NOTES ON THE BACTERIOLOGY OF SCARLET FEVER

with special reference to the Streptococci.

By

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INTRODUCTION. -

That Scarlet Fever is an infectious disease is admitted by everyone. That it is due to the action of a specific micro-organism however, there is at present only presumptive evidence, based principally on the close analogy of the disease to such others of the infectious group as Diphtheria and Typhoid Fever, in which the microbial cause has been fully demonstrated.

In all three, the disease has a definite incubation period, following on infection from a previous case, either by direct contact, or indirectly from fomites or discharges, and runs a definite course. The power of transmitting the disease to other persons is also retained, frequently for long periods, after the sufferer is himself apparently quite recovered, and presents no appearance of local abnormality, and with no relation whatever to the original severity of the disease.

Between Diphtheria and Scarlet Fever the analogy is still closer, for not only are the throat and nose the parts usually most severely affected at the onset, together with the glands in their vicinity, but in each the poison exerts a definitely selective action upon certain tissues and organs in the latter stages; in Scarlet Fever the kidneys, and in Diphtheria the heart muscle and peripheral nerves are infected specifically.

Again, Scarlet Fever in many points bears a very close resemblance to ordinary septic infections, the most striking point perhaps being the similarity of the rash in Scarlet Fever to that so frequently met with after burns and scalds, usually, but not necessarily, of large extent, and to the rash seen sometimes in puerperal septicaemia. This resemblance may be a source of very grave trouble when it appears in a surgical ward, for it is occasionally impossible, in a case of true Scarlet Fever, to discover any means whatever by which scarlatinal infection could have been introduced, as in two cases of which I shall make mention later; and even where all possibility of such infection can be absolutely excluded, the patient may pass through what is to all appearances a typical attack of the disease.

A more or less similar rash may follow the administration of a simple soap and water enema (I have myself seen a number in which the eruption was identical with that typical of Scarlet Fever), and certain drugs, such as belladonna, turpentine, chloral, opium, quinine, or (rarely) salicylates.

FROM WHAT PART OF THE BODY DOES THE INFECTION EMANATE ?

The first step in a search for the causative organism of Scarlet Fever is to determine where the source of infection in a patient lies. Our most reliable data are obtained from the statistics of the condition of patients discharged from hospital, who have infected other persons with the same disease, after their return home.

Of these we have a plentiful supply. The first extensive series of observations published are those of C. KILLICK MILLARD, who gives the records of 158 Scarlatinal patients, who, of a total of 4,810, were the carriers of the disease to 171 "return" cases (Brit: Med: Journ: Sept. 3, 1898). The special points to which he drew attention were. -

(1) " The length of the interval between discharge of the primary case, and onset of the disease in the "return". He showed that infection took place most frequently within one week, and gradually diminished up to 6 weeks, after which he knew of no cases".

(2) "RECRUDESENCE OF INFECTIVITY. No figures are given, but Millard simply states generally that without doubt many cases which leave hospital apparently sound and free from infection, become infectious again, as evidenced by the recurrence, or even primary development, of a rhinorrhoea or otorrhoea."

(3) "PERIOD OF ISOLATION OF THE INFECTING CASE. His general conclusions from this are that it is not specially the cases discharged early which cause "returns", but equally those of long duration which are complicated by sequelae of a more or less chronic nature. After 12 weeks' isolation he finds the chance of infectivity very small, in all cases, and, generally, that the chance of infecting others is at its maximum in the 8 and 9 week periods, falling rapidly after."

(4) AGE DISTRIBUTION. - He finds no greater tendency to convey infection at any one age period than at any other.

(5) TYPE OF INFECTING CASES . - Millard finds that it is not necessarily the severe cases which cause the "returns", mild ones being as powerful in that respect as any. But he specially notes the preponderance of certain sequelae among them. In the subjoined table a comparison is made between the condition on discharge of the infecting cases, with that of all cases, excluding such as were the only children in their household, and all that had been in hospital for over 13 weeks, as he is satisfied that it was practically impossible for these to carry infection :-

	Desquamating	Fauces Abnormal	Adenitis	Sores & Excoriations	Skin Eruptions	Albuminuria	Otorrhoea	Rhinitis	Quite Clear in every way
Infecting cases. %	7.0	3.1	2.1	3.1	3.1	0.6	2.2	22.9	58.2
All cases. %	8.2	5.4	2.3	2.2	1.0	0.5	1.5	6.0	72.6

The only desquamation was on the feet, and of the late variety. That on the body and hands was usually completed within the minimum period of isolation. "Fauces abnormal" includes congested mucosa and enlarged tonsils;"sores and excoriations" includes cracks,abrasions, and raw surfaces elsewhere than about the nostrils; "adenitis" means enlarged cervical glands;"skin eruptions" includes all forms of eruption liable to occur during convalescence; "otorrhoea" only refers to purulent, but "rhinitis" to all discharges from the middle ear and nose respectively; "rhinitis" also includes cases in which there may be no actual rhinorrhoea, but in which an ~~xxxxxx~~ unhealthy condition of the nostrils exists, no matter how slight.

Millard deduces from these results that it is the "rhinitis" that is the most active condition in the spread of infection in his series of "return" cases, "otorrhoea" also figuring to some extent. In the last 60 of his "infecting" cases, Millard made special enquiries as to the onset of otorrhoea and rhinorrhoea after their return home; out of 33 of them which were free from such discharges on leaving hospital, two developed otorrhoea, and no less than ten had nasal discharge sufficient to attract the mother's attention. He does not consider late desquamation of the feet to be infectious. The large proportion of "infecting" cases apparently quite clear in every way, seems ~~xx~~ significant, as ~~xxxxxxxxxx~~ pointing to persistence of the infecting agent in the mucous secretion of an apparently healthy throat, a point not mentioned by Millard.

Four years later the same observer,(Lancet,Apr.5,1902) writing on the "Supposed Infectivity of the Desquamation in Scarlet Fever", summarises the principal arguments against this supposition as follows :-

i. Absence of evidence supporting it. It is difficult to believe but that if the old supposition were correct, strong evidence of it would ere this have been forthcoming as is now the case with the discharges from the nose and ears.

ii. The fact that infectivity begins prior to the onset of desquamation, and frequently continues long after desquamation has ceased.

iii. The fact that Scarlet Fever wards, though abounding in desquamating epithelium, are not a danger to neighbouring houses.

iv. The fact that the proportion of "return" cases does not appear to be increased among patients sent out from hospital still desquamating.

On the other hand, the principal argument ~~xxxxxxxx~~ in favour of the view that desquamation is infectious is the fact that patients still desquamating, but otherwise apparently free from infection, have frequently been known to convey the disease to others.

The whole force of this argument disappears, however, when we consider that patients apparently quite free from infection, in whom desquamation has entirely ceased, have also been known to convey the disease; moreover, patients still desquamating have frequently mixed freely with others without untoward result.

Other striking evidence against the infectiousness of the desquamation is given by Dr. J. Priestley (Trans. Epidem. Soc. Vol. xiv. 1894-5) who records that during an outbreak of Smallpox at Leicester, about 180 children in various stages of desquamation after Scarlet Fever, were sent home, and no secondary cases occurred at any of their homes.

Dr. R. E. Lauder also, at Southampton (Lancet. Mar. 12. 1904) discharged among others, 204 patients who had not suffered from complications while in hospital, after an average detention of 28 days. These were all desquamating; from them there resulted only two "return" cases. From 88 others, who had suffered from various complications in hospital, and who were detained for an average of 50 days, he states that there were five "return" cases.

The next important investigation was that of Prof. W. J. R. Simpson, for the Metropolitan Asylums Board (1901). He investigated 90 "infecting" cases which occurred among 6507 discharged patients during the six months October 1898 to March 1899. The following were his findings :-

25 had colds in the head.
5 had colds in the chest;

these two conditions point to mucous from the pharynx and respiratory tubes as the source of infection.

49 had rhinorrhoea,
5 had sores about the nose;
15 had colds in the head with nasal discharge.

Otorrhoea being often accompanied by rhinorrhoea, was not regarded as being of itself a proven source of infection.
Desquamation of the late type appeared to be non-infectious.

This investigation was followed by another, for the same authority, and on the same subject, by Dr. A. G. R. Cameron (1905). As he dealt with a larger number of cases, extending over a longer period (1901-2) than Simpson, his results are even more valuable. In this exhaustive piece of work, 688 "return" outbreaks are dealt with and Cameron's conclusions are supplemented by the critical opinions of the Superintendents of the various hospitals of the Board. The following are among the most noteworthy general deductions :-

i. Only 46.5 per cent of the alleged "infecting" cases were definitely proved to be responsible for "return" cases.

ii. 46.8 per cent of "return" cases occurred in the first week after the discharge from hospital of the "infecting" case, 26.5 per cent in the second week, 11 per cent in the third week, and so on, the proportion gradually diminishing till the end of the eighth week, when "return" cases practically ceased.

iii. The period of detention in hospital of "infecting" cases was practically the same as for all cases. Of those detained under 8 weeks, and over 8 weeks, the proportion of "infecting" cases is the same, but of the latter, those detained the longest give the best results.

iv. During the months, from November to April, when the largest number of "return" cases occurred, the "infecting" cases suffered from Mucous discharges, after leaving hospital, much more frequently than during the remaining months.

v. Between the ages of 4 and 10 years, patients carry home infection more frequently than at any other age period.

vi. At this age period an unduly large proportion (56.8 per cent) suffer from morbid affections of the nose. Adults, who seldom suffer from nasal affections after discharge, rarely cause "return" cases.

vii. The mortality of "return" cases was 5.8 per cent, as compared with 3.6 per cent for all cases, thus showing increased virulence of the disease.

viii. Apart from their condition on discharge, patients who suffer from complications in hospital are more prone than uncomplicated cases to cause "return" cases, the effect of the complications being to prolong the period of infectiousness.

The complication most common in "infecting" cases after discharge is a morbid condition of the nose; in such, infectiousness may continue after the objective signs of the complications have disappeared. When the discharge is purulent, the case should certainly be regarded as infectious.

ix. Recurrence of a complication, such as rhinorrhoea or tonsillitis, or its primary onset, after discharge of the patient, is apt to be attended by a recrudescence of infection, and "return" cases result. In over 50 per cent of "infecting" cases examined after occurrence of a "return" case, nasal discharge was found to be present, having commenced, in most instances, after leaving hospital.

x. Patients discharged with otorrhoea, apart from any nasal complication, are seldom associated with the occurrence of "return" cases.

xi. Late desquamation can not be regarded as evidence of infectiousness.

xii. Persons suffering from sore throat, or other anomalous illnesses, may convey Scarlet Fever infection to others.

Dr.F.M.TURNER, in January 1906, published an ivestigation, supplementary to that of Cameron, and his results coincided in the essential details with those just enumerated.

Dr. WILLIAMS, in his Report on the Sheffield Infectious Hospitals (1904), describes 70 cases which were associated with "return" cases. On discharge from hospital all were healthy but 13, which had otorrhoea, of whom 7 had no further treatment at home. Of the rest, 14 developed rhinorrhoea, and 5 a sore throat or nose; the remainder had no abnormal conditions.

Dr.A.Knyvett Gordon, in the Reports of the Monsall Infectious Hospital, Manchester, for 1902-3 and 4, gives the following account of the condition of "infecting" cases on leaving hospital, and subsequently. There were altogether 302 alleged "infecting" cases, and 312 "returns". Of these 302 "infecting" cases

272 had no abnormal conditions on discharge;
179 had no abnormal conditions subsequently;
31 developed otorrhoea after their return home;
63 developed rhinorrhoea;
12 developed late desquamation;
17 developed sores about the nose or lips;
30 suffered from abnormalities when discharged, of which 8 had otorrhoea;
10 had rhinorrhoea;
4 were still desquamating; and
8 had sores about the nose or lips.

Such lists of hospital statistics might be multiplied indefinitely, but those quoted above serve to show the lines along which the general concensus of experience trends.

CONCLUSIONS. - The possible sources of infection therefore are the mucous or purulent discharges from the throat, nose, and ear, and the desquamating skin particles. That the urine and faeces may contain the infecting agent is also possible, but there is no evidence at all to support the theory; the fact that Courtois, a number of years ago, found streptococci in the urine in several cases of Scarlatinal nephritis can hardly be accepted as proof of anything further than the presence of a streptococcal infection of the kidney, probably secondary to the original disease.

From the list we may, I think, eliminate the desquamation. There is no proof at all that it, of itself, can convey the disease. If such were the case, surely the fine branny scales, shed profusely by most cases, and wafted freely about by every breath of air, would carry infection to large numbers of the susceptible individuals in the immediate neighbourhood of houses or hospitals containing Scarlet Fever patients. Yet the striking distance of Scarlet Fever is surprisingly small, and such centres do <u>not</u> act to any extent as foci of

infection. A moist eczematous condition of the skin appears to be xxable to harbour infection, and Kidd (Lancet, July 17, 1905) even reports a "return" case infected by a discharged patient whose only visible abnormality was a moist eczema round the anus, which developed after his return home. In regard to purulent ear discharges, the case is, as yet, not proven. We do not know for certain the relationship of otorrhoea to "return" cases, as so large a proportion of "infecting" cases, with otorrhoea, have also some faucial or nasal sequela as well.

That the naso-bucco-pharyngeal discharges contain the infection is abundantly proved. Though how long such infection persists in a chronic watery discharge from the nose we have no means as yet of determining, for it is often impossible to cure such, and patients have to be sent home with the condition still persisting. Only a relatively small proportion of these act as "infecting" cases.

The first and only, but absolute proof of the infectious nature of the bucco-pharyngeal mucous was established by J.W. STICKLER (New York Med. Rec. Sept. 9, 1899), and published from his posthumous papers. He inoculated ten healthy individuals with mucus from the mouth and throat of a recovered mild case of Scarlet Fever, his idea being to produce an artificial immunity. The mucus was diluted with weak carbolic lotion. The result was a typical attack of Scarlet Fever in each case, with vomiting, sore throat, moderate rise of temperature, and general desquamation, with intense desquamation, and sometimes abscess formation, at the seat of inoculation. In several cases nephritis occurred. The incubation period varied from 12 to 72 hours from the time of inoculation.

Many experiments have been made in the endeavour to produce the disease in animals, but with no definite results, owing to the fact that Scarlet Fever does not appear to attack them at all. Class (Med. Rec. Sept. 2, 1899) quotes Behla (Centralblatt für Bakteriologie), who, noticing that some pigs belonging to an infected family developed a Scarlet rash, to prove a connection injected some blood from an infected child into a healthy pig. The pig, after a few days, developed a Scarlet rash round the wound, and this was followed by desquamation. A similar condition, however, often follows inoculation of the human subject with simple horse-serum, apart altogether from the action of disease-immunising constituents.

Class also inoculated white swine with an organism believed by him to be specific for Scarlet Fever, and produced an erythema and desquamation; but this cannot be taken as proving anything for man.

In connection with the investigation by Crookshank and Axe of the organism found by Klein on the teats of cows during the much discussed Hendon outbreak, many attempts to inoculate cows with Scarlet Fever from various human sources were made, but failed.

As Mervyn H.Gordon pointed out in his article on "the Cause and Prevention of Return Cases of Scarlet Fever" (Brit.Med.Journ.Aug.16,1902) the aural and nasal discharges issue from passages which communicate directly with the pharynx. It is therefore possible that the pharynx,and region of the fauces,is the chief seat of, at any rate, the infection that clings so persistently to the scarlatinal patient;and the large proportion of "infecting" cases in which no abnormal condition can be discovered form a factor,by no means negligible,which certainly does not tend to disprove this theory.

From Stickler's experiment we know for certain that the pharyngeal mucus from a convalescent ~~xxxxxx~~ case contains the specific virus. From Koenigers' experiments (Zeit.für Hygiene,Bd.xxxiv,Hefl,1), and from those by Mervyn H.Gordon (Rep.of M.O,to L.G.B.1902-3) we know that in the acts of talking,sneezing,coughing,etc., finely disseminated particles of mucus and saliva are expelled to considerable distances from their source, in a sheltered room. The experimenters rinsed out their mouths with an emulsion of Bacillus Prodigiosus culture before commencing to talk,or read aloud, having first excluded the presence of the organism in the air of the room by control experiments. Agar plates placed about the room at various points were found to become inoculated, up to a distance of 40 feet. These results probably explain the occurrence of "return"cases of Scarlet Fever where the supposed "infecting" case was suffering from no abnormal discharges,and show that the infection can be aerially, without direct contact of the individuals,or even the agency of fomites.

W.T.Gordon Pugh (Lancet Feb.4,1905) objects to this mode of transmission of Scarlet Fever the fact that fluid sprayed from the mouth during the act of talking comes from the neighbourhood of the incisor teeth,and that as the general flow of the saliva is backwards to the pharynx,it can only occasionally happen that such particles contain organisms from the faucial region. Now Stenson's Duct enters the buccal cavity opposite the second lower molar tooth; and in the ordinary movements of the tongue and cheeks,and in mastication, one may surely assume that a certain amount of the mucus from the fauces becomes admixed with the buccal saliva in the mouth.

From all this evidence it appears that in a search for the specific germ (if such exists) the most likely road to success lies through a thorough examination of the pharyngeal bacteria. Much work has already been done,and though a large accumulation of information has accrued, we are still in ignorance of the actual causative organism.

HISTORY OF OBSERVATIONS

Before proceeding to describe in detail the more recent investigations on the subject, it is of interest to recall the earlier observations.

The first work of importance was that of KLEIN (1885) who investigated the Hendon milk epidemic, and showed that this was due to Streptococci from pustules on the teats of cows supplying the milk. With Klein was associated POWER.

A similar organism was isolated from throats in 5 out of 11 cases of Scarlet Fever in Man (Rep. of M.O, to L.G.B. 1886-7).

CROOKSHANK AND AXE reinvestigated this epidemic, and showed that the condition in the cows had really been Vaccinia, and that the streptococcus described by Klein was identical with Strep. Pyogenes; also that there had been a possible cause of contamination of the milk from human sources (Trans. Path. Soc. London, on Hendon outbreak 1888).

EDINGTON (1887) next described a bacillus which he obtained from the desquamation scales in cases of Scarlet Fever (Brit. Med. Journ. June 1887). This Klein identified with his own "scurf" bacillus, a variety of the ubiquitous Bacillus Mesentericus (Rep. of M.O. to L.G.B. 1896-7). Edington also first isolated the micrococcus capsaeformis from the scales.

