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Studies on the Gram-negative Cocci ; with special reference to the Isolation and Differentiation of the Gonococcus, the Meningococcus and the Micrococcus catarrhalis.

C O N T E N T S .

Otto Fr. Müller, Vermium terrestrium et fluviatilium Historia, 1774. *

Animalcula infusoria fluviatilia et marina, 1786. *

Ehrenberg, Die Infusionstierchen als vollkommene Organismen, 1833. *

F. Dujardin, Histoire naturelle des Zoophytes, Infusoires. Suites a Buffon 1841.

The refrences marked thus * have not been available in the original.

Studies on the Gram-negative Cocci:- with special reference to the Isolation and Differentiation of the Gonococcus, the Meningococcus, and the Micrococcus catarrhalis.

INTRODUCTION.

Bacteriology, in spite of much magnificent work, is still a science in evolution. In a comparatively short history it has no doubt accomplished much, but perfection is a long way from attainment. At present, when organisms are being "discovered" on all hands, it greatly lacks definite criteria of species.

The Classification of Bacteria has always presented peculiar difficulties. The great Swedish naturalist, Carl Linnaeus, contented himself with creating the genus "Chaos," to which he relegated all the then known minute forms of animal life. The first step in differentiation was taken by O.F.Muller (1774) who on morphological grounds divided them into two genera - "Monas" and "Vibrio". This crude classification was followed with only minor modifications by all systematists till 1833 when Ehrenberg, with much more perfect instruments, further subdivided the Vibriona into four genera - Bacterium, Vibrio, Spirillum and Spirochaete; but Dujardin, having observed the development of bacteria in infusions, by merging the Spirochaetae with the Spirilla, reduced the number to three. It is worthy of note that even at this time the spherical forms were, for the most part, unknown although a

Goodsir, History of a case in which a fluid periodically ejected from the stomach contained vegetable organisms of an undescribed form, with a chemical analysis of the fluid by George Wilson M.D. Lecturer on Chemistry in Edinburgh. Edinburgh Medical & Surgical Journal Vol.LVII, p430, 1842.

E.Hallier, Die pflanzlichen Parasiten des menschlichen Korpers, Fur Aerzte, Botaniker & Studierende; zugleich als Anleitung in das Studium der niederen Organismen. Leipsig, 1866.

Zopf, Die Spaltpilze. 3rd Auflage, Breslau, 1885. *

F.Cohn, Untersuchungen über Bacterien. Beitrage zur Biologie der Pflanzen Band I, Heft II, p.127, 1872.

W.Migula, System der Bakterien. Band II, 1900.

few species were no doubt included among the Monades. Goodsir's discovery of the Sarcina ventriculi in 1842 is therefore a classic one, but it was Hallier in 1866 who introduced the term Mikrokokkus, which Cohn later (1872) changed to Micrococcus. The name Coccaceen was first applied in 1885 by Zopf to those bacteria which generally appear in spherical form. He was a firm believer in the transformation of bacterial species; but gradually with the progress of time the general stability of bacterial characters has been established and now the spherical bacteria are recognised as constituting a well marked natural group. The family is defined by Migula (1900) as follows;-

"Cells, in their free condition, spherical; during division somewhat elliptical. In the latter condition, division has already set in, although it may not be apparent. Division in one, two, or three planes, without previous elongation of the cells. If the cells remain in contact after division they are frequently flattened in the plane of division. Motility is present only in a few forms. Formation of endospores appears to be absent or very rare."

The great difficulties arise when an attempt is made to subdivide the group further. While in the main it has been amply demonstrated that e.g. typhoid bacilli descend from typhoid bacilli and tubercle bacilli from tubercle bacilli and that there is no process known whereby these fundamental types can be transformed into one another, still there is ample evidence to show that bacterial variation does exist in a less crude form. Recent work

E.K.Dunham, The Influence of Physical Conditions on the Character of Colonies on Gelatin Plates. Science,N.S.,Vol.XVII,p.372,1903(abstract).

L.Buerger & C.Ryttenberger, Observations upon certain properties acquired by the Pneumococcus in the human body.
THE Journal of Infectious Diseases Vol,4,p.609, 1907.

has shown that the typhoid bacillus for example is not an isolated type but that several bacteria exist which form a group of closely related organisms, differing not so much in morphology as in physiology. Morphological differences, indeed, are so slight in the typhoid-colon group as to be valueless for differential purposes and reliance must be placed on physiological properties, which again are frequently extremely variable as in the case of pathogenic power. Indeed with organisms which multiply so rapidly as the bacteria, where great numbers of generations succeed one another in a very short space of time and where, in the absence of demonstrated sexual processes, there is no amphimixis to bring back exceptional variations to the specific mean the possibilities are theoretically all in favour of environment impressing itself on strains and the transmission of acquired characters. The comparative stability of bacterial types is therefore all the more remarkable.

In the main three kinds of variation are recognisable -

(1) that due directly to the environment "false variation"; e.g. Bacillus prodigiosus produces red pigment at 20° C. but not at 37° C. Many minor differences in the appearance of surface colonies again have been shown to be due to the conditions of temperature and moisture under which the cultures have been grown (Dunham, 1903). (See Plate 1, fig.5 & 6; Plate 6, figs.35 & 36.)

(2) that due to the impress of environment in the past or "impressed variation"; e.g. the modification of virulence by passage through animals or by long continued artificial cultivation.

H.G.Dyar, On Certain Bacteria from the Air of New York City.
Annals of the New York Academy of Sciences,Vol.VIII,p.322, 1895.

M.Neisser, Ein Fall von Mutation nach de Vries bei Bakterien und andere Demonstrationen. Centrbl. f. Bakt.,Abt.I,Refer.,Bd.XXXVIII,p.98, 1906.
see also
K.Massini, Über einen in biologischer Beziehung interessanten Kolistamm (Bacterium coli mutabile). Ein Beitrag zur Variation bei Bakterien.
Archiv f. Hygiene, Bd.LXI,p.250, 1907

F.W.Twort, The Fermentation of Glucosides by Bacteria of the Typhoid-coli group & the acquisition of new fermenting powers by Bacillus dysenteriae & other micro-organisms.
Proc.Royal Society,B.Vol.LXXIX,p.329, 1907.

H.M.Goodman, Variability in the Diphtheria group of Bacilli.
The Journal of Infectious Diseases Vol.5,p.421, 1908.

(3) "True racial variations" which differentiate related bacterial strains under identical environmental conditions. There are two types of this among higher animals and plants continuous or fluctuating variations and discontinuous sports or mutations. Dyar in 1895 believed he had observed both forms among bacteria, but great care is necessary in accepting such statements as in bacteriology there is always the danger of contamination. An interesting example of the sportive type of variation was, however, demonstrated by Neisser in 1906. He used a bacillus of the paratyphoid group which did not ferment lactose and therefore gave colourless colonies on Endo's medium. Successive plate cultures made from young colonies always gave colourless colonies, but he observed that raised nodules appeared on the surface of old colonies grown in this medium and when plates were made from these two kinds of daughter colonies appeared - colourless ones and red ones. In other words from this paratyphoid bacillus a strain had developed with the power of fermenting lactose. The red colonies bred true but old white colonies could always be made to yield white and red ones. Other instances of altered fermentation powers have since been demonstrated by Twort (1907) in the case of typhoid, paratyphoid and dysentry bacilli, and by Goodman (1908) who worked with the diphtheria group. Starting with a virulent Bacillus diphtheriae he succeeded ultimately in obtaining a bacillus with pseudo-diphtheria characters, the change in this instance being gradual and the result of careful selection of the variants which produced the least degree of acid in glucose bouillion (36 generations).

W.Kruse, Variabilität der Mikroorganismen. Die Mikroorganismen, C.Flügge III auflage p.475, 1896.

F.W.Andrewes & T.J.Horder, A Study of the Streptococci Pathogenic for Man. The Lancet Vol. II p.708, 1906.

A.Quetelet, Lettres - sur la théorie des probabilités, appliqué aux sciences morales et politiques. Bruxelles, 1846. *

F.Galton, Co-relations and their Measurement, chiefly from Anthropometric Data. Proc. of the Royal Society Vol, XLV, p.135, 1889.

Natural Inheritance. London, 1889.

K.Pearson, The Grammar of Science. London, 1900.

Variations however are much less common than one might expect. The education of organisms to ferment unusual carbohydrates or glucosides is no easy matter and in point of fact has only been found possible in a few instances. Variations particularly occur, as emphasized by Kruse (1896), when organisms are grown under unfavourable conditions or under circumstances which permit only of slow multiplication as, for example, new environment. Andrewes and Horder (1906) pointed out that they are most common in "dominant" genera, groups that are succeeding in the struggle for existence. The very fact of their occurrence at all enormously increases the difficulties of bacterial classification. If a specific name is to be allotted to every discernible degree of variation hopeless confusion alone can result as is seen in the enormous numbers of species collated by Migula, and on the other hand not to take cognisance of some variations leads to vagueness.

Here it would appear that the statistical method of investigation offers the best solution to difficulties. First suggested for the study of human characteristics by Quetelet (1846) and specifically applied to the biological problems of variation and heredity by Galton (1889) Biometrics has been extended and developed in detail by Pearson (1900) and his pupils with excellent results. Briefly its method may be explained by an anthropological illustration: men vary much in stature, hue of complexion and shape of head. All sorts are met with in any community but when these three characters are measured, the results tabulated and

W.Z.Kipley, The Races of Europe. New York, 1899.

C.-E.A.Winslow & A.F.Rogers, A Revision of the Coccaceae. Science, N.S. Vol.XXI p.669, 1905.

C.-E.A.Winslow & A.K.Winslow, The Systematic Relationships of the Coccaceae. New York, 1908, page 13.

statistically collated then of associated characters in certain areas three main European types or races are distinguishable - the tall, long-headed, fair in Scandinavia; the broad-headed in Central Europe; and the short, long-headed, dark on the Mediterranean as has been shown by Ripley (1899).

Winslow and Rogers (1905) and Andrewes and Horder (1906) have independently applied the method to the study of bacteria; the former to elucidate the relationships of the Coccaceae in general and the latter to those of the Streptococci in particular. The following extracts from their writings are worth quotation:-

"If individual strains only are considered, an infinite series of differences appear. If the same strains are considered statistically, that is if the frequency of a given character be taken into account, it is apparent that certain combinations of characters are much more common than others. Measurement of almost any character by quantitative methods shows that the bacteria examined group themselves on a simple or complex curve of frequency. The modes of this curve indicate centres of variations about which the individuals fluctuate; and these centres of variation are the real systematic units of the group.

The grouping of specific types is an even more important problem than the definition of the types themselves; and here the correlation data obtained by biometrical study are of assistance. A true natural classification is tree-like and includes branches and twigs of varying grades of importance. Genera of bacteria should be aggregates of those specific types which are most nearly

Andrewes & Horder, loc. cit. p.711.

related; and the basis of the relationship will differ in each individual case."

Andrewes and Horder after pointing out the difficulties in the way of classifying the Streptococci say:-

"There was, however, one guide which, as in all such taxonomic problems, proved of the greatest help - namely, the numerical frequency of occurrence of any given type. When any arbitrary set of characters is taken as a basis for the classification of a group of natural objects the same phenomena are usually seen - large groups of like objects connected by small groups which differ from them in only one or two particulars. If the numerical frequency of each individual like the group is represented by the proportional height of a vertical line and the lines are arranged in series the commoner types stand out boldly above the rarer ones. Only in nature they are plotted out, not in linear series, but in space of two dimensions, as it were, so that the common types stand out as mountain tops above their fellows, each mountain connected by valleys of intermediate groups with many of its neighbours. If now the mountains were cut off by a horizontal plane half way up their sides and attention were paid only to the mountain tops, disregarding the valleys, we should have the popular conception of pieces. The biologist, on the contrary, is more concerned with the intermediate types in the valleys, as illustrating variation and the connection between allied species. In some groups of plants and animals the mountains are few and high and the valleys very deep. These are the groups which are, so to speak, in a

loc. cit. p.30.

loc. cit. p.263.

stationary condition - which are not rapidly varying and adapting themselves to new conditions. In other groups, which biologists call "dominant genera", the mountain tops are numerous but not so high and separated by only shallow valleys; these are the groups which are at the moment succeeding in the struggle for existence."

The Winslows summarise thus:-

"Bacteria show, in many groups at least, great variations. A comparative study of a considerable number of strains shows that certain characters, or combinations of characters, occur with special numerical frequency. These frequency types represent the centres about which related organisms are varying. Each type centre which is distinguished from other type centres by the exhibition of a definitely measurable character may be given the rank of a species. Genera may be constituted for nearly related groups of species which exhibit characters in common. Such species and genera represent real, natural groups of bacteria, which owe their similarity either to community of descent (phylogenetic species) or to similar pressure of environment (ontogenetic species).

Below is given Winslows' key to the Genera and Species of the Coccaceae which combines their own and Andrewes and Horder's work.

CELLS SPHERICAL: FAMILY, COCCACEAE.

A. Subfamily, PARACOCCACEAE.

Parasites. Growth not abundant, (or, one Species, Zoogloea-forming saprophytes. Growth abundant in saccharose media). Generally Gram -positive. Acid formers.

I. Genus, DIPLOCOCCUS.

Cells in capsulated pairs. Parasites. Growth very meagre. Inulin fermented. No pigment.

A. Cells lanceolate, in pairs, no chains. Gram-positive.

1. In lungs and sputum. D. pneumoniae.

B. Cells flattened, in pairs, no chains. Gram-negative.

2. In pus cells in gonorrhoeal infection. Growth meagre. D. gonorrhoeae.

3. In cells in cerebro-spinal exudate. Growth meagre. D. Weichselbaumii.

4. In normal throat. Growth on media fair. D. catarrhalis.

C. Cells in chains of pairs, surrounded by zoogloea,

5. D. involutus.

II. Genus, ASCOCOCCUS.

Cells in chains occurring in masses of zoogloea in sugar refineries. Aberrant saprophytic forms. Growth abundant in saccharose media. No pigment.

1. A. mesenteroides.

III. Genus, STREPTOCOCCUS.

Cells in chains. Parasites. Growth meagre. Inulin not fermented. No pigment.

A. Does not liquefy gelatine.

a. Does not acidify lactose.

1. Chains medium. Growth of 20 C. feeble. Habitat, intestines of Herbivora. Acidifies saccharose, salacin, and coniferin. Str. equinus.

b. Acidifies lactose, saccharose, and salicin but not raffinose. Does not clot milk.

2. Chains short. Habitat, human saliva and faeces. Str. mitis.

3. Chains long. Haemolytic power marked. Found in pathological conditions. Str. pyogenes.

c. Acidifies lactose, saccharose, and raffinose but not salicin. Clots milk. Reduces neutral red.

4. Chains short. Habitat, normal mouth and intestine. Str. salivarius.

5. Chains long. Haemolytic power marked. Found in cases of sore throat. Str. anginosus.

d. Acidifies lactose, saccharose, salicin, coniferin, and mannite but not raffinose. Clots milk. Reduces neutral red.

6. Chains short. Habitat, human intestine. Str. faecalis.

B. Liquefies gelatine.

7. Str. gracilis.

IV. Cells in irregular groups. Parasites. Growth fair. Orange pigment.

Genus, AUROCOCCUS.

A. Nitrates not reduced.

1. Gelatine strongly liquefied. Aur. aureus.

2. Gelatine not liquefied. Aur. aurantiacus.

B. Nitrates reduced.

3. Aur. mollis.

V. Genus, ALBOCOCCUS.

Cells in irregular groups or in fours. Parasites. Growth good. White pigment.

A. Cell groups irregular.

a. Gelatine liquefied.

1. Nitrates not reduced. Alb. pyogenes.

2. Nitrates reduced. Alb. epidermidis.

b. Gelatine not liquefied.

3. Alb. candidus.

B. Cells in the body in capsulated groups of four. Growth viscid.

4. Alb. tetragenus.

B. Subfamily, METACOCCACEAE.

Saprophytes. Growth abundant. No Zooglea. Generally Gram-negative. Not acid formers.

VI. Cells in irregular groups. Pigment generally yellow. Genus, MICROCOCCUS.

A. Pigment typical, yellow.

a. Gelatine liquefied.

1. Nitrates not reduced. M. flavus.

2. Nitrates reduced. M. citreus.

b. Gelatine not liquefied.

3. M. luteus.

B. Pigment white. Gelatine not liquefied. Nitrites not reduced.

4. M. candicans.

V. Genus. ALEUCOCOCCUS.

Cells in irregular groups or in fours. Parasites. Growth good.
White pigment.

A. Cell groups irregular.

a. Gelatine liquefied.

1. Nitrates not reduced. Alb. proteus.

2. Nitrates reduced. Alb. conglomerata.

b. Gelatine not liquefied. Alb. candidus.

3.

B. Cells in the body in separated groups of four. Growth viscid. Alb. tetragenus.

4.

B. Subfamily. METACOCCACEAE.

loc. cit. p.14

Saprophytes. Growth abundant. No Zooglea. Generally Gram-negative. Not acid formers.

VI. Cells in irregular groups. Pigment generally yellow.

loc. cit. p 92.

Genus. MICROCOCCUS.

A. Pigment typical, yellow.

B. Gelatine liquefied.

1. Nitrates not reduced. M. flavus.

2. Nitrates reduced. M. citreus.

b. Gelatine not liquefied.

3. M. luteus.

B. Pigment white. Gelatine not liquefied. Nitrates not reduced.

4. M. candicans.

VII. Genus, SARCINA.

Cells in packets. Pigment yellow.

A. Gelatine liquefied.

1. Nitrates not reduced. S. flava.

2. Nitrates reduced. S. citrea.

B. Gelatine not liquefied.

3. S. lutea.

VIII. Genus, RHODOCOCCUS.

Cells in irregular groups or packets. Pigment red.

1. Nitrates reduced to nitrites. R. roseus.

2. Nitrates not reduced. R. fulvus.

The Winslows are quite alive to the fact that "it cannot of course be expected that the correlation of characters in bacteria shall be absolute;" that some members of a group often differ radically in a single property, e.g., Gonococcus and Gram's stain, from the general characteristics of the group as a whole but, as they put it, "Classification however must be phylogenetic rather than logical." In many respects, however, their findings are valuable and much in advance of anything previously attained. The generalisation of the following characters with the parasitic habit is particularly striking - pathogenicity; poor growth on artificial media; morphology, chains, pairs or masses (never packets); Gram-positivity and pigment production, if any, white or orange. These characters contrast markedly with those of organisms found in other situations than in or on the animal or

VII. Genus. SARCINA.

Cells in packets. Pigment yellow.

A. Gelatine liquefied.

1. Nitrates not reduced. B. flava.

2. Nitrates reduced. B. citrea.

B. Gelatine not liquefied.

B. lutea.

VIII. Genus. MICROCOCCUS.

Cells in irregular groups or packets. Pigment red.

1. Nitrates reduced to nitrites. M. rosea.

2. Nitrates not reduced. M. ruber.

loc.cit.p.42.

The Winslows are quite alive to the fact that "it cannot of course be expected that the correlation of characters in bacteria shall be absolute: that some members of a group often differ materially in a single property, e.g., Gonococcus and Gram's stain, from the general characteristics of the group as a whole but, as they put it, "Classification however must be symptomatic rather than logical." In many respects, however, their findings are valuable and mark an advance of hitherto previously attained. The generalization of the following characters with the exception of habit is particularly striking - pathogenicity, poor growth on artificial media; non-motile, chain, pairs or masses (never packets); Gram-positivity and fermentation. If not, white or orange. These characters contrast markedly with those of organisms found in other situations than in or on the animal or

plant body (the saprophytes) - non-pathogenicity; abundant growth on artificial media; morphology, often packets; Gram-negativity and yellow or red pigment production.

SCOPE OF STUDY.

The present work deals particularly with Group B. of Winslow's Genus, DIPLOCOCCUS; sub-family, METACOCCACEAE, which embraces the Gonococcus, the Meningococcus and the Micrococcus catarrhalis. Here it must be noted that they specifically state their personal studies did not extend to the genus Diplococcus, but were limited to the forms of cocci which can be found in ordinary environments and which may be cultivated on ordinary laboratory media, i.e., to the genera -Streptococcus, Micrococcus and Sarcina as ordinarily understood. "The organisms belonging to the genus Diplococcus do not easily lend themselves to comparative study on account of the difficulty with which they may be cultivated." Their recognition of species was purely from the early literature on the subject. Of late, however, owing to the recent widespread epidemics of Cerebro-spinal Meningitis the organisms of this group have attracted much attention. An enormous literature has grown up, but the conclusions are in some cases so much at variance that it appeared worth while undertaking a personal investigation, the results of which are embodied below.

At the outset it may be stated that faulty technique is in large part responsible for the discrepancies in the literature, and the delicacy of the organisms themselves has further militated

Martha Wollstein, Biological Relationships of Diplococcus intracellularis & Gonococcus. The Journal of Experimental Medicine Vol.IX,p.588, 1907.

Th.Vannod, Contributions à l'etude du gonocoque.
Centrbl. f. Bakt.I Orig.Bd.XLIV p.10, 1907.

I.McKenzie & W.B.M.Martin, Serum-therapy in Cerebro-spinal Fever.
The Journal of Pathology & Bacteriology Vol.XII p.547,1908.

against their accurate study.

Briefly put the following chief questions have to be answered - What do we understand by the terms Gonococcus, Meningococcus and Micrococcus catarrhalis? Do they stand for distinct entities (bacterial species) or not?

The questions are of some urgency if the subject of the Gram-negative cocci is not to remain in a chaotic state, for example, Wollstein (1907) as the result of her work in Flexner's laboratory (the source of the largely used anti-meningitis serum, Flexner-Jobling) has concluded that, apart from their pathogenic effects on Man, the Gonococcus and the Meningococcus mainly differ in the less abundant growth of the former and its restriction to particular culture media. Vannod (1907) however has indubitably shown that the Gonococcus can be cultivated, and even isolated, on plain agar thus levelling one distinction between Gonococcus and Meningococcus: and then Josselin de Jong (1908), who states he knows a Meningococcus when he sees it, has described a case of gonococcal Meningitis. (It will be afterwards shown, however, that he by no means has proved his point and that it must be seriously questioned whether a true gonococcal meningitis has ever occurred.) (see below p.79)

No one has described a meningococcal urethritis and yet, as I have demonstrated (1908), Meningococci can frequently be isolated from the urine of Cerebro-spinal Fever cases in which there is not the slightest evidence of renal or genito-urinary inflammation. (Compare Enteric Fever) Undoubted instances

C.Fräenkel, Zeitschr. f. Hyg. u. Infektionskr., Bd.XXXI 1899.

Haglund, Klin. Monatsbl. f. Augenh., Bd.XXXVIII 1900.

Heller, Ueber experimentelle Blennorrhoe im Augen neugeborener Kaninchen nebst Erfahrungen über die Cultur des Gonokokkus.
Charité-Annalen Bd.XXI 1896.

Th.Axenfeld, The Bacteriology of the Eye. (Macnab's translation) p.126,1908.

Turro, Gonokokkenzüchtung und künstlicher Tripper.
Centrbl.f.Bakt. Bd.XVI p.17, 1894.

