, although absolute proof cannot be obtained, we may consider the active cause of the disease, this Streptococcus conglomerates.