KURTH (1891), with RASKIN, FRÄNKL, AND FREUDENBERG, observed an organism in Scarlet Fever which Kurth named Streptococcus Conglomeratus (Arb. aus dem Kaiserlichen Gesundheitsamte, B. vii). This germ was found in 4 out of 12 cases of Scarlet Fever, but not in other diseases. In 1 out of 7 cases it was isolated from the tonsil; on two occasions from the pus of one cervical abscess; it was not found in the only aural discharge examined. Post-mortem Kurth obtained the Strep-Conglom, in 2 out of 6 spleens examined; in 2 out of 3 specimens of pus from the region of the neck or throat; and in 1 out of 3 cases in which the kidney was examined. Other Streptococci were found in 4 spleens, 1 kidney, 1 abscess of the neck and 1 liver. Thus in the autopsies the strep-conglom. was not found to be present so frequently as other forms of streptococci.

BOHM (1892) found Strep-Pyogenes in the tonsils only of a rapidly fatal case.

SIDNEY MARTIN (1894) found only large diplococci in the late angina of Scarlet Fever cases.

BELLINGHAM SMITH and MARY D. STURGE (Lancet Nov. 16, 1895) found streptococci in pus from a suppurating elbow joint, and in the kidney, in an autopsy on a scarlatinal case; also a pure culture of streptococci in pus from a suppurating knee joint; and pneumococci in pus from an empyema, in Scarlet Fever.

SYMES (Lancet, Aug. 24, 1895) published the results of the examination of cultures on blood serum from 68 Scarlatinal throats. Of these :-

14 showed pure streptococci;
25 streptococci with staphylococci;
2 staphylococci only;
1 staphylococci, and Bac. Diphtheriae (long form) and Bac. Diphtheriae (short form);
14 cocci and other forms of bacilli;
3 various forms of bacilli only.

In two further cases he obtained streptococci with Staph. Pyog. Aur., from the tonsils; Streptococci, Staph. Pyog. Aur., and Strepto-Bacilli" (Bac. Mesentericus ?) from nasal discharge, and streptococci from the pus of a cervical abscess.

As controls, he examined 5 cases of Tonsillitis. 4 showed streptococci, 1 of which had also "large oval cocci" 1 showed staphylococci only.

CRAJKOWSKI (1895) found Gram-negative diplococci in the blood of 15 cases.

L. PFEIFFER, about the same time, as the result of a somewhat cursory histological examination, asserted that coccidia were the cause of cancer, and all eruptive diseases.

CROOKSHANK in his text book (Bacteriology and Infectious diseases, 1896) mentions the following :-
COZE AND FELTZ found cocci in the blood.

CROOKE found bacilli, cocci, and streptococci in the throat organs; also cocci in the internal organs. He was not sure that the latter were a primary infection.

LÖFFLER found the same streptococci in the throats of scarlatinal cases as in Diphtheria.

BABES proved the constant presence of a streptococcus in inflammatory products secondary to Scarlet Fever.

HEUBNER and BAHRDT found a streptococcus identical with Strep. Pyog., in a fatal case with pericarditis and secondary joint suppurations.

FRÄNKL and FREUDENBERG he says, examined 3 cases, and in each found and cultivated cocci from the submaxillary glands, spleen, liver, and kidney; these were indistinguishable culturally or by inoculation into animals from Strep. Pyog., and the streptococcus found in Puerperal Fever. Therefore they concluded that this Streptococcus was not the specific organism of the disease, and that its presence was due to a secondary infection through the throat.

CROOKSHANK, RASKINE, HOLMES, and others agreed with this, and believed that the streptococci in suppuration, puerperal septicaemia, pyaemia, septicaemia, certain cases of Scarlet Fever, Measles, and Diphtheria, are identical.

GRUNBAUM (1897) obtained a non-motile diplococcus from the blood of a Scarlet Fever case, which organism agglutinated with serum from another case of Scarlet Fever.

CHABADE. (1898) working in St. Petersburg, in 214 cases of Scarlet Fever found streptococci, Staphylococci, and (in 13) Bac. Diphtheriae present in the throat.

RASKINE found streptococci in the blood in 2 out of 64 cases examined.

O. VIERORDT found streptococci in secondary pleuritic exudations.

PEARCE (1899) found secondary lesions to be due to streptococcus Pyogenes, Staph-Pyog. Aur., and Pneumococcus, in this order of frequency. He found Streptococci present in the nose, throat, and general infection.

KLEIN AND MERVYN H. GORDON (1896) began to publish the results of a further investigation on the subject, which lasted till 1903. The general trend was to show that the causative organism is the streptococcus Scarlatinae, or conglomeratus (hereafter described as Strep. Conglom., for convenience) of Klein, though the case for this germ is not satisfactorily proven. Their work was of a very complete and exhaustive nature, and is by far the best extant on the subject.

W. J. CLASS (New York Med. Rec. Sept. 2, 1899) announced his discovery of a diplococcus, polymorphous in character, and resembling a large Gonococcus, which was constantly present in the blood, throat, and desquamation scales of Scarlatinal cases. This organism he claimed as specific.

BAGINSKY AND SOMMERFELD (Berliner Klin. Woch July 2 and 9 1900), working independently of Class, found constantly present in the blood and throat secretions of Scarlet Fever patients a polymorphous streptococcus varying in the length of its chains from 2 units in the blood up to long chains in broth cultures, resembling the organism of Class very closely in many respects, but differing from it in its chain formation. In this faculty it approximated more closely to the Strep-Conglom, described by Kurth, and that of Klein and Gordon; the organisms of all these observers were distinguished by their property of growing in glutinous dense masses in artificial media. Further reference will be made to these organisms later.

RUEDIGER (Journ. Amer. Med. Assoc., Oct. 13, 1906) published the results of a comparative examination of the pharyngeal bacteria in 154 cases of persons in health, or suffering from one of the infectious diseases. No very conclusive results were obtained.

SIEGEL (Berlin Med. Klin. 1905) found in the blood of Scarlatinal patients a protozoon, in the form of small, nucleated, gelatinous discs, showing division, from simple bisection to disintegration into mobile spores, and closely resembling the corpuscles of vaccine lymph.

MALLORY (Journ. Med. Research, Vol. x, No. 4, p. 483, 1906) describes a protozoon - Cyclasterion Scarlatinale - found by him in the blood in Scarlet Fever.

OBSERVATIONS OF

KURTH, KLEIN AND GORDON, CLASS, BAGINSKY AND SOMMERFELD

I. KURTH (Arb. aus dem Kaiserlichen Gesundheitsamte, B. viii, 1891) first, after Klein, drew attention to the association with Scarlet Fever of an organism distinguished by its peculiar prowth in broth. This was a streptococcus, which in broth forms agglomerations of streptococcal chains, matted together. He therefore named it Streptococcus Conglomeratus. He mentioned that occasional individuals in the chains are larger than their neighbours; the broth cultures are clear, with a peculiar, nebulous, white-grey mass at the bottom of the tube. Milk is coagulated. Kurth gives little information beyond this, his observations having been confined chiefly to cultural characteristics in bouillon. As mentioned above, only 12 cases of Scarlet Fever were examined, and this organism was found in 4. It was found in the aural discharge of Scarlatinal cases. Kurth's work therefore was strictly limited in scope.

II. KLEIN in 1885, in an investigation into the causes of an outbreak of Scarlet Fever at Hendon, isolated from pustules on the teats of cows supplying most of the infected households a streptococcus apparently identical with one which he also found present in the blood of Scarlatinal patients. The special characteristics of this organism were its formation in broth culture of a nebulous, coherent mass of long chains at the bottom of the tube, with clear medium, together with rapid coagulation of milk in milk culture, these two features differentiating it from other varieties of streptococcus (Rep. of M. O, to L. G. B., 1885-8). Though Klein and Gordon call this organism Streptococcus Scarlatinae or Conglomeratus, as mentioned above, I shall for convenience refer to it as Strep. Conglom., to distinguish it from the non-conglomerate streptococci also dealt with. Inoculation of this organism into mice usually proved fatal.

In 1896, in conjunction with Mervyn H. Gordon, Klein again took up the bacteriology of Scarlet Fever. In the Report of the Medical Officer to the L. G. B. for 1896-7 are given the results of (1) the examination of the desquamating cuticle of 8 cases of Scarlet Fever, (2) of the urine of 3 cases, and (3) of the throat secretions in 6 cases, together with the throat secretions from 20 cases of sore throat, and 17 normal throats. The following organisms were found :-

(1) Desquamating cuticle -

Bacillus Mesentericus (Scurf Bacillus) the most common, in various types. This probably is the bacillus described by Edington in 1887.

Xerosis Bacillus, in 2 out of the 8 cases.
Staphylococcus Albus non-liquefaciens, in 4 cases.
Staphylococcus Albus, slowly liquefying gelatin, in 2 cases.
Sarcina, in 2 cases.
Streptococci were not found at all.

(2) Urine .-

Micrococcus Urea, in 1 case.
Staph. Alb. Liquescens, and
Bac. Coli Communis, in the second case.
Proteus Vulgaris, and
Staph. Alb. Non. liquescens, in the third.

(3) Throat - tonsillar secretion. -

(a) Scarlatinal, 6 cases -
Streptococci, including strep. conglom, with
Staphylococci, in every case.

(b) Simple sore throat, 20 cases -
Staph. Alb. in all cases.
Staph. Pyog. Aureus, in 5 cases.
Streptococci, in 16 cases - no strep conglom.
Bac. Buccalis Minutus, in 2 cases
Hofmann's Pseudo-diphtheria Bacillus, in 3 cases.

(c) Normal throat, 17 cases -
Staph. Alb in every case, Staph. Alb. Liquesc in 9.
Staph. Citreus, in 5 cases; no staph aureus.
Streptococci, in 14 cases; mostly Strep. Pyog.
and Strep. Brev., and 1 liquefying gelatin.
Sarcinae, moulds, etc, in 9 cases.
Bacilli of various descriptions in most cases,
including 1 Bac. Coli Communis.

In the following report (1897-8) Klein describes his method of collection of specimens from scarlatinal throats by sterilised swabs, which were washed in sterile normal saline, and a few loopfuls of this smeared over 6 agar plates. Incubation at 37 deg. C., for 24 to 48 hours resulted in the appearance of numerous small, round, translucent, knobbed colonies, subcultures of which in broth produced the typical conglomeratus growth, and in litmus milk caused rapid clotting with acid formation. Inoculation into mice caused death from septicaemia in a large proportion of cases, in from a week to a month; in rabbits only temporary oedema and rubor resulted. He cites 4 cases of early Scarlet Fever in which large quantities of Strep-Conglom, were found in the throat.

Eleven cases, varying from the 4th week to the 7th month from onset of the disease, are also described. Of these, the throat secretions were examined in 9, the nares in 4, ear discharge in 2, desquamating cuticle in 5, and urine in 5, with the following results :-

(1) Throat _ 9 Cases

Strep. Conglom. in 8 including that in the 7th month
Strep. Pyog. in 4
Strep. Brev. in 1. No Streptococci at all, 1case
Staph. Pyog. Alb. in all 9
Staphy. Pyog. Aur. in 4.
Bac Hofmann. in 1.

(2) Nares - 4Cases -

Strep. Conglom. in 2 No Streptococci at all. 1 case
Staphy. Pyog. Alb. in 4.
Staphy. Pyog. Aur. in 4.

(3) Ear discharge - 2 cases -
Staph. Pyog.Alb. in 2.
Strep. Pyog. in 1, No Streptococci at all, 1 case.
Strep. Conglom. not found at all.

(4) Urine - 5 cases

Strep. Conglom. not found.

(5) Desquamating Cuticle - 5cases -
Strep. Conglom. not found.

From this it appeared that the Strep. Conglom persisted till very in convalescence.

At this point Klein left the further investigation of the subject in the hands of Mervyn H Gordon, who continued his researches first on the lines of more definitely establishing the relationship of the Strep. Conglom. to Scarlet Fever, and secondly of differentiating that organism from others of the Streptococcus group.

In the Report of the Medical Officer to the L.G.B. for 1898-9 Gordon commences with a comparison and contrast of the cultural characteristics of Streptococcus Longus (as found in examination of Strep. Long. Buccalis), Streptococcus Medius (as obtained from pus), and the Streptococcus Conglomeratus of Scarlet Fever.

The following table summarises briefly the chief points;-

Culture Medium.	Strep. Long.	Strep. Med.	Strep. Conglom.
Broth (24 hrs, 37deg, C)	Fluid clear; stringy mucus like growth at bottom, of long straight chains; little or no coherency.	Fluid clear; deposit of flakes, flocculi, or powder, at bottom, without coherency. Medium curling chains occasionally showing tangled masses.	Fluid clear; one or more very coherent masses at bottom. Chain formation sometimes difficult to make out.
Gelatin (24 hrs - 37deg, C)	- - Very similar to growth in broth - - -		
Litmus Milk. (24 hrs, 37deg, C)	Acid reaction; no clotting; growth as in broth.	Faint acid production; no clotting; growth as in broth.	Rapid, strong acid production and clotting. Growth much as in broth, but with more chain formation.
Agar Colonies (24 hrs 37 deg, C)	(1) Flat, feathery-edged, and granular; interminable network of chains. (2) Smaller number more heaped up and limited; occasionally no chains visible at edges; colonies dark and coarsely granular, and very like those of Strep. Conglom.	(1) Round, firm edges, grey and smooth or finely granular. Edge may be slightly irregular, and show few medium chains. (2) Minority show network of medium disjointed chains.	(1) Grey, granular, irregularly outlined, and tuberculated. (2) Similar, but of coherent appearance, and without tubercles (3) Younger colonies, with dark compact, coherent centre, with surrounding frilling of chains. All 3 have granular, glossy, coherent centre, and are smaller, more opaque, and more irregular than those of other Streps.
Gelatin Colonies (2 or 3 days 20 degC)	Grey colonies of same type as on agar; chain formation always present; cocci sometimes of larger size.	Round or oval, greyish blue spots, composed of cocci in group (Staphylococcal) formation; chains may appear later. Cocci sometimes of larger size.	Circular, or oblong grey dots, with firm edge, and consisting of closely set, coherent masses of cocci; chain formation sometimes more marked than in case of Strep. Med. Cocci sometimes of larger size.

All stained well by Gram's method & by the ordinary aniline dyes.

Then follows the results of the examination of 12 Aural discharges, pus from 1 submental abscess, and 27 examinations of the tonsils of 18 patients, in all stages of the disease, from the 4th, day to the 9th week. The organisms found were as follows :-

(1) Aural discanrges - 12 cases. -

Strep. Conglom., not found at all.
Strep. Med., in 5 cases.
Strep. Brev., in 1 case.
Staph. Aur., in 7 cases.
Staph. Alb. Liquescens in 7,
Staph. Alb. in 2.
Xerosis Bacillus in 5,
Friedlander's Pneumo-bacillus in 1,
Diphtheria Bacillus in 1.

(2) Nasal Discharges - 12 cases. -

Strep. Conglom. in 2,
Strep. Med. in 5
Strep. Brev. in 2,
Staph. Aur. in 8,
Staph. Alb. Liq. in 6
Staph. Alb. in 6.
Staph. Citreus Liquesc. in 2,
Staph. Citreus in 2.
Bacillus Xerosis in 3,
Hofmann's Bacillus in 1,
Bac. Mesentericus in 1,
Sarcinae in 2.

(3) Submental abscess Pus - 1 case. -
Strep. Conglom., and
Staph. Alb. Liquesc.

(4) Tonsillar Mucus - 18 cases; 27 examinations.

Strep. Conglom. in 20,
Strep. Long. in 10,
Strep. Med. in 19,
Strep. Brev. in 11,
Staph. Aur. in 8,
Staph. Alb. Liquesc. in 14,
Staph. Alb. in 18 and
Staph. Citreus in 10 specimens.

In the throat examinations it was a noteworthy fact that in only two cases Strep. Conglom., was absent throughout; in the remaining 16 it was found constantly, if not at the first examination, at a later period.

On injection into mice on 10 occasions, Strep. Conglom, caused illness in every case, and death occurred in 8 of the animals, from 4 to 37 days after inoculation.

In the next Report (1899-1900) Gordon draws special attention to the variation in form of the individual organisms of the Strep.Conglom. All Streptococci show bacillary forms occasionally, but in Strep Conglom., this phenomenon was noted so frequently as to cause it to be regarded as characteristic of the species. The bacilli which occur may be bullet-shaped, wedge-shaped, pear-shaped, like dumb-bells, segmented spindles or actual rods; in the last case they correspond in outline to segments of chains, showing the curves, and often lying parallel to one another. They often closely resemble Diphtheria Bacilli. Great care was taken to ensure that the cultures used were absolutely pure, so as to exclude all possibility of bacillary contamination. The bacillary forms were most readily found in impression films of a one-night's agar culture, or of colonies on a 10 day gelatin plate; also after passage of the organism through mice. a small one, in which conglomeration is best seen, and a larger one, which tends to form chains. The two types are interchangeable, a mouse inoculated with the one showing pure culture of the other in the organs post mortem. Sometimes the organisms recovered consists entirely of the bacillary form.

Notes two typical types of organism,

Acid formation, and clotting of milk he found to vary, though alkali formation never occurred, in any form of the organism. Culturally, in broth, litmus milk, agar, and gelatin, all the forms produced exactly the same growths and reactions as the original from which they developed, the only variation being in the form and arrangement of the individual elements. Special stress is laid on examination of the water of condensation at the bottom of culture tubes.

A Streptococcus obtained from the pleuritic fluid of a case of Scarlet Fever is described. It tallied in all respects culturally with the Strep.Conglom.

EXAMINATION OF THE SCARLATINAL CADAVER. - 10 CASES - were examined altogether, and a Streptococcus of conglomerate type, and identical in most respects with Strep. Conglom., was obtained from the following organs: -

Cervical Glands	in 6	out of 7,	and in pure culture in 4.
Tonsil.....	3	" - 3,	in mixed culture.
Heart's Blood.....	8	" - 9,	& in pure culture in 5.
Lung	4	" " 4,	in mixed culture.
Bronchial Gland	1	" - 1,	in mixed culture.
Spleen	7	" " 9,	in pure culture in 5.
Kidney	6	" "10,	-------do------ 5.
Liver	6	" " 9,	-------do------- 5.
Pus (Cervical abscess and ankle joint)	2	" " 2,	in pure culture,
Blood from vessel ...	1	" " 1,	in mixed culture.