Friedberger & Frohner, Veterinary Pathology. (Hayes' translation)p.327,1898.

of meningococcal conjunctivitis are, however, on record (Fränkel, 1899; Haglund, 1900) and I have personally observed two cases during the course of Cerebro-spinal Fever where the features were profuse watery discharge with marked injection of the whole conjunctival sac. Meningococcal and gonococcal arthritis and endocarditis are well accredited, so that, even in their pathogenicity in Man, the Gonococcus and the Meningococcus are not absolutely differentiated.

The common laboratory animals are useless for estimating the pathogenicity of these organisms. In spite of the statement of Heller (1896) I have repeatedly failed to produce a conjunctivitis in even very young rabbits with inoculations either of recently isolated gonococcal strains or of pure acute gonorrhoeal pus and indeed this is in accordance with the experience of the best ophthalmic bacteriologists (Axenfeld,1908).

Turro (1894) has stated that he easily produced a urethritis in dogs, but as his organisms grew on acid gelatine they cannot have been Gonococci at all. Though some degree of vaginitis and urethritis accompanies Dourine and the Vesicular exanthema of horses and cattle there appears to be no disease in the domestic animals comparable to gonorrhoea in Man. Friedberger and Fröhner indeed say that a true gonorrhoea does not exist in the domestic animals the "clap" of dogs being merely a purulent catarrh of the prepuce in which the mucous membrane of the urethra does not participate.

Councilman, Mallory & Wright, Report of the State Board of Health of Massachusetts. Boston, 1898. Epidemic Cerebro-spinal meningitis and its relation to other forms of meningitis. *

Von Lingelsheim & Leuchs, Klinisches Jahrbuch, p. 489, 1906. Tierversuche mit dem Diplococcus intracellularis (Meningococcus).

S. M'Donald, Observations on Epidemic Cerebro-spinal Meningitis. The Journal of Pathology and Bacteriology Vol.XII p.442, 1908.

M. Weinberg, Un cas de méningite cérébro-spinale chez le chimpanzé. Bull. de l'Inst. Pasteur Tome VII p.432, 1909. (reference in)

J. Marcq, Recherches sur la méningite cérébro-spinale entozootique du cheval. Bull. de l'Inst. Pasteur Tome VII p.432, 1909. (reference in)

E. Bertarelli, Ueber einem pathogenen Keim der Iguana und interessante, von ihm erzeugte Verletzungen (Diplococcus iguanae n.sp.) Centrbl.f.Bakt. Abt. I, Orig. Bd.XL, p.458, 1906.

Councilman, Mallory and Wright (1898) produced a meningitis in goats by intraspinal injection of Meningococci and Flexner (1906), von Lingelsheim & Leuchs (1906) and McDonald (1907) have all succeeded in inducing meningitis in monkeys, but only on intraspinal injection of Meningococci. Even then these animals are less susceptible than Man. Injections of either organism into the blood stream of rabbits, guinea-pigs, mice, or monkeys does not lead to a septicaemia or to arthritis; intraperitoneal injection gives rise, at the most, to slight peritonitis; subcutaneous or intramuscular injections may cause local induration and muscular rigidity but not suppuration; a transient arthritis may be produced by direct inoculation into joints; but there is little or no proliferation of organisms within the animal tissues and they soon die out. The same results, indeed, can be obtained by the bacterial endotoxines as by living cultures and when the inoculated animals succumb it is usually to toxaemia.

Spontaneously meningitis occurs in the lower animals and diplococci resembling the Meningococcus have been isolated from them - Weinberg (1909) from a chimpanzee, Marcq (1909) from the Maladie de Borna of horses and Bertarelli (1906) from an iguana. Young animals specially suffer and horses and sheep more than cattle or dogs. A particularly fatal epizootic of cerebrospinal meningitis decimated the stock of Egypt in 1876.

For my own part, however, I am firmly convinced that pathogenicity in Man is of prime importance and that human urethral inoculations, were they permissible, would be practically crucial.

F.Göppert, Über Genickstarre.
Ergeb. d. Inn. Med. u. Kinderheilkunde Bd.IV, p.165, 1909.

J.Comby, Méningite Cérébrospinale Épidémique.
Archives de Medicin des Enfants T.XIII,p.161, 1910.

L.E.Holt, Gonococcus Infections in Children,with especial reference to their prevalence in Institutions and means of prevention.
New York Medical Journal Vol.LXXXI p.521, 1905.

* See below p.82.

† British Medical Association, Discussion on Cerebro-spinal Meningitis.
British Medical Journal 31st.Oct.,1908,p.1341-3.

A.E.Garrod, in Allbutt & Rolleston's System of Medicine, Vol.III p.51,1907.

That the Meningococcus should single out the cerebro-spinal meninges, particularly of the young; and the Gonococcus, the urethra of the adult (or the conjunctiva, urethra and vagina of the child) are not mere chances of accidental inoculation but are definite evidences of a relation between susceptibility of the host on the one hand and pathogenicity of the invading organisms on the other. The circumstances in childhood are particularly instructive. If Holt's observations are reliable (he gives no detail of his bacteriological criteria in any of his papers) and there is little reason to doubt them, gonorrhoeal vaginitis may be a very serious menace in children's hospitals. Its infectivity is remarkable and quite a number of arthritis cases have been observed even in male children who have not presented any evidence of local eye or urethral mischief.* Here, whatever the portal of entry, the spread must have been by the blood; but that is just the means whereby, according to the most accredited theory†, infection of the pia-arachnoid system occurs in Cerebro-spinal Fever, yet Holt has never met with a genuine case of gonorrhoeal meningitis though some of his cases have succumbed to the infection. In adults gonorrhoeal metastases are notorious but here it might be objected that meningitis of any kind is less common than earlier in life. (That e.g. every metastasis in gonorrhoea is not gonococcal but may be due to secondary infection is of course recognised. Garrod.)

In the absence, therefore, of a suitable susceptible host upon which to test pathogenicity we are driven back to

See p.76. Also plate 2.

See plate 1, fig.5.

bacteriological laboratory methods. I hope to be able to show that, though closely related, these organisms are differentiable by such means and are also, according to the principles enunciated in the introduction, worthy of specific rank.

The work has comprised a survey of :-

1. The occurrence of Gram-negative cocci in human pathological conditions arthritis, conjunctivitis, bronchitis, meningitis, urethritis &c.
2. The clinical bacteriological diagnosis embracing the bacteriology of allied conditions.
3. Methods of isolation and culture media.
4. Differential diagnosis of members of the group by bacteriological laboratory methods.

It should be clearly understood that no attempt has been made to study all the Gram-negative cocci which may be found in or on the human body. In addition to the Micrococcus catarrhalis a number of similar organisms are commonly to be found in sputum. They are probably derived from the atmospheric dust and may have no pathogenic significance at all; but for the sake of completeness they are included in the present study. Then chronically inflamed vaginae sometimes contain hosts of organisms of diplococcal form which do not retain the stain by Gram's method. A number of these are really short diplo-bacilli while others are streptococci. They are referred to under the heading of vaginitis. Gram-negative bacilli are sometimes so short as to give the appearance of cocci. In suitable fluid culture media, however, their true nature is readily apparent. In some

Gram, Ueber die isolirte Färbung der Schizomyceten in Schnitt und Trockenpraeparaten
Fortsch. der Med. II, 1884, p. 185.

situations e.g. the urine or peritoneal exudates, one is always on guard for such appearances but it is perhaps worth mentioning that there is a very fatal type of cellulitis met with in infancy, characterised by an extensive sero-purulent exudation, which is due to a very short Gram-negative cocco-bacillus. I saw two cases in the summer of 1907 at Ruchill Hospital but unfortunately the strains died out before complete identification of the organisms was possible. (see below p.28)

GRAM'S DIFFERENTIAL STAINING PROCESS.

Ever since its introduction Gram's method has been the subject of controversy and modification. In view of the remarks on bacterial variation made above it is unnecessary to re-open the discussion here as to whether some organisms do or do not retain the stain in Gram's process. No one doubts that a pyogenic Staphylococcus aureus is Gram-positive or that the Bacillus coli is Gram-negative because the latter destains practically instantaneously while the former is unaffected even by long treatment with the decolourising agent. Equally, anyone who has studied the streptococci in the saliva will be prepared to admit that Gram-positive and Gram-negative elements are to be found in the same chain and that the older the culture the greater the tendency to negativity. It is useless now quibbling whether this type of streptococcus is Gram-positive or Gram-negative, depending on the relative duration of the stages

A.Neisser & W.Scholtz, Handbuch der pathogenen Mikroorganismen, Band III,p.158, 1903.

Weinrich, Die Farbarkeit des Gonococcus. Centrbl.f.Bakt. Bd.XXIV,p.258, 1898.

of the staining process now, far better to admit that it is both, or irregular in its reaction with Gram's stain. Some authorities have laid great stress on the duration of the stages, e.g. the Committee on Standard Methods of Water Analysis to the Laboratory Section of the American Public Health Association in 1905 suggested one minute for staining, two minutes for iodine and five minutes for decolourising, but for present purposes an organism is Gram-negative if it loses the stain within seconds of trestment by the decolourising agent. But this holds good only for culture films and for thin films of pus as Neisser & Scholtz have recognised. The thick parts of pus films not infrequently cannot be destained by any reasonable length of treatment with the decolouriser. In cases where the cell nuclei or the ground work retains the stain no reliance can be put on the findings and the films should be discarded. In great part such a result is obviated by making the pus film with a tiny drop of water as in the ordinary technique for culture films; and I have also found it a considerable advantage, as Weinrich (1898) first suggested, not to wash the films with water between the application of the gentian violet and the iodine stages of the process. Even with these precautions a few Gram films are unsatisfactory, particularly those from pus rich in mucinous substances e.g. from joints and chronic gleets. Here I have found that after fixation with gentle heat exposure to the action of a weak acid solution (a twentieth normal HCl) for a minute before the staining process proper is begun often gives quite sharp differentiation in films which are refractory

Muir & Ritchie, Manual of Bacteriology, 4th. Ed. p. 100, 1907.

to all other methods. Absolute alcohol on the whole is a more rapid decolouriser than aniline-oil xylol but it is less uniform in its action and in the end gives no sharper differentiation.

The following is the method in detail which has been uniformly used throughout. Essentially it is the Weigert modification recommended by Muir and Ritchie.

1. Stain in carbol-gentian-violet for about two minutes.

2. Treat the section or film with Gram's iodine solution till its colour becomes a purplish black, generally about half a minute or a minute is sufficient for the action to take place.

3. The preparation is dried either in air in the case of films or by means of blotting in the case of sections then treated by aniline-oil-xylol. 30 seconds is usually sufficient time for perfect decolourising in the case of culture films but with pus films the time may be extended with advantage in some instances to a minute. (Carbol fuchsin is an excellent counter-stain, 1 in 10)

When proper precautions in the preparation and staining of films are taken the Gonococcus, the Meningococcus and the Micrococcus catarrhalis have always proved in my experience Gram-negative. That some elements even in culture films should retain the stain longer than the vast majority of the organisms is only what one would expect and indeed the phenomenon has presented itself where, when destaining was very rapidly carried out, one element of a pair appeared violet and the other red. The point of importance, however, is that such an appearance has only very rarely been met with - one violet to countless reds - and even then a very slight extension of the decolourising stage

M'Donald, loc. cit. p.443.

Fonseca, Le Gonocoque; morphologie, reactions colorantes, inoculations Soc. de Biol., 16 juillet 1898. *

Wahl, New York Medical Record, May 23rd., 1903, p.844.

Pappenheim, "Ueber Gonokokken farbung." Centralb. f. Bakt. Abt. I Ref.Bd.XXXIV, 1903.

Leeuwenhoeck, Arcana naturae detecta. Lugduni Batavorum, 1680. *

gave a perfectly uniform red film. This degree of Gram-positivity is therefore minimal and in no wise approaching that of a Staphylococcus aureus. In pus films the decolourisation of some cell nuclei is usually taken as a guide to the successfulness of the staining process. This, however, is not an absolutely reliable one, particularly where the films have been over-heated in fixation and treated with water between the violet and the iodine stages of the process.

Jäger's Gram-positive coccal strains from meningitis cases (Diplococcus crassus, von Lingelsheim) can no longer be considered to be genuine meningococci. I have not met with the phenomenon described by McDonald (1908) and others - negativity in films from exudates, positivity in cultures. Neither can I subscribe to Fonseca's (1898) conclusions that the Gonococcus is Gram-positive when grown on neutral or alkaline media and negative on faintly acid media.

The many other staining processes devised for the detection of the Gonococcus in pus e.g. Wahl's (1903) and Pappenheim's (1903), are not nearly as valuable as Gram's, for morphologically intracellular staphylococci can quite mimic gonococci and they are not infrequently present in chronic gonorrhoeal discharges as proven by culture. **(Plate 1, fig.3, and plate 10, fig.58)**

GENERAL HISTORICAL SKETCH.

Though the Dutch Naturalist Leeuwenhoeck (1632-1723) may be said to have been the discoverer of Bacteria as he probably observed some of the larger saprophytic ones in the faeces, putrid

Pollender, Mikroscopische und microchemische Untersuchung der Milzbrandblutes, 1855. *

Rayer & Davaine, Inoculation du sang de rate
Mem. de la Soc. de Biol., 1850, p.141. *

Davaine, Recherches sur les infusoires du sang dans la maladie connue sous le nom de sang de rate. C.R.de l'Acad.des sc. 1863,1864 & 1865.

Obermeier, Vorkommen feinster,eigene Bewegung zeigender Fäden im Blute von recurrentkranker. Centrbl. f. die med. Wissensch. 1873.

Hansen, Norsk. Mag. f. Loegevidensk, 1874 *

Neisser, Ueber ein der Gonorrheae eigenthümliche Micrococcenform
Centrbl. f. die med. Wissensch. 1879.

Hallier, Zeitschr. für Parasitenkunde, Bd.I,p.179, 1872.

infusions &c. which he examined with his magnifying glasses (1675), it was not till the middle of the 19th century that, mainly through the brilliant researches of Pasteur (1822-1895), the rôle of micro-organisms in disease was established.

Anthrax was the first pathogenic micro-organism to be recognised, Pollender (1849) and Davaine (1850) both having observed it in the blood of animals dying from this disease. The latter, by repeated inoculation experiments, claim to have demonstrated it to be the materies morbi of the disease in 1863.

Next, in 1873 Obermeier found the causal spirillum in the blood of patients suffering from Relapsing Fever.

A year later Hansen (1874) announced the finding of bacilli in the cells of leprous tissues; then in 1879 Neisser made his communication on the Gonococcus, which he had found with great constancy both in gonorrhoeal pus and in the discharges of ophthalmia neonatorum. It is no doubt true that Hallier in 1872 observed the Gonococcus in gonorrhoeal pus, but the great credit for demonstrating the association of an organism, of very definite morphology, with gonorrhoeal infections is undoubtedly Neisser's.

With the improved methods of technique which had by this time been elaborated (e.g. the compound microscope, Schroeder & van Dusch's cotton-wool plugs, Pasteur's sterile culture fluids, Koch's isolation methods with solid media, Ehrlich and Weigert's aniline stains) other discoveries soon followed in rapid succession.

The pyogenic micrococci (staphylococci and streptococci)

A.Ogston, Report upon Micro-organisms in surgical Disease.
The British Medical Journal Vol.I, p.369, 1881.

Weichselbaum, Ueber die Aetiologie der akuten Meningitis cerebrospinalis
Fortschr. der Med., 1887, p.573.

Sternberg, The American Journal of Medical Sciences April 1881.

Bordoni-Uffreduzzi, Centralbl. f. Bakt. Abt. I Bd.VII, p.188, 1890.

Jaeger, Ztschr. f. Hyg. u. Infectionskrankh. Bd.XIX, p.351, 1895.
Die Cerebrospinalmeningitis als Heeresseuche. Berlin, 1901. *

Lehmann & Neumann, Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik. 1907.

were demonstrated by Ogston in 1881, the year before Koch's discovery of the tubercle bacillus.

Weichselbaum discovered the Meningococcus in 1887, having isolated it post mortem from the meningeal exudate of six cases of primary Cerebro-spinal Meningitis. To him it clearly differentiated itself from the Diplococcus pneumoniae (the Pneumococcus of Fraenkel) an organism first described by Sternberg (1880) under the name of Micrococcus Pasteuri and which had already been recognised as the causal agent in some cases of meningitis; but for many years the two organisms were confused and the Meningococcus was regarded by some e.g. Bordoni-Uffreduzzi (1890) as a mere variety of the Pneumococcus.

The subject of meningococci was further complicated and obscured by Jäger in 1895 (and several others later) who published descriptions of organisms which he had obtained from genuine cases of Cerebro-spinal Meningitis and which differed materially from Weichselbaum's. At first he described his cocci as capsulated but in 1901 he discarded that belief. He also asserted that it grew sometimes in long chains and that it was at one time Gram-positive, at others Gram-negative, and that growth might occur on gelatine at room temperature. Subsequent research has, however, shown that Jäger's findings on the causal virus of Cerebro-spinal Meningitis are inaccurate, the result of faulty technique; yet his work has had a great influence and strangely enough the description of the Meningococcus by Lehmann & Neumann (1907) is really one of Jäger's diplococcus.

Still, The Journal of Pathology Vol.V, 1898.

Councilman, Mallory & Wright, loc. cit.

Albrecht & Ghon, Wien. klin. Woch, 1901.

Bettencourt & Franca, Ztschr. f. Hyg. u. Infectionskrankh. Bd. XLVI, p.463, 1904.

Von Lingelsheim, Klin. Jahrb. Bd.XV, p.373, 1906.

Shennan & Kitchie, The Journal of Pathology & Bacteriology Vol.XII, p.456, 1908.

Elser & Huntoon, The Journal of Medical Research, Vol.XX, p.371, 1909. "Studies on Meningitis". (Particularly pp.521 and 535)

Kutscher, Kolle und Wassermann, Handbuch der pathogenen Mikroorganismen Erster Ergänzungsband, p.497, 1907.

The best of recent work and my experience in Glasgow has all gone to confirm Weichselbaum's original description of the characters of the Meningococcus and to demonstrate that it is the real cause of the great epidemics of Cerebro-spinal Meningitis and of Sporadic or Posterior-basic Meningitis.

In the opinion of Elser & Huntoon (1909), which they have formed from a lengthy review of the literature and from many personal observations, there is only one other organism - the encapsulated streptococcus of Bonomè - which from its special predilection for the meninges can be considered as a likely cause of epidemics of meningitis. Unfortunately their single strain isolated post mortem from the lung of a case of lobar pneumonia, which showed symptoms of "meningismus" during life, with which they had induced a meningitis in rabbits by intravenous injection, died out before its exact relationship to the Streptococcus mucosus could be determined. They are clear, however, that, though closely resembling the Streptococcus mucosus, it was not identical to it and that it equally was not a Pneumococcus. But one might here add that Jäger's Diplococcus, which is common in the naso-pharynx, also shows a certain affinity for the meninges mostly however when they are already the seat of disease (Kutscher,1907)

Many organisms of course can cause a meningitis in certain circumstances. How far head symptoms in acute diseases are merely toxic or are due to the actual presence of bacteria in the pia-arachnoid system is difficult to determine, but in a fair proportion of septicaemias micro-organisms can be obtained post mortem from the cerebro-spinal fluid. It is not uncommon

Lesné & Simon, Deux cas de méningite microbienne sans reaction cellulaire. Archiv. de Med. des Enfants Vol.XIII, p.239, 1910.

e.g. to find pneumococci post mortem in the cerebro-spinal fluid of acute lobar pneumonia cases and the striking thing is that the brain often shows only a trifling hyperaemia with no evidence of purulent lepto-meningeal exudation. It is difficult to account for the presence of these by post mortem invasion alone because they are not present in every case although pneumococci are constantly present in the blood during some stage of pneumonia. If one depends on the presence of a visible purulent exudate alone, then not a few cases of meningitis will be missed post mortem. In an overwhelming infection the cellular reaction may be practically nil. Lesné & Simon (1910) have observed during life two such pneumococcic cases in nurslings. A very acute type of Cerebro-spinal Fever, fatal before exudation has time to appear, has of course long been recognised and the haemorrhagic lesions in anthrax lepto-meningitis are also well known.

Again mixed meningeal infections are common enough. They are usually the result of gross lesions (wounds of the skull, middle-ear disease &c.) but there are others which do not come under this category. I have recently seen one case which teemed with organisms - streptococci, Gram-positive bacilli, thin Gram-negative bacilli and B. coli - yet no gross lesion was demonstrable. It is just a question if many of the chained Gram-irregular cocci, which grow on gelatine, the Jäger-Heubner type of meningococcus are not terminal infections with streptococci which outgrow the more delicate Meningococcus. Such at all

*See plate 3, fig.14. Also the chart of the case with the details of the cerebro-spinal fluid examinations, plate 11.

events is the direction in which my opinion tends. One case in particular, carefully observed for a period of six weeks, substantiates this attitude. It was a typical one of Cerebro-spinal Fever and came under observation on the third day of illness. Lumbar puncture was repeatedly performed for diagnostic and therapeutic purposes (13 occasions in all). Typical Meningococci were at first obtained from the cerebro-spinal fluid, then, as the patient improved, they could no longer be demonstrated in films or by culture. After a period of comparative well-being, though the leucocyte counts and a slight though progressive emaciation showed that all was not well, a relapse occurred and again Meningococci were demonstrated in pure culture in the cerebro-spinal fluid. On this occasion the response to treatment was not so good and after a short interval the symptoms became aggravated and the patient died. On the last occasion upon which lumbar puncture was performed (3 days before death) the fluid yielded numerous Meningococci in pure culture. At the post mortem the gross lesions were typical of Cerebro-spinal Fever but microscopically films of the meningeal exudate showed a great variety of micro-organisms. Meningococci could be recognised but streptococci of very varied size, staphylococci, morphological pneumococci and a large sporing Gram-negative bacillus were much more abundant. Meningococci and an atypical streptococcus* were isolated by culture; the latter closely simulated in its characters Jäger's coccus. Two possibilities occur to one to account for these findings - either we introduced the mixed bacterial flora on the occasion of the last lumbar

M'Donald, loc. cit. page 454, 1908.

Eve & Clements, Discussion on Cerebro-spinal Meningitis, loc. cit. 1908.

see also
Symmers & Wilson, The Journal of Hygiene Vol.IX,p.9, April 1909.
Some points bearing on the Bacteriology of Cerebro-spinal Meningitis.

puncture or they were the result of a terminal or post mortem invasion say from the gastro-intestinal tract, as not uncommonly occurs ante mortem in debilitated subjects. The absence of a "septic spleen" is rather in favour of the latter but it must be admitted that in the vast majority of post mortems on acute cases Meningococci are present in a pure condition. It may be noted that McDonald (1908) has suggested, though on no very good evidence, the possibility of a primary intestinal route of infection in the naturally acquired disease.