In the 10 cases, cultures were made from 56 sources, 45 of which yielded Streptococci, on 26 occasions in pure culture.

10 of these Streptococci clotted milk, and 35 failed to do so. 6 of the ten were otherwise identical morphologically and culturally with the 35; the remaining 4 were identical in all respects with Strep.Conglom., giving the typical

appearances in broth, and on gelatin and agar, - very coherent conglomeration, ~~xxxxx~~ bacillus-formation, "lace work" like arrangement round the colonies, with rapid acid formation and clotting in litmus milk. The cultural characteristics of the other 41 differed as follows :- in broth there was clear, or slightly turbid fluid with a conglomerate growth, but not so firmly coherent as in the case of Strep. Conglom., at the bottom; chain formation was also evident, and bacillary forms were rarely present; on agar the growth was more profuse than that of Strep. Conglom., and showed less chain work, and none of the tubercles in the colonies. In agar condensation fluid either conglomeration or long chain formation was the more prominent feature, both being present; marked bacillus-formation was noted, as was also "lace-work", unlike cultures of Strep. Conglom., from the Scarlatinal throat.

On gelatin the growth was rather faster than in the case of Strep. Conglom., from the Scarlatinal throat, though not faster than the latter when passed through mice. Bacillary forms were very rarely met with on prolonged culture, unlike Strep. Conglom., Nodulation was sometimes seen.

Litmus Milk was made acid, but not clotted. In pathogenicity to mice, the organism from the cadaver greatly exceeded Strep. Conglom., from the Scarlatinal throat. On passage of Strep. Conglom. through animals, and its recovery from their organs post-mortem, an exact cultural correspondence was still unobtainable, with that found in the cadaver, though there was a slight approximation in some points.

In the identification of these Streptococci with Strep. Conglom., the special points noted were formation of conglomerate masses in broth, appearance of bacillary forms on continued growth in gelatin, and in agar condensation fluid, and an appearance of a "lace-work" arrangement of the chains in the latter. The power of clotting milk varied greatly, as did also, in proportion to the number of organisms inoculated, the pathogenicity to mice. Anaerobic cultivation gave the same results as aerobic.

Sections of organs showed the Streptococci in situ in the following instances :-

Tonsil in 3 out of 3 cases, with infiltration and invasion from the surface inwards.

Enlarged Cervical Glands in 5 out of 5 cases; most marked in the outer edges, in the "lymph channel".

Spleen in 2 out of 9 cases examined.

Kidney in 1 out of 9 cases.

Liver in 1 out of 5 cases.

Heart's Blood Smears in 5 out of 8 cases.

In one Heart's Blood Bac.Coli alone was seen; otherwise Strep.Conglom. was the only organism seen in any instance in situ. Other organisms found culturally were Staph.Aur., Staph.Alb., Pneumococcus, Bac.Coli., and Bac. Diphtheriae, but they exhibited none of the constancy of the Strep. Conglom.

In two further cases of Scarlet Fever, a Strep.Conglom., virulent to mice, and a Strep.Pyog.Long., non-virulent, were obtained from each throat.

For comparison, Streptococci from 30 different sources in diseased conditions were examined. None corresponded to Strep.Conglom., but were all of the type Strep. Medius or Pyogenes. They were obtained as follows :-

	Ear discharge - Scarlatina	4
Nasal	Nasal discharge --do--	3
	Tonsil ..... --do--	1
	Follicular Tonsillitis	1
	Tonsillitis	1
	Diphtheria	1
	Phlegmon	1
	Abscess	2
	Bubo	2
	Joint suppuration	2
	Empyema	1
Pleural Effusion (serous) in Erysipelas ...		1
	Acute Pleurisy	1
	Peritonitis	2
	Meningitis	2
	Heart's Blood - Pyaemia	1
	Spleen in Septicaemia	2
Heart's Blood - puerperal septicaemia		2

In examination of broth cultures from these, he found that turbidity was of no account, as the same organism showed variations at different times. Growth occurred as flocculi, granules, or scattered flakes, mostly at the bottom of the tube, and sometimes adherent to the sides. No conglomeration appeared as a rule, though occasionally it occurred to a slight extent. Bacillary forms were rare. In agar condensation fluid Strep. Medius or Pyogenes shows short or medium chains and flocculi of cocci, rarely bacilli, and very occasionally slight "lace-work" arrangement of the chains. In litmus milk there was usually acid formation, without clotting. In fact, examination of these gave results almost identical with those described in the previous year's report. Pathogenicity to mice varied, but often exceeded that of Strep.Conglom.

Compared with Strep.Conglom., Strep.Pyog. or Medius showed different appearance in broth, less marked chain-formation on agar and gelatin, and greater uniformity of size and shape of the individuals in all media, the rarer occurrence of bacillary and spindle forms being specially noticeable.

In the following year (Report of M.O.to L.G.B. 1900-1) Gordon published the results of further researches on the same subject. The special points to which he directed attention were in connection with the examination of aural discharges in Scarlet Fever, the comparison and relative proportions of streptococci present in the throats of mild,uncomplicated cases of Scarlet Fever, and in Diphtheria, and a further comparative investigation of Strep. Pyogenes. The effects of succesive passages through mice on Streptococci,virulent for these animals, obtained from the Scarlatinal tonsil, was also noted. Measured dilutions of the tonsillar mucus were used in all cases, and the culture medium was solidified blood-serum (horse) instead of agar.

i. Mucus from 10 scarlatinal tonsils (mild cases) was first examined; Strep.Conglom.was obtained from all, and in 7 of these it was in pure culture; 6 specimens were pathogenic for mice.

Strep.Pyog. was obtained from 3 cases, and these were all in a very early stage (2nd,3rd,and 4th,days); this organism was fatal to mice in each instance. The occurrence of a virulent Strep.Pyog. in early stages, and in abundance, Gordon regarded as pointing to the fact of its bearing an important rôle in this stage of the disease; but its absence in the other 7 cases negatived anything but a subsidiary rôle in the causation of the strictly scarlatinal process.

ii. A similar examination was next made of 3 suspected cases of Scarlatina. In 2, no typical Strep.Conglom. was found, though several were present which resembled it slightly, but were non-pathogenic for mice; and there were no subsequent symptoms. In the third,however, Strep. Conglom., and Strep.Pyog., both pathogenic for mice, were found, and the case subsequently showed slight desquamation. The source of the epidemic during which these 3 cases occurred was traced to a girl at a dairy farm; she had no rash, but showed slight sore throat, and pin-point desquamation; a typical,pathogenic Strep.Conglom., was recovered from her throat.

iii. The tonsillar mucus from 6 cases of Diphtheria was next examined, and streptococci noted. In 2 cases a non-virulent,conglomerate streptococcus was obtained, and this usually clotted milk, and occasionally showed bacillary forms on agar. In the remaining 4 cases virulent streptococci were obtained, 3 of which resembled Strep. Conglom., morphologically and culturally, showing bacillary forms, and clotting milk; and the other resembled Strep.Pyog., showing a few bacillary forms, but not clotting milk. One nasal discharge was examined, but it only showed diphtheria bacilli.

iv. 7 cases of Scarlatinal ear discharge were examined, and none of the bacilli found were identifiable with Strep.Conglom., but appeared to approach Bac.Diphtheriae in some instances, and Bac.Hofmann,or Bac.Xerosis, in others. 5 out of the 7 showed streptococci, and one of these was virulent,and identical with Strep.Conglom., while the others corresponded to Strep.Pyog.

v. 8 Streptococci, identical with, or closely resembling Strep.Conglom., and isolated from Scarlatinal ~~throats~~ throats, were each successively inoculated into a series of mice. 5 were recovered unchanged from the third mouse post mortem; 3 had undergone some modification, and one of these was now indistinguishable from Strep.Pyog. This last, when first isolated from the throat of the Scarlatinal patient, differed from Strep.Conglom. in not clotting milk, and only slightly acidifying it, otherwise corresponding. The specimens of true Strep.Conglom. underwent no change nor modification at all.

vi. Finally comes a further comparative study of Strep.Pyog., specially carried out with a view to finding whether the organism ever exhibited characters which could make it identifiable with either Strep. Conglom., or the organism recovered post-mortem from the organs of fatal cases of Scarlet Fever, as described in the previous year's report, and at that stage of the investigations supposed to be merely a modified form of the Strep.Conglom., found in the throats of patients suffering from the disease. The specimens used were obtained from (1) the heart's blood of a patient dead from a non-scarlatinal septicaemia, originating from the region of the larynx. This organism on culture was indistinguishable from that obtained most frequently post-mortem from the scarlatinal organs, showing "lace-work", bacillary forms, and conglomeration (though not quite ~~xxxxxxxxx~~ to the same extent as Strep.Conglom.) in agar and broth, and slight acid-formation, but no clotting, in litmus milk. (2) and (3), from the spleens of two cases dead from Pneumonic Plague, gave exactly the same results as the first. Therefore Gordon concluded that the organism he isolated most frequently from the viscera of fatal cases of Scarlet Fever was only an unusual form of Strep.Pyog, and not, as he thought previously, a modified form of Strep.Conglom. He believes also that its rôle in Scarlet Fever, as in the cases of Pneumonic Plague just cited, is subsidiary or secondary to which the disease must be primarily attributed.

Gordon's FINAL CONCLUSIONS are therefore that there are two organisms which play an important part in Scarlet Fever - the Streptococcus Conglomeratus and the Streptococcus Pyogenes.

Strep.Pyogenes may be present on the surface of the tonsil in the early ~~xxxx~~ stages of the disease, with Strep.Conglomeratus, which is always present. In the nasal and aural discharges Strep.Pyogenes is much more frequently present than Strep.Conglom. Finally Strep.Pyog., in some cases invades the blood and organs and so appears to be directly responsible for the death of the patient.

Strep.Conglomeratus, however, probably plays a more important part. The streptococcic invasion, to which the death of the patient is often due, has been traced to the tonsil, on the surface of which Strep.Conglom

is always present in Scarlet Fever, frequently in pure culture. Its absence in non-scarlatinal conditions is noteworthy. It occurs comparatively rarely in the aural and nasal discharges of Scarlet Fever. On inoculation into mice it was found to undergo no cultural nor morphological change in 5 out of 8 instances, and remained absolutely as distinct from Strep. Pyogenes as originally; and it appears to be capable of producing a general disease in the mouse and guinea pig, with a fatal termination, while itself remaining restricted to the site at which it was inoculated. This last point may explain the occurrence of those exceptionally rapidly fatal cases of "toxic" scarlatina, in which no organism is recoverable from the blood or organs after death.

In the more slowly fatal cases Strep. Pyogenes seems to outstrip Strep. Conglom. and may even seem to have led to the complete suppression of the latter - the original cause of the disease.

Gordon is of opinion that Strep. Conglomeratus occupies a place in the bacterial kingdom between Strep. Pyogenes and Bac. Diphtheriae, but that, in spite of its bacillary forms, it is to be regarded as a peculiar Streptococcus rather than as a Bacillus.

III. W. J. CLASS announced (Chicago Medical Recorder, May 1899) his discovery of a large diplococcus, which he claimed as the specific organism of Scarlet Fever. A full description appeared in the New York Medical Record Sept 2. 1899.

During the course of an examination of blood serum cultures from between 700 and 800 throats he noticed that about 1 in every 20 showed large diplococci mixed with the other germs present, and that these most frequently occurred in cultures from scarlatinal throats.

Examination was next made, on the same medium, of cultures from the desquamation scales of Scarlet Fever, and it was found that the same germ was constantly present, though it exhibited a very marked polymorphous character.

Class next substituted for the blood serum a special medium of glycerin-agar, to which was added 5 per cent of black garden soil. On this pabulum the diplococcus grew in the form of a very large Gonococcus, best seen in old cultures. When lightly stained it was of biscuit shape, with a slightly cupped appearance, the division line between the two cocci being often very indistinct. By its subdivision tetrads, and then groups of smaller cocci, were produced, the latter not showing diplococcal characteristics in their early stages, but occurring firmly bunched together, owing to the presence between them of a glutinous intercellular substance. The appearances varied in each successive subculture, and daily in the original. The organism showed no capsule, spores, nor flagella, and had no independent motion in a hanging drop.

Streptococcal forms were occasionally, but very rarely, met with. It was recovered in pure culture from the blood in early stages of Scarlet Fever as a diplococcus, the two individuals being globular. The organism stained well by the ordinary aniline dyes, but was decolourised by Gram's method, though not to the same extent as the Gonococcus.

On Glycerin-soil-Agar at 35 deg.C., (with which the best results were obtained,) growth from the scales occurred as slowly forming, small, whitish-grey, semi-transparent colonies, isolated at first, but coalescing later. The colonies were of very glutinous consistency, being drawn out, when removed by the needle, in strings, like glue. Later they lost their viscidity, and became darker in colour.

The presence of the blood seemed to exert an inhibitory influence on their growth, and they were not obtained from this source in the later stages of the disease. There was no growth on gelatin, agar-agar, glycerin-agar, or in bouillon, and milk was not coagulated. The organism was slow growing and of poor vitality, being readily killed by heat. The cultures obtained by the plate method grew very poorly. On Glycerin-soil-agar alone was the growth at all plentiful.

Class obtained his diplococcus from the scales in 74 cases of Scarlet Fever, from the throat in large numbers in about 50 cases, and from the blood in 22 cases, but he does not state how many were examined altogether; the inference is that it was present in all.

As a control, he examined 23 specimens of scales (mostly dandruff from the scalp) from healthy persons, and obtained his diplococcus in 3; he found it in very small numbers in 8 out of 36 throats, both in health and disease (not Scarlet Fever); blood from 10 healthy persons gave absolutely negative results. He appends a list of 14 common cocci of the skin.

Class also states that he only found Streptococci in the throat in Scarlet Fever in 30 per cent of the cases in the later stages, and usually when a severe angina was present; they never formed the bulk of a culture; they were rarely found in the early stages (1st or 2nd day), and were never present in the blood. Occasionally his diplococcus was found in chains of 4 individuals in the blood, but never more. The diplococcus was non-pathogenic, rapidly losing its virulence, for rabbits and guinea-pigs, but produced what appeared to be a form of Scarlet Fever in white swine, which exhibited an erythematous rash, and subsequent desquamation round the site of the inoculation.

Jacques, who experimented with specimens of Class' diplococcus, reported (Journ. of Americ. Med. Assoc, May 26, 1900) that his results confirmed those of Class. He also notes that occasionally the conditions seemed to favour the production of a streptococcal form of organism, short chains appearing on the ordinary culture media.

IV. BAGINSKY AND SOMMERFELD (Berliner Klin. Woch. July 2 and 9, 1900) described the results of an investigation carried out by them on the same subject. First, a previous investigation of Baginsky on the bacteriology of Diphtheria is mentioned. In the course of this he noted that in cases which subsequently developed Scarlet Fever as a new infection, the diphtheria bacillus was supplanted by streptococci. This served as the basis for the investigation of the bacteriology of Scarlet Fever.

To begin with, a microscopical examination of smear preparations (in some cases also of cultures) was made from the pharyngeal mucus of 366 cases of the disease. The results were as follows :-

Cocci only, chiefly streptococci, were found in 336.
Klebs. Löffler's Bacillus, with streptococci, in 22 cases which were complicated with Diphtheria.
Cocci, with various other bacteria, in 5 cases.
Cultures on Löffler's Blood-serum were made from 62 cases, and

Streptococci were found in pure culture in 4;
Streptococci and Staphylococci in 29 cases ; and
Streptococci, with diplo: or pneumo-cocci, and other organisms, in 29 cases.

Lumbar puncture was performed in 1 case, with coma and convulsions, and the fluid examined.
It contained Streptococci in pure culture. Blood from the same case also gave a pure culture of streptococci.

42 Fatal cases were next examined post mortem. In 8 of these, death occurred early (in 2 to 5 days from onset of symptoms), so precluding the possibility of a secondary affection having developed; in the remaining 34, secondary affections were present. Cultures were made from the Heart's blood, bone marrow (generally tibia) and in the majority of cases from the lungs, spleen, kidney, liver, bronchial and mesenteric glands, and gall bladder.

In every instance a Streptococcus was obtained, and in the cases examined immediately after death, in pure culture. It occurred in pure culture in all cases in the heart's blood and bone marrow; occasionally it was noted in tetrads. In pathogenicity to guineapigs the organisms were very variable; agglutination experiments were negative.

Culturally, the morphology varied according to the medium used, the chains consisting of from 3 or 4 to 50 or more units, which also showed variations in size. The organisms stained well by Gram's method. In bouillon

the fluid was usually clear, with a thick, heavy adherent, precipitate; occasionally it was turbid. Litmus milk was acidified and clotted. Growth on agar was in small, round, yellowish brown colonies, with finely punctuated, but not quite regular, border. Growth on gelatin was slow. Apart from the tendency to form conglomerate masses, no special characteristic was detected by which the organism might be differentiated from the streptococci.

Baginsky and Sommerfeld claimed a specific role in Scarlet Fever for this organism, on the strength of its constant presence in the body in the disease; its variable virulence for animals, the virulence being increased by successive passage through animals; and the fact that artificial media on which they grow acquire toxic properties.

They deduced that all the clinical manifestations of Scarlet Fever may be explained by the presence of the Streptococcus in the internal organs (infection), and by the poisonous action of its metabolic products (toxicity).

COMPARISON OF THE ORGANISMS OF

KURTH, CLASS, BAGINSKY & SOMMERFELD, AND KLEIN & GORDON.

Owing to the differences in the methods of examination used by the different observers, it is not possible to identify all, or any two, of the organisms as being the same. Kurth's observations were very limited in extent; Class gives insufficient data on several points, notably the results of growth of his diplococcus on agar and gelatin (which he described as negative); Baginsky and Sommerfeld seem to have made the most of their observations on organisms obtained from the cadaver; none of the first three examined impression preparations of colonies, nor the appearance of the organisms in the condensation fluid of their solid media, so that Gordon's special points of "lace-work" arrangement, and appearance of bacillary forms of the organisms, were not observed.