Atypical organisms, more or less closely simulating the Meningococcus, have been described occurring either alone or with the Meningococcus in the cerebro-spinal exudate of meningitis cases. For example, Eve & Clements isolated an organism, from a sporadic case of meningitis in a child of a year and two months, which closely resembled a Micrococcus catarrhalis but showed in addition a few rod-like forms in older cultures. In this instance, however, there was a preceding abscess of the neck, an associated bronchitis and a terminal pneumonia. They also isolated in pure culture two other organisms (Hull No.2 and No.3) from apparently primary meningitis in infants. These more closely simulated the Micrococcus catarrhalis only they did not grow at room temperature. Then McDonald (1908) has described five cases where Gram-negative bacilli, which often grew to a great length in culture, were found in the cerebro-spinal exudate alone, with meningococci or with Diplococcus crassus. By describing a pyaemic case in a child of one and a half years

Kitchie & M'Donald, A Case of Pyaemia and Meningitis, associated with a Pathogenic Leptothrix Bacillus.
The Journal of Pathology and Bacteriology Vol.XIII, p.19.1908.

K. Stoevesändt, Erfahrungen bei der bakteriologischen Untersuchung meningitisverdachtigen Materials.
Centralbl. f. Bakt. I Abt. Orig. Bd. XLVI, p.259. 1908.

compare also
V.kuata, Ueber einen Kokkobacillus der menschlichen Bindehaut.
Centralbl.f.Bakt. I Abt. Orig.Bd.II,p.630 1909.

Von Siefert, Volkmanns klin. Vorträge, Nr. 240. *

M. Kirchner, Zeitschr. f. Hyg. Bd. IX. 1890.

Frosche & Kolle, In Flugge's Die Mikroorganismen. II, p.96. 1896.

which developed meningitis, the causal organism being a Gram-negative leptothrix bacillus, Ritchie & McDonald (1908) have linked together McDonald's earlier cases of Gram-negative bacilli with those such as have been referred to already on page 19 associated with cellulitic infections. The interesting fact is that all these diplo-lepto-bacillary organisms have been isolated from young subjects. Stoevesandt (1908) has also described Gram-negative cocco-bacillary organisms in meningitis cases but he was doubtful of their etiological importance.

The organism which goes by the name of the Micrococcus catarrhalis was probably first observed by von Siefert who noticed it in the nasal secretion and sputum during a small epidemic of infective bronchitis, and first isolated by Kirchner (1890) but it was Pfeiffer who gave it its name, his description, verbally communicated to Frosch & Kolle, being published in 1896. He had observed it in and cultivated it from the sputum of some cases of acute infectious bronchitis which ran a less severe course than influenza. He also found it in the bronchioles and alveoli in broncho-pneumonia of children; very often with other organisms e.g. the B. influenzae. From its intracellular grouping, coffee-bean appearance and Gram-negativity it closely simulated both the Gonococcus and the Meningococcus. It grew on ordinary media and on gelatine at room temperature slowly without liquefaction, though it grew best on serum media it could also be grown even from the first on plain agar. On agar the growth resembled a delicate Staphylococcus albus one. It was

A. Ghon, K. Pfeiffer & H.Sederl, Der Mikrococcus catarrhalis (K.Pfeiffer) als Krankheitserreger. Zeitschr. f. klin. Med. Bd.XLIV, p.262. 1902.

Bezançon & de Jong, Bull. Soc. Méd. Hop. de Paris, March 2 & 16, 1905.*

K.A.Bunn & M.H.Gordon, Remarks on the clinical & bacteriological aspects of an epidemic simulating influenza. (in East Herts.district) The British Medical Journal Vol.II, p.421. 1905.

Lord, Centralbl. f. Bakt. I Abt. Orig. XXXIV, p.641. 1903.

J.A.Arkwright, On the occurrence of the Micrococcus catarrhalis in normal and catarrhal noses and its differentiation from the Gram-negative cocci. The Journal of Hygiene Vol.VII, p.145. 1907.

Vannod, loc. cit. page 10.

Bumm, Der Mikroorganismus der gonorrheischen Schleimhaut Erkrankungen. Wiesbaden, 1885.

further studied by Ghon,Pfeiffer & Sederl in 1902. Later Bezançon & de Jong (1905) in France and Dunn & Gordon (1905) in England described epidemics due to this organism; but Lord in 1903 pointed out that it occurred in ordinary bronchitic sputa and from a survey of the literature he came to the conclusion that it had frequently prior to that been mistaken for the Meningococcus. Arkwright (1907) has concluded from his work that it was not commoner in catarrhal than in healthy noses except in the case of children. Gordon in 1905 applied fermentative tests and differentiated three types of catarrhalis organisms, but this method was more extensively applied by von Lingelsheim (1906) who differentiated six types of Gram-negative cocci in nasopharyngeal secretions.

THE GONOCOCCUS.

Ever since its discovery by Neisser in 1879 Gonococcus has been the subject of much discussion. Indeed as Vannod put it in 1907 "la question du gonocoque est toujours a l'ordre du jour". Bumm (1885) who first succeeded in cultivating the organism (he used coagulated human serum) began the controversy of the differentiation of the Gonococcus by describing five other kinds of micrococci simulating it which he had isolated from the genitalia in health or disease (Micrococcus subflavus, Micrococcus citreus conglomeratus, Micrococcus lacteus faviformis, Micrococcus albicans amplus and Micrococcus albicans tardissimus). As all five,however,

G. Roux, Procédé technique de diagnose des Gonococci
C. R. de l'Acad. des sc., 8 Nov., 1886.

Löderlein, Archiv. f. Gynaec. Bd.XXXI, p.412. 1887.

readily grew on gelatine and were Gram-positive, they have never presented serious difficulties in diagnosis since Roux (1886) drew attention to the diagnostic value of Gram-negativity and intracellular disposition as characteristics of the Gonococcus. Complications further arose when it was shown that all ophthalmia neonatorum cases were not gonorrhoeal as Neisser had at first thought; but time has abundantly shown that a genuine infectious urethritis in Man is always associated with the presence of gonococcus-like organisms:- the Gonococci of Neisser.

(That other organisms e.g. the colon bacillus and the staphylococci, may be the basis of a urethritis is possible but such conditions are very rare and the infection is not in any sense of the word contagious. With the vagina on the other hand non-gonorrhoeal infections are comparatively common. The reason for this difference is obvious. The male urethra is frequently being flushed with urine and indeed save for the extreme anterior end it is normally devoid of micro-organisms. The vagina lacks such flushings and, having a wider aperture, soon after birth becomes inhabited by bacteria. In health, from puberty to the climacterium, the common organism present is the vaginal bacillus of Döderlein. To it the secretion owes its acid character during this period, an acidity which goes far to inhibit the development of other organisms and prevents the vagina becoming a breeding ground for pathogenic bacteria; but at each menstrual period this natural defence is weakened by admixture of the alkaline uterine secretion. Any

Dr. C. Menge & Dr. B. Krönig, Bakteriologie des weiblichen Genitalkanales 1897.

Axenfeld, In Kolle & Wassermann Bd.III, p.496. 1903. Also Wilbrand, Saenger, Staehlin, Jahrb. d. Hamburger Staatskrankenanstalten, 1894. *

W.B.I.Pollock, The Bacteriology of Conjunctivitis. Trans. of the Ophth. Soc. of the United Kingdom. Vol.XXV,p.28,1905.

Krukenberg, Klin. Monatsbl. f. Augenheilk.,Bd.XXXVII,p.271. 1899.

Morax, do. 1900.

Schanz, Über die Variabilitat der Gonokokken. D. med. Woch. 1904.

cause, therefore, which tends to augment the uterine secretion also tends to alter the normal vaginal flora and when such occurs the chances of bacteria gaining admission to the uterine cavity are considerably increased. Whatever be the primary cause or causes of endometritis there is no doubt that bacteria are demonstrable in a very large proportion of cases sooner or later and this contrasts markedly with the healthy uterine cavity, which is normally devoid of micro-organisms. Menge & Kronig (1897) have shown that the vaginal secretion does not reach a maximum acidity till several years after birth and this may in part be the explanation of the frequency of vaginitis in infancy.)

The great discussions have arisen on the characters of this "Gonococcus", whether it is a single clearly defined species or whether there are several varieties of it. Indeed the term pseudo-gonococcus has long been in use particularly by ophthalmic bacteriologists. At first it was used as a synonym for staphylococcus in mixed conjunctival infections e.g. by Axenfeld (1894) and by Pollock (1905), but its continued use in such a sense is much to be deprecated;for though intracellular staphylococci can mimic very closely gonococci in pus (see plate 1. fig. 3) the two organisms are otherwise vastly different and there is never any real difficulty in distinguishing them. Later by it Krukenberg (1899) has indicated Gram-negative cocci such as are occasionally found on conjunctivae presenting often no trace of inflammation. Morax (1900) and Schanz (1904) both considered these to be genuine gonococci but as the organisms

C.Brons, Beitrage zur Frage der gram-negativen Diplokokken der Bindehaut. Klin. Monatsbl. f. Augenheilkunde Bd.XLV p.1, 1907.

Axenfeld, loc. cit. p.122.

Compare also
Neisser & Scholtz, in Kolle - Wassermann Bd.III p.166, 1903.

Thalmann, Zuchtung der Gonokokken auf einfachen Nährböden. Centralbl.f.Bakt. Bd.XXVII 1900.

Wildbolz, Bakteriologische Studien über Gonococcus Neisser. Arch.f.Dermatol.u.Syph. 1902.

Vannod, L'agar ordinaire comme milieu de culture du gonocoque. Centralbl.f.Bakt. Bd.XL 1905.

proved non-pathogenic when inoculated on the latter's own conjunctiva they were probably not gonococci but members of the Micrococcus catarrhalis class (or even meningococci) as has since been demonstrated in the case of other eye strains by Brons (1907). Axenfeld (1908) indeed holds the view that gonococci only very rarely become latent on the conjunctiva - which is in marked contrast to the condition of affairs obtaining in the urethra or vagina.

For a study of the organisms in gonorrhoeal infections they must first be isolated and this is by no means an easy matter, or the many different gonococcus media which at present exist would not have been from time to time proposed. If it be realised, as is indeed the case, that strains vary much in their adaptability to culture media, then the subject of the cultivation of the Gonococcus will not be as grotesque as it appears at first sight. The dictum of Neisser (1899) regarding growths on ordinary agar that "Alles was wachst, sind sicher keine Gonokokken" must go. The true nature of the organisms isolated on ordinary media by some of the early workers may be in doubt, but Thalmann(1900) undoubtedly obtained primary cultures - sub-cultures failed - on his agar. Wildbolz (1902) succeded in maintaining growth on agar after several sub-cultures on serum-agar but Vannod (1905) both isolated and maintained a number of strains on plain agar, and I have been able to confirm his results. Even Vannod, however, does not claim his agar to be the best medium for the gonococcus and indeed in mixed infections

Wertheim, Reinzuchtung des Gonococcus Neisser mittels des Plattenverfahrens. Deutsche med. Wochensshr. Bd.XVII, 1891.

A.Wassermann, Ueber Gonokokkenkultur und Gonokokkengifte. Berlin. klin. Wochenschr. No.32 1897.

Finger,Ghon & Schlagenhaufer, Beitrage zur Biologie des Gonococcus. Arch.f.Dermat.u.Syph. 1894.

it is imperative to use other media. Vannod considers Wertheim's (1891) and Wassermann's (1897) the best of these and he concurs with Neisser & Scholtz in preferring the former. Wertheim's medium is in essence an ascitic-fluid-agar medium while Wassermann's is a pig serum-agar medium containing in addition a small proportion of glycerine and nutrose (casein sodium phosphate).

From a survey of the many suggested media it appears that the essential constituent of a good gonococcus medium is a proportion of uncoagulated albumin, derived either from animals or Man. Haemoglobin is no advantage and urine is of questionable value apart from the phosphates it contains. These again are better added in a purer condition.

Of late the reaction of culture media has been recognised to be of great importance though the early compounders of gonococcus media (and even, media in general) paid little attention to this point and described the failures with certain batches of medium rather to the lack of nutritive qualities in particular constituents. Finger, Ghon & Schlagenhaufer (1894) postulated a reaction frankly acid to litmus as essential. Thalmann in 1900, however, laid the optimum reaction between neutrality to litmus and neutrality to phenolphthalein. Vannod always uses a medium faintly alkaline to litmus and with serum media he also takes the very necessary precaution of adjusting the reaction of the ascitic fluids with sodium carbonate, for exudates vary notoriously in composition and in the amount of hydrochloric acid necessary to make them neutral to litmus. The neglect of the

E.Cohen, Physical Chemistry for Physicians & Biologists.(Fischer's translation) London, 1903.

L.J.Henderson, Equilibrium in solutions of Phosphates. Amer.Journ.of Physiology Vol.XV p.257, 1906.

reaction of the albuminous fluids used in such media may largely explain the lack of uniform success in the employment of Wertheim's and Wassermann's media in different hands.

What is the optimum reaction for the growth of the gonococcus and which indicator should be used?

In the early days of culture media Koch used litmus as an indicator but the modern tendency has been to replace it with a sharper indicator e.g. phenol-phthalein, which has the advantage of being colourless in acid solutions and pink in alkaline ones. That litmus is alkaline to some salts which are acid to phenol-phthalein is really beside the point. In using phenol-phthalein, however, care must be taken to eliminate the effect of carbon di-oxide (which is acid to phenol-phthalein) and this is best accomplished by making titrations of media as nearly at 100°C. as possible so that afterwards in the process of sterilisation the reaction of the medium will not be altered by the driving off of dissolved carbon di-oxide. The temperature at which the reactions of acid salt solutions are estimated is also of importance, because elevation of temperature leads to more dissociation of dissolved compounds into their constituent ions and acidity, according to the theory of electrolytic dissociation, is merely an expression of the degree of preponderance of hydrogen ions over hydroxyl ions; so that is another reason for choosing the temperature of boiling water.

Having chosen the indicator the next point is the choice of the degree of the reaction to it. For this a short consideration of the behaviour of blood serum is first necessary. As is well

B.Moore, in Further Advances in Physiology, Ed. by L.Hill, p.21, 1909.

L.J.Henderson, Concerning the neutrality of protoplasm.
Proc. Socy. Exper. Biol. & Med. N.Y., Vol.IV, p.87, 1906-7.*

L.J.Henderson & H.B.Webster, The preservation of neutrality in culture media with the aid of phosphates.
The Journal of Medical Research Vol.XVI p.5 1907-8.

H.Noguchi, Onthe Influence of the Reaction & Dessication upon Opsonins.
The Journal of Experimental Medicine Vol.IX 1907.

known serum reacts acid to phenol-phthalein and alkaline to methyl orange or to litmus. Pure aqueous solutions of acids or alkalies never react so but salt solutions e.g. phosphates, may. Blood, in addition to salts, possesses proteins and these also can act either as acids or bases. Now, while blood serum reacts differently to different indicators or in other words is amphoteric to indicators, that does not mean it is at one and the same time both acid and alkaline, which would be absurd. On the contrary, as Moore (1909) points out, the actual reaction of the serum is almost that of exact neutrality, in the sense of physical chemistry; that is to say, the concentrations of hydrogen ion and hydroxyl ion are about equal. The explanation of the behaviour of serum is that none of the indicators in use change colour just at the chemical or exact reutral point. They only do so when the ratio of the concentrations of the two ions reaches a certain point, different for each; but it must still be a low one if the indicators are to be of any practical value. (Alizarin and Congo red for example are quite insensitive to the weak organic acids.) It is the capacity of the proteins for combining with either acid or alkali, as the case may be, which regulates the reaction of the serum in health and prevents the occurrence of free acid or alkali in the blood. This is of the greatest importance to the tissue cells as they cannot bear any appreciable degree either of acidity or alkalinity (Henderson, 1906-7; Noguchi 1907). The phosphates act similarly as powerful neutralisers of free acid as is illustrated by the following equation:-

$$Na_2HPO_4 + HCl = NaH_2PO_4 + NaCl$$

In these experiments 13.5% acidity was taken as the maximum because it was the highest grade of acidity experimentally attained in the human urine by the oral administration of acid sodium phosphate (60grains 6 hourly).

These results in great measure explain the failures in the treatment of uro-genital tract infections by mere variations of the reaction of the urine.

Could one introduce free acid into the urinary tract the results would be vastly different.

In the case of alkalies orally administered (sodium or potassium carbonate or citrate) it is impossible to reduce the acidity to actual neutrality to phenolphthalein, although this point is approached.

The best direction in which to modify the reaction of the urine in gonorrhoeal infections is towards greater acidity.

Incidentally it may be remarked that the reaction of the urine in health varies directly with the specific gravity, which again is dependent upon the concentration of contained salts (largely in this connection, the phosphates).

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* Other recent Gonococcus strains, however, did grow on the 0.6% medium although only slowly. When the tubes were smeared with serum good growths took place on the 0.6%, the 1.5% & the 2.5% acid ones within 24 hours. No growth could be obtained on any of the more acid ones, even when sub-cultured from the 2.5% tube.

Di-sodium phosphate is slightly alkaline to phenol-phthalein, a 1% solution being 0.25% alkaline; sodium chloride is just acid to phenol-phthalein, on the neutral point, and mono-sodium phosphate is distinctly acid to phenol-phthalein, a 1% solution being 7% acid. That acid phosphate acidity is little noxious to bacteria in general is shown by the fact that good growths of B. coli, B. typhosus, B. diphtheriae and Staphylococcus pyogenes aureus can all be obtained on agar media in which sodium chloride is replaced by sodium phosphate (NaH_2PO_4) and having an acidity as high as 13.5% to phenol-phthalein. (5 grams per litre give 5% acidity to phenol-phthalein). V. cholerae which is notoriously sensitive to small amounts of free acid grew well up to an acidity of 2.5% acid to phenol-phthalein: faintly at 5% acid and it ceased to grow at 13.5% acid. The bacillus of plague gave good growth up to 2.5% acid and slight growths at the higher degrees. (The morphology on the less acid media was the typical short bi-polar form, but in the higher degrees the organism grew in long thin uniformly staining rods.) A recently isolated streptococcus and a recently isolated gonococcus both failed to grow on any of the tubes varying from 0.6% to 13.5% acid, but neither of them had ever been grown on a serum free medium.*

The acidity of an average specimen of serum when titrated to phenol-phthalein is equivalent to about 0.65% of hydrochloric acid of which about 0.51% has been estimated by Moore (1909) to represent the combining power of the serum proteins, the remainder

F.D.Chester, A Manual of Determinative Bacteriology. London,1901,p.27.

F.B.Gurd, A contribution to the bacteriology of the female genital tract with special reference to the detection of the Gonococcus. The Journal of Medical Research Vol.XVIII p.291, 1908.

being that of the salts.

Now as gonococci flourish among human tissue cells it appears not unreasonable to choose the reaction of the blood serum for that of gonococcus media and in practice this indeed has proved the most suitable. In this connection it is interesting to note that though the American Public Health Association in 1898 adopted 1.5% acid to phenol-phthalein as the standard Chester 1901 observed this was unsuitable for the growth of many delicate organisms and he suggested 0.5%.

The following is the medium which has given the most uniform results in my hands. It is a modification of Gurd's (1908) It possesses the advantages of transparency, simplicity in manufacture and suitability for either plates or sloped tubes. It is also very economical in serum, a point of importance where an extensive research has to be done.

A beef meat-extract is prepared as usual. To it are added 0.5% of sodium phosphate (Na_2HPO_4), 1% Witte's peptone and 2% of powdered agar. (Note that sodium phosphate replaces the sodium chloride which is so commonly used in making culture media. This for two reasons; first, the toxic action of sodium chloride on delicate unicellular organisms is well known, and second, while practically neutral to phenol-phthalein it has not the neutralising power of a phosphate compound. Further it is well recognised that, in making extracts of meat, chlorides are much more readily dissolved out of the tissues than are phosphates and yet phosphates are essential to ~~the~~ perfect nutrition.

The mixture is placed in a Koch's steriliser and after the agar has melted the medium is titrated while still hot. I use 5 c.cs. of the medium, 2 drops of a 1% phenol-phthalein solution and a 1/20th Normal Sodium Hydrate solution. The end point is taken when a faint, but permanent, pink colour appears. If the agar is prepared with care it is practically colourless (a faint yellow) and the changing colour is easily noted, but if the medium has been too long heated it darkens to a colour which makes the end point difficult to determine. Dilution, largely overcomes this difficulty, but over-heating should be avoided in any case as in general better growths are obtained the less the medium has been cooked. If 0.6 c.c. of the soda has been used to obtain this end point then (with the above proportions) the medium is of the correct degree of acidity. Always, however, more alkali is at first required; suppose 2 c.cs. were used then the medium is 1.4% too acid. To each 100 c.cs. of medium 1.4 c.cs. normal Soda solution must therefore be added; in practice usually a little more than the calculated figure is requisite. The reaction having been adjusted the medium is cleared, filtered, tubed and sterilised as usual. It should possess a moderate amount of water of condensation. Too dry a medium is a fault.

For use:- on to the surface of each sloped tube 3 or 4 drops of sterile heated (57°C) human serum are run and then the tubes are incubated over night to ensure that they are still sterile. For plates the agar is melted, cooled in a thermostat to 45°C and the serum added in the proportion of 0.2 c.c. to 5 c.cs. of agar. Before being inoculated the mixture is further

See plates 4 & 5.

cooled to at least 40°C as the Gonococcus is very easily killed by heat.

I prefer to isolate by successive strokes on slopes rather than by plating. It is truly remarkable how even from chronic cases in the female, which swarm with other micro-organisms, isolated gonococcus colonies develope and may with a little practice be distinguished from those of diphtheroids, streptococci and staphylococci which are the usual contaminating organisms. Fortunately a number of bacteria which occur in hosts in such conditions do not grow on this medium. In recent ophthalmia cases and urethral cases in the male the cultures are often pure from the beginning and well grown in 24 hours. To obtain the purest cultures it is necessary in the female to avoid the labial surfaces which teem with bacteria. I have found it sufficient to wipe the parts clean with dry sterile gauze and pass the platinum loop into the urethra or vagina. With women a vaginal speculum is necessary if it is desired to investigate the cervical secretion. Cultures may often be obtained from it in chronic cases where it is impossible to isolate from the vaginal secretion.

Although the latency of gonorrhoeal genital infections has been noted by several observers the fact does not seem to be sufficiently recognised that gonococci may be demonstrated with comparative ease in many cases at a time when they present little or no sign of inflamation and infinitesimal discharge. This is particularly so in males (see plate 2, fig.12) but it is also true of a proportion of cases in young female

Compare also
P.W.Nathan, The diagnosis & treatment of chronic polyarticular disease in children. New York Medical Record Vol.XXIX p.733, 1906.

* See plate 1, figure 4 & plate 10, figure 58.

children.

From joints the best procedure is to plate direct or with the sediment after centrifugalisation. Here the results are often negative which may be due to the fact that cases rarely come under observation early enough or that the organism does not flourish in the joint (the reaction of such effusions is often markedly acid to phenol-phthalein) but rather in the synovial membrane as is the case with Beattie's Streptococcus rheumaticus. I have rarely been able to demonstrate gonococci in films of joint effusions even after prolonged careful search though in some of these instances cultures have been positive.* It is often very difficult to say whether one is dealing with degenerate nuclear fragments or degenerate cocci when regarding some intracellular bodies not uncommon in the polymorpho-nuclear leucocytes of such effusions. It is of importance to recognise. however, that in some circumstances several days (even five) may lapse before colonies are visible in such primary plates. Perhaps this may also be coupled with the fact that the organism does not grow at all well anaerobically.

It is of great practical importance especially in winter to place the inoculated media at once in the incubator as the Gonococci readily die out at room temperature (they are often quite dead in a couple of hours). Further, care should be taken to prevent dessication of the medium. The small amount of water of condensation in the tubes may be conserved for weeks by the use of india-rubber caps.

On first isolation it is best to subculture every second day;

de Christmas, Contribution à l'étude du gonocoque et de sa toxine. Annales de l'Inst.Pasteur T.XIV, 1900.

Compare the plates shown at the end.

later the period may safely be extended to a week. Beyond this time the results are irregular: in one exceptional instance I have obtained sub-cultures after six weeks. Inoculations from the supergrowths often yield viable and well stained elements when the rest of the culture does not.

Fluid media even that of de Christmas (1900), have proved less satisfactory in my hands than solid ones and they are not to be recommended for isolation purposes.

Cultural Appearances.

At the first appearance on this medium (18 to 24 hours) the gonococcus colonies are minute semi-transparent, slightly elevated discs presenting a moist-looking, glancing surface to the naked eye. When examined with a low power lens they are almost transparent, a light greyish yellow colour with transmitted light. They are homogenous, the ground substance being finely granular, and they have definite uniform margins which, under a higher power, are very slightly toothed. As the colonies enlarge they tend to remain discrete; the centre thickens and gets more opaque, owing to the development of numerous ovoid coarse granulations, and the margins become scalloped instead of remaining circular (48 to 72 hours). Then owing to a radial plication of the colony radial striations develope and concentric rings, due to zones of different degrees of opacity also appear. Finally (about a week) still coarser granulations become visible as points of supergrowth throughout the colony and may attain a considerable size. These are often so white and opaque in contrast to the rest of

the colony as to suggest to the uninitiated contaminations. When examined at an early stage with a low power the appearance is exactly that of a superimposed daughter colony. When touched with a platinum loop the growths are readily removed from the medium and they have a distinctly viscous consistence, but they are neither slimy on the one hand nor tenaciously viscid on the other. Cultures fairly readily emulsify.

On agar the growth is less luxuriant and supergrowths appear as a rule earlier.

In sub-culture strokes on Löffler's serum after a week's incubation two types of growth may be seen:-most commonly, transparent flattened minute discrete discs with rounded or slightly undulating margins; more rarely, pale brownish yellow nail-head-like colonies. They have rounded raised centres, narrow flat more transparent peripheral zones and undulating margins.

In serum bouillon the common appearance is a finely granular sediment with clear fluid above. The size of the granules is variable as is their consistence and occasionally there is a transient faint uniform turbidity.

No growth occurs on gelatine at room temperature as growth only takes place at near incubator temperature. This statement requires a little modification because I have obtained a slight growth at 24°C on serum agar with 2 genuine gonococcus strains which had been under cultivation for 9 and 12 months respectively. Four other equally old strains did not show any growth at all and neither did 12 more recently isolated strains which were all

C.T.McClintock & L.T.Clark, Autolysis of the Gonococcus.
The Journal of Infectious Diseases Vol.6 p.217, 1909.

Vannod, loc.cit. p.21, 1907.

tested at the same time. Cultures readily succumb to higher or lower temperatures and they soon die out if dessicated, though they may remain viable for several weeks if drying is prevented. Frequent sub-inoculations (weekly) are necessary to maintain growth as the organisms undergo spontaneous autolysis, swelling to many times their original size and losing their affinity for stains. In physiological salt solution at incubator temperature this takes place with particular rapidity, no viable organisms being obtainable after 3 hours.

Anaerobically growth is much inhibited but not entirely suspended. Vannod found no growth at all to take place in vacuo but such a method is open to the criticism that the delicate cells may have been ruptured by the rapidly reduced external pressure consequent upon attachment to an air pump. My results were obtained in an atmosphere of pure Hydrogen.

Morphologically the coffee-bean diplococcus forms is the commonest, but tetrads and even appearances of chains of four elements are occasionally seen. There is much variation in size of individuals, even among successive generations of the same strain.

From the above description and a scrutiny of the plates shown at the end it will be noted that my colonies differ considerably from the classical description of Neisser & Scholtz and even from that of Gurd in that they have attained a larger size and shown more differentiation. All my strains have not presented identical appearances in culture. As colonies of the same strain on different parts of the same plate

Elser & Huntoon, loc.cit. p.402, 1909.

S.Flexner, Contributions to the Biology of the Diplococcus intracellularis. The Journ. of Expr. Medicine VOL. IX, p.105, 1907.

are not always alike it is hardly to be expected that on different plates they will be the same. The thinner and the drier the layer of medium the smaller and more opaque are the colonies, development often ceasing in 48 hours. Transparency of margin is not constant, some strains which on the whole show a less extensive growth being opaque to the edge. Other colonies again after some days develope a thickened margin leaving the more transparent zone between it and the centre. The granular centre is occasionally lacking and so are radial and concentric striations. The margin at a later date is sometimes more serrated than undulating.

No single character is absolute: but the combination of characters - greyish bluish white moist appearance, slight viscidity, transparency and scalloping of margin, granularity of centre, and concentric and radial striation-is of great importance for the diagnosis of gonococcus colonies. In spite of what Elser & Huntoon (1909) say many strains of Gonococci can be absolutely differentiated from cultural appearances alone particularly if their colonies are observed in their successive development for a few generations.

THE MENINGOCOCCUS.

This organism like the Gonococcus is a delicate one, but while it multiplies more rapidly and is more prone to spontaneous autolytic degeneration it is on the whole less susceptible to unfavourable influences e.g. cold, heat, sodium chloride, carbolic

P.H.Hiss jnr. & H.Zinseer, A report of 24 cases of Epidemic Meningitis treated with leucocyte extract.
The Journ.of Medical Research Vol.XIX p.429, 1908.

acid &c., and it is much less particular about the reaction of the culture medium. The range of reaction is a fairly wide one. For isolation purposes a reaction of about 1.5% acid to phenol-phthalein (slight alkalinity to litmus) is best, but in sub- cultures the organism readily accommodates itself to other degrees of acidity.

Undoubtedly the best method of isolation is by plating direct from the cerebro-spinal fluid with a serum agar mixture. (Mutatis mutandis the technique is identical to that used for the isolation of the Gonococcus from joints. See page 39.) Colonies are usually visible within 24 hours, though it may be 48 hours before they appear. Delays in plating are to be guarded against. The exact reason why meningococci should die out in tapped exudates which have stood for a time is not quite clear. They do not always do so, as I have on two occasions obtained abundant cultures where the fluid was kept over night in ice. Perhaps leucocytic ferments liberated by the disintegration of polymorphs (Hiss & Zinsser (1908)) may have some share in the process although, as Flexner (1907) has shown, the autolytic ferments produced by the cocci themselves are also noxious.

It is of considerable importance with recently isolated strains to sub-culture daily for a time, as at first they tend to die out very rapidly and of course dessication must be guarded against. Sub-inoculations on ordinary agar grow well and primary isolations are also possible with it, but stocks are best maintained on serum agar slopes (compare page 39)

P.Esch, Ein Beitrage zur Zuchtung des Meningococcus.
Centrbl.f.Bakt. I Abt. Orig. Bd.LII p.150, 1909.

R.M.Buchanan, On the differentiation of the Meningococcus from other Gram-negative Diplococci in the naso-pharynx of Cerebro-spinal Fever contacts. The Lancet, p.1590 June, 1907.

See below p.78.

Sub-cultures should be made every 4 or 5 days for safety, although strains can sometimes be revived in serum bouillion at the end of a fortnight.

Chapoteaut's peptone agar, with or without, 1-2% glucose, has given in Wassermann's hands better growths that Witte's peptone agar but of this peptone I have no experience.

The medium (sheep's blood-maltose-ascitic agar) suggested by Esch (1909) for the rapid isolation of the Meningococcus for diagnostic purposes has quite a good formula but its serviceability is not so great as Esch supposes. In the first place it is no easier to prepare than Buchanan's modification of Löffler's serum (glucose and neutral red). Secondly, in many cases, excellent primary growths are obtainable on other media within 18 hours from cerebro-spinal exudates, and finally the presence of a Gram-negative diplococcus in such an exudate is presumptive evidence that it is a meningococcus and serum treatment should be begun at once without waiting for cultural confirmation. The accurate identification of any organism cannot be condensed into a few hours.

The meningococcus grows readily in bouillion, a small proportion of serum materially aiding growth, forming a uniform fairly dense turbidity; later a finally granular sediment is deposited.

No growth occurs on gelatine at room temperature.

A delicate microscopical growth may sometimes be obtained on potato.

Under anaerobic conditions growth is inhibited, but not

entirely suspended.

Cultural Appearances.

In sub-cultures on serum agar meningococcus colonies are sometimes visible within six hours. After 18-24 hours there is usually an abundant growth. The colonies are minute semi-transparent greyish bluish white slightly elevated discs with a moist looking surface. On the whole they are more opaque, naked eye, than the average minute gonococcus colonies. Examined with a low power the most striking and highly diagnostic feature (it is rarely absent) is a very transparent outer zone, the actual margin being almost invisible (see Plate 5, fig.27). The general groundwork of the colony is semi-transparent and of a faint yellowish white colour when viewed with transmitted light. With a higher power it is seen to be made up of rather coarser granules than those of gonococcus colonies (see Plate 3, fig.18).

As the colonies increase in size they tend to remain circular and discrete. Fine radial striae do occur in older (2-3 days) colonies and the margin may become irregular (more notched than undulating, however), but they are not usually associated with a plication of the colony or with concentric striations, the colonies being usually uniformly opaque to very nearly the margin, where occasionally a thickening may be met with. Old meningococcus colonies presenting these features are little likely to be confounded with equal sized but, of course, older gonococcus colonies. Naked eye their flat uniformly opaque bluish white enamelled appearance when viewed by reflected

light is only rarely simulated by a Gonococcus. A narrow, transparent almost invisible marginal zone further gives the clue to the meningococcic nature of the colony, but it often requires a lens for its detection. Then the very coarse ovoid secondary central granulations so common with gonococcus colonies, which give the contrast between centre and periphery in them, are very rare in meningococcus colonies though supergrowths are of course common.

Occasionally old colonies (4 days and more) take on a sprawling type of growth. Rounded buds project from the circular central portion and give the margin a deeply bayed appearance which when slightly magnified shows secondary scallopings very similar to those of the gonococcus colonies, but as a rule they are more angular than undulating. The margin nearly always remains delicate though frequently there is a thicker more opaque zone running concentrically just within it. Supergrowths may appear anywhere on old colonies but they do not offer the same contrast as the gonococcus ones do on account of the already present comparative opacity of the whole colony.

The growths are like the gonococcus ones slightly viscid. They are easily removed from the surface of the medium and readily emulsify.

Morphologically in stained films of cultures the Meningococcus exactly simulates the Gonococcus. The fact that meningococcic diplococci are sometimes very minute is of no diagnostic value.

MICROCOCCUS CATARRHALIS.

This organism is usually described as growing readily on agar and as flourishing at room temperature on gelatine. The majority of strains do but I have met with undoubted ones which could not be grown on plain agar, of varying degrees of acidity, until they had been cultivated for several generations on serum media and some could not be coaxed to grow at all on gelatine at room temperature (see also Gordon, 1905). A medium slightly alkaline to litmus is best as in the case of a meningococcus.

The best isolation method is by serum agar plates, with or without the addition of an indicator as in Buchanan's medium, the sputum or naso-pharyngeal mucus having been emulsified with bouillion and diluted to an appropriate degree.

One rarely fails to obtain them from any sputum:- bronchitic, phthisical, or resolving pneumonic. Personally I have never met with a genuine Micrococcus catarrhalis infection as described by Dunn & Gordon.

Cultural Appearances.

The colonies are circular somewhat raised glistening discs of a greyish to creamy white colour; as a rule they are thicker more opaque and less moist than ~~the~~ meningococcus colonies, indeed from their opaque white varnished appearance they closely simulate a delicate Staphylococcus pyogenes albus growth. Viewed by a low power of the microscope they are brownish yellow in colour with distinct thick circular uniform margins. The ground-work shows minute granulations at the margin, but very coarse opaque ones towards the centre.

AS the colonies enlarge the centre appears darker and is definitely raised. The margin usually, sooner or later, (2 days onwards) becomes markedly notched and finally presents a jagged outline naked eye (see Plate 9, fig.54).

The raised central portion gives a fairly diagnostic appearance of a round-headed nail flattened peripherally; thus in cross section. Catarrhalis strains vary and sometimes, by taking on a less luxuriant type of growth, simulate a Meningococcus in gross appearance on slopes but there is little difficulty in differentiating them when single colonies are observed in detail.

The growths are readily removed from the surface of solid media and are more friable than viscid in consistence as a rule. Emulsions are not readily made in salt solution as agglutination spontaneously occurs.

In bouillion the commonest appearance is a dense granular sediment without turbidity, though on shaking a dense uniform turbidity results but it speedily settles into the granular deposit again. Occasionally, if the inoculation has been made just at the junction of fluid and tube and the tube kept still in the incubator, a gelatinous thick pellicle with stalactites forms on the surface but this, too, readily precipitates when the tube is shaken and allowed to stand.

On gelatine at room temperature growth usually slowly occurs without liquefaction (but see also previous page).

A true Micrococcus catarrhalis is never pigmented although the central portions of old colonies when viewed with transmitted

W.von Lingelsheim, Die bakteriologischen Arbeiten der Kgl. Hygienischen Station zu Beuthen O.-Schl. während der Genickstarreepidemie in Oberschlesien im Winter 1904-5.

Klinisches Jahrbuch Bd.XV 373p. 1906.

light appear brownish.

Regarding viability, it is often stated that the Micrococcus catarrhalis is a resistant organism but some of my strains have been nearly, if not quite as delicate, as some Meningococci and required as frequent sub-culturing; so that the atatement is only true in a general way.

Morphologically the Micrococcus catarrhalis presents as a rule larger elements in films than either the Gonococcus or the Meningococcus. The large spherical forms that appear in 24-48 hour cultures are rather more retentive of the stain in Gram's process than are the corresponding forms in the case of the other two organisms.

(The presence of crystalline masses in colonies upon which some authorities have laid stress for diagnostic purposes is quite of no value.)

OTHER GRAM-NEGATIVE COCCI FOUND IN THE RESPIRATORY TRACT.

Von Lingelsheim (1906) described five other organisms which differed from the Micrococcus catarrhalis in pigment production, agglutination and fermentation reactions:- (1) Micrococcus pharyngis siccus, (2) Micrococcus pharyngis cinereus, (3) Diplococcus pharyngis flavus I, (4) Diplococcus pharyngis flavus II, and (5) Diplococcus pharyngis flavus III.

Gordon (1905) had also noticed the occurrence of atypical fermenters, describing three varieties in all of catarrhalis organisms.

The colonies of (1) are in the early stages like the Micrococcus catarrhalis but have a slightly yellower tint and usually present a corrugated surface later, not unlike that of some of the acid-fast bacterial growths. The growth distinguishes itself by its extreme toughness and its very firm adhesion to the surface of the medium.

The others are sometimes also distinguishable naked eye by the colour of their colonies, particularly on Loffler's serum or on glucose containing media. (2) is greyish white, (3) greenish yellow, (4) golden yellow and (5) light yellow.

Pigment production is very variable even in the case of such an organism as the Staphylococcus pyogenes aureus so that further differential tests are necessary. The following table taken from von Lingelsheim shows the fermentative capacities of his varieties :-

24 hour results on solid media.

Organism	Number of strains	Dextrose	Laevulose	Galactose	Mannite	Dulcite	Saccharose	Maltose	Lactose	Inuline
Meningococcus	83	+	0	0	0	0	0	+	0	0
Micrococcus catarrhalis	21	0	0	0	0	0	0	0	0	0
" cinereus	4	0	0	0	0	0	0	0	0	0
Diplococcus pharyngis flavus I	6	+'	+	0	0	0	0	+	0	0
" " " II	4	+	+	0	0	0	0	+	0	0
" " " III	8	+"	0	0	0	0	0	+"	0	0
" " cinereus	4	+	+	0	0	0	0	+	0	0

+ indicates fermented ; +' indicates stronger fermentation ; +" indicates weaker fermentation.

The Micrococcus catarrhalis & the Micrococcus cinereus were further distinguishable from one another by agglutination tests.

If pigment production were slight D.ph. flav.III might be superficially mistaken for the Meningococcus.

Elser & Huntoon, loc.cit. p.398, 1909.

* To such the term Pseudo-meningococci is often applied.

A. Lieberknecht, Ueber Pseudomeningokokken aus dem Rachen gesunder Schulkinder, verglichen mit echten Meningokokken, unter besonderer Berücksichtigung des Wachsthums dieser Arten auf hämatinhaltigen Nährböden.
Archiv. f. Hyg. Bd. LXVIII, 143p. 1908.

For comparison's sake Gordon's findings are also tabulated:-

Organism	Number of strains	Dextrose	Sclactose	Maltose	Saccharose	Growth on Peptone at 20°C. after 3 days
Catarrhalis group	22	=	=	=	=	+
	2 (+1 from urine)	+	+	+	+	—
	1	+	=	+	=	+
Meningococcus	1	+	+	+	=	—
Gonococcus	1	+	+ acid	= alkaline	=	— none

Elser & Huntoon (1909) divided the chromogenic Gram-negative cocci of the naso-pharynx into three groups. The members of their Chromogenic group I have the same fermentation reactions (producing acid in dextrose, maltose, laevulose and saccharose) but differ among themselves in agglutination reactions. The pigment is greenish yellow. The chromogenic group II comprises organisms of the same species (fermenting dextrose, maltose and laevulose) and it is apparently synonymous with von Lingelsheim's Diplococcus pharyngis flavus I. The members of the chromogenic group III differed only in minor inconstant features from those of the chromogenic group I.

One great feature of these catarrhalis-like organisms is their tendency to revert on long cultivation to a scarcely pigmented rather moist semi-meningococcic type of growth already mentioned (page 51) as occasionally assumed by the Micrococcus catarrhalis itself.* On first isolation, however, they are readily distinguishable by cultural appearances from the Meningococcus.

They too should be isolated on a serum medium, preferably a glucose-containing one, to accentuate pigment production.

The organisms of this class which I have encountered have all given deposits in bouillion, without turbidity. On shaking

For fermentation test results see Table on p.62.

the sediment has either arisen as a tenacious column or been disseminated throughout the fluid in the form of granules of varying size. Supergrowths have been much less commonly observed than in the case of Gonococcus, Meningococcus or Micrococcus catarrhalis colonies.

None liquefied gelatine at room temperature and some did not grow on it.

As already mentioned (page 18) these organisms are of doubtful pathological importance; are fairly easily differentiated naked eye from the more important three and really only are noted here because of their behaviour with Gram's stain.

GENERAL CONCLUSIONS from CULTURAL APPEARANCES.

The Gonococcus, the Meningococcus and the Micrococcus catarrhalis are in a large proportion of cases readily differentiable from one another by cultural appearances alone.

The delicacy of growth and its comparative restriction to particular serum media; the granularity of centre, the transparency scalloping and striation of the margin of colonies are all highly characteristic of the Gonococcus when grown on the above described (page 38) serum medium and may be coupled with poor growth in serum bouillion.

The Meningococcus gives a more luxuriant and more rapid growth, growing well on a wide variety of media; the delicate almost invisible margins of young colonies (Plate 5, fig.27) which are bluish white to the naked eye are most characteristic

while the organism also gives an abundant growth (uniform turbidity with deposit later) in serum bouillion.

The Micrococcus catarrhalis is the most opaque and active grower of the three, it being the only one which grows on gelatine (without liquefaction) at room temperature soon after isolation. Its growth in serum bouillion - scum and granular deposit without general turbidity - and the opaque white varnished-looking colonies, with crenated margins and raised centre, on (serum) agar are also highly characteristic.

From the foregoing details it will be seen that the Micrococcus catarrhalis is the most, and the Gonococcus the least, vegetative organism of the three; that occasionally the vegetative capacity of the Micrococcus catarrhalis is very much less; and that with prolonged artificial cultivation a greater degree of it can be impressed on the Gonococcus than it possesses at isolation (see page 43).

So with practically every other cultural character, variations occur: while the rule is - only a granular deposit in serum bouillion with the Gonococcus, occasionally a slight turbidity is produced; this may be merely an expression of the different rates of growth in the two instances, as the different cultural appearances of surface colonies most probably are also.

Related as these three organisms undoubtedly are the frequency of the occurrence of certain cultural characters in strains isolated from different sources (but of course from similar cases) convinces me that they are representatives of

types which are sufficiently definite to be fairly readily recognised even when the organisms are encountered in unusual situations. That variations occur on each side of the mean does not in my opinion invalidate the claim of each to a specific name as Goodman (1908) on principle maintains.

It must not be imagined, however, that I would build species on minor cultural variations alone, for a mere glance at the gonococcus colony plates would indicate the possibility for several new names. A true species requires to be erected upon a summation of characters and of necessity the definition implies the presence of varieties within it.

Beyond cultural appearances and pathogenic properties, which have been already referred to in detail (pages 14-17) there are other properties which can be investigated with a view to the lucidation of the frequency (i.e. constancy) of characters in these organisms, such as - enzymatic activities upon fermentable substances and immunity reactions, and they will be discussed in this order.

ENZYMATIC ACTIVITIES upon FERMENTABLE SUBSTANCES.

As in everything else, difference of opinion exists in the choice of method best suitable for the elucidation of fermentative activities. Some vaunt solid media and others liquid. I have used both. The latter is easier to prepare, but I much prefer the former for use with this group and fluid media for the typhoid-colon group where it is an advantage to estimate the amount of gas produced. Whatever may be the case with other

J.A.Arkwright, Varieties of the Meningococcus with special reference to a comparison of strains from Epidemic & Sporadic Sources.
The Journal of Hygiene Vol.IX,p.104, 1909.

H.Trautmann & W.Fromme, Beiträge zur Epidemiologie und Bakteriologie der epidemischen Genickstarre.
München. med. Wochnschr. p.791, 1908.

groups, I have found little tendency, unlike Trautmann & Fromme (1908) and Arkwright (1909), for the tubes to be alkaline at one part and acid at others if drying is prevented by india-rubber caps; and I have uniformly observed my strains for the period of a month. For such a delicate organism they possess this great advantage that one can always on solid media see the nature and extent of the growth from the beginning and be certain that growth has taken place and also that no contaminations have occurred. The point is of considerable importance where serum is used, because as serum cannot certainly be sterilised by heat, on account of coagulation ensuing, one can never be sure of its sterility. It can only be obtained with asceptic precautions and then tested to prove its sterility. Then, Gonococci can be sub-cultured on plain agar of reaction almost neutral to litmus (better when serum has been smeared on the surface, but this is not necessary in a great proportion of cases) and this is a further advantage that the basis of the medium containing the fermentable substances is of definite and constant composition as is not the case in fluid media which contain a large proportion of added serum or albuminous fluids.

A further point of the greatest importance in the preparation of these media is to avoid alteration of the composition of the fermentable substances in the process of sterilisation. The proclivity for complex carbohydrates and glucosides to undergo hydrolytic dissociation when heated with acids, alkalies etc. is well known e.g. lactose yields dextrose and galactose.

L.Maquenne, Les Sucres et Principaux Derives. Paris, 1900, p.752.