The principal points of difference and resemblance are shown in the following table :-

	KURTH	CLASS	Baginsky and Sommerfeld.	Klein and Gordon.
Morphology.	Streptococcus, tending to form conglomerate masses; a few individuals exceed the rest in size.	Large diplococcoccus, very polymorphous in size and shape; varying in successive generations; markedly conglomerate in arrangement. Occasional very short chains.	Chains vary greatly in length; longest in broth. Appears to be a diplococcal organism, even in the chains. Individuals vary in size. Some conglomeration.	Cocci in pairs, varying in size, and occurring in chains, groups, and conglomerate masses of chains. Bacillary forms frequent. Smaller cocci favour conglomeration, and large ones chain formation.
Staining.		Almost completely decolourised by Gram.	Well by Gram's method.	Well by Gram's method.
Found in.	Tonsillar mucus, cervical abscesses, kidney and spleen.	Blood, throat and desquamation.	Blood, throat secretions, and internal organs and marrow.	Throat and nose secretions. Blood, rarely in aural discharge.
Bouillon.	Clear fluid; grey flocculent mass of chains at bottom	Clear fluid; short chains occasionally, (Jacques). No growth at all, (Class).	Clear fluid, occasionally turbid; thick heavy adherent precipitate of long chains.	Fleecy mass of long twisted chains at bottom, very coherent. Medium clear.
Agar.	Not described.	No growth (Class) Glycerin-soil-agar:- small whitish grey semi-transparent colonies, which coalesce and darken later; very glutinous.	Small, round, slightly yellowish brown colonies, with very finely punctuated but not quite regular border.	Small, round, grey, semi-transparent colonies, becoming brownish by transmitted light; with irregular outline due to "lace-work" and tuberculated surface; sometimes coherent, without tubercles; always a granular glossy, coherent centre.

Continuation of Table.

	KURTH.	CLASS.	Baginsky and Sommerfeld.	Klein and Gordon.
Gelatin.	Not described.	No growth. (Class)	Very Slow growth; fine colonies.	Small colonies, like crenated, knobbed grey discs; cocci slightly larger, and in longer chains, at lower temperature.
Milk.	Coagulated.	Not coagulated.	Coagulated and acidified.	Usually, but not always coagulated and acidified rapidly.
Pathogenesis.	Not described.	Non-pathogenic or only feebly virulent for rabbits and guinea pigs; rapidly loses the little virulence it may possess.	Very variable.	Variable.

RUEDIGER (Journ. Amer. Med. Assoc., Oct 13,1906) described the results of the examination of the throats of 154 persons, viz:-

51 normal & healthy	5 cases of Tonsillitis;
75 cases of Scarlet Fever;	5 of Pneumonia; and
14 cases of Measles:	4 of Pharyngitis.

He found Streptococcus Pyogenes constantly in great abundance on the tonsils in Tonsillitis, and Scarlet Fever, before the inflamation in the throat has subsided, the number rapidly decreasing with subsidence of the local condition.

In normal throats Strep. Pyogenes was found only in small numbers in 58 per cent of the cases examined.

Pneumococci of low virulence were found in 135 of the 154 throats.

A large group of organisms intermediate between typical Strep. Pyog, and Pneumococcus was found in all the normal, and nearly all the diseased throats. They have little virulence for rabbits, and appear to be normal inhabitants of the throat. Those taken from scarlatinal throats had slightly more virulence for rabbits than Strep. Pyogenes.

DIFFERENTIATION OF STREPTOCOCCI (Gordon)-

In the Report of the M.O. to the L.G.B. 1903-4, Mervyn H. Gordon describes a further investigation for the differentiation of streptococci by their bio. chemical reactions with various carbon compounds.

He found that, as the result of their growth in neutral-litmus- tinted broth, of very slightly alkaline reaction, and containing a small quantity of various reagents of the carbo. hydrate, glucoside, and higher alcohol series, different streptococci caused the decomposition of different reagents, setting free acid, which was indicated by the litmus becoming red.

Experimenting with a very large number of reagents, he found that some had to be discarded at the outset, owing to changes they underwent during preparation of the media; others gave more or less constant results with most streptococci; while a smaller number gave varying results with different organisms.
The following were selected as most likely to be of service in distinguishing the various strains (of streptococci) from one another:-

CARBOHYDRATES.

MONOSACCHARIDES.-

Pentoses $C_6 H_{12} O_5$ - Rhamnose was tested, but gave only slightly differential reactions.

Hexoses $C_6 H_{12} O_6$ - Glucose, fructose, mannose, and galactose.- These were all decomposed by all the streptococci experimented with, so were discarded.

DISACCHARIDES. Maltose $C_{12}H_{22} O_{11}$ ~~and~~ + H_2O - gave constant positive (acid) reactions, so was discarded.

Saccharose $C_{12} H_{22} O_{11}$ and Lactose $C_{12}H_{22}O_{11}$ ~~and~~ + $H_2 O$ - proved to be of useful differential value, some streptococci decomposing them, and others having no action on them.

TRISACCHARIDES. - Raffinose $C_{18} H_{22} O_{16}$ ~~and~~ + $5H_2 O$ - also gave variable results.

POLYSACCHARIDES - $(C_6 H_{10} O_5)n$

Dextrin gave constant acid reactions, and starch (which proved difficult to prepare),, Arabin, and Glycogen were constantly unaffected, so were discarded.

Inulin brought out variations, so was retained.

GLUCOSIDES.

18 of these were tested, and variable results were obtained with 8 - Amygdalin, Arbutin, Digitalin, Helicin, Populin, Syringin, and Coniferin $C_{16} H_{22} O_8$ ~~and~~ + $2H O$, and Salicin $C_{13} H_{18} O_7$. The last two, as showing the greatest variation were retained.

Polyatomic Alcohols - 6 of these were tested, and differential results obtained with Glycerin $C_3 H_8 O_3$, Sorbite $C_6 H_{14} O_6$, and Mannite $C_6 H_{14} O_6$. The last one, as giving most variation, was retained.

Other tests which were investigated were - decomposing action on Acid Amides - Caffein, Asparagin, and Urea were examined, and Oils - Sperm, and Cod Liver, but negative results were obtained with all.

Also the following further tests:-

Reduction of Nitrate to Nitrite - this proved negative in the case of the streptococci, but positive with staphylococci. Production of sulphuretted Hydrogen from lead Acetate - this gave only slight differentiál results.

Change of colour in broth containing an Aniline dye - Indigo sulphate, Methylene Blue, and Azur, aerobically and Neutral Red a anaerobically, were examined. Of these the last one gave the best differential results, so was retained.

The Test Substances finally selected as giving the best differentiál results were therefore:-

1 Saccharose
2 Lactose
3 Raffinose
4 Inulin
5 Salicin
6 Coniferin
7 Mannite

all in litmus-tinted broth.

8 Neutral Red, in plain broth (anaerobically)

9 Litmus Milk.

All at 37°C.

10 Gelatin, at 20°C.

The character of the growth in ordinary broth was also noted, and that on agar, on blood serum, or on "Nasgar" (nutrose-ascitic-agar).

The broth used was, of necessity, sugar free.

This condition was first obtained by inoculating it with Bacillus Coli, incubating it for 3 days at 37°C., sterilising, and filtering; but Houston found that by using broth made from 1 per cent Lemco (with 1 per cent Peptone) instead of the usual fresh beef, much trouble was saved, and the substitute being of constant composition, as well as sugar-free, gave excellent results.

Neutral litmus, 10 per cent in aqueous solution, was added in the proportion of 10 per cent of the broth.

Sodium Bicarbonate 0.1 per cent, and the Test substance 1 per cent, completed the medium.

Formula - Lemco 1 per cent.

Peptone 1 per cent.

Sod. Bicarb. 0.1 per cent.

Test substance 1 per cent.

10 per cent Aqueous Neutral Litmus 10 per cent.

Distilled water 87 per cent.

10 streptococci from widely different sources were severally inoculated on the above test media, and reinoculated twice subsequently, identical results being obtained in each of the 3 series, and all the streptococci giving different combinations of reactions.

It was found that though in some cases an organism gave positive reactions in various media in 24 hours, a considerably longer incubation was necessary to bring out the maximum powers of the less active germs, so first 3 days, and subsequently 7 days, was adopted as the standard period of incubation.

Making use of these tests, Gordon examined

(1) 300 Streptococci from 22 specimens of saliva of healthy persons. These were found to be of 48 different types; the only characteristics common to all were a positive reaction with Saccharose, and a negative with mannite.

(2) 18 streptococci from diseased conditions, viz;-
(a) 13 from various septic and septicaemic processes, including Scarlet Fever. These showed 6 types; none of them reacted with Raffinose or Neutral Red, and 5 failed to change Coniferin or clot Litmus Milk; 3 gave a positive reaction with Mannite.

(b) 5 from cases of ulcerative endocarditis. These showed 4 types. All clotted milk, and none reacted with Mannite; 3 gave positive reactions with Raffinose and Neutral Red, and 2 were positive with Coniferin.

None of group (2) reacted with Inulin.

Several streptococci were found in group (2) which gave similar reactions to members of group (1).

(3) 10 Streptococci from various septic conditions and 1 air streptococcus, before and after passage through mice.

9 of these gave the same results in both cases; 2 showed slight differences, 1 having acquired a positive effect with salicin, the other having lost its power to decolourise Neutral Red.

(4) 14 streptococci from various sources - typhoid stool, horse-dung, oyster liquor, milk, dust, and air.

These all differed from one another to a greater or less degree.

An investigation on similar lines was made in regard to the differentiation of Staphylococci, a series of test substances being found which served to bring out marked differences between individual specimens in a way similar to the foregoing method for Streptococci.

INVESTIGATION BY F. W. ANDREWES & T. J. HORDER.-

Working on the lines suggested by Mervyn H. Gordon, Andrewes and Horder made an investigation of the streptococci Pathogenic for man (Lancet, Sept 15,22, & 29, 1906) with the purpose of finding any specific characters by which the streptococci occurring in various morbid conditions might be identified.

First they dismiss Marmorek's theory that all streptococci pathogenic for man are fundamentally identical, (as shown by the fact that bonillon in which one sort of streptococcus has grown is incapable of serving as a culture medium for any other sort, and that all alike show similar haemolytic power), as not supported by the results of more varied tests. Next they describe as inadequate Von Lingelsheim's (1891) classification according to the length of chain formation, agreeing ~~to~~ with him that as a general rule the more virulent streptococci form the longer chains, with a flocculent or granular deposit in a clear broth, and the short chained forms, with clouded medium are usually of low, or no virulence. Chains frquently vary greatly in length in one culture, and depend entirely on the degree of cohesion between the individual cocci - a variable factor.

Schottmüller's classification of streptococci according to their haemolytic action on human blood in plates of blood-agar, is insufficient, as it only relies upon a single characteristic.

In describing streptococci morphologically, they find that growth in broth is the only one which furnishes data which are of any real value, the differences on such media as agar-agar and gelatin being too variable for purposes of differentiation. The formation of bacilli they admit may be of use, but too little is known of this faculty yet for any final verdict to be pronounced.

They adopt the following as the most convenient terminology in regard to growth characteristics in broth:-

1. Brevissimus - forms occurring chiefly as diplococci.

2. Brevis - slightly longer chains (4 to 8 elements).

   Both these render broth ~~of moderate-lengt~~ uniformly turbid.

3. Medius - chains of moderate length (8 to 16 elements). Macroscopically this type is usually intermediate between the short and long streptococci.

4. Longus - long chained forms.

5. Longissimus - chains of extreme length. Macroscopically these two forms usually show clear broth with stringy or granular deposit.

6. Conglomeratus - long-chained forms contorted into dense balls, and similar in macroscopic appearance to the long streptococci.

As regards the temperature at which growth occurs, they find that many of the pathogenic organisms which as a class thrive best at blood temperature, will not grow at all at 20°C.

A very few liquefy gelatin.

Staining properties are of no value, as all are Gram-positive, and stain well with the ordinary aniline dyes, and none are acid-fast.

Pathogenic power for rodents they also discard, as this fluctuates so markedly according to cultural conditions, virulence being very readily lost or gained.

Serum reactions they find do not discriminate accurately between different streptococci.

Agglutination tests show varied, but graduated differences, and both they, and determination of opsonic indices are very difficult and unreliable.

They therefore decide that in the present state of our knowledge, Gordon's tests form the only reasonable means of differentiation.

In carrying out these tests on 228 specimens of streptococci from all available varieties of diseased conditions, special attention was directed to the constancy of the tests with the same organism, by testing several colonies from one original culture; by testing specimens obtained from different persons who had become infected from the same source; by retesting organisms after very prolonged subculture, and also after passage through animals. The results

invariably showed remarkable constancy, and except in a small number of instances in which the Salicin and Neutral Red reactions were lost or gained, and in two cases in which the Mannite reaction was lost, the reactions in all the media remained identical in every subsequent examination with those in the original one.

Variations in virulence were sometimes accompanied by variations in the intensity with which different test substances were attacked. Sometimes a positive reaction could be obtained under anaerobic conditions where none occurred in aerobic culture.

In order to form an opinion of the value of Gordon's tests as to correspondence between differences revealed by them and differences of other kinds, such as distribution in nature, pathogenesis, and other biological characters, Andrewes undertook the correlation of all the published records obtainable in which streptococci had been tested by Gordon's method. These included:-

Normal flora of the mouth	(Gordon)	300
Normal flora of the intestine	(Houston)	300
Streptococci of milk	(Houston)	172
Streptococci of air	(Gordon)	200
Pathogenic Streptococci	(Gordon)	18
Various sources - air, milk, sewage, Animal's intestines	(Andrewes & Horder)	Number not stated.
Pathogenic Streptococci, human	(Andrewes & Horder)	228

At first the results were very confusing, for no definite distinction could be made out by which the organisms could be sorted into well marked groups, distinct from one another.

On the contrary, they gradually merged into each other, so that nowhere could a hard and fast line be drawn.

By using Gordon's tests alone, they found it impossible to classify the different species at all adequately, but by taking into consideration the other differences mentioned above, it was possible to arrange them roughly into seven groups, all of which, however, still showed the same merging into the others as in the case of the individual strains. Generally speaking, groups A,B,D, & F consist of Saprophytic streptococci, F being a facultative parasite; C comprises the "pyogenes" class, E the "anginosus" class (having a special connection with inflamation of the fances, and Scarlet Fever), and

G the "pneumococci". C & E consist as a rule of long chained streptococci, the remainder being chiefly short chained forms.

They therefore conclude that though no one of Gordon's tests is of absolute and constant worth, while several are certainly of little value, taken together, and in conjuction with other characters, the tests afford a clue to the nature of any given form of streptococcus which is invaluable, and form the most important advance which has been made in recent years in the study of the streptococci.

In their examination of the streptococci obtained from diseased conditions, Andrewes and Horder found the same diversity of type as was seen in the results of the correlation mentioned above. They were able, however to arrange them in 5 main type groups in each of which was a number of variants from what they adopted as a type form. In all, some 85 variations were obtained amoung the 228 individual specimens, and of these 160 were definitely pathogenic, and 68 were associated with disease. Of the 68, Streptococci from scarlatinal throats accounted for 33.

In order to appreciate the results in regard to Scarlet Fever, it is necessary to briefly summarise the characteristics of the 5 groups;-

GROUP 1. STREPTOCOCCUS PYOGENES- Negative reactions with milk, neutral red, raffinose and inulin, vigorous growth on gelatin at 20°C, long to medium chains in broth and a speedily fatal result when inoculated into a mouse soon after isolation from the patient's blood or tissues.

12 of the streptococci from scarlatinal throats fell into this group, 8 being variants from the type form.

79 were from other sources.

GROUP 2. STREPTOCOCCUS SALIVARIUS. - Short chains in broth, which becomes uniformly turbid; frequent but not constant inability to grow on gelatin at 20° C., positive reactions with milk, neutral red, saccharose, lactose, often raffinose, but not Mannite, nor as a rule inulin; often also with salicin and coniferin. Virulence feeble or absent.

1 Streptococcus from a scarlatinal throat, and it a variant from the type form, came under this heading.
32 were from other sources.

GROUP III STREPTOCOCCUS ANGINOSUS. - This is merely a long-chained form of strep. salivarius, of somewhat greater virulence, and resembling strep. Pyogenes in its haemolytic powers. It is most commonly associated with inflamed throats.

20 Streptococci from scarlatinal throats came under this group, 17 being variants from the type form, 34 were from other sources.

GROUP 1V - STREPTOCOCCUS FAECALIS - A positive Mannite reaction was adopted as an artificial, though useful, distinction between this, and the Salivarius group. The chains varied in length, as did also the power to grow on gelatin at 20°C., and to clot milk. The inulin reaction was usually negative.

No Streptococci from Scarlet Fever fell into this or the following group.
16 Streptococci from other sources were classified in group 1V.

GROUP V PNEUMOCOCCI - This group includes all streptococci occurring with a well-defined capsule. Chains in broth are usually short, with uniform clouding of the medium, though in agar condensation fluid the chains are often of considerable length. These were characterised by great difficulty in getting them to grow in artificial media.

34 Streptococci from other diseased conditions than Scarlet Fever belonged to this group.

All the types were connected with one another by gradual transitions.

Referring to the result of their examination of the 33 Streptococci from scarlatinal throats, and comparing them with the streptococci from other sources, Andrewes and Horder find themselves unable to draw any definite conclusions as to the causative organism of that disease, it being still unproven that streptococci do more than play an important part in the secondary infections, though frequently they are the actual cause of death. In no disease do they figure more prominently in the secondary complications.

Their method was to make agar plate cultures from dilutions of the tonsillar mucus, and after examination of individual colonies under the low power of the microscope, to subculture specimens of all the varieties noticed in Gordon's series of test media.

The first obvious point was that that "Anginosus" and "Pyogenes" forms occurred by far the most frequently, and almost to the exclusion of other kinds.

The short chained forms of Streptococci, which predominate in normal saliva, appeared to be entirely replaced in the scarlatinal throat by long forms, even in the earliest stages of the disease.

Of the two longed chained forms "Anginosus" appears to be the more frequent, and it resembles Klein's

Streptococcus Scarlatinae or Conglomeratus in clotting milk, but differs in growing on gelatin at 20°C. only with great difficulty.