L.Rivas, An improved and rapid test for Indol in broth cultures and for the presence of this substance in Meat Sugar-free broth.
The Journ. of Infectious Diseases Vol.IV p.641, 1907.

This is avoided by sterilising them separately in distilled water for 10 minutes at 110°C and not when they are in the agar medium, For careful work all vessels must be of Jena glass well steamed before use. Ordinary glass yields a considerable amount of alkali to the contained fluids during sterilisation.

Then of course the sugars &c. must be of the purest: dextrine e.g. must not contain dextrose as it commonly does (Maquenne (1900)) I have used both Merck's & Khalbaum's products.

The litmus is often decolourised when boiled in carbohydrate-containing media. This is also avoided by separate sterilisation.

The following is the method of preparation in detail. A beef meat extract is prepared as usual. It is inoculated with B.coli and the latter allowed to grow in it for 36 hours to exhaust the fluid of muscle carbohydrates. (As shown by Rivas (1907). B.coli does not completely exhaust a meat extract of its fermentable substances. It does so for members of its own class, for the organisms of this group and of some others but, as I have been able to confirm, there still remain substances fermentable by e.g. Streptococci. It is, therefore, necessary in the case of these latter organisms to use a pure peptone water agar medium. Controls in any case should always be employed. One batch of coli-exhausted (24 hours) medium, for example, had its controll~~ed~~ tubes fermented by a single meningococcus strain while 10 other strains did not ferment them.) Peptone and salt are now added and the medium heated to 100°C. It usually can be filtered clear through filter paper by the adoption of this device, which is a great saving of time over the old tedious

method of passing it through a Chamberland candle in order to remove the B.coli. Powdered agar (2%) is added to the clear filtrate, melted in it, the reaction adjusted to faint alkalinity to litmus, the medium cleared with egg-white, filtered and measured into separate flasks, which are sterilised in the Koch for short periods on three successive days.

The litmus (Kubel-Tiemann's solution was used throughout) is sterilised alone in the autoclave at 110°C for 10 minutes.

The carbohydrates &c. are made up in 10% solutions in previously sterilised distilled water and also autoclaved at 110°C for 10 minutes.

When the fluids are all hot they are mixed in the following proportions :- 85 c.cs. agar, 5 c.cs. litmus and 10 c.cs. of the respective sugar solutions, and then carefully run into previously sterilised tubes. The medium is immediately sloped.

The medium thus obtained is perfectly clear and sufficiently tinted with litmus to indicate its faintly alkaline reaction. (1/1000th normal soda solution produces no change in litmus but 1/100th is distinctly alkaline) It also possesses a moderate amount of water of condensation. The tubes of medium should be sterile if carefully prepared but they ought always to be incubated for 24 hours to ensure that they are sterile. With this medium I have obtained remarkably uniform and very definite results.

All my 25 strains, isolated from gonorrhoeal cases (eyes, joints, urethrae and vaginae), fermented with the production

of acid, but not of gas, glucose and glucose alone out of the following list of 19 substances :-

(One organism isolated from a knee joint of a case clinically diagnosed as gonorrhoeal arthritis by Sir George T. Beatson was an exception. It is referred to below, see page 79.)

Glycerine	triatomic alcohol	$C_3H_5(OH)_3$
Erythrite	tetratomic alcohol	$C_4H_6(OH)_4$
Adonite	pentatomic alcohol	$C_5H_7(OH)_5$
Arabinose	aldehyde of above	$C_5H_{10}O_5$
Xylose	" " "	$C_5H_{10}O_5$
Mannite	hexatomic alcohol	$C_6H_8(OH)_6$
Dulcite	" "	$C_6H_8(OH)_6$
Sorbite	" "	$C_6H_8(OH)_6 + \frac{1}{2}H_2O$
Dextrose, Laevulose, Galactose	mono-saccharides	$C_6H_{12}O_6$
Amygdaline	glucoside	$C_{20}H_{27}NO_{11} + 3H_2O$
Salacine	"	$C_6H_{11}O_5OC_6H_4CH_2OH$
Saccharose, Lactose, Maltose	di-saccharides	$C_{12}H_{22}O_{11}$
Raffinose, Dextrine, Inuline	poly-saccharides	$C_6H_{10}O_5$

The meningococcic strains examined, 30 in all, (isolated from Cerebro-spinal Fever cases) have all fermented maltose and glucose but no others.

Of true catarrhalis strains, 6 in all, (isolated from sputum and urine)none fermented any of the above mentioned substances; on the contrary they rapidly made the media alkaline.

Of the catarrhalis-like organisms the following types have been encountered :-

2 which fermented laevulose and maltose

2 which fermented glucose and maltose and

6 which fermented glucose, laevulose, maltose and saccharose

For comparison's sake the fermentative reactions of some other micro-organisms are given below : **(only acid is indicated)**

	glucose	galactose	lactose	laevulose	maltose	mannite	saccharose	adonite	dulcite	inulin	salacin	dextrin
Staphylococcus pyog. aureus	+	+	+	+	+	+	+	-	-	-	-	-
" " albus	+	+	+	+	+	-	+	-	-	-	-	-
" from a case of rheumatoid arthritis	+	±	+	+	±	-	-	-	+	-	-	-
Bacillus diphtheriae	+	+	-	+	-	-	-	-	-	-	-	-
" pseudo-dip.(Hoffmann)	-	-	-	-	-	-	-	-	-	-	-	-
Xerosis bacillus	-	-	-	-	-	-	-	-	-	-	-	-
Diphtheroid from a knee joint ? a skin contamination	+	+	-	+	+	+	+	-	-	+	-	+
Streptococcus from vaginitis	+	+	-	+	+	-	+	-	-	-	+	+
" rheumaticus	+	+	-	+	+	+	-	-	-	-	+	+
Jäger's Diplococcus (D.crassus) et von Lingelsheim.	+	+	+	+	+	-	+	-	-	-	-	-
Gonococcus	+	-	-	-	-	-	-	-	-	-	-	-
Meningococcus	+	-	-	-	+	-	-	-	-	-	-	-
Micrococcus catarrhalis	-	-	-	-	-	-	-	-	-	-	-	-

C.Brons, Beiträge zur Frage der gram-negativen Diplokokken der Bindehaut. Klinische Monatsbl.f.Augenh. Bd.XLV p.1, 1907.

kothe, Ueber d ie Verwendung verschiedener Zuckernahrboden zur Differentialdiagnose der Gonokokken.
Centrbl.f. Bakt. IAbt. Orig. Bd.XLVI,p._645, 1908.

As regards the rate at which the changes occur; meningococcus and catarrhalis results are usually well marked in 48 hours while the Gonococcus requires 4 to 5 days. At the end of a month's incubation the results are the same. The rapidity with which non-fermented tubes in the case of meningococcus and catarrhalis strains become markedly alkaline is strikingly in contrast with the very slow development of a much less alkaline reaction in the gonococcus tubes and is of diagnostic value.

Some gonococcus strains ferment glucose more feebly than others.

Some meningococcus strains also ferment glucose less rapidly than they do maltose.

The definiteness of these carbohydrate findings gives another set of characters of great specific importance and they may be added to those given on pages 55 and 56.

It must, however, be stated that all observers have not attained such uniform results. If one excludes galactose from Gordon's original results they essentially confirm mine. (I have prepared his medium with all the precautions outlined above - pp. 58 to 60 - designed to prevent alteration of the sugars and I have used a pure galactose. The results obtained were then identical to my own obtained on solid media). Von Lingelsheim (1906) did not study the Gonococcus but his Meningococcus results are confirmed by mine. More recently Brons (1907), Rothe (1908), and Elser & Huntoon (1909) are all in essential agreement.

It is a striking thing that the least consistent results

Andrewes, The Lancet. 1906, I, p.1172.

Dunham, The Journal of Infectious Diseases 1906, suppl.ii, p.10.
Goodwin & von Sholly, " p.21.

have been obtained by the users of fluid media, e.g. Arkwright (1909) found laevulose fermented by several meningococcic strains when it was dissolved in broth but not when in plain peptone water.

The vast majority of workers agree that both glucose and maltose are fermented by the meningococcus :- Gordon (1905), von Lingelsheim (1905), Andrewes (1906), Dunham (1906), Goodwin & von Sholly (1906), Kutscher (1907), Stoevesandt (1908), Symmers & Wilson (1909) and Arkwright (1909). Differences exist regarding galactose, laevulose and dextrine but if the reports are scanned it will be found that due care has not been taken to procure pure substances or to avoid alterations consequent upon sterilisation to which galactose and laevulose are particularly prone. The accounts of fermentations of galactose, laevulose and dextrine can therefore be passed by. Arkwright's descriptions of alterations of fermentative properties with artificial cultivation are highly interesting (compare, page 4) e.g. he isolated one strain which fermented no sugars on isolation, then maltose alone for some months and finally, after 10 months artificial cultivation, glucose and maltose; another strain fermented both on isolation but lost the capacity to ferment either; he has also obtained strains which at no time fermented either glucose or maltose, but I have not any parallel observations of my own, though I have had some strains in cultivation for nearly two years.

Regarding the Gonococcus:- Of two strains tested by Arkwright (1909) the one fermented glucose and maltose as had been recorded by Wollstein (1907) and by Gurd (1908) - he also got fermentation of mannite with five strains! -and the other

Watabiki, The behaviour of the Gonococcus in carbohydrate media.
The Journal of Medical Research Vol.XX,p.365, 1909.

J.W.Fisher, A study of agglutination.
The Journal of Medical Research Vol.XVI,p.208, 1907.

For a discussion of the criteria of potency of anti-meningitis sera (opsonins v. agglutinins & complement deviation) see:-

J.W.Jobling, Standardization of the Antimeningitis Serum.
The Journ. of Exper. Medicine Vol.XI, p.614, 1909.

R.Kraus & St.Baecher, Ueber Meningokokkenserum.
Ztscrh. f. Immunitatsforsch. u. exper. Therapie
I.Teil Orig. Bd.III p.9 1909.

fermented glucose and galactose as Gordon (1905) and Sheenan & Ritchie (1908) had also found for one strain each. Watabiki (1909) studied fifteen strains in a 50% horse serum peptone water medium and found they all behaved alike - glucose, maltose, dextrine and laevulose being fermented while galactose, lactose, saccharose, mannite, dulcite and inulin were unaffected. No reliance, however, can be placed on results obtained in a medium prepared as his was. Rothe (1908) and Elser & Huntoon (1909) - fifteen strains - found as I have done that the Gonococcus only fermented glucose.

IMMUNITY REACTIONS.

If diversity of opinions and results can exist regarding fermentative capacities there is even more scope for them among immunity phenomena.

Agglutination, bactericidal action, complement deviation and opsonic action have all been studied by various observers.

It ought to be borne in mind that one type of immunity reaction may be of differential value in some groups and useless in others. Agglutination, for example, is of great service in the typhoid-colon group, but valueless in the Dysentery group (Fischer, 1907). Then, anti-substances do not by any means vary pari passu e.g. a serum may be actively opsonic yet not possess much complement deviating power. Finally, a marked degree of immunity may exist without demonstrable agglutinin, bactericidal action or anti-infectious (which could confer passive immunity)

R.P.Strong, Protective inoculation against Plague.
The Journal of Medical Research VolXVIII,p.325, 1908.

T.Houston, & J.Kankine, The Opsonic & Agglutinative action of blood serum in Cerebro-spinal Fever.
The Lancet 1907, p.1213.
The British Medical Journal Novr.1907,p.1414.

A.S.M.Macgregor, Cerebro-spinal Meningitis Immunity Phenomena with reference to Clinical Aspect.
The British Medical Journal 31st.Octr. 1908.

being present in the blood serum as has been pointed out by Strong (1908) in the case of immunity to Plague. (Although there was a slight degree of complement deviating power and a triflingly increased opsonic action, they were infinitessimal compared to the degree of immunity the animals possessed.)

Regarding agglutination and opsonic action much has been made of them by Arkwright (1909) and by Houston & Rankin (1907) for the differentiation of epidemic and sporadic strains of meningococci; but as demonstrated by McGregor (1908) Meningococci may suddenly in the course of artificial cultivation lose their susceptibility to opsonic action and old cultures as Houston & Rankin themselves confessed are often phagocyted by normal serum. Then as regards agglutinins Symmers & Wilson (1909) and Elser & Huntoon (1909) note that old cultures are as a rule twice as easily agglutinated as recent ones and Arkwright acknowledges this in general. Indeed, a close scrutiny of the latter's table VII, p.112, shows agglutination to vary with the same strain and sera at different times. Stoevesandt (1908) has pointed out that the sera of patients suffering from other diseases may agglutinate the Meningococcus as actively as that of many Cerebro-spinal Fever patients' do (1 in 30 - 1 in 100 within 24 hours). Still more to complicate matters Elser & Huntoon (1909) point out that the nature of the medium cultures are grown on exerts an effect upon their agglutinability (serum acting deleteriously) and that under certain circumstances centrifugalisation of an immune serum causes a reduction of its agglutinating value.

J.Bruckner & C.Cristéanu,
Agglutination du gonocoque par un sérum specifique
" " meningocoque(deWeichselbaum)par un sérum gonocoque
and other papers in C. R. soc. biol., T.LX, p.846 &c 1906.

J.C.Torrey, Agglutinins & Precipitins in Anti-gonococcic Serum.
The Journ. of Med. Research Vol.XVI,p.329, 1907.

O.Teague & J.C.Torrey, A study of Gonococcus by the method of"fixation of complement". The Journ. of Med. Research Vol.XVII p.223, 1907.

R.Muir & W.B.M.Martin, On the Deviation of Complement by a Serum and its Anti-serum, and its Relations to the Precipitin Test. 1906.

Enough has perhaps been said to emphasise the extreme complexity of the subject of agglutinins and opsonins in connection with this group and to show the futility of using small differences to subdivide the families with and also the necessity for many controls if these methods are to be used for clinical diagnostic purposes. Bruckner & Christéanu (1906) found that an anti-gonococcus serum from a horse agglutinated gonococci and meningococci equally in a dilution of 1 in 2000 but in the opinion of Vannod (1906), Torrey (1907) and Elser & Huntoon (1909) who have studied this in great detail, provided that one uses active sera and the results are properly controlled (the Gonococcus is particularly susceptible to normal rabbit and group agglutinins) and that the strains chosen are not highly inagglutinable, agglutination tests served to differentiate the three great groups from one another.

Complement Deviation.

Wollstein (1907) obtained no difference between gonococci and meningococci by this method but Tague & Torrey (1907) and Vannod (1906) have on the other hand found it to be of service in differentiating them and I can confirm this latter opinion. The following figures which are typical of many experiments made with anti-sera derived from rabbits, which had been immunised over a long period with (dead and later, living) cultures, illustrate the point.

The method used was essentially that described in detail in the Journal of Hygiene, 1906, vol. VI., p. 265.

Constant amounts of organism emulsion and anti-serum were used and varying amounts of guinea pig complement. This appears to me to be a much better method than that employed by Teague & Torrey who kept emulsion and complement constant and varied immune body. As the rabbits had been injected with cultures grown on human serum-smeared tubes the emulsions for deviation purposes could not be used from cultures grown on these. The meningococci were therefore grown on agar and the gonococci on cat serum agar. Emulsions of approximately equal density were made in salt solution and heated to 80°C for fifteen minutes before use. A luxuriant 24 hour agar growth of the Meningococcus was emulsified with 1 c.c. of salt solution and made the standard of density. The amounts used for the tests were 0.1 c.c. of emulsion and 0.05 of anti-serum. The volumes were made up to 0.5 c.c. with saline solution and then guinea pig serum was added in increasing amounts. The mixtures were incubated for an hour and a half before adding the test 1 c.c. of sensitised ox red blood corpuscles. The following figures were obtained from a series simultaneously estimated :-

Dose of complement alone	less than .01 c.c.
" " " + IB v Mgccs.	.01 c.c.
" " " + Mgccs. emulsion	.04 c.c.
" " " + Mgccs.+ IB v Mgccs.	.3 c.c.
" " " + IB v Gccs.	.01 c.c.
" " " + Gccs. emulsion	.03 c.c.
" " " + Gccs.+ IB v Gccs.	.1 c.c.
" " " + Gccs.+ IB v Mgccs.	.04 c.c.
" " " + Mgccs.+ IB v Gccs.	.06 c.c.

D.J.Davis, Studies in Meningococcus Infections.
The Journal of Infectious Diseases Vol.IV,p.558, 1907.

Ivy McKenzie & W.B.M.Martin, Serum-therapy in Cerebro-spinal Fever. 1908.

J.C.Torrey, Bacteriolysis of the Gonococcus & of the Meningococcus with Normal & Specific Immune Rabbit Serums.
The Journ. of Med. Research Vol. XIX, p.471, 1908.

W.B.M.Martin, Bactericidal action in relation to gonococci & meningococci. 1909.

They show that the immune sera alone do not appreciably deviate; that the organism emulsions alone deviate 4 and 3 doses respectively; that the combination of organism emulsion plus the specific immune body deviates 30 and 10 doses respectively; that the combination of organism emulsion plus non-specific immune body deviates 6 and 4 doses respectively which indicates the presence of both specific and group deviating immune-bodies in these anti-sera.

Similar results were obtained by the use of commercial anti-meningococcic sera; Flexner's being more powerful than Ruppel's or Burroughs Wellcome & Co's.

Bactericidal Action.

The first account of the bactericidal action of serum on the Meningococcus was published by Davis in 1907 but I had independently observed and studied the phenomenon with normal and meningitic patients' sera in the summer of 1907. The methods, the findings and the results of their practical application to the treatment of Cerebro-spinal Fever by Dr. Ivy McKenzie and myself are given in detail in the Journal of Pathology and Bacteriology Vol. XII. pp.539-548 (1908)

Then Torrey in December 1908 published his results with rabbit sera by which he had divided the gonococci into groups. I have since further studied the action of normal and immune sera obtained from rabbits on both the Gonococcus and Meningococcus; Proceedings of the Pathological Socy. of Gt. Britain,p.2,Jly,1909.

The technique is difficult on account of the tendency of the Gonococcus to undergo rapid autolysis in salt solution,to die even

at room temperature and to succumb to temperatures very slightly over 40°C. The necessary precautions and controls make determinations very laborious. Comparative tests, to be of any value, must be simultaneously carried out on account of the variations that occur in sera even of normal animals from day to day; so that it is practically impossible to compare a large series of strains by this method. Though Torrey is no doubt right in the main that the gonococcus group is variable in this particular as in agglutination and complement deviation reactions, the fact that he used different amounts of rabbit serum for activation with different strains and compared results on different days robs his results of much of their value.

The conclusions deduced from a large series of experiments are given below. They afford further evidence of important differences between gonococci and meningococci.

1. Normal sera may be bactericidal towards gonococci and meningococci. Of those tested (guinea-pig, rabbit, cat, human) cat's serum has been most active on both organisms.

2. A normal serum may be distinctly bactericidal towards meningococci and yet have practically no effect on gonococci, e.g. guinea-pig and human.

3. The serum of a normal rabbit may vary within short periods of time, on one occasion being actively bactericidal towards the meningococci, while on another almost without action. Simultaneous observations on the gonococcus showed comparatively little variation in the serum.

4, From rabbits inoculated with living cultures of gonococci

and meningococci, bacteriolytic immune-bodies have been obtained which can be reactivated by feebly acting normal sera and a marked bactericidal action result. These immune-bodies are relatively specific; thus a reactivated rabbit v.gonococcus serum which has a marked bactericidal effect on the gonococcus has only a slight effect on the meningococcus.

The following tables show the results in more detail.

At first experiments were tried with commercial anti-meningococcic sera, but as they contain, on an average, 0.4% of added phenol (for preservative purposes) which of itself is bactericidal, even in the presence of much albuminous matter (heated normal serum) , true serum reactions were impossible.

The meningococcus emulsions were made with bouillion, 1% acid to phenolphthalein, 1 c.c. to an 18 hour serum agar culture. 0.05 c.c. of a 1/100 dilution was added to each tube. The volume was made up to 0.5 c.c. in each case by the addition of bouillion (not salt solution). After 3 hours incubation 0.03 c.c. of each mixture was plated in 5 c.cs. of a serum agar mixture and the results read after 2 days incubation. In the case of the Gonococcus a 0.6% acid bouillion was used.

Effect of Phenol (0.4% aqueous solution) on Gonococcus & Meningococcus.

1.	0.2cc Human serum +	no phenol	20000	25000	colonies
2.	"	+0.025cc phenol solution	less	4000	"
3.	"	+0.05cc " "	less	1200	"
4.	"	+0.1 cc " "	less	600	"
5.	"	+0.2 cc " "	2000	2	"
6.	"	+0.3 cc " "	300	1	"

In a similar experiment these strengths of phenol had even, in plain bouillion, less action on either B.typhosus or V.Metchnikoff.

R.Muir & C.H.Browning, On the Bactericiral Action of Normal Serum.
The Journal of Path. & Bacteriology Vol.XIII p.76,1908

Effect of Guinea-pig's serum on Gonococcus & Meningococcus.

1. o.3cc Guinea-pig's serum	practically	0	colonies
2. o.15cc " "	equal	1	"
3. o.05cc " "	about	800	"
4. o.025cc " "	1000 in each	8000	"
5. control, no serum	50	2000	"
6. control,+ o.1cc heated human serum	1000	16000	"

The gonococcus emulsion when plated at once gave 1000 colonies, when allowed to stand at room temperature 600 colonies & when incubated 50 only. This illustrates the difficulties which have to be overcome in such experiments. The influence of even small proportions of heated serum on growth is also demonstrated.

Effect of Cat's serum on Gonococcus & Meningococcus.

1. o.3cc Cat's serum	0	0 colonies
2. o.15cc " "	0	0 "
3. o.05cc " "	0	0 "
4. o.15cc heated cat's serum	thousands	tens of thousands
5. o,05cc " " "	thousands	tens of thousands
6. control (plated at once)	thousands	thousands

This Cat serum is particularly active. Note also the different rates of growth of the two organisms.

Effect of human serum on Gonococcus, Meningococcus & Bacillus typhosus.

1. o.3cc human serum	1000	800	0 colonies
2. o.15cc " "	1000	2500	"
3. o.05cc " "	1000	5000	countless "
4. o.3cc " " (heated)	4000	5000	countless "

This indicates well how much sharper bactericidal action is in the case of the B.typhosus, all the organisms being killed off at the highest proportion of serum. It also shows how a serum may be actively bactericidal towards one organism and not to others. Whether this is an indication of multiplicity of bacteriophilic complements or of the presence of natural-immune-bodies for some bacteria and not for others is at present undetermined. (In this connection see Muir & Browning,1908).

So far illustrations have been given of the action of normal sera; on the next page some typical results obtained with immune rabbit sera are tabulated. They are from a series simultaneously carried out. The same normal rabbit serum was used for both organisms. The immune sera were used both fresh and after they had been heated (to 57°C for 1½hours); this degree of heating destroys any complement but leaves any immune-bodies they may contain.

Bactericidal experiment: effect of normal, immune & reactivated immune sera from the rabbit upon gonococci & meningococci.