Streptococcus Pyogenes being so commonly the agent of suppuration, it is unlikely that it is the primary cause of a specific, communicable disease like Scarlet Fever, though quite able to be a very important factor in the causation of the accompanying complications.

Streptococcus Anginosus, on the other hand, was found in many other conditions than Scarlet Fever, viz:- Malignant endocarditis, pericardial effusion of acute rheumatism, simple tonsillitis, membranous stomatitis, peritonitis secondary to appendicitis, mastoid abscess in otitis, suppurative pachymeningitis, empyema, septic finger, sinus in a tuberculous knee-joint, etc.

Sometimes it was not found at all in scarlatinal throats.

Finally, Andrewes and Horder offer the following suggestions as to the nature of Scarlet Fever:-

1. The disease may be of primary streptococcal origin, but not due to any one specific streptococcus.

   Such a view would explain the occurrence of Scarlet Fever after burns, and surgical operations, without known exposure to specific infection. It would be in harmony with the occurence of scarlatiniform rashes in septic conditions due to streptococci (septicaemias etc). It would be in accord with the varied nature of their results in the application of Gordon's tests to streptococci from the scarlatinal throat. But it is a view very difficult to maintain in face of the strong clinical evidence that Scarlet Fever habitually breeds true, and spreads from case to case as a specific entity.

2. The disease may be primarily due to a specific streptococcus, as maintained by Klein, Gordon, Kurth, and others.

   Much may be said in favour of this view, and if it be maintained, the specific organism will in all probability be found within the limits of the group described as Streptococcus Anginosus. Nevertheless, their results fail to indicate any one variant as constant in, or peculiar to, the Scarlet Fever throat. A much larger series of cases must be examined before the matter can be settled on such lines as these.

3. Scarlet Fever may be due primarily to some non-streptococcal cause, perchance ultra-microscopic, such as Mallory's protozoon "Cyclaster Scarlatinalis".

Even on such a view, streptococci might still retain an overwhelmingly important rôle as causes of the graver symptoms, and complications of the disease, and antistreptococcal therapy would remain perhaps the most important element in treatment. There is always the risk that an organism, held out to be the primary cause of a disease, may prove in the end to be merely a secondary invader.

PERSONAL OBSERVATIONS.

Primarily with the idea of finding out the frequency with which the Diphtheria Bacillus was to be found in in the throats of Scarlet Fever patients at the time of admission to hospital, I made and examined cultures on blood serum from the throats of 160 consecutive cases sent into the Bury & District Joint Infectious Hospital, during the winter and spring of 1905 - 6. All were in the first week of the disease.

At the same time, as a control experiment, I made similar cultures from 40 healthy throats of country children who, so far as I knew, had had no opportunity of coming in contact with scarlatinal cases.

The results were as follows:-

Organisms found.	In Scarlatinal Throats.	In Healthy Throats.
Streptococci & Diplococci	30	14
Streptococci & Staphylococci	13	1
Strep., Diplococc., & Staph.	29	5
Strep., Diploc., Staph., & Bacilli	28-(1 Bac.Diph.)	4
Strep., Staph., & Bacilli	9-(3 Bac.Diph.)	2
Strep., & Bacilli	3-(1 Bac.Diph.)	0
Strep., Diploc., & Bacilli	16-(2 Bac.Diph.)	6
Strep., Diploc., & Yeasts	1	0
Strep., Staph., & Yeasts	4	0
Strep., Staph., Bacilli & Yeasts	1	1
Strep., Diploc., Bacilli, & Yeasts	1	0
Strep., Diploc., Staph., Bacilli & Yeasts	2-(1 Bac.Diph.)	0
Strep., Diploc., Staph., & Yeasts	6	0
Diplococci	5	0
Diplococci & Staphylococci	4	0
Diplococci & Bacilli	6-(2 Bac.Diph.)	1
Diploc., Staph., & Bacilli	5-(1 Bac.Diph.)	3
Diploc., Staph., Bacilli & Yeasts	1-(1 Bac.Diph.)	2
Staph & Bacilli	5-(2 Bac.Diph.)	1
	160	40

SUMMARISING.-

	Scarlatinal	Healthy
Streptococci	134, or 84 per cent	33, or 83 per cent
Diplococci	125, or 78 per cent.	35, or 88 per cent
Streptococci or Diplococci	155, or 97%	39, or 98%
Staphylococci	98, or 61%	19, or 48%
Diphtheria Bacilli	14, or 9%	0

It will be seen from this that as regards the presence of Streptococci and Diplococci (many of which prove to be streptococci on cultivation in fluid media) scarlatinal and healthy throats closely correspond. Staphylococci are rather more numerous in the scarlatinal throats.

The presence of the Diphtheria Bacillus, which corresponded both in morphology, and staining reactions (Gram's, Neisser's, and Pugh's methods), and in cultural appearances with the Klebs-Löffler Bacillus, was unattended in any case by clinical signs of Diphtheria.

One difference between the two conditions was that, as a general rule, the streptococci were in much greater numerical proportion to all the other organisms present in the scarlatinal than in the healthy throats.

During the same period I examined in the same way blood-serum cultures from the purulent nasal discharges of 67 cases of Scarlet Fever. Only 6 normal healthy noses were similarly examined, there being no suspicion of infectious disease about any of them.

The following were the organisms found:-

Organisms found	In Scarlatinal Noses.	In Healthy Noses.
Streptococci & Diplococci	2	0
Streptococci & Staphylococci	6	0
Strep., Diploc., & Staph.	5	1
Strep., Diploc., & Bacilli	2-(2 Bac.Diph.)	1
Strep., Staph., & Bacilli	8-(5 Bac.Diph.)	0
Strep., Staph., & Yeasts	1	1
Strep., Diploc., Staph., & Bacilli	4-(1 Bac.Diph.)	2
Diplococci	1	0
Diplococci & Staphylococci	8	0
Diplococci & Bacilli	9-(7 Bac.Diph.)	1
Diploc., Staph., & Bacilli	3-(1 Bac.Diph.)	0
Staphylococci	3	0
Staphylococci & Bacilli	12-(9 Bac.Diph.)	0
Bacilli	3	0
	67	6

Summarising.-	Scarlatinal	Healthy
Streptococci	28, or 42%	5
Diplococci	34, or 51%	5
Streptococci or Diplococci	49, or 73%	6
Staphylococci	50, or 75%	4
Diphtheria Bacilli	25, or 37%	0

It would be obviously inaccurate to take percentages from the small number of healthy noses examined, but roughly speaking, the streptococci and Diplococci again correspond in the two, and staphylococci are relatively more frequently present in the scarlatinal noses.

The Diphtheria Bacillus appears much more frequently in the discharging nose of Scarlet Fever than in the throat.

17 cases of Scarlet Fever with ear discharges were next taken, and cultures made from the discharge in each, and the following is a description of the organisms obtained:-

Streptococci & Diplococci	found in	3	cases.
Strep., Diploc., & Staph.	"	" 2	"
Strep., Staph., & Bacilli	"	" 1	" (1 Bac.Diph)
Strep., Bac.Diph., & Bac.Pyocyaneus		1	" (1 Bac.Diph)
Diplococci & Staphylococci	found in	3	"
Diploc., Staph., & Bacilli	"	" 2	"
Staph., Pyog., Aur.	"	" 1	"
Staphylococci & Bacilli	"	" 2	" (1Bac.Diph)
Bac. Diphtheriae	"	" 1	" (1Bac.Diph)
Bac. Diphtheriae & Leptothrix	"	" 1	" (1Bac.Diph)
		17	

SUMMARISING.-

Streptococci	7, or 41 per cent
Diplococci	10, or 59 per cent.
Streptococci or Diplococci	12, or 71 per cent.
Staphylococci	11, or 65 per cent.
Diphtheria Bacilli	5, or 29 per cent.

Streptococci and Diplococci were present in much the same proportion of cases as in the nose, and in considerably less than in the throat.

Staphylococci were more frequent than in the throat, and less frequent than in the nose.

The Diphtheria Bacillus (which possibly should be regarded merely as a diphtheroid type, though indistinguishable from the true Bac. Klebs-Loffler) was relatively much more commonly found in the ear discharge than in either the nose or the throat.

Cultures were also made on blood-serum from other sources in Scarlet Fever cases, with the following results:-

Desquamation Scales - - 2 cases - -
Diplococci, & Staphylococcus Albus, found in each.

Fine pustular skin eruption, present with the rash - 2 cases -

Diplococci	found in	2
Staphylococcus Albus	" "	1
No growth	in	1

Blood (2nd. day) - - 6 cases - (cultures in a number of media, both solid and liquid)

Streptococcus medius (made up of Diplococci)	in	1 case,
Diplococci (no chain formation)	in	1 case.
No growth	in	4

Pus from a cervical gland - - 6 cases -
Streptococci found in 6

Pus from suppurating fingers - - 4 cases -
Staphylococcus Albus or Aureus in 4

Pus from a large gumboil - - 1 case -
Streptococci, Diplococci, and Staphylococci.

Pus from the mastoid antrum - - 2 cases -
Streptococci in 2.

Thus in the Adenitis, in the blood, and in the mastoid infections, the organisms found in pure culture were very similar to those most frequent numerically in the throat. From their constant presence in so true secondary conditions as adenitis and mastoiditis, the evidence that streptococci play an important part in the etiology of Scarlet Fever is very strong.

A series of 20 cases of Scarlet Fever in all stages of the disease was next examined in greater detail, with special reference to the Diplococci present.

The method adopted was as follows:-

A swab was taken (with sterilised apparatus) from the throat or nose, washed in 10cc. of sterile normal saline, and a very small loopful of this smeared over the surface of an agar plate. The plate was then incubated at 37°C for 24 hours, after which the resulting colonies were examined under the low power of the microscope, and their appearance noted; a

specimen of each type of colony which showed only diplococci was then examined microscopically in a spread film or an impression preparation; sub-cultures were next made fromall the different types of Diplococcal colonies present, on agar-agar (stroke; a few by the stab method), in bouillon, litmus milk, gelatin (at 37°C), and on gelatin (stroke) at 20°C. Class' glycerin-soil-agar medium was also.made use of in a number of instances.

The first point noted was the great variety of form, within certain limits, exhibited by the various colonies on agar, even of what appeared to be the same organism. This difference was most noticeable in the early stages of growth, of nearly all the organisms, before the relatively small ultimate size of the streptococcal colonies had become evident.

The second point brought out was the impossibility in many instances of recognising a growth as that of a streptococcus until its appearance in bouillon culture had been examined microscopically, the organism so frequently appearing only as a diplococcus on solid media, or at most in short series which, from their appearance might merely be due to accidental arrangement in the preparation of the film, rather than the process of development.

The chief efforts were directed towards identifying, if possible, the Diplococcus of Class, and determining, so far as might be, its relation to other Diplococci. As, according to Class, the organism grows readily on his glycerin-soil-agar medium, but not at all on plain peptone agar, I first made a series of about 50 parallel cultures on agar, glycerin-soil-agar, and blood-serum, from scarlatinal throats and noses, and very carefully compared the growths obtained on the three. As was to be expected, streptococci, diplococci, and staphylococci grew slightly less readily on the blood-serum than on the two agar media, but I could detect no difference whatever between the growths on the agar and glycerin-soil-agar tubes. Nor did the individuals among the various diplocccci display any greater tendency to grow to an unusually large size on the soil-agar than on the plain agar.

The glycerin-soil-agar medium being difficult to prepare and sterilise, and inconvenient to work with, it was therefore discarded for routine work, and only used occasionally, when it was thought advisable to determine the nature of the growth of a particular organism on it.

The cultural characteristics of the diplococci found were as follows:-

1.- Present in the throats of 16 cases.-

Agar - 24 hours at 37°C - creamy white colonies, smooth, shiny, with raised centre, and thin, well-defined, opalescent border; under the low power

the colonies appeared brownish and granular, with a dark brown core-like centre, usually round, but often spindle shaped, with pointed extremities; the colonies varied a good deal in appearance according to age, in some only the "core" being present, and in others the "core", surrounded by a thin, even band of granular consistency.

Under the high power the colonies were found to consist of small diplococci of the same shape as the Gonococcus, the adjacent surfaces being ~~feathered~~ flattened. In some instances individual organisms were noticed which were of very much larger size than the rest; these were usually absent after the first or second day's incubation. On lifting a portion of a colony with the needle, the mass was seen to be coherent and sticky, and microscopically the organisms arranged themselves in groups, difficult to break up. There was no chain formation visible. When examined on subsequent days the organisms were seen to vary somewhat in size, but not in shape or arrangement.

Gelatin.- 4 days at 20°C - Fine bluish-grey colonies, similar microscopically to those on agar; no liquefaction. In stab culture, a larger colony, round, smooth, white, shiny, and raised, formed over the point of entrance, and round the needle track were many small grey colonies in the substance of the medium.

Bouillon.- 24 to 48 hours, at 37°C.- The medium was usually, but not always, slightly clouded, and at the bottom, and adherent to the bottom of the tube, was a large coherent, woolly mass, of greyish-white colour; only on vigorous shaking could this be disturbed, when thick, tough, ropy masses rose sluggishly, and subsided again.

Microscopically, the growth was found to consist of diplococci of rather larger size than on the agar; the division between the individuals was more marked, and the same coherent grouping was seen. A few short chains were usually visible, but as in many of them all the individual pairs lay with their septa in the long axis of the chain, these series may have been merely due to an accidental arrangement, and not to the process of development. In one instance only the short chain arrangement was found throughout the medium.

Gelatin.- 24 to 48 hours at 37°C- showed similar appearances both macroscopically and microscopically to the bouillon.

Litmus Milk - 3 days at 37°C - In every case an acid reaction was obtained, though this varied somewhat in degree, and the milk was coagulated.

ii Present in the throats of 4 cases.-

Agar - 24 hours at 37°C - Smaller, very irregular colonies with deeply indented outline, which corresponded roughly to the lobulations of a very, uneven centre or "core". The border was thin, broad, and well-defined, and in some colonies did not completely surround the thick centre. In the earlier stages an appearance of "dehiscence" from the "core" was produced. Microscopically, diplococci of large size, with some individuals much in excess of the rest in calibre, were found. Some of the latter showed division into four, and they stained much more deeply than those of smaller size. Subcultures on agar from these colonies showed none of the very large organisms at any period of their growth.

Bouillon - 24 ~~hrs~~ to 48 hours at 37°C - The medium was clear, except for a few suspended tags. At the bottom was a large white, woolly mass, similar to that in the first type, but not quite so coherent. Microscopically it showed similar organisms to those seen in the agar culture, with the same arrangement as in the previous type. None of the very large individuals was found in any of the short chains. The other cultures gave similar results to the previous type.

iii Present in the throats of 6 cases.-

Agar - 24 hours at 37°C - Raised, round, white, thick colonies, granular, with thickened centre, brownish by transmitted light. Microscopically they consisted of small diplococci, mostly in masses, and a few of them forming short chains. The organisms were uniform in size.

Bouillon. - 24 to 48 hours, at 37°C - Clear, with coherent woolly mass at the bottom, from which a few fragments were detachable on shaking. Microscopically, diplococci of rather large size were found, all arranged in short chains, except in one case, in which both masses and short chains appeared.

Gelatin. 24 to 48 hours, at 37°C - Similar to Bouillon.

Other cultures similar to the preceeding types.

iv Present in the throats of 2 cases -

A Diplococcus resembling the first type in all but the following points.-

In Bouillon the ropy mass disintegrated after 2 days' growth into smaller flocculent, but individually coherent masses. Microscopically it appeared the same as type I.
Litmus milk was unaffected.

V Present in the throat of 1 case.-

Agar - thin, whitish, translucent, and opalescent colonies, with uneven spreading border, and a "core" which presented a distinctly radiating appearance. Microscopically there were diplococci of various sizes, though none of the very large type, arranged in groups, singly, and a few in short chains.

Bouillon.- Finely granular deposit in a slightly clouded medium, showing microscopically small diplococci singly and in short chains.

Gelatin at 20°C - Small opalescent colonies, no liquefaction.

Litmus Milk. - coagulated, with acid reaction.

VI Present in the throat of 1 case.-

Agar - Pale, thin, granular colony, with spotty centre. Microscopically - diplococci of various sizes, singly and in groups, but no chains.

Bouillon.- Clouding, with a fine granular deposit at the bottom. Microscopically - similar to the growth on agar, with a very slight tendency to chain formation.

Litmus Milk. - 3 days at 37°C - a gelatinous, mucus-like clot formed, but there was no pink reaction, the blue colour being only slightly bleached.

In one case, in which diplococci corresponding to type II were found, a further examination was made after the patient had had a fortnight's vigorous local treatment by spraying and swabbing with antiseptics. A diplococcus was recovered which gave, in all but the primary agar culture, similar appearances; but in the first agar colonies it appeared microscopically in a great variety of irregular shapes, frequently the individuals forming a single diplococcus differing markedly from one another.

In one case cultures were made from the desquamating cuticle, and 3 different diplococci were obtained:-

i corresponded to Type III described above.

ii showed small, reddish, coarsely granular colonies on agar; a large ropy, coherent mass at the bottom of slightly clouded bouillon; no liquefaction of gelatin;

coagulation of milk, with formation of acid; and microscopically small diplococci singly and in groups, with no chain formation.

iii Produced large, spreading, white, moist colonies on agar; did not liquefy gelatin; did not affect litmus milk; and microscopically consisted of large coarse-looking diplococci, singly and in groups; in bouillon a few (accidental?) chains were noticed.

All the diplococci found were well stained by Gram's method.

Several were inoculated on glycerin-soil-agar but in each instance the growth differed in no respect from that on ordinary agar.

SUMMARY

From these results it will be seen that no diplococcus was found which at all resembled that described by Class.

All grew readily in bouillon, whereas his did not; from only 2 throats was a diplococcus isolated which did not coagulate milk; all grew well on agar as well as the glycerin-soil-agar, while that of Class only grew with difficulty on agar: and finally, unlike that of Class, all the above diplococci stained readily by Gram's method.

The outstanding points in common with the diplococcus of class were the conglomerate arrangement so commonly found among the specimens examined, and the varying sizes, often in the one culture.

That each type described above would have been found to represent many sub-types if further differential test cultures had been made, is highly probable, but only those tests used by Class himself were adopted.

No control examinations of healthy throats were made in this series, but one important point in regard to scarlatinal throats was brought out incidentally, viz.- the fact that though the commonest diplococcus was only obtained in 16 (80 per cent) of the 20 throats examined, streptococci of various kinds were present in all, and in a considerable proportion the streptococcal colonies of themselves constituted a majority in the agar plates.

In the course of a later investigation of throat streptococci in health and disease, the diplococcus described as Type I was met with frequently, in all conditions of the throat.

SPECIAL CASES-

Of the above series, two cases are worthy of mention as being of special interest. Both were boys of about 9 years of age, who had been inmates of the

Bury Infirmary, - W.M. for 5 months, and S.R. for 3 weeks before infection occurred.

W. M. was under treatment for Morbus Coxae, and had several discharging sinuses about the left hip, and also showed unmistakable signs of early pulmonary ~~[illegible]~~ tuberculosis. He was in a general surgical ward with 15 or 16 men and 2 other children. Without any discoverable source of infection, and many weeks after operation, he suddenly developed all the classical signs of Scarlet Fever, the "strawberry" tongue, congested fances, and punctiform rash being very well shown.

The eruption commenced round the region of the sinuses, and remained more intense there than elsewhere on the body; after removal to the Infectious Hospital it was noticed that one sinus in particular had begun to discharge more freely and offensively than before, while the temperature rose and fell at night, and in the morning with much greater emphasis than formerly. Desquamation followed in due course, and the progress of the case became comparatively uneventful, and except for the presence of the hip disease, typical of an ordinary, moderately severe type of Scarlet Fever.

Bacteriological examination of the throat on the first day after the appearance of the rash showed the presence of streptococci, large and small diplococci, and ~~[illegible]~~ staphylococci.

Pus from the hip showed streptococci similar in cultural and morphological appearance to those from the throat; some chain-forming bacilli were also seen.

On the 9th day of the disease, otorrhoea commenced on the left side; the discharge on its first appearance, was examined, and found to contain a few very short chains of streptococci, similar to those mentioned above; a number of short thick, Gram-negative bacilli, and a very large number of bacilli indistinguishable from the Diphtheria Bacillus, either culturally, or by staining methods. As the patient was absolutely isolated from all other cases, and attended by a special nurse, he could hardly have become diphtheritically infected in the Infectious Hospital. On the day flowing the appearance of the otorrhoea, Diphtheria Bacilli, ~~[illegible]~~ were recovered from both the pharynx and the nasal passages, and remained there for about 4 weeks; after which it dried up in response to treatment. 8 weeks later it recommenced, the pus being of a bluish green colour this time; microscopically streptococci were again found, but this time with a very large proportion of typical Bacillus Pyocyaneus. This second discharge only lasted for a few days, after which it dried up, and the patient was shortly returned to the care of the surgeons.

the otorrhoea persisted for 3 weeks,

S.R., after being three weeks in the same ward in the Bury Infirmary, but a year after W.M. had left, was operated on for the radical cure of hernia. Four days after the operation, he complained of sore throat, vomited, and the region of the wound became red and tender, a typical scarlatiniform rash appearing the same day, and spreading from the vinicity of the wound all over the body. The stitches were removed and next day pus appeared. The patient was sent to the Infectious Hospital, where he was isolated, and attended by a special nurse. Desquamation commenced in a week, and the patient passed through a typical moderately severe attack of Scarlet Fever. On admission, there were found in the pharyngeal mucus short chains of streptococci, diplococci, staphylococcus Pyogenes Aureus, and mouth bacilli.

In the wound pus, and in the pus of a small scrotal abscess which was opened on the third day, were found diplococci [redacted] and short chains of streptococci. In the fifth week the throat showed diplococci of various sizes, and mouth bacilli.

In each of these cases every effort was made to determine a possible source of infection, but none could be found. None of the hospital staff had been exposed to scarlatinal infection, nor had any of the visitors to any of the patients in the ward. At the time that each occurred there were no cases of Scarlet Fever in the neighbourhood of the Infirmary nor of the patient's home, nor had there been any notified from these localities for some time previously.

The question therefore suggests itself - Can [redacted] true Scarlet Fever arise as the result of a change in the function of a septic organism such as Streptococcus Pyogenes, already present?

EXAMINATION OF STREPTOCOCCI.

From my first series of examinations of scarlatinal and healthy throats it was seen that Streptococci or Diplococci were present in about 97 per cent of each, i.e. were practically constant, and streptococci in 83 or 84 per cent of each, but relatively more numerous in Scarlet Fever.

In the second series, consisting of scarlatinal cases alone, in which the organisms were cultivated in bouillon as well as on solid media, streptococci were obtained in every case, and in most , in larger numbers than all the other organisms together.

Consequently it seemed to me that a more thorough comparison of the individual streptococci found in scarlatinal and normal throats might lead to the discovery of some general point of difference, possibly denoting a tendency towards a higher functional evolution of the streptococcimin Scarlet

Fever, whereby they are enabled to attack the tissues in their characteristic manner.

Accordingly I decided to follow up the preceding results with a further examination on the lines suggested by Mervyn H. Gordon, [1.28] in the scheme previously described for the differentiation of Streptococci by their cultural reactions with the series of organic substances found by him to bring out the differences best, so little having been done so far in this direction in the investigation of Scarlet Fever.

For comparative purposes I was handicapped at the outset by being unable to obtain any Coniferin, either from any of the home or any of the continental manufacturers, there being, it appeared, a dearth of the raw material. So that I was obliged to restrict myself to the following tests:-

Growth in
(1) Litmus Milk,
(2) Neutral Red broth (anaerobically)
(3) Saccharose broth,
(4) Lactose broth.
(5) Raffinose broth.
(6) Inulin broth.
(7) Salicin broth.
(8) Mannite broth.
(9) Nitrate broth (for production of Nitrite)

all incubated at 37°C for 7 days.

(10) Character of growth, if any, on Gelatin at 20°C, and
(11) Macroscopic & Microscopic appearances in ordinary "Lemco" broth, grown at 37°C for 48 hours.

All the Media were prepared in strict accordance with the methods laid down by Gordon, "Lemco" being used instead of fresh meat, in order to obtain sugar-free pabula.

100 separate streptococci were taken from the following SCARLATINAL sources:-

80	specimens	from 26	different	throats.
6	"	" 2	"	noses.
8	"	" 2	"	discharging ears. (different patients)
6	"	" 2	"	cases of mastoid infection (from Pus)

i.e. 100 specimens from 27 different Scarlatinal cases.

To compare with these, there were
50 specimens from 20 different Healthy Throats.

And to make the comparison more complete, 50 specimens from OTHER DISEASES (not Scarlet Fever) were taken, as follows.-

30 specimens from 6 different Diphtheria Throats.
13 " " 5 " Catarrhal "
7 " " 1 case of Follicular Tonsillitis with abscess formation.

i.e. 50 specimens from 12 diseased throats.

METHOD.-

In each case a swale, or large platinum loop-ful of mucus (or pus), from the part to be examined was well washed (or diluted) in 10ccm of sterile normal saline solution, and a very small quantity of this inoculated on agar, and blood serum or "Nasgar" (nutrose-ascitic-agar) plates or tubes, and the resulting colonies, after incubation for 24 hours at 37°C examined macroscopically, and with the hand lens, and when possible, microscopically; a number of as different appearances as possible were then inoculated into tubes of "Lemco" broth, and incubated at 37°C for 48 hours.

These were then examined microscopically and macroscopically, and such as showed morphological differences, however slight, were inoculated from the bouillon into the test-media; the latter were then arranged in rows in tins fitted with partitions, and incubated for 7 days at 37°C, excepting, of course, the gelatin tubes, which were incubated at 20°C.

Gordon advised the use of blood-serum instead of agar, as the former excluded many of the non-pathogenic organisms. Owing to its opacity, however, I found it a most inconvenient medium to work with, and accordingly substituted "Nasgar", which Gordon also recommended. This, when well made, is almost indistinguishable in appearance from agar, but I cannot say that I found much difference between the growths on the two media, though in almost every case both media were used.

"Nasgar" is prepared as follows.-

A. Ascites fluid 15ccm.
Distilled water 35c.cm
Nutrose 1 gm.

Put into a flask, and bring to the boil, constantly shaking till the fluid boils. Filter.

B. Ordinary Peptone Agar.

Mix 1 part of A with 2 parts of B, steam for 30 minutes, filter, and put into tubes.

Whether or not it was owing to the lack of sugar in the plain "Lemco" broth, I had great difficulty in getting streptococci to grow in it, frequently finding a batch of a dozen or more tubes absolutely

sterile after the 48 hours' incubation.

The organisms grew readily enough in the test media as a rule, though a number of series had to be discarded owing to the lack of growth in one or two individual tubes.

This uncertainty of growth, taken together with the fact that I was obliged to prepare all my fluid media personally, rendered this observation a most laborious business..Altogether it involved the making and examination of over 4000 cultures.

Owing to bleaching of the media in some cases, when examined after or during the week's incubation, some difficulty was experienced in determining whether or not acid had been produced. As a rule a definite colour reappeared on thoroughly shaking up and so aerating the medium, and leaving on the bench for 24 hours; but occasionally it was necessary to resort to the use of litmus papers to discover the present reaction. Anaerobic incubation had no effect in restoring colour to a bleached specimen.

All the streptococci stained well by Gram's method.

Having finally obtained the results of this detailed examination, the next question was - how to classify them?.

The first method adopted was according to the origin or source of the organisms, i.e. under the headings "Scarlet Fever", "Healthy", "Diphtheria" etc, and the following set of tables show this, all the organisms from each case being shown in detail, and the different case groups being separated off from one another by heavier lines:-

Scarlet Fever.

No.	Reaction to Gordon's Tests: Litmus Milk: Acid	Litmus Milk: Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate	(+ or − refers to Liquefaction.) Growth on Gelatin at 20°C	Morphology	Source	Stage
1	+	+	−	+	+	+	−	+	−	−	Small Grey Colonies −	Strep. Long.	Throat	4th day
2	+	−	−	+	+	+	−	+	−	−	−	Strep. Med.	Throat	3rd day.
3	+	+	−	+	+	−	−	+	−	−	−	Strep. Brevis	Throat	3rd day
4	+	+	−	+	+	+	−	+	−	−	−	Str. Med.	Throat	13th day.
5	+	+	−	+	+	+	−	+	−	−	−	Str. Long.	Throat	5th week
6	+	+	+	+	−	−	−	−	−	−	−	Str. Med. to Long	Throat	13th week
7	+	−	+	+	+	+	−	−	−	−	−	Str. Conglom.	"	"
8	+	+	+	+	+	+	+	+	−	−	−	Str. M. to L.	"	"
9	+	+	+	+	−	−	−	−	−	−	−	Str. Long.	Nose	"
10	+	+	+	+	−	−	−	−	−	−	−	Str. Long.	"	"
11	−	+	−	+	−	−	−	−	−	−	Fine Colonies −	Str. Long.	"	"
12	−	+	−	+	−	−	−	−	−	−	−	Str. Long.	"	"
13	+	+	−	+	+	+	−	−	−	−	−	Str. Med.	Throat	4th day.
14	+	+	+	+	+	−	−	+	−	−	−	Str. M. to L.	Throat	6th week
15	+	+	−	+	+	−	−	+	−	−	−	Str. M. to L.	"	"
16	+	−	−	+	+	−	−	+	−	−	−	Str. M. to L.	Throat	7th week
17	+	+	−	+	+	+	−	−	−	−	−	Str. M. to L.	"	"
18	+	+	−	+	+	+	−	+	−	−	−	Str. M. to L.	Throat	6th week
19	+	+	−	+	+	+	−	−	−	−	−	Str. M. to L.	Throat	2nd day.
20	+	+	−	+	+	+	−	−	−	−	−	Str. Med.	Throat	2nd day
21	+	+	−	+	+	−	−	+	−	−	−	Str. Long.	"	"
22	+	+	−	+	+	+	−	+	−	−	−	Str. Longiss.	Throat	6th week
23	+	+	−	+	+	−	−	+	−	−	Grey Colonies −	Str. Long.	Throat	12th week.
24	+	+	−	+	+	−	−	+	−	−	Grey Colonies −	Str. Long.	Throat	7th day
25	+	+	−	+	+	−	−	+	−	−	−	Str. Long.	Throat	4th day.
26	+	+	+	+	+	−	−	−	−	−	Fine Colonies −	Str. Long.	Throat	9th day
27	+	+	+	+	+	−	−	−	−	−	Fine Colonies −	Str. Long.	"	"
28	+	+	+	+	+	+	+	−	−	−	−	Str. Long.	Throat	9th week.
29	+	+	+	+	+	+	+	+	−	−	−	Str. Longiss.	"	"
30	+	+	+	+	+	−	−	+	−	−	−	Str. M. to L.	"	"
31	+	+	+	+	+	+	−	+	−	−	−	Str. M. to L.	"	"
32	+	+	+	+	+	−	−	+	−	−	−	Str. Med.	Throat	2nd day
33	+	+	+	+	+	−	−	−	−	−	−	Str. Long.	"	"
34	+	−	−	+	+	−	−	−	−	−	−	Str. Longiss.	"	"
35	+	−	−	+	+	−	−	−	−	−	−	Str. Long.	"	"

Scarlet Fever.-

No.	Reaction to Gordon's Tests. Litmus Milk: Acid	Litmus Milk: Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate	(+ or − refers to Liquefaction) Growth on Gelatin at 20°C.	Morphology	Source	Stage
36	+	-	-	+	+	-	-	-	-	-	Fine Colonies –	Strep. Conglom.	Throat	7th day
37	+	+	+	+	+	+	-	+	-	-	Grey Colonies –	Str. M. to L.	Throat	4th day
38	+	+	+	+	+	+	-	-	-	-	–	Str. Long.	"	"
39	+	-	+	+	+	-	-	+	-	-	–	Str. Long.	"	"
40	-	-	+	+	-	+	-	-	-	-	–	Str. Med.	"	"
41	+	-	-	+	+	-	-	-	-	-	Fine Colonies –	Str. Long.	"	"
42	+	+	+	+	-	-	-	-	-	-	Fine Colonies –	Str. Longis.	Throat	2nd day
43	+	+	+	+	+	-	-	-	-	-	–	Str. Long.	Ear	8th day
44	+	+	+	+	+	-	-	-	-	-	–	Str. Long.	"	"
45	+	+	+	+	+	-	-	-	-	-	–	Str. Longis.	"	"
46	+	+	-	+	+	-	-	-	-	-	–	Str. Long.	"	"
47	+	+	-	+	+	-	-	-	-	-	–	Str. Longis.	"	"
48	-	-	-	+	-	-	-	-	-	-	Small Colonies –	Str. Longis.	Pus from Mastoid	3rd week
49	-	-	+	-	+	-	-	-	-	-	Small Colonies –	Str. Longis.	"	"
50	-	-	-	+	+	-	-	-	-	-	Small Colonies –	Str. Longis.	"	"
51	+	+	+	+	+	+	-	+	-	-	–	Str. Med.	Throat	3rd day
52	+	-	+	+	+	-	-	-	-	-	–	Str. Long.	"	"
53	+	+	-	-	+	-	-	-	-	-	Small Colonies –	Str. Long. to Med.	Ear	3rd week
54	+	+	-	-	+	-	-	-	-	-	Small Colonies –	Str. Long. to Med.	"	"
55	+	+	-	+	-	-	-	-	-	-	Small Colonies –	Str. Long. to Med.	"	"
56	-	+	-	-	-	-	-	-	-	-	–	Str. Long.	Throat	3rd day
57	+	+	-	+	+	+	-	-	-	-	Fine Colonies –	Str. Long.	"	"
58	+	+	-	+	+	-	-	-	-	-	–	Str. Long.	"	"
59	+	+	-	+	-	-	-	-	-	-	–	Str. Long.	"	"
60	-	-	-	+	+	-	-	+	-	-	Fine Colonies –	Str. Med.	Throat	2nd day
61	+	+	-	+	+	-	-	-	-	-	Fine Colonies –	Str. Long.	"	"
62	+	+	+	+	+	+	-	-	-	-	Fine Colonies –	Str. Long.	Throat	3rd week
63	+	+	+	+	+	+	-	-	-	-	–	Str. Long.	"	"
64	+	+	+	+	+	+	-	+	-	-	Fine Colonies –	Str. Long.	"	"
65	+	+	+	+	+	+	-	+	-	-	–	Str. Long.	"	"
66	+	+	+	+	+	+	-	+	-	-	–	Str. Long.	"	"
67	+	+	+	+	-	-	-	-	-	-	–	Str. Longis.	Nose	7th week
68	+	+	+	+	-	-	-	-	+	-	Fine Colonies –	Str. Longis.	"	"
69	+	+	+	+	-	-	-	-	-	-	Fine Colonies –	Str. Long.	Throat	18th day

No.	Reaction to Gordon's Tests										(+ or − refers to Liquefaction) Growth on Gelatin at 20°C.	Morphology	Scarlet Fever.	
	Litmus Milk		Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate			Source	Stage
	Acid	Clot												
70	−	−	−	+	−	+	−	−	−	−	−	Shep. Long.	Throat	4th day
71	+	+	−	+	−	+	−	−	−	−	−	Shep. Long.	"	"
72	+	+	+	+	−	+	−	+	−	−	−	Str. Longiss.	"	"
73	+	+	−	+	−	−	−	−	−	−	Fine Colonies −	Str. Longiss.	"	"
74	+	+	−	+	+	+	−	−	−	−	−	Str. Long.	"	"
75	+	+	−	+	+	−	−	−	−	−	−	Str. Long.	Throat	2nd day
76	+	+	−	+	+	−	−	−	−	−	−	Str. Long.	"	"
77	−	+	−	−	−	−	+	−	−	−	Fine Colonies −	Str. Long.	"	"
78	+	+	+	+	+	−	−	+	−	−	Fine Colonies −	Str. Conglom.	"	"
79	+	+	+	+	+	−	−	+	−	−	Fine Colonies −	Str. Conglom.	"	"
80	+	+	−	+	+	−	−	+	−	−	Fine Colonies −	Str. Conglom.	"	"
81	+	+	+	+	−	−	−	+	−	−	−	Str. Conglom.	Throat	8th day
82	+	+	+	+	−	−	+	−	−	−	Fine Colonies −	Str. Longiss.	"	"
83	+	+	−	+	−	−	+	−	−	−	Fine Colonies −	Str. Longiss.	"	"
84	+	+	−	+	−	−	−	−	−	−	Fine Colonies −	Str. Longiss.	"	"
85	−	+	−	−	−	−	−	−	−	−	Fine Colonies −	Str. Longiss.	"	"
86	+	+	−	+	+	−	−	+	−	−	Fine Colonies −	Str. Longiss.	"	"
87	+	+	+	+	−	−	−	−	−	−	−	Str. Longiss.	Throat	4th day
88	+	+	−	−	−	−	−	−	−	−	Fine Colonies −	Str. Conglom.	"	"
89	+	+	+	+	−	−	−	−	−	−	−	Str. Longiss.	Throat	4th day
90	−	+	−	+	−	+	+	−	−	−	Fine Colonies −	Str. Long.	"	"
91	+	+	+	+	−	+	−	−	−	−	Fine Colonies −	Str. Conglom.	"	"
92	+	+	+	+	+	+	+	−	+	−	−	Str. Long.	"	"
93	+	+	+	−	−	+	+	−	−	−	−	Str. Long.	"	"
94	+	+	+	+	+	−	−	−	−	−	−	Str. Conglom.	"	"
95	+	+	+	+	+	+	−	−	−	−	−	Str. Long.	"	"
96	+	−	−	+	−	+	−	−	−	−	−	Str. Long.	"	"
97	−	+	−	+	−	−	−	−	−	−	−	Str. Longiss.	"	"
98	+	+	−	+	+	−	+	−	−	−	Fine Colonies −	Str. Long.	Pus from Mastoid	5th week
99	+	+	+	+	+	−	+	+	−	−	Fine Colonies −	Str. Long.	"	"
100	+	+	+	+	+	−	+	−	−	−	Fine Colonies −	Str. Long.	"	"

Healthy Throats.