Plate	Sera	Colonies	
1.	o.2cc fresh normal	10000	650
2.	o.15cc " "	15000	2000
3.	o.1cc " "	15000	25000
4.	o.2cc heated "	15000	12000
5.	o.2cc fresh immune (homologous)	10000	18000
6.	o.1cc " " "	15000	38000
7.	o.2cc heated " (i.e. homologous Immune-Body)	10000	12000.
8.	o.2cc fresh immune (heterologous)	10000	25000
9.	o.1cc " " "	15000	38000
10.	o.2cc heated " (i.e. heterologous Immune-Body)	15000	38000
11.	o.2cc fresh normal + o.2cc heated immune (homologous)	0	0
12.	o.2cc " " + o.1cc " " "	0	100
13.	o.2cc " " + o.05cc " " "	600	1000
14.	o.1cc " " + o.2cc " " "	400	600
15.	o.1cc " " + o.1cc " " "	8000	18000
16.	o.1cc " " + o.05cc " " "	15000	25000
17.	o.2cc " " + o.2cc " " (heterologous)	10000	500
18.	o.2cc " " + o.1cc " " "	15000	700
19.	o.2cc " " + o.05cc " " "	15000	300
20.	o.1cc " " + o.2cc " " "	15000	25000
21.	o.1cc " " + o.1cc " " "	15000	25000
22.	o.1cc " " + o.05cc " " "	15000	25000
23.	o.1cc " " + o.2cc heated normal	10000	1500
24.	control, no serum; organism emulsion incubated in bouillion	10000	40000
25.	" " " " " plated at once	15000	20000

gonococcus meningococcus

First observe the numbers in the control plates 24 & 25; the gonococci have shown a slight tendency to die out in simple bouillion, but the meningococci have doubled their numbers within the incubation period of 3 hours.

Then observe the action of the fresh sera - plates1,2,3; 5,6; 8 & 9. On the Gonococcus even the immune serum has little action. With the meningococcus, on the other hand, fresh sera have more action: the normal serum is more potent than the immune. As complement is supposed to vary with the health of the animals,it would appear that the long process of immunisation of these animals had weakened their natural complement,although the general condition & weights of both remained good.

Consider next the action after heating the sera - plates 4,7,10; the bactericidal action of the fresh serum is in great part destroyed, but it will be observed (plates 1 & 4) that large amounts of heated serum still possess some bactericidal property. The theory of this is unknown.

The effect of combining fresh and heated normal serum is seen by comparing plates 3 & 23. There is some enhancement of the action particularly

A.Hamilton & J.M.Cooke, Inoculation treatment of gonorrhoeal vulvo-vaginitis in children. The Journ.of Infect.Diseases Vol.5,p.158,1908.

E.E.Irons, The treatment of Gonococcus Arthritis by injections of dead Gonococci,and the clinical reaction which follows the injections. The Journ.of Infect.Diseases Vol.5,p.279,1908.

W.K.Jack, Four cases of Gonorrhoeal Arthritis treated by vaccine therapy. The Glasgow Med. Journal,April 1910.

J.Rogers & J.C.Torrey, The treatment of Gonorrhoeal Infections by a Specific Antiserum. The Journ.of the Amer.Med.Ass. Vol.XLIXp.918 1907.

in the case of the Meningococcus (second column figures).

A much more enhanced effect is, however, obtained by combining heated immune serum with the fresh serum - compare plates 1,7,11,12,13 and 3,7,14, 15,16.

Then if the plates 11,12,13 and 14,15,16 be compared with 17,18,19 and 20,21,22 respectively it will be seen that a heterologous heated immune serum, while it has some action, is not nearly so powerful as the homologous heated immune serum.

Put in other words, we have here evidence of bactericidal immune bodies in the sera of the immunised animals which can be activated by complement (which is present in normal serum) . These immune bodies are also relatively specific.

If these bactericidal results indicate anything it is the importance of complemented immune sera. The immunity to any infection must be a very complex thing and lie far deeper than in mere alterations of serum or of polymorphonuclear leucocytes. As one cannot study all the factors that may have a bearing on the problem one can only deduce from the known ones and test any such theories practically. In the case of meningococcic infections everyone is agreed that treatment of whatever sort must be applied directly to the meninges e.g. by lumbar puncture. Further, all are agreed that if treatment is to be of any avail it must be begun early in the course of the disease. Bearing these provisos in mind,I cannot but be convinced that,by using suitably complemented highly immune (preferably polyvalent) serum,the death rate might be considerably reduced from what it is at present with the use of uncomplemented sera.

In the case of gonococcus infections the necessity is even greater for a specific immune serum. How this can best be attained in practice, whether by active immunity (vaccines) or passive immunity (polyvalent sera from animals) must be left for the future to decide. As there is a considerable body of evidence

D.J.Davis, Immune-bodies in Urinary Infections with Colon Bacilli & treatment by Inoculation. The Journ.of Inf.Diseases Vol.6,p.224 1909.

C.H.Browning, Chemo-Therapy in Trypanosome Infections. The Journ.of Path.& Bact. Vol.XII p.166, 1908.

E.Legrain, Recherches sur les rapports qu'affecte le Gonococcus avec les les éléments du pus blennorragique. Arch. de Physiol. No.6 1887.

K.vonHofmann, Bacterienbefunde bei chronische Gonorrhöe. Ztralbl.f.d.Harnkrankh. No.11, 1904. *

K.Stanziale, Die Bakterien der Harnröhre unter normalen Verhältnissen und bei Gonorrhöe. Centrbl.f.Bakt. I Abt. Orig. Bd.XLII p.19, 1906.

even now accumulated to show that organisms can acquire a high degree of resistance to antagonistic substances of all kinds when they have been subjected to their influence gradually over a period, chronic infections must be more difficult to eradicate than recent ones. Here it would appear that if, for example, vaccine therapy is to be carried out, the strain which is used will be of prime importance.

CLINICAL FINDINGS AND PRACTICAL DEDUCTIONS.

Acute conditions (with active inflammation and abundant muco-purulent discharge) in the urethra, vagina or eye can be diagnosed as gonorrhoeal with almost absolute precision from the examination of carefully prepared Gram stained films alone - the morphology and disposition of the Gonococcus and its presence in a practically pure state being highly characteristic. No other organism presents such a combination of clinical and microscopical appearances in these situations.

In chronic conditions there is more difficulty. As Legrain (1887) pointed out the Gonococci are often extra-cellular in this stage (as they are at the very commencement) and pus corpuscles relatively few in proportion to epithelial cells. Still, in the male urethra a diagnosis can often be positively made from films alone, even after many months (see plate 2, fig.12) . The commonly associated organisms in my experience are diphtheroids (of the Xerosis type) and staphylococci (mostly albus); see also Hofmann (1904) and Stanziale (1906). The frequency of the occurrence of

W.F.Robertson & D.Mckae, Rev.Neurol. & Psychiat. Edinb. 1907,p.455.

J.P.Candler, A Bacteriological Investigation of General Paralysis. The Archives of Neurology & Psychiatry Vol.IV, 1909.

Menge & Kronog, loc. cit. p. 22

the former (and their almost ubiquitous disposition on the body surface) goes far in my opinion to invalidate Ford Robertson's theory of a causal relationship of such organisms with General Paralysis of the Insane (see also Candler, 1909). Although I have obtained catarrhalis-like organisms from normal urines I have never met with the Micrococcus catarrhalis in cultures from the urethra. In this respect my experience differs from that of Gurd.

A chronic gonorrhoea in the female urethra is practically always associated with the presence of numerous other organisms - streptococci, staphylococci, Gram-negative coli-form bacilli (which are often diplococcal) and minute thin Gram-negative bacilli (influenzoid) which are frequently intracellular, see plate 2, fig.11. Much more care is necessary in diagnosis. It should not be made on the presence of a single pair of Gram-negative even coffee-bean cocci, for these might be degenerate non-pathogenic staphylococci and culture is here frequently necessary before a certain opinion can be given. The statement of Menge & Kronig (1897) that the Gonococcus is little inclined to symbiosis with other bacteria and is the predominating organism even in chronic cases is hardly in accord with my experience of female cases even when the secretion has been carefully obtained.

The vagina of adults is rarely (unlike that of infants) the seat of a primary pure gonococcal infection (but see plate 2, fig.8), but Gonococci and hosts of other organisms may be encountered in chronic gonorrhoeal cases with an involved cervix (compare plate 2, fig.10).

Veillon & Hallé, Étude bactériologique des vulvo-vaginites chez petites filles, et du conduit vulvo-vaginal à l'état sain.
Arch. de med.exper., p.281, 1896.

Sub-acute vulvo-vaginitis cases in infants are a diagnostic pitfall for the unwary. While Holt (1905) Veillon & Hallé (1896) Hamilton,(1908) and Gurd (1908) all maintain that Vulvo-vaginitis in children is,quite apart from any question of sexual intercourse,usually gonorrhoeal in origin and emphasise the chronicity of some cases, yet there is undoubtedly a class due to other micro-organisms. The children are frequently unusually fretful "off colour" and have a slightly elevated temperature for which there is no very obvious cause (cystitis may give the same clinical picture). Pruritis may or may not be present. The secretion is from the onset thinner and less purulent than that of the true gonorrhoeal variety. On microscopical examination epithelial cells are seen to predominate. The flora is mixed and organisms are as a rule numerous. Gram-irregular (often negative) minute diplococci not unlike small pneumococci have frequently been encountered. On culture these prove to be atypical (Gram-positive in young, Gram-negative in old cultures) short chained streptococci. These organisms can be differentiated from the Gonococcus in films on two grounds:- the great numbers of organisms present and their minute lanceolate,rather than coffee-bean shape. The Gonococcus is never observed in such masses and numbers as are shown in plate 2, fig.9. The non-gonorrhoeal nature of these cases is confirmed by the rapidity with which they respond to ordinary hygienic measures.

In the eye as has already been mentioned (page 32) sub-acute conditions cannot be diagnosed as gonococcal by Gram films alone. Thorough investigation of the characters of the isolated

organisms is necessary and in the overwhelming proportion of cases which have been so investigated the organisms have not been gonococci.

In the cerebro-spinal exudates Gram-negative extra- or intra-cellular cocci of coffee-bean shape are most probably Weichselbaum's meningococci and the case may be for practical and therepeutical purposes so regarded. The atypical Gram-negative cocci which have been isolated from this source have, after all, been very few in number. The mere finding of a few Gram-negative diplococci (particularly intra-cellular ones) after a long search in films should not be relied upon to establish a diagnosis. Cultures should always be made and tubercle bacilli also searched for; e.g. in an early stage of streptococcic meningitis when the organisms may be exceedingly scanty and in very short chains a single phagocyted pair, partially digested, may appear Gram-negative. Then I have on one occasion had an exudate submitted to me where undoubted minute Gram-negative intra-cellular cocci but no tubercle bacilli were visible in films. The case passed from observation and rapidly proved fatal. At post mortem a lepto-meningitis was found but there were also definitely tubercular lesions present (histologically confirmed) in the meninges, brain and organs generally. (I am indebted to Dr. M. J. Stewart of Ruchill Hospital for these post mortem data). There was no opportunity for making cultures and the exact nature of these Gram-negative organisms is therefore obscure (compare page 26). The case, however, illustrates

Josselin de Jong, Centralbl.f. Bakt.I Abt. Orig. Bd.XLV, p.501, 1908.

one of the rarer possibilities of mixed infection.

With regard to bacteriological findings elsewhere in the body - throat, blood, joints &c., a complete study of the organisms is necessary to establish their identity.

The vast number of "meningococci" described in the throat have been called so on insufficient data.

Josselin de Jong's (1908) organism from a case of meningitis, which spontaneously recovered, was considered to be a gonococcus on account of the fact that the patient was suffering at the time from a gonorrhoea and the cultural appearances did not appear typical of the meningococcus (the details given, however, are by no means characteristic of the Gonococcus).

As already stated the findings from arthritic lesions in gonorrhoeal subjects are often negative (see page 41). I have, however, on several occasions, six in all, obtained Gram-negative cocci from such cases, which completely conformed to the gonococcus type isolated from the urethra. But one strain already referred to was atypical (page 61). It was isolated on 30th January, 1909, in pure culture (several colonies grew) from a knee joint (the only affected joint) of a woman, aged 31; the illness dated from 21st December, 1908. Superficially from cultural characteristics, appearances in films and conditions of growth (meagre save on gonococcus media) it looked, and was regarded as, a gonococcus. A vaccine was prepared. The patient improved and left hospital in March, but did not return to report. Six months later the fermentative reactions were studied and the organism gave those of a Micrococcus catarrhalis.

F.B.Mallory & J.H.Wright, Pathological Technique, 3rd. edition, Philadelphia, 1904, p.148.

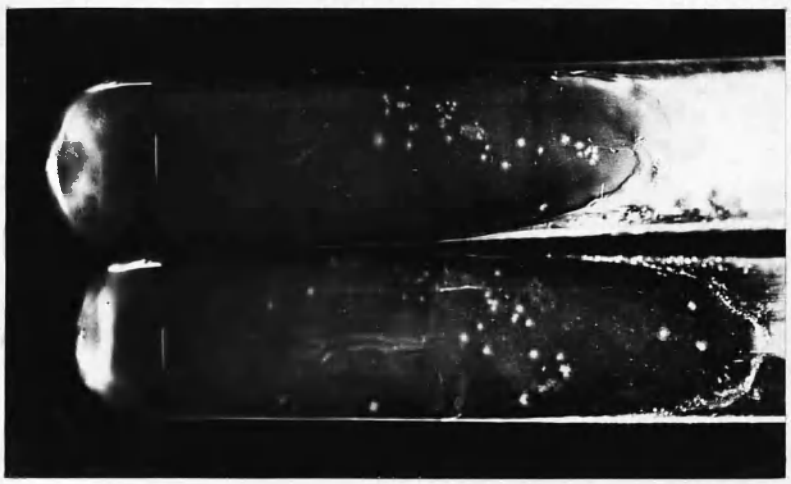
It was then also found to grow on a variety of media and slowly but well on serum agar at 24° C (not on gelatine) and that its viability was on the gonococcus medium at 37°C was considerable (sub-cultures took after six weeks). Its growth was more viscid than friable, however, and stable suspensions could be made in salt solution. Its growth in serum bouillion was in the form of a granular deposit. Up to the present it has not given an actively complement-deviating serum in a rabbit but like a genuine gonococcus strain it deviates comparatively little with an active meningococcic serum. It is unfortunate that this organism's characters were not studied at length immediately on isolation and that the genitalia were not also bacteriologically investigated. The fact remains, however, that it is an atypical Gram-negative organism from a joint.

The only other reference in the literature to the possibility of such a finding is that given by Mallory & Wright (1904) who state that "we have met with a Gram-decolourising coccus in an arthritis of the knee, clinically of gonorrhoeal origin, which, in cover-glass preparations from the exudate, was regarded as the gonococcus, but which was found not to be that organism by the study of it in cultures." Dr. J. Homer Wright has kindly furnished me with the following details of the case, which have not been published elsewhere.

"The patient was a woman, 39 years of age. Two days before delivery of a child her right ankle and a joint of her right thumb became red, swollen and painful. The next day the right knee became swollen. The child developed gonorrhoeal ophthalmia. Three weeks after delivery she entered the Hospital for operation on knee which had become flexed and fixed on account of pain. It was swollen, hot and extremely tender. She had a profuse vaginal discharge, which contained micrococci decolourising by Gram's method and intra-cellular.

At the operation on the knee a few drops of a cloudy, pinkish fluid, with flocculi in suspension, was obtained for examination. Microscopical examination of this showed numerous pus cells. In a few of these diplococci, decolourising by Gram's method were present. From this fluid three blood-agar slant culture tubes were inoculated. On the surface of each of these 20 to 30 colonies appeared after 48 hours. The photograph shows the gross appearances of the colonies. The colonies were composed of micrococci, which as a rule were made up of paired hemispheres; in some instances tetrads were formed. There was great variability in the size of the individual micro-organisms. They decolourised by Gram's method of staining. Degenerate forms also early appeared in the cultures as in the case of the gonococcus. Further study of the micro-organism in sub-cultures showed that it would grow on plain agar and also on Loeffler's firmly coagulated blood serum, such as is used for cultures of the diphtheria bacillus, in the form of minute colourless colonies. It did not die out after seven days in the incubator. In glucose bouillion it grew in the form of a viscid sediment with slight clouding of the medium. In the course of some days there was a tendency to the formation of a delicate pellicle at the surface.

As will be seen from the foregoing and from the photograph this micro-organism differs from the gonococcus in its ability to grow on the ordinary culture media mentioned and in the morphology of its colonies. The borders of the colonies were more lobulated than those of the gonococcus and furthermore the colonies did not show the granulation of their central portions which is so characteristic of colonies of the gonococcus. Another peculiarity of the colonies distinguishing them from the gonococcus colonies was the presence of dark lines or fissures extending peripherally from the centre of the colonies."



As will be seen from Dr, Wright's description and from his photograph it is by no means excluded that his organism is not a gonococcus but as he did not apply fermentation tests the exact nature of his organism must remain undetermined.

A. Wadsworth, The isolation of the Meningococcus from a case of Scarlet Fever. Ref. in Schmidt's Jahrbuch Bd.305, p.72, 1910.
Studies from the Dept. of Path. of the Colls. of Phys. & Surg. Columbia University, XI. 2. 1909. *

Wadsworth (1909) has described an organism from the knee exudate of a Scarlet Fever patient, which microscopically was not differentiable from the meningococcus but as I have been unable to obtain the original paper I cannot say how far he substantiates his point or whether his strain more properly belongs to this class of atypical Gram-negative organisms in joints. The occurrence of meningococcic arthritis during the course of Cerebro-spinal Fever is well accredited and it is also established even an ophthalmia may be the primary source of infection of a gonococcal arthritis; but in the light of the above findings, the true nature of Gram-negative organisms in joints, apart from definitely gonorrhoeal or meningococcic lesions, has still to be demonstrated. Likewise, with the knowledge of the existence of an extensive group of saprophytic catarrhalis-like organisms, the occurrence of such conditions as gonorrhoeal stomatitis and proctitis must be seriously questioned and it must be left to the future to establish their reality.

I trust that sufficient evidence has been adduced in the foregoing pages to bring conviction that the subject of the Gram-negative cocci pathogenic to Man bristles with difficulties, and to show that if <u>single</u> characters of organisms are alone considered, strains from different sources (and even from the same class of source) vary much and almost innumerable variations

occur: but that when the several characters of organisms from various sources are studied, certain fairly well-defined groups of associated characters are found to pertain to organisms from certain localities; that a Gonococcus type, a Meningococcus type and a Micrococcus catarrhalis type exist, the organisms of the first being associated chiefly with gonorrhoeal, those of the second chiefly with meningeal and those of the third chiefly with catarrhal (respiratory) conditions in Man; and that, while border-land organisms do exist, it has yet to be proved that a typical organism of one class can be artificially converted to a typical organism of another; that on the theory of probabilities a diagnosis of an organism may be made in some circumstances from a few characters with almost absolute precision, but that in other situations every resource of Bacteriology must be taxed before one is able to recognise an organism with any degree of certainty.

Plate 1.

Fig.1.
Gonococcus, 24 hours growth.
Primary culture from urethra showing tetrads.
Gram & Carbol fuchsin. x 1000 diameters.

Fig.2.
Gonococcus, 24 hours' growth.
First sub-culture (same strain as fig.1) showing variation in size.
Gram & Carbol fuchsin. x 1000.

Fig.3.
Staphylococcus pyogenes aureus, pus film from knee joint.
To show the mimicking of Gonococci.
Gram & Carbol fuchsin. x 1000.

Fig.4.
Gonorrhoeal Arthritis, film of exudate.
No organisms were seen in films, but Gonococci were obtained by culture.
This is the common appearance, contrast with Plate 10, fig.58.
Gram & Carbol fuchsin. x 1000.

Fig.5.
Bacillus coli in a urinary sediment.
From a case of pyelo-cystitis. The organism was present in pure culture. To show diplococcal forms.
Gram & Carbol fuchsin. x 1000.

Fig.6.
Bacillus coli in a urinary sediment.
From the same case as fig.5. The organism was present in pure culture. To show filamentous forms. This was obtained when symptoms were more acute, but this relationship does not always hold.
Gram & Carbol fuchsin. x 1000.

PLATE 1.

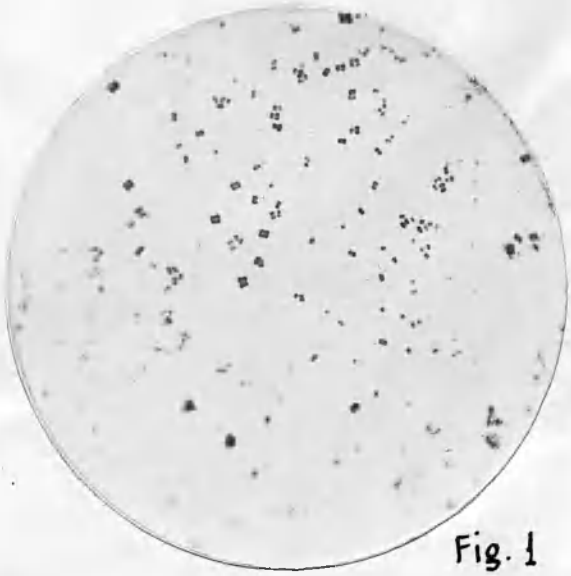


Fig. 1

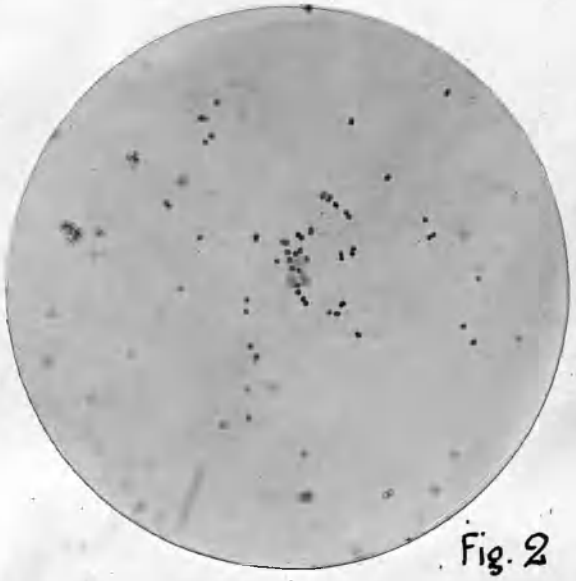


Fig. 2

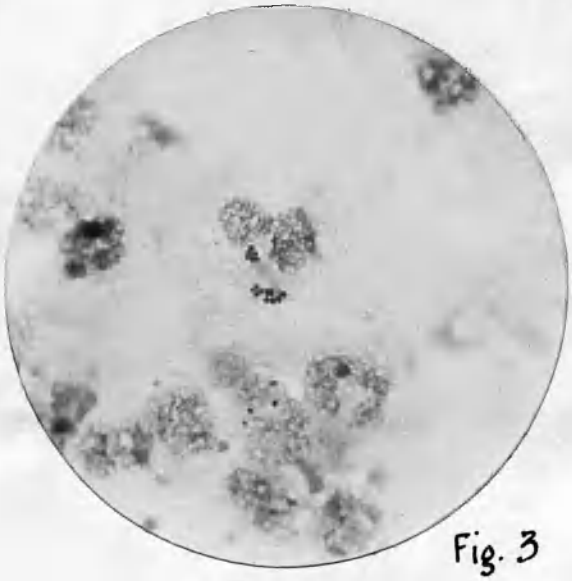


Fig. 3

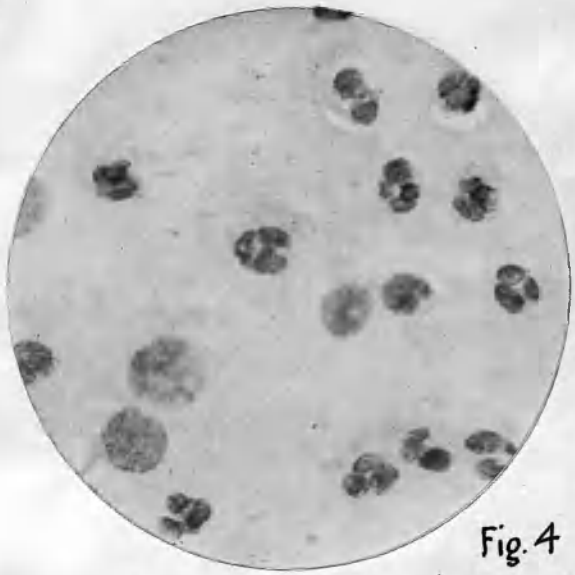


Fig. 4

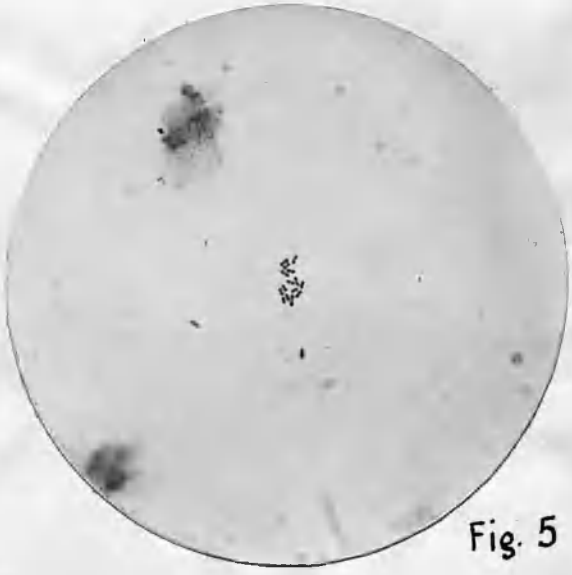


Fig. 5

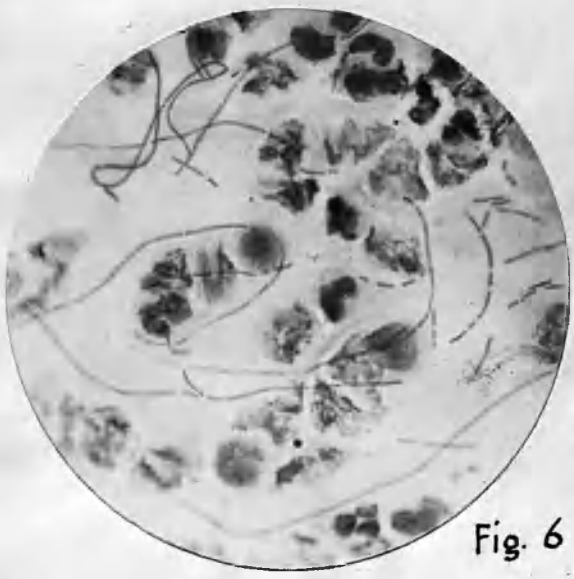


Fig. 6

PLATE 2.

Fig.7.

Vaginal discharge normal, showing epithelial cells and vaginal bacilli (Doderlein). Gram & Carbol fuchsin. x 1000 diameters.

Fig.8.

Vaginal discharge gonorrhoeal, showing gonococci, vaginal bacilli and many pus cells. Gram & Carbol fuchsin. x 1000 diameters.
From a patient aged 19.

Fig.9.

Vaginal discharge non-gonorrhoeal, showing epithelial cells, few pus cells and enormous numbers of minute diplococci.
From a patient aged 3 years (vulvo-vaginitis)
Carbol fuchsin. x 1000 diameters.

Fig.10.

Vaginal discharge, chronic gonorrhoea, showing pus and epithelial cells, and a large variety of micro-organisms - minute and large bacilli mostly Gram-negative, many varieties of cocci and a large straptococcus chain. No gonococci are visible in the field. From a patient aged 39.
Gram & Carbol fuchsin. x 1000 diameters.

Fig.11.

Urethral discharge, chronic gonorrhoea, showing pus and epithelial cells streptococci in chains and minute Gram-negative bacilli within a pus cell ("influenzoid" bacilli). No Gonococci are visible in the field. Culture was positive. From the same patient as fig.10. Female 39 years.
Gram & Carbol fuchsin. x 1000 diameters.

Fig.12.

Urethral discharge, chronic gonorrhoea, 18 months duration, showing pus and epithelial cells and extra-cellular Gonococci in tetrad formation. From a male of 30 years.
Gram & Carbol fuchsin. x 1000 diameters.

PLATE 2.

Fig. 7

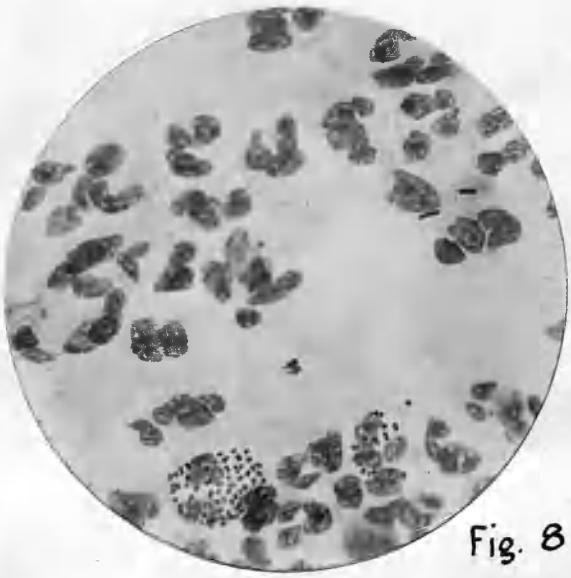


Fig. 8

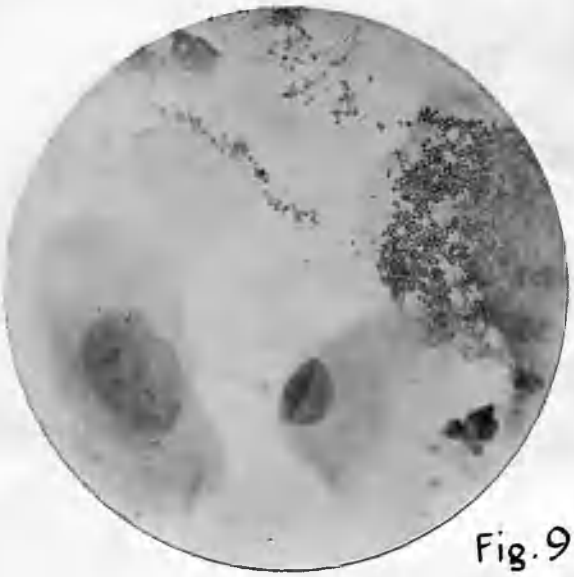


Fig. 9

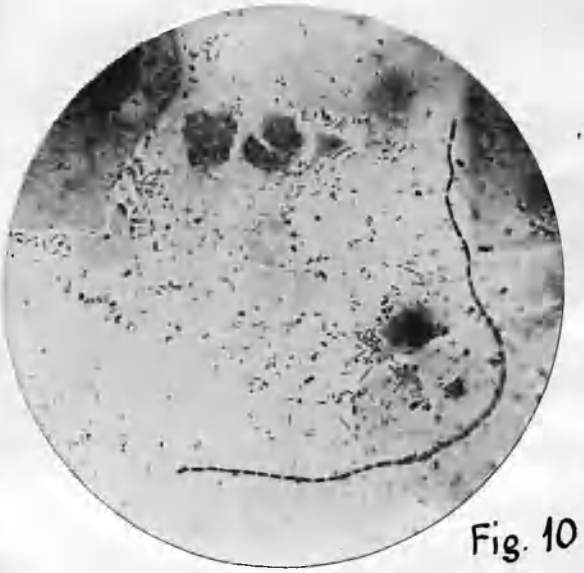


Fig. 10

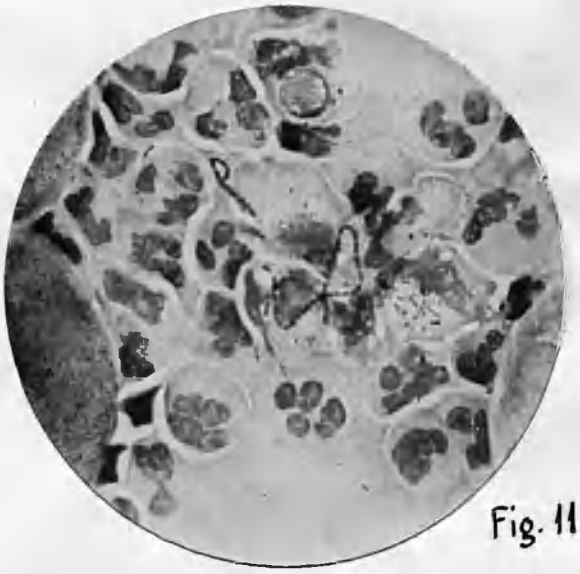


Fig. 11

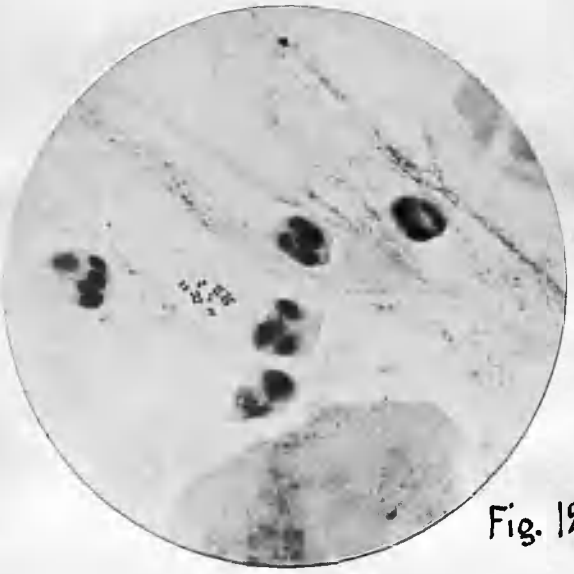


Fig. 12

PLATE 3.

Fig.13.
Meningeal exudate, post mortem, from a case of post operative meningitis, showing numerous Pneumococci mainly extra-cellular.
Gram & Carbol fuchsin. x 1000 diameters.

Fig.14.
Meningeal exudate, post mortem, from the case of Cerebro-spinal Fever referred to on page 27, showing diplococci, two short-chained streptococci well stained and some other chains more faintly stained (Gram-negative ones). Typical memingococci are not shown in the field.
Gram & Carbol fuchsin. x 1000 diameters.

Fig.15.
Meningeal exudate, during life, from a case of streptococcic meningitis secondary to otitis media, early stage. The organisms were extremely scanty. Note the proportion of mono-nuclear cells.
Gram & Carbol fuchsin. x 1000 diameters.

Fig.16.
Meningeal exudate, during life, from the same case as fig.15, obtained shortly before death. Note the polymorpho-nuclear character of the cells and the large numbers of organisms, some being diplococcal but the majority long-chained. Pure cultures were obtained.
Gram & Carbol fuchsin. x 1000 diameters.

Fig.17.
The margin of a gonococcus colony, 3 days' growth, x 200
To contrast with fig.18 the margin is delicate but visible and is very finely toothed.

Fig.18.
The margin of a meningococcus colony, 2 days' growth, x 200
To contrast with fig.17 the actual margin is invisible, the most striking feature is the coarse granulation of the groundwork.

PLATE 3.

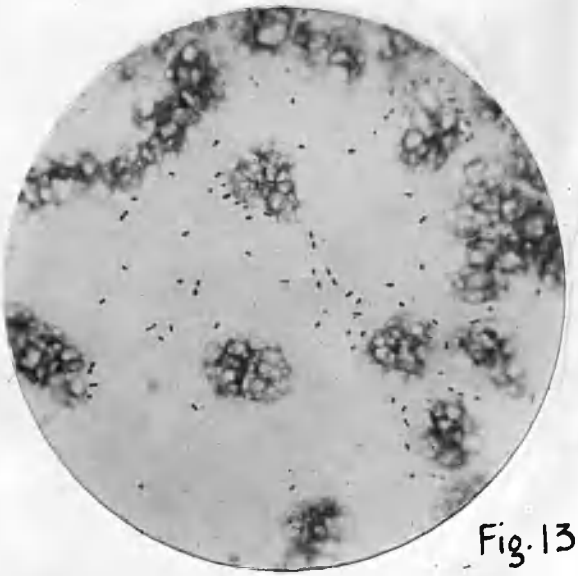


Fig. 13

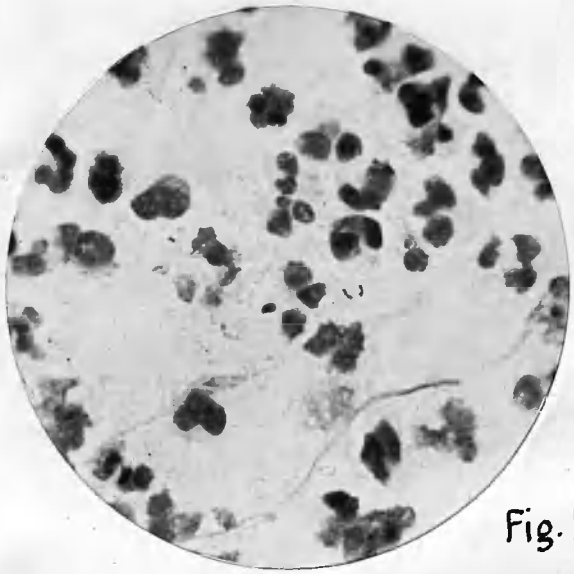


Fig. 14

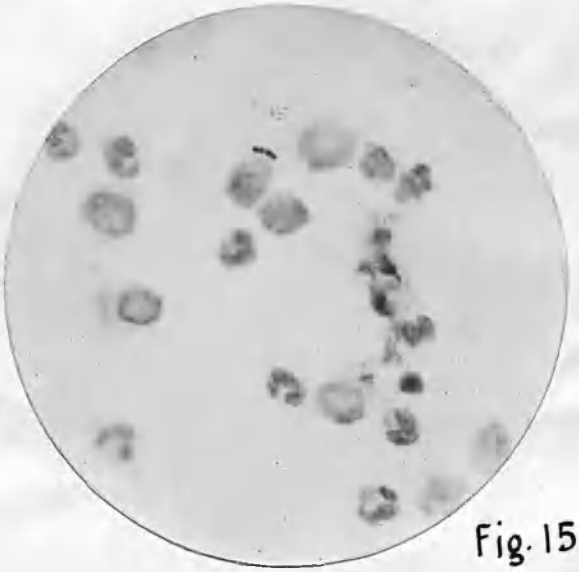


Fig. 15

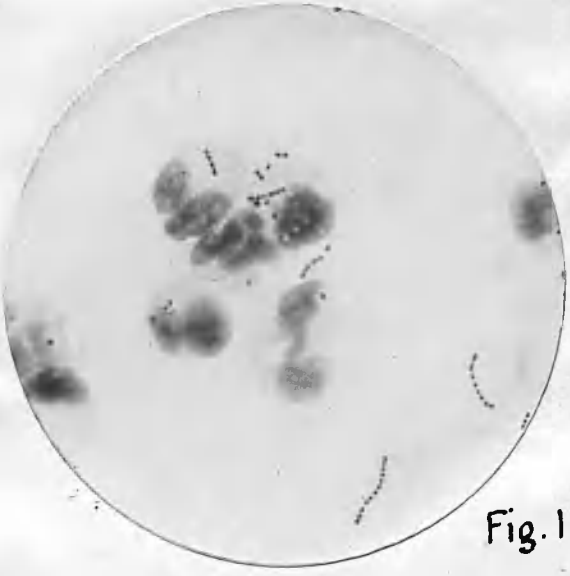


Fig. 16

Fig. 17

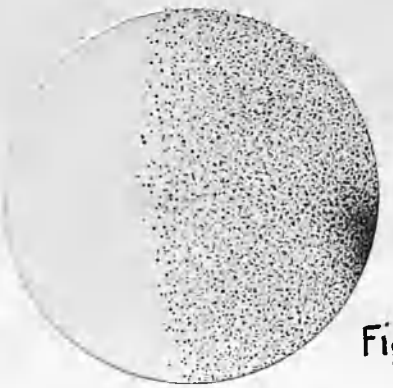


Fig. 18

Plate 4.

Fig.19.
Gonococcus stroke culture 1 day's growth.

Fig.20.
Gonococcus stroke culture 1 day's growth. From the same plate as fig.19. Note the extreme transparency.

Fig.21.
Streptococcus stroke culture 1 day's growth. Note the tendency for the central colonies to fuse, also the fuzzy margins. The strain was from an endometritis.

Fig.22.
Bacillus diphtheriae stroke culture 1 day's growth. Naked eye the growth is drier and more opaque than either the streptococcus or the gonococcus growths. To a high power lens the colonies are, like the streptococcus colonies, composed of coarse granules, even more so than is shown in plate 3,fig.18. The margins are also distinctly fuzzy though less so than a streptococcus.

Fig.23.
A chromogenic Gram-negative coccus 1 day's stroke culture. Naked eye colonies were a light greenish yellow and fairly opaque.

Fig.24.
Micrococcus catarrhalis stroke culture 2 days' growth. The colonies are, for the most part, heaped up in the centre and consequently very opaque.

These photographs are all taken by transmitted light and are x 9 diameters.

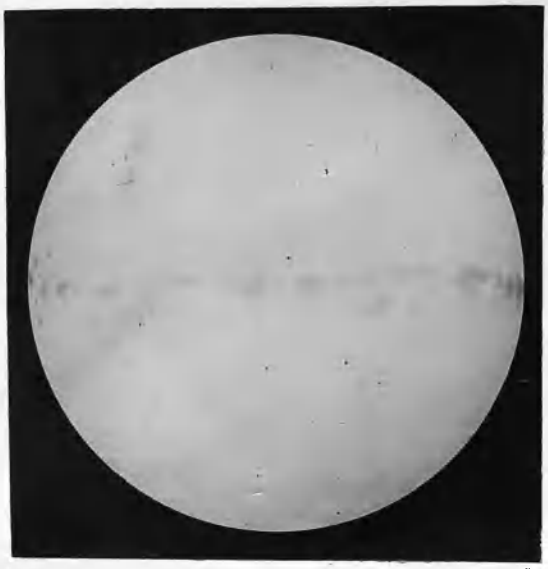


Fig. 19

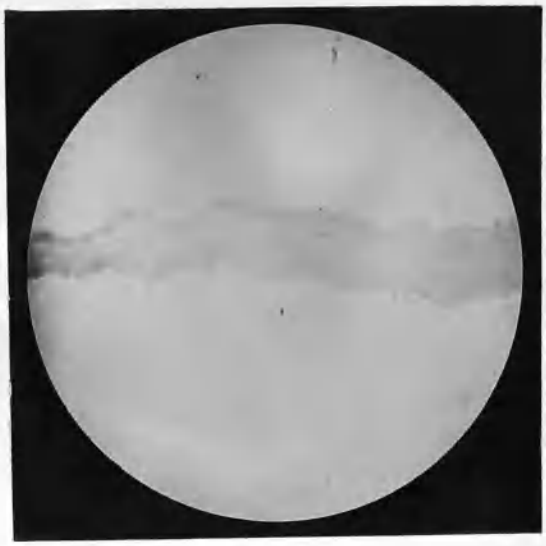


Fig. 20

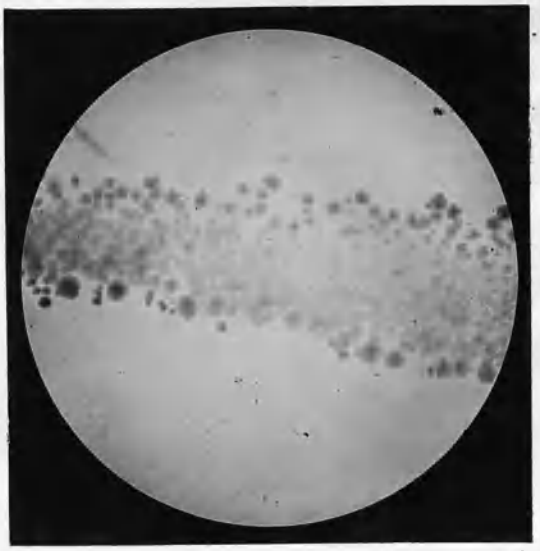


Fig. 21

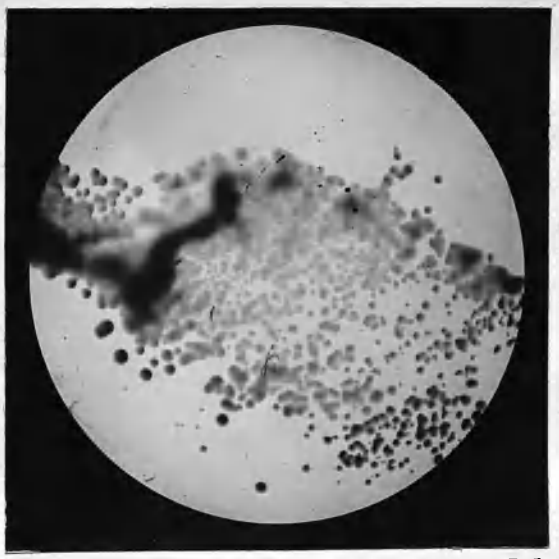


Fig. 22

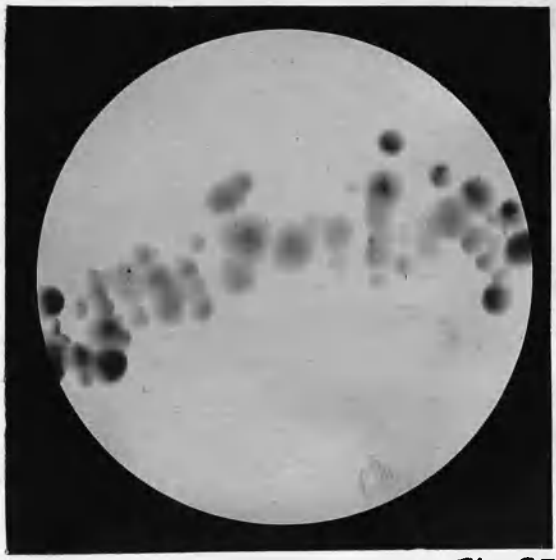


Fig. 23

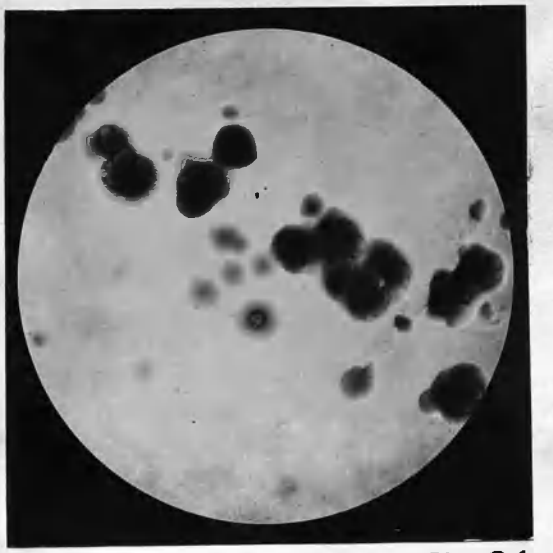


Fig. 24

Plate 5.

Fig.25.

Gonococcus stroke culture 5 day's growth.
The semi-confluent nature of the growth is well shown.
The super-growth granules are also well shown.

Fig.26.

A diphtheroid organism of the Xerosis type 1 day colony.
From a urethra (chronic gonorrhoea). The extreme opacity even at this stage is shown by the density of the object.
At a later date the margins would become very notched.

Fig.27.

Meningococcus colonies 2 days' stroke culture.
The opacity of the centre and the transparency of the margin are well shown.

Fig.28.

Gonococcus (W) 3 days' growth.
Note the granular centre, the scalloped margin and the radial and concentric striation.

Fig.29.

Gonococcus (ON) 3 days' growth.
Note the granularity of centre. There is slight radial striation, but the margin is comparatively circular still.

Fig.30.

Gonococcus (KC) 3 days' growth.
Note the granular centre and the general plicated nature of the colony (better seen naked eye)

These photographs were all taken by transmitted light and are x 9 diameters.

PLATE . 5.

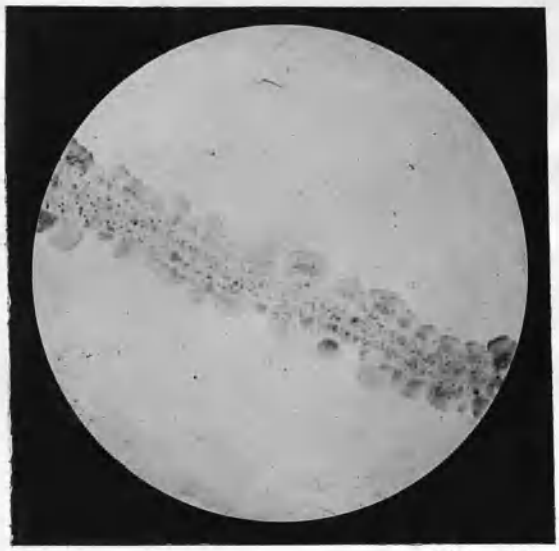


Fig. 25

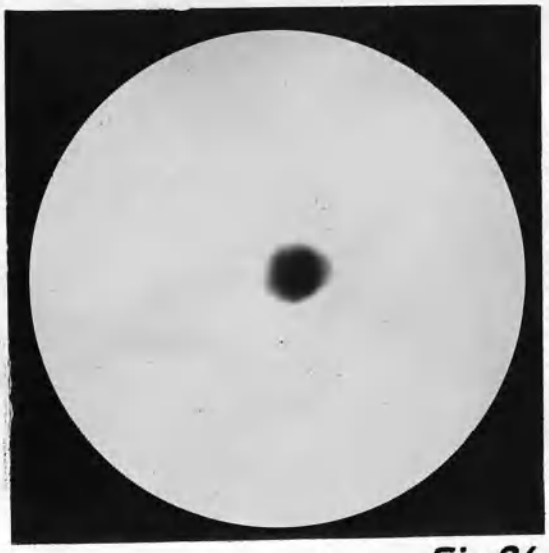


Fig. 26

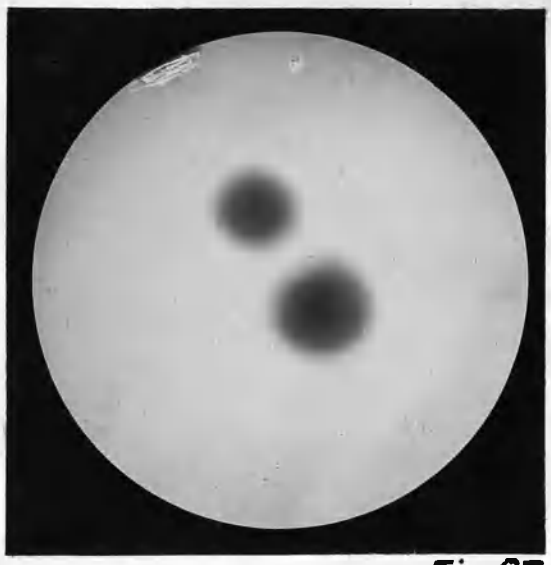


Fig. 27

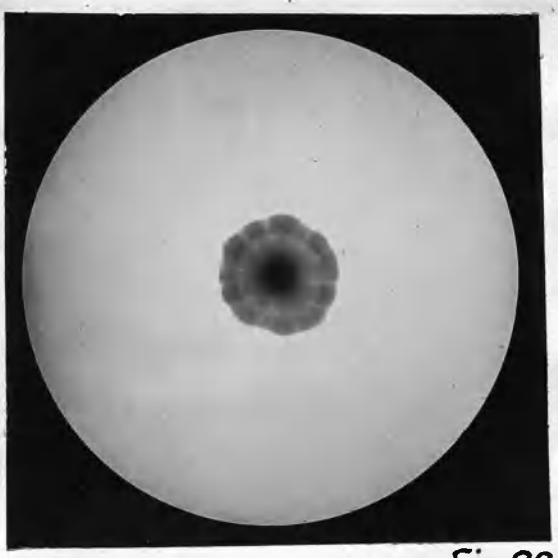


Fig. 28

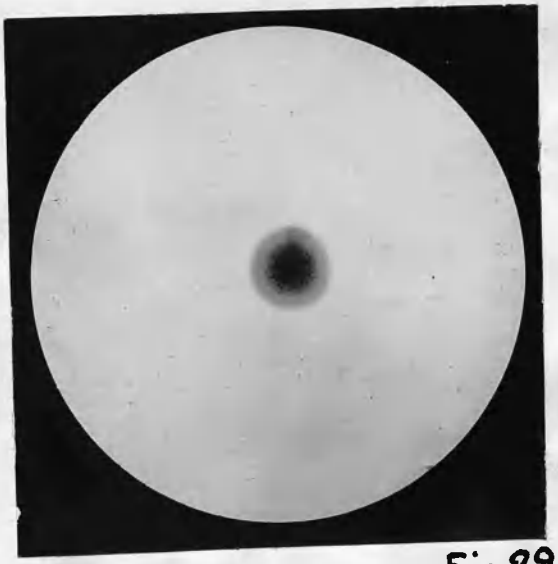


Fig. 29

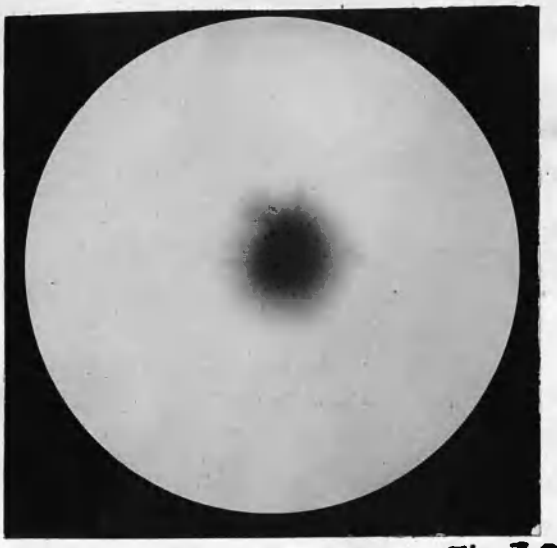


Fig. 30

Plate 6.

Fig.31.
Gonococcus (C) 3 days' growth.
Note granularity of centre and extreme plication of colony.

Fig.32.
Gonococcus (KS) 5 days' growth.
There is scarcely any granular centre and little radial striation. The scalloping of the margin is extreme.

Fig.33.
Gonococcus (H) 5 days' growth.
To contrast with fig.34, which is from another colony of the same strain on the same plate.

Fig.34.
Gonococcus (H) 5 days' growth.
To contrast with fig.33. Naked eye the colony appeared more opaque and radial striation was less marked.

Fig.35.
Gonococcus (V) 5 days' growth.
A highly typical gonococcus picture is presented.
To contrast with another colony of the same strain grown on the same plate. Fig.36.

Fig.36.
Gonococcus (V) 5 days' growth.
To contrast with colony shown in fig.35.
There is much less differentiation and the colony naked eye appeared more opaque.

These photographs were all taken by transmitted light and are x 9 diameters.

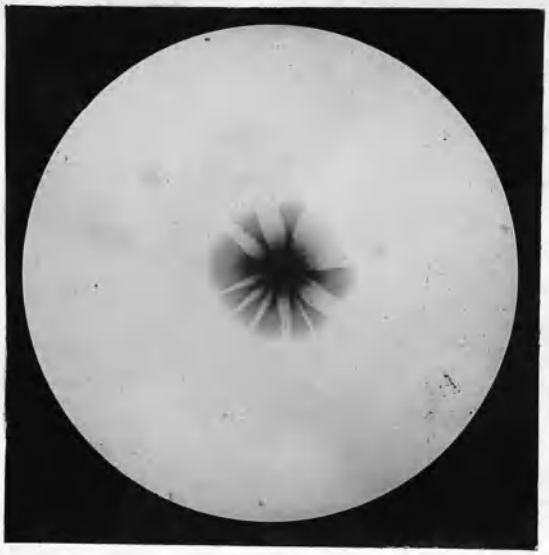


Fig.31

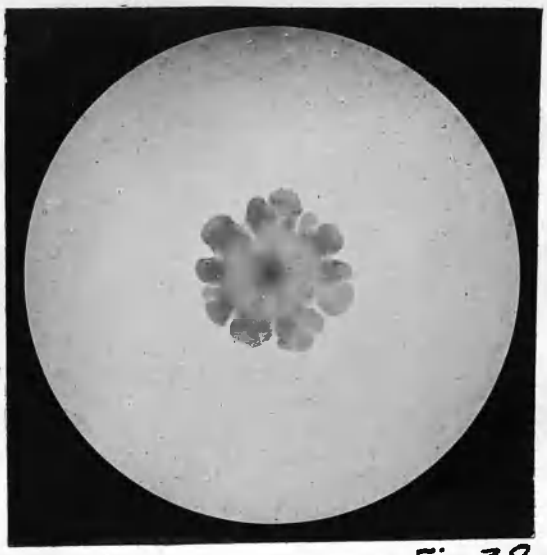


Fig.32

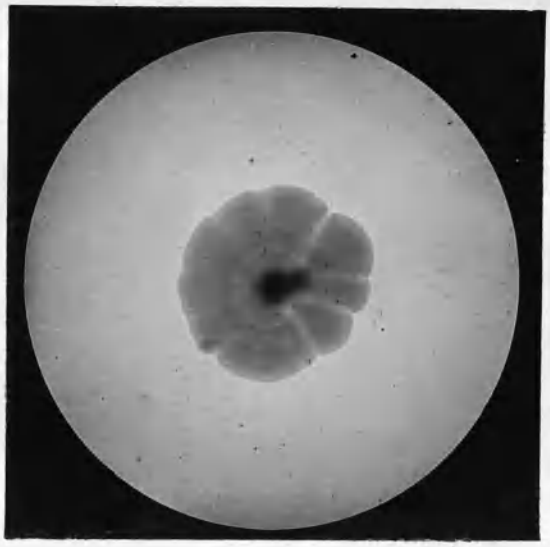


Fig.33

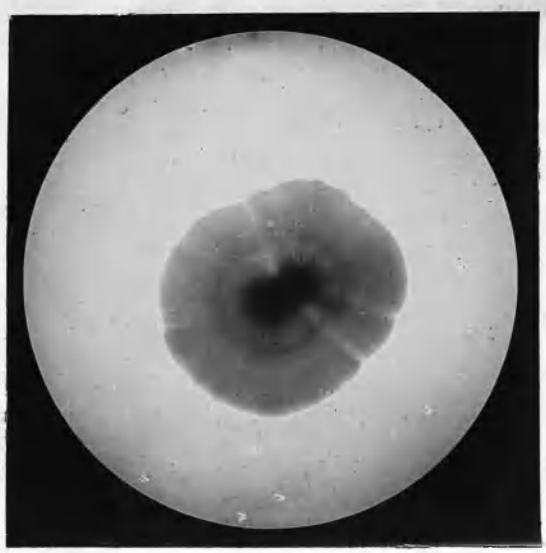


Fig.34

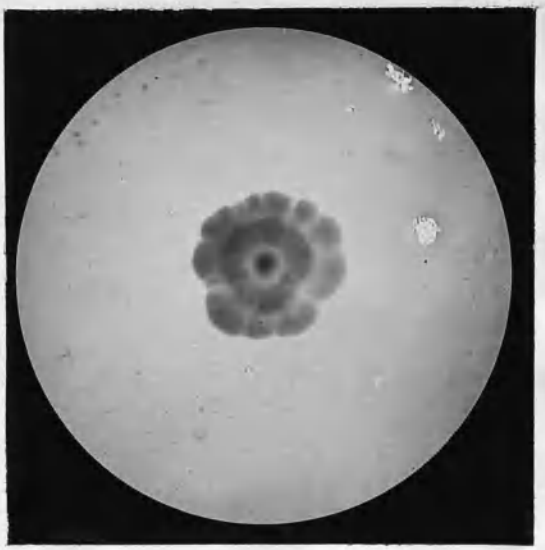


Fig.35

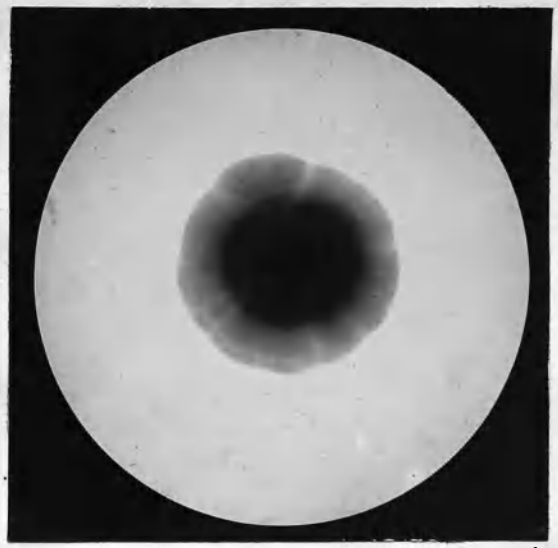


Fig.36

Plate 7.

Fig.37.

Gonococcus (KC) 13 days' growth.
The granular centre, the plication of the colony, the radial and the concentric striations are perfectly shown, and also the scalloped margin. Note commencing super-growth granulations forming a zone where concentric striation is most marked. x $7\frac{1}{2}$ diameters.

Fig.38.

Gonococcus (KC) 13 days' colony.
The granularity of centre causing opacity is more marked than in fig.37. The radial plication is marked. Concentric striation is less in evidence. Note the rim of coarse super-growth granulations. These were grown on different plates. x $7\frac{1}{2}$ diameters.

Fig.39.

Gonococcus (ON) 9 days' growth.
A fairly typical appearance is presented at an earlier stage than is shown in fig.38. x 9 diameters.

Fig.40.

Gonococcus (ON) 13 days' growth.
The photograph shows well the coarsely granular centre and the more transparent marginal zone concentrically and radially striated. x 9 diameters.

Fig.41.

Gonococcus (KC) 9 days' growth.
The photograph shows the general characters, but it is not so well taken as some of the preceding. x 9 diameters.

Fig.42.

Gonococcus (C) 10 days' growth.
Note the granular centre is limited. The radial plication of the colony has produced the optical effect of lily leaves radiating from the centre. The dark circular shadow is due to a large super-growth (out of focus). x $7\frac{1}{2}$ diameters.

These photographs were all taken by transmitted light.

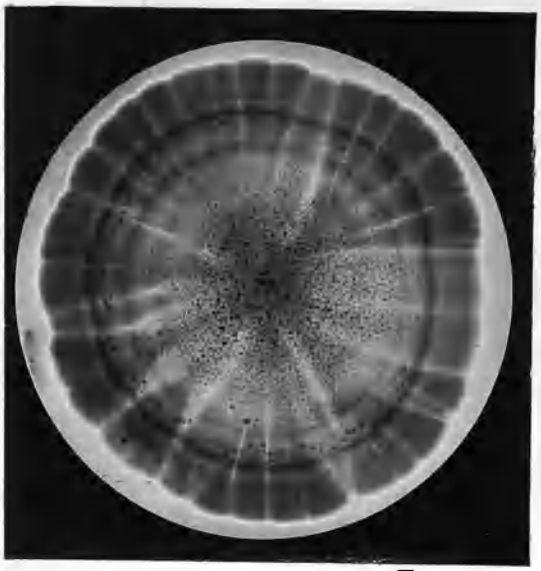


Fig. 37

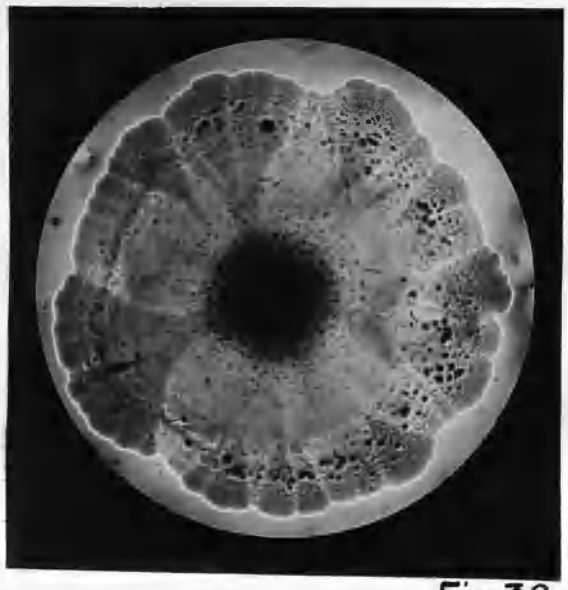


Fig. 38

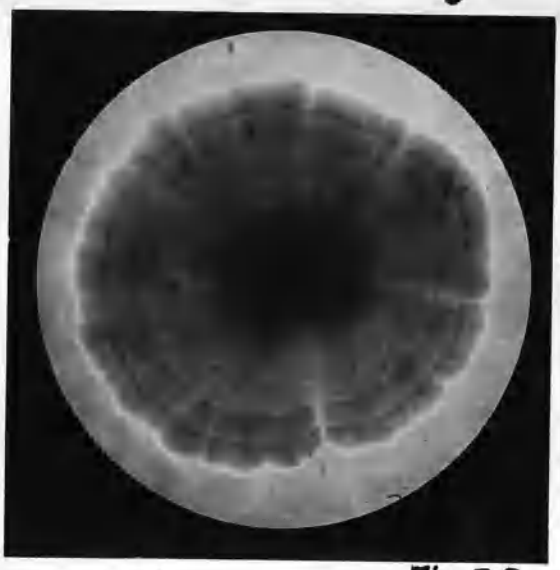


Fig. 39

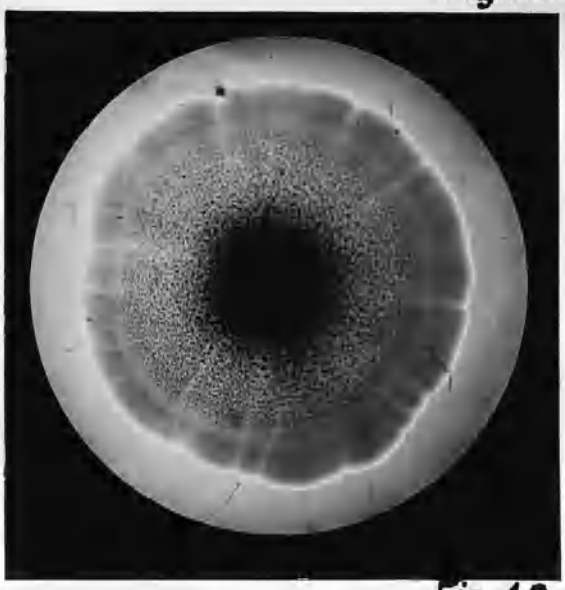


Fig. 40

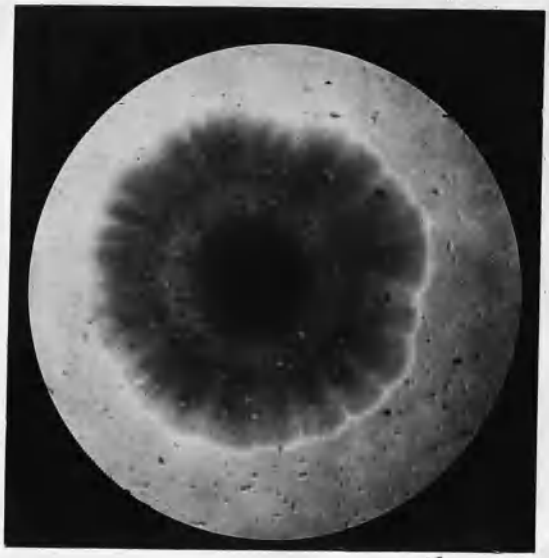


Fig. 41

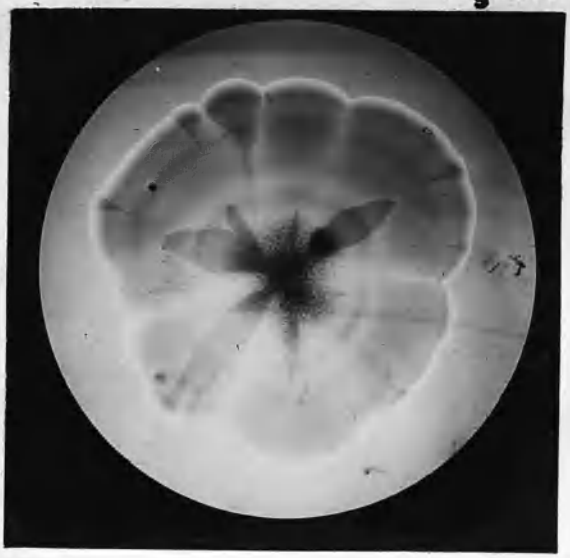


Fig. 42

Plate 8.

Fig.43.
Atypical arthritis organism (page 79) 6 days' growth.
Note the peppering of coarse granules, the scalloped margin and the radial plications. Contrast with fig.44 which is another colony from the same plate. x 9 diameters.

Fig.44.
Atypical arthritis organism (page 79) 6 days' growth.
To contrast with fig.43. The colony as a whole is more opaque and the granular centre more marked. x 9 diameters.

Fig.45.
Atypical arthritis organism (page 79) 7 days' growth.
Note the extreme plication of the colony and its comparative opacity. Coarse granulations are visible on the surface.
x 9 diameters.

Fig.46.
Atypical arthritis organism (page 79) 13 days' growth.
Contrast with fig.45. Note the well marked coarse granulation of the centre portion. The margin is less opaque than that of the colony shown in fig.45. x $7\frac{1}{2}$ diameters.

Fig.47.
Gonococcus (KS) 6 days' growth.
Note the comparative translucence of the whole colony. A series of super-growth granulations