No.	Reaction to Gordon's Tests.										(+ or − refers to Liquefaction) Growth or Gelatin at 20°C.	Morphology
	Litmus Milk		Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate		
	Acid	Clot										
1	+	+	-	+	+	+	-	-	+	-	-	Str. med. to Long.
2	+	+	-	+	+	-	+	-	-	-	-	Str. Brev. to Med.
3	+	+	-	+	+	-	-	+	-	-	-	Str. Med.
4	+	+	-	+	+	+	-	-	-	-	-	Str. Med.
5	+	+	-	+	+	+	-	+	-	-	-	Str. Long.
6	+	+	-	+	+	-	-	+	-	-	-	Str. Long.
7	+	+	-	+	+	-	-	-	-	-	-	Str. Long.
8	+	+	-	+	+	-	-	-	-	-	-	Str. Longis.
9	+	+	+	-	-	-	-	-	-	-	White Growth +	Str. Long.
10	+	+	-	+	+	+	-	+	+	-	Grey Colonies -	Str. Med. Conglom.
11	+	+	+	+	+	-	-	-	-	-	Fine Colonies -	Str. Long.
12	+	+	+	+	+	+	-	-	-	-	-	Str. Med. Congl.
13	-	-	+	+	+	-	-	-	-	-	Fine Colonies -	Str. Med.
14	+	+	+	+	+	+	-	+	-	-	Fine Colonies -	Str. Med.
15	+	+	-	+	+	+	-	-	-	-	-	Str. Long.
16	-	-	-	+	-	-	-	-	-	-	Fine Colonies -	Str. Med.
17	+	+	-	+	+	+	-	+	-	-	Fine Colonies -	Str. Med.
18	+	+	-	+	+	+	+	+	+	-	Fine Colonies	Str. Med.
19	+	+	+	+	+	+	-	+	-	-	Grey Colonies -	Str. Long.
20	+	+	-	+	+	+	-	+	-	-	-	Str. Long.
21	-	-	-	-	+	-	-	-	-	-	Fine Colonies -	Str. Med.
22	+	+	-	+	+	+	-	+	-	-	Fine Colonies	Str. Brev.
23	+	-	-	+	+	-	-	-	-	-	Fine Colonies -	Str. Longis.
24	+	+	-	+	+	+	-	-	-	-	Fine Colonies -	Str. Longis.
25	+	+	-	-	-	-	-	-	-	-	Fine Colonies	Str. Med.
26	+	+	-	+	+	-	-	-	-	-	-	Str. Long.
27	+	+	-	+	+	-	+	+	-	-	Grey Growth +	Str. Brev. Congl.
28	+	+	-	+	-	-	-	-	-	-	Fine Colonies -	Str. Long. Congl.
29	+	+	-	+	+	+	-	-	-	-	Fine Colonies -	Str. Med.
30	-	-	-	+	-	+	-	-	-	-	Fine Colonies -	Str. Med. to Long.
31	-	-	-	-	-	-	-	-	-	-	Fine Colonies -	Str. Long.
32	+	-	+	+	+	+	-	+	-	-	Fine Colonies -	Str. Med. to Long.
33	+	-	-	+	+	-	-	-	-	-	Fine Colonies -	Str. Med. to Long.
34	-	-	-	+	+	-	-	-	-	-	-	Str. Med. to Long.
35	+	+	-	+	+	+	-	+	-	+	Fine Colonies -	Str. Med. to Long.

No.	Reaction to Gordons Tests.										(+ or − refers to Liquefaction) Growth on Gelatin at 20°C.	Morphology	Healthy Throats.	
	Litmus Milk: Acid	Litmus Milk: Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate				
36	+	+	−	+	+	−	−	−	−	−	Fine Colonies −	Str. Conglom.		
37	−	−	−	−	+	+	−	−	+	−	Fine Colonies −	Str. Long.		
38	+	+	−	+	−	−	−	−	−	−	Fine Colonies −	Str. Conglom.		
39	+	+	−	+	+	+	−	+	−	−	−	Str. Long. Congl.		
40	−	−	−	+	−	+	−	−	+	−	−	Str. Long.		
41	+	−	−	+	+	+	−	+	−	−	−	Str. Long.		
42	+	−	−	+	−	+	−	−	−	−	−	Str. Long. to Med.		
43	+	−	−	+	+	−	−	+	−	−	−	Str. Long.		
44	−	+	+	+	−	−	−	−	+	+	Fine Colonies −	Str. Long.		
45	+	+	+	+	+	+	−	+	−	−	Grey Growth +	Str. Beav.		
46	−	−	−	+	−	+	−	−	+	−	Fine Colonies −	Str. Long. Congl.		
47	−	−	−	+	−	+	−	−	−	−	−	Str. Long.		
48	+	+	−	+	+	−	−	−	−	−	Fine Colonies −	Str. Longis.		
49	+	+	+	−	−	+	−	−	−	−	Fine Colonies −	Str. Med.		
50	+	+	−	−	−	−	−	−	−	−	−	Str. Long.		

No.	Reactions to Gordon's Tests.										(+ or − refers to Liquefaction) Growth on Gelatin at 20°C.	Morphology.	Diphtheria Throats	
	Litmus Milk													
	Acid	Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate			Stage	Disease
1	+	+	−	+	+	−	−	−	−	−	−	Strep. Med.	Convalescence	Diphtheria
2	+	+	−	+	+	−	−	−	−	−	−	Strep. Med.	"	"
3	+	+	+	+	+	−	−	+	−	−	−	Strep. Med.	"	"
4	−	−	−	+	+	−	−	+	−	−	−	Str. Long.	2nd day	Diphtheria
5	+	+	+	+	+	+	−	+	−	−	−	Str. Med.	3rd week	"
6	+	+	+	+	+	+	−	−	−	−	Fine Colonies	− Str. Longis.	"	"
7	+	+	+	−	+	−	−	−	−	−	−	Str. Longis.	5th week	"
8	+	−	−	+	+	−	−	−	−	−	−	Str. Longis.	"	"
9	+	+	+	+	+	+	−	+	−	−	−	Str. Med. to L.	"	"
10	+	−	−	+	+	−	−	+	−	−	Fine Colonies	− Str. Long.	"	"
11	+	+	−	+	+	−	−	−	−	−	−	Str. Long.	6th week	"
12	+	+	−	+	+	−	−	+	−	−	White Growth +	Str. Long.	"	"
13	+	+	+	+	+	−	−	+	−	−	−	Str. Longiss.	3rd day	Diphtheria.
14	+	−	+	+	+	+	+	−	−	−	−	Str. Med. to L.	8th day	Diphtheria.
15	+	+	+	+	−	−	−	+	−	−	−	Str. Long.	2nd day	Diphtheria
16	+	+	−	+	+	−	−	+	−	−	−	Str. Long.	"	"
17	+	+	−	+	+	−	−	+	−	−	−	Str. Long.	"	"
18	+	+	−	+	+	−	−	+	−	−	−	Str. Long.	"	"
19	+	+	−	+	+	−	−	+	−	−	−	Str. Long.	"	"
20	+	+	+	+	+	−	−	−	−	−	−	Str. Long.	"	"
21	+	+	+	−	−	−	−	−	−	−	−	Str. Long.	2nd day of each	Diphtheria & Measles
22	+	+	+	+	+	−	−	−	−	−	−	Str. Long.	"	"
23	+	+	+	+	+	−	−	−	−	−	−	Str. Conglom.	"	"
24	+	+	−	−	+	+	−	−	−	−	−	Str. Long.	"	"
25	+	+	−	+	+	−	−	−	−	−	Fine Colonies	− Str. Conglom.	"	"
26	+	+	−	+	+	−	−	−	−	−	Fine Colonies	Str. Long.	"	"
27	+	+	+	+	−	−	−	−	−	−	−	Str. Conglom.	"	"
28	+	+	−	+	−	−	−	−	+	−	−	Str. Long.	2nd week	"
29	+	+	−	+	−	−	+	−	−	−	−	Str. Longis.	"	"
30	+	+	+	+	−	−	−	−	−	−	−	Str. Longis.	"	"

Catarrhal Throats.

No.	Reaction to Gordon's Tests. Litmus Milk: Acid	Litmus Milk: Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate	(+ or − refers to Liquefaction) Growth on Gelatin at 20°C	Morphology
1	+	+	-	+	+	-	-	-	-	-	-	Strep. Long.
2	+	+	+	+	+	+	-	+	-	-	-	Strep. Long.
3	+	-	-	+	+	-	-	+	-	-	-	Strep. Long.
4	+	+	-	+	+	+	-	-	-	-	-	Str. Long. Congl.
5	-	-	-	+	+	-	-	-	-	-	Fine Colonies -	Str. Conglom.
6	-	-	-	-	-	-	-	-	-	-	Fine Colonies -	Str. M. to L.
7	-	-	-	-	-	-	-	-	-	-	Fine Colonies -	Str. Med. Congl.
8	+	+	+	+	+	+	+	-	-	-	-	Str. M. to L.
9	+	+	+	+	+	+	+	-	-	-	-	Str. Long.
10	+	+	+	+	-	-	-	-	-	-	Fine Colonies -	Str. Longis.
11	+	+	+	+	+	+	+	+	-	-	-	Str. Longis.
12	+	+	+	+	-	-	+	+	+	-	-	Str. Longis.
13	+	+	-	+	-	-	-	-	-	-	-	Str. Conglom.

Quinsy Throat

No.	Reaction to Gordon's Tests. Litmus Milk: Acid	Litmus Milk: Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Nitrate	(+ or − refers to Liquefaction) Growth on Gelatin at 20°C	Morphology
1	+	+	+	+	-	-	-	-	-	-	Fine Colonies -	Str. Conglom.
2	+	+	+	+	-	-	-	-	-	-	Fine Colonies -	Str. Conglom.
3	+	+	+	+	-	-	-	-	-	-	Fine Colonies -	Str. Conglom.
4	+	+	+	+	+	-	-	-	-	-	-	Str. M. to L.
5	+	+	+	+	+	-	-	-	-	-	-	Str. Conglom.
6	+	+	+	+	+	-	-	-	-	-	Fine Colonies -	Str. Conglom.
7	+	+	+	+	+	-	-	-	-	-	-	Str. Conglom.

In these tables it will be seen that frequently the the same set of reactions is given by several organisms from a single case. The specimens in question all differed slightly in their morphological appearances in the original plate or tube culture, hence their inclusion in the list.

The first point noticeable is the extreme variety of the combinations shown. In all there are 64 different types among the 200 specimens, and all differing in one or more points from one another, but showing very slight gradations, which renders it extremely difficult to separate out distinct classes.

A second point is that 2 specimens from the same case often differ from one another in only a single reaction.

It is also noteworthy that no coincidence was found in any Scarlet Fever case between organisms obtained from the ear, when discharging, or from the mastoid antrum, and any that were obtained from the throat. In the forth case examined, an organism was obtained from the nasal discharge which gave the same reactions as one of those found in the throat.

Taking into account only the morphological differences as seen in the growth in bouillon, the following classification may be made according to the length of the chain.-

Chains	Healthy		Scarlet Fever		Other Diseases		All Diseases.	
	No.	%	No.	%	No.	%	No.	%
Longissimus	4	8	20	20	9	18	29	19
Longus	17	34	48	48	19	38	67	45
Medius to Longus	7	14	14	14	5	10	19	13
Medius	11	22	8	8	4	8	12	8
Brevis to Medius	1	2	-	-	-	-	-	-
Brevis	2	4	1	1	-	-	-	-
Conglomeratus	8	16	9	9	13	26	22	15

The longer forms of Streptococci then appear to be relatively more numerous in the diseased than in the healthy conditions, a steady tendency to the reverse appearing as one passes down the scale towards the short forms, i.e. the short forms appear relatively more numerous in the healthy throats.

As compared with "Other Diseases","Scarlet Fever" shows a considerably higher percentage of the longer forms (though not as marked a difference as is shown between "All Diseases" and "Healthy"), pointing to what Andrewes & Horder referred to as a replacement of the common short forms by long varieties, quite early in the disease.

The conglomerate forms appear to be about as numerous in diseased conditions as in health, but considerably less numerous in Scarlet Fever (which is quite contrary to the findings of Mervyn H. Gordon), and correspondingly more numerous in "Other Diseases" than in "Health".

An organism was classed as Conglomerate if, in broth culture, it appeared in a clear medium as one or more masses which were only divisible into their component chains by the exercise of considerable force, such as pressure between a slide and cover-slip, and not at all by shaking the culture tube.

As regards cultural appearance, macroscopically, there was close correspondence among the individuals in each class, the longest forms of all showing a cloudy, stringy, loose mass at the bottom of a clear medium, the long forms showing small loose flakes or granules at the bottom, or sticking to the sides of the tube near the bottom, of a clear medium; the short forms produced general clouding, usually with no deposit; and the medium forms came somewhere between the long and the short.

In size, the individual cocci forming the chains exhibited some slight variation. Some, more especially the "Longus" type, were frequently made up of very small cocci; most, however, corresponded pretty closely to an average size. Nearly all cultures showed, in their chains, occasional individuals of nearly double the size of the rest; from these there often arose branch chains.

Such variations in size and form were not found to be specially indicative of any particular source, as regards disease or health.

The next method of arrangement of the specimens is in accordance with their reactions, which are roughly classed after the plan adopted by Andrewes & Horder, as described above. No specimens were found which could be classed in Group V (Pneumococci), but each of the other four groups is represented, both by streptococci corresponding to the type forms, and

No.	Andrewes & Horder's Classification	Reaction to Gordon's Tests. — Litmus Milk: Acid	Litmus Milk: Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannite	Nitrate	Growth in Gelatin	Morphology	Scarlet Fever: No. of Specimens	Scarlet Fever: No. of Cases	Healthy: No. of Specimens	Healthy: No. of Cases	Other Diseases: No. of Specimens	Other Diseases: No. of Cases
1	I 1a	−	−	−	+	+	−	−	+	−	−	−	±	Med. to Long.	1	1	1	1	2	2
2	1c	+	−	−	+	+	−	−	−	−	−	−	±	Conglom. + Med. to Long.	5	4	3	3	2	2
3	1f	−	−	−	−	+	−	−	−	−	−	−	+	Med.	−	−	1	1	−	−
4		−	−	−	−	−	−	−	−		−	−	+	M. to L. + Congl.	−	−	1	1	2	1
5		−	−	−	+	−	+	−	−		−	−	±	M. to L.	1	1	2	2	−	−
6		+	−	−	+	−	+	−	−		−	−	−	Long.	1	1	1	1	−	−
7	1h	−	−	−	+	−	−	−	−	−	−	−	+	M. + Longiss.	1	1	1	1	−	−
8	1i	+	−	−	+	+	−	−	+	+	−	−	−	M. to L.	1	1	−	−	1	1
9	II 2d	+	+	−	+	+	−	−	+	±	−	−	−	Brev. to Med.	1	1	1	1	−	−
10	2f	+	+	−	+	+	+	−	+	+	−	−	+	Brev.	−	−	1	1	−	−
11	2i	+	+	+	+	+	+	−	+	+	−	−	+ lig.	Brev.	−	−	1	1	−	−
12	2l	+	−	+	+	+	+	−	+	−	−	−	+	M. to L.	−	−	1	1	−	−
13		+	−	+	+	+	+	+	−		−	−	−	M. to L.	−	−	−	−	1	1
14	III 3a	+	+	+	+	+	+	−	−	−	−	−	±	L. to Longiss. Mon.	4	3	1	1	1	1
15		+	+	+	+	−	+	−	−		−	−	+	Conglom.	1	1	−	−	−	−
16	3b	+	+	+	+	+	−	−	−	−	−	−	±	M. to Longiss.; Con.	7	4	1	1	7	3
17		+	+	+	−	+	−	−	−		−	−	−	Longiss.	−	−	−	−	1	1
18	3c	+	+	+	+	−	−	−	−	−	−	−	±	M. to Longiss.; Con.	8	6	−	−	6	3
19		+	+	−	+	−	−	−	−		−	−	±	L. to Longiss. Con.	4	4	2	2	1	1
20		+	+	+	−	−	−	−	−		−	−	+ lig.	Long.	−	−	1	1	1	1
21		+	+	−	−	−	−	−	−		−	−	±	M. to L.; Congl.	1	1	2	2	−	−
22		−	+	−	+	−	−	−	−		−	−	±	L. to Longiss	3	2	−	−	−	−
23		−	+	−	−	−	−	−	−		−	−	±	L. to Longiss.	2	2	−	−	−	−
24	3d	+	+	−	+	+	−	−	−	−	−	−	±	M. to Longiss.; Con.	6	4	5	4	6	4
25	3e	+	+	−	+	+	+	−	−	−	−	−	±	M. to Longiss. Con.	6	6	4	4	1	1
26	3f	+	+	−	+	+	−	−	+	±	−	−	±	M. to L.; Congl.	7	7	1	1	4	1
27		+	+	−	+	+	−	−	+		−	−	+ lig.	Long.	−	−	−	−	1	1
28		+	+	−	+	+	−	+	−		−	−	+	Long.	1	1	−	−	−	−
29		+	+	−	−	+	−	−	−		−	−	+	M. to L.	2	1	−	−	−	−
30		+	+	−	−	+	+	−	−		−	−	−	Long.	−	−	−	−	1	1
31	3i	+	+	−	+	+	+	−	+	+	−	−	±	M. to Longiss.; Con.	5	4	5	4	−	−
32	3j	+	+	+	+	+	−	−	+	−	−	−	±	M. to Longiss; Con.	5	4	−	−	2	2
33		+	+	+	+	−	−	+	−		−	−	+	Longiss.	1	1	−	−	−	−
34		+	+	+	+	−	−	−	+		−	−	−	Long.; Congl.	1	1	−	−	1	1
35	3k	+	+	+	+	+	+	−	+	±	−	−	±	M. to L.	6	4	2	2	3	2
36		+	+	+	+	+	+	+	+		−	−	−	M. to Longiss.	2	2	−	−	1	1
37		+	+	+	+	+	+	+	−		−	−	−	M. to L.	1	1	−	−	2	1
38		+	+	+	+	+	−	+	+		−	−	+	Long.	1	1	−	−	−	−
39		+	+	+	+	+	−	+	−		−	−	+	Long.	1	1	−	−	−	−
40		+	+	+	+	−	+	−	+		−	−	−	Longiss.	1	1	−	−	−	−
41		+	+	+	−	−	+	+	−		−	−	−	Long.	1	1	−	−	−	−
42		+	+	+	−	−	+	−	−		−	−	+	Med.	−	−	1	1	−	−
43	3m	+	+	−	+	+	−	+	−	−	−	−	−	Brev. to Med.	−	−	1	1	−	−
44		+	+	−	+	+	−	+	+		−	−	+ lig.	Conglom.	−	−	1	1	−	−
45	3n	+	+	−	+	−	−	+	−	−	−	−	±	Longiss.	1	1	−	−	1	1
46		+	+	−	+	−	+	−	−		−	−	−	Long.	1	1	−	−	−	−
47		−	+	−	+	−	+	+	−		−	−	+	Long.	1	1	−	−	−	−
48		−	+	−	−	−	−	+	−		−	−	+	Long.	1	1	−	−	−	−
49	3o	−	−	+	+	−	+	−	−	−	−	−	−	Med.	1	1	−	−	−	−
50		−	−	+	−	+	−	−	−		−	−	+	Longiss.	1	1	−	−	−	−
51	3s	−	−	+	+	+	−	−	−	−	−	−	+	Med.	−	−	1	1	−	−
52		+	−	+	+	+	−	−	−		−	−	−	Long.	1	1	−	−	−	−
53		+	−	+	+	+	+	−	−		−	−	−	Conglom.	1	1	−	−	−	−
54	3t	+	−	+	+	+	−	−	+	+	−	−	−	M. to L.	2	2	1	1	−	−
55	IV	+	+	+	+	−	−	+	+		+	−	−	Longiss.	−	−	−	−	1	1
56		+	+	+	+	−	−	−	−		+	−	+	Longiss.	1	1	−	−	−	−
57		−	+	+	+	−	−	−	−		+	+	+	Long.	−	−	1	1	−	−
58	4b	+	+	−	+	+	+	+	+	−	+	−	+	Med.	−	−	1	1	−	−
59	4c	+	+	−	+	+	+	−	−	−	+	−	−	M. to L.	−	−	1	1	−	−
60		+	+	−	+	+	+	−	+		+	−	+	Conglom.	−	−	1	1	−	−
61		+	+	+	+	+	+	+	−		+	−	−	Long.	1	1	−	−	−	−
62		+	+	−	+	−	−	−	−		+	−	−	Long.	−	−	−	−	1	1
63		−	−	−	+	−	+	−	−		+	−	±	Long.; Congl.	−	−	2	2	−	−
64		−	−	−	−	+	+	−	−		+	−	+	Long.	−	−	1	1	−	−
															100		50		50	

by Variants from them. Many of the latter are similar (allowing for the absence of the Conifern-broth test) to streptococci found by Andrewes &Horder.

As was to be expected, much the largest number come under Group iii , the "Anginosus" class, individuals from all sources being represented.

In the large table (Page 61) will be found both the number of specimens from each source which corresponded to the various types, and also the number of separate cases or individual persons from whom such organisms were obtained.

Where a series of reactions corresponds to one given in any of the tables in Andrewes & Horders' paper (Lancet, Sept 15,22,&29 1906) the number they assign is shown in red ink, as is also the reaction they obtained with Coniferin.

The power of growing on Gelatin at 20°C having proved very variable in all specimens, from whatever sources they were obtained, this has not been regarded as a distinguishing test for purposes of classification.

As regards the Groups.-

1 Streptococcus Pyogenes.

11 Streptococcus Salivaruis.

111 Streptocuccus Anginosus

1V Streptococcus Faecalis.

the following is the numerical distribution of my 200 specimens, from the several sources.

Group	Healthy No.	%	Scarlatinal No.	%	Other Diseases No.	%	All Diseases No.	%
1	10	20	10	10	7	14	17	11
11	4	8	1	1	1	2	2	1
111	29	58	87	87	40	80	127	85
1V	7	14	2	2	2	4	4	3

This table shows that the majority of the Streptococci obtained from each of the sources belonged to Group 111 but that those ~~from~~ from healthy throats showed a much lower percentage than in the case of diseased conditions, of which Scarlet fever showed the

highest percentage.

In Groups I, II, & IV all the diseased sources show practically similar per-centages - very low; while in healthy throats the percentage of organisms which belong to these groups is nearly three times as high.

The following tables shows a comparison of the group distribution of my series of 150 organisms from Scarlet Fever and three other diseased conditions (the latter of throats only), with the 228 of Andrewes and Horder, from a very wide range of diseased conditions, including Scarlet Fever. ~~are strictly comparable.~~ Only the figures for Scarlet Fever are strictly comparable.

Percentages alone are given.

	Scarlet Fever.		Other Diseased		All Diseases.	
Groups.	Mine	Andrewes & Horder.	Mine	Andrewes & Horder	Mine	Andrewes & Horder.
I.	10	36	14	42	11	40
II.	1	3	2	16	1	14
III.	87	61	80	17	85	24
IV	2	0	4	8	3	7
V	0	0	0	17	0	15

It is difficult to decide in many instances to what group an organism should be assigned, hence, possibly the difference between our figures.

Not only do we show a difference in Group classification, but if the morphology of our organisms ~~obtained from the various sources, we get the following results~~ be compared, a divergence is found in the forms of the Streptococci which we obtained from the throats of Scarlet Fever patients. This is brought out in the following table, in which again percentages only are given.

	Long forms.	Medium to long.	Medium	Short	Conglomerate
Mine	68	14	8	1	9
Andrewes & Horder.	76	12	9	3	0

This shows that a number of specimens appeared in my liquid media in tough conglomerate masses, whereas Andrewes and Horder apparently found none of these in Scarlet Fever; I only found one short form among my 100 specimens, while they got one among their33.

Taking the numerical frequency of occurrence of a positive reaction in each test medium of the organisms obtained from the various sources, we get the following results:- (my own specimens)

	Healthy.		Scarlet Fever		Other Diseases		All Diseases	
Test.	No.	%	No.	%	No.	%	No.	%
Milk-Pink.	39	78	88	88	46	92	134	89
Milk Clot.	34	68	83	83	42	84	125	83
Neutral Red	11	22	48	48	27	54	75	50
Saccharose	43	86	92	92	45	90	137	91
Lactose	36	72	68	68	36	72	104	69
Raffinose	25	50	34	34	10	20	44	29
Inulin	3	6	12	12	6	12	18	12
Salicin	17	34	33	33	16	32	49	33
Mannite	7	14	2	2	2	4	4	3

From this table it will be seen how little difference there is between the four columns. The chief variations shown by the organisms <u>from healthy sources</u> are as follows:-

i Less power to clot milk.
ii Less power to reduce Neutral Red.
iii Less power to decompose salicin.
iv Greater power to decompose Raffinose, &
v Much greater power to decompose Mannite, than organisms from diseased sources.

The only appreciable difference between the Scarlet Fever germs and those from other diseases is a rather greater power on the part of the former to decompose Raffinose, though this power is considerably

below that shown by the organisms found in the throat in health.

One Streptococcus from a healthy throat reduced Nitrate to Nitrite; and three from healthy throats, and one from "Other Diseases" liquefied gelatin.

Not one reaction is constant among streptococci from any one source - e.g "Healthy", or Scarlatinal"; that with Saccharose most nearly approaches constancy but shows approximatly the same frequency of occurrence in each source group.

Obviously, then, there is little to be learnt from the consideration of single reactions.

The following table shows a comparison between the reactions given by my series of 150 streptococci from Scarlet Fever, and three other diseased conditions, and those given by the 228 streptococci from various diseased conditions, of Andrewes & Horder, percentages only being shown:-

Test.	Scarlet Fever.		Other Diseases		All Diseases.	
	Mine	Andrewes & Horder	Mine	Andrewes & Horder	Mine	Andrewes & Horder.
Milk-Clot.	83	48	84	34	83	36
Neutral Red	48	36	54	18	50	21
Saccharose	92	94	90	85	91	86
Lactose	68	85	72	87	69	87
Raffinose	34	36	20	26	29	27
Inulin	12	12	12	9	12	10
Salicin	33	42	32	59	33	57
Mannite	2	3	4	15	3	10

The figures for Scarlet Fever are alone comparable, because, as mentioned above, Andrewes' & Horder's "Other Diseases" included a selection from extremely varied sources, whereas mine were limited to throat conditions, and they were only represented by Diphtheria, Tonsillitis, and Catarrh.

On comparing the two sets of reactions for Scarlet Fever organisms, there is seen to be a general

rough correspondence between them, with the exception of those for the clotting of milk, in which mine show nearly twice the proportion of positive reactions that Andrewes' & Horder's series gave.

CONSTANCY OF REACTIONS.

A number of specimens (about a dozen) in my series were subcultured in duplicate, in order to test the constancy of reaction of individuals. In every case the two sets of reactions agreed exactly.

A few more (about six) were retested after lying in the incubator for a month or more. These gave unchanged reactions, except that two had lost the power of decomposing Salicin.

No fatal cases of Scarlet Fever having occurred among my patients during the period covered by these observations, I have been unable to examine the condition of the organs bacteriologically after death from the disease.

SUMMARY OF RESULTS.

From my three series of observations then, I find that:-

i Streptococci were present in 83 to 84 per cent of all throats, and in the same proportion of Scarlatinal throats.
Streptococci were present in 83 per cent of healthy noses, and in 42 per cent of discharging noses in Scarlet Fever, and in 41 per cent of ear discharges in Scarlet Fever.

ii Streptococci or Diplococci (many of the latter proving to be streptococci when grown in liquid media) were present in 97 to 98 per cent of all throats, Scarlet Fever again showing the same proportion.
In Scarlatinal noses they appeared in 73 per cent of cases, and in 100 per cent of the healthy noses (six) examined.
In Scarlatinal ear discharges they were found in 71 per cent of cases.

iii Staphylococci occurred rather more frequently in the throat and nose in Scarlet Fever than in health, in the throat in 61 per cent of Scarlatinal, and 48 per cent of healthy subjects, and in the nose in 75 per cent of scarlatinal, and 66 per cent of healthy subjects.

Staphylococci were found in 65 per cent of ear discharges in Scarlet Fever.

iv Diphtheria Bacilli appeared in 9 per cent of scarlatinal throats, in 37 per cent of scarlatinal

nasal discharges, and in 29 per cent of scarlatinal ear discharges. They were not found at all in healthy throats or noses.

v. Streptococci were obtained in pure culture from the pus in 6 cases of cervical adenitis, and 2 cases of infection of the mastoid antrum in Scarlet Fever, i.e. in 100 per cent of cases.

vi Of the Diplococci present in the Scarlatinal throat, none was found which at all corresponded to that described by Class.
That which occurred most frequently was present in 80 per cent of the cases examined, but in later observations it was noticed to occur frequently in healthy throats.

vii Detailed examination of the Streptococci present in Scarlet Fever, and in the throat in health, Diphtheria, Catarrh, and Tonsillitis, showed that in the diseased conditions, the short-chained streptococci, which are commonly present in health, are early replaced by long forms, and that this is particularly marked in Scarlet Fever.

Conglomerate Streptococci were found least frequently of all in Scarlet Fever, being much more common in Health, and very much more common in Other Diseases.

viii Very great diversity indeed is shown by the Streptococci to Gordon's tests. From their reactions (taken collectively for each specimen) the majority from all sources appeared to belong to the "Anginosus" Group (of Andrewes & Horder), the scarlatinal streptococci showing the highest proportion.
None of the streptococci I found resembled any of the "Pneumococcus" Group.
No correspondence was found between any of the organisms of the throat, and of the true secondary complications in any case.

ix As regards the frequency of occurrence of a positive reaction in each particular test medium, it was found that, as a class, the "health" streptococci showed (1) less power to clot milk, reduce Neutral Red, and decompose Salicin; and (2) greater power to decompose Raffinose and Mannite than organisms from diseased sources.
Scarlatinal organisms showed a rather greater power to decompose Raffinose than Streptococci from the other diseased sources, but less power than those from healthy throats.

x. The tests were found to be almost constant for each organism at all stages of cultivation.

Serum therapy gives us little assistance yet in the elucidation of the Scarlet Fever problem. In spite of the favourable - but nevertheless distinctly vague - results published by A.Knyvett Gordon (Lancet, June 3, 1905) from the use of various Antistreptococcus sera in the treatment of the disease in Monsall Hospital, Manchester, the general concensus of medical opinion appears to be that such sera, whether "polyvalent" (and consequently of the "blunderbuss" denomination) or otherwise, have not yet justified their existence. Isolated cases undoubtedly have occurred in which a markedly beneficial result has followed the use of such a serum. I have myself used the "polyvalent" serum of Burroughs, Wellcome & Co., in about a score of severe cases of Scarlet Fever, but in not one could I conscientiously credit the serum with having, of itself, done any good.

The preparation of special sera from the patient's own particular organism is usually obviously impossible, on account of the time required. A case of non-scarlatinal endocarditis reported by Horder (Lancet, July 16, 1904) in which this was done, was absolutely unaffected by the serum, and pursued the even tenor of its way to a fatal conclusion quite unchecked.

Opsonic investigations are extremely difficult and unreliable with streptococci, but a successful case of treatment of an infective (non-scarlatinal) endocarditis by inoculation of a vaccine prepared from organisms obtained from the patient's blood, regulated by periodical examination of the opsonic power of the patient's serum, reported by Barr, Bell, and Douglas (Lancet Feb.23,1907) offers some slight hope of our obtaining light by similar means in Scarlet Fever. Such a successfully treated Scarlet Fever case would give much information on the etiology of the disease from examination of the organism from which the successful vaccine was prepared. Success in this direction seems more possible on account of the short time required to prepare the vaccine.

FINAL CONCLUSIONS:-

It is disappointing, in view of the enormous amount of time spent by so many observers in the investigation of the etiological factor in Scarlet Fever, to find no evidence at all which can be regarded as conclusively pointing to a particular organism as the specific cause of the disease.

Each fresh investigation, however, provides some information, even if it be only of a negative description. Possibly by the tedious process of

exclusion, we may finally arrive at the solution of the mystery.

A number of organisms have been put forward in their turns, but each has been discredited by subsequent investigators. Even Mervyn H. Gordon, whose magnificent piece of work, which seemed so conclusively to indicate that Klein's Streptococcus Scarlatinae or Conglomeratus was the missing link between infection and noninfection, had his convictions shaken by his own later observations. In a letter dated Oct.24. 1906, he states that he will only go so far as to say that "it is streptococci which kill the patient in Scarlet Fever, that I regard as absolutely proved," claiming no specific primary action for any member of the group. And indeed, Gordon's results, the outcome of the observations of a man who is probably more familiar with the appearance of, and the peculiar variations shown by, the members of the great streptococcus group of micro-organisms than any other living bacteriologist, do not seem to have been at all generally confirmed by other workers, who, in all probability,, were less experienced in this branch of work than himself.

Gordon suggested that good results might follow a detailed systematic examination of the Staphylococci in the tonsillar mucus; this work, owing to lack of time, I have been unable so far to take up, but certainly, from the greater frequency with which I have been able to demonstrate their occurrence in the scarlatinal than in the healthy throat, and nose, much information might be gained by an examination of them on similar lines to that described above for the streptococci.

As regards the protozoon theories, recently advanced, I am not in a position to criticise them, having done no work in that direction myself.

Consequently it appears that we can only fall back on the opinion that Scarlet Fever is due either to

(1) A septic organism, as yet quite undiscovered, which, by its action on the tissues, lowers their power of resistance to the septic (Streptococcal) organisms, which undoubtedly cause most of the secondary complications. This is the theory which has held ground for some years past, and is fully stated and supported in Nothnagel's Encyclopaedia of Practical Medicine (Vol. on Infectious Diseases, page 469) by Von Jurgensen, who also points to the frequent extension of a general constitutional disease as the result of an attack of Scarlet Fever.

Or that Scarlet Fever is due to

(2) A Streptococcus, or other organism present in the throat - for the one outstanding fact in the

whole business is that the pharyngeal mucus does contain the infective agent - which by some process of evolution has acquired special powers to produce the disease.

Gordon's series of tests certainly brings out differences between individual data which can be regarded as the best possible criteria of the actual physiological capabilities of the organisms, when acting among, or on, the living animal tissues? (streptococci; but do they provide

My own results hardly point to any such acquired power in regard to the test substances, hence my idea that these tests may not be the most appropriate under the circumstances.

One thing is certain, that there is little chance of a definite result being obtained without prolonged and patient research, preferably on the part of observers who are able to devote their entire time to the prosecution of that research.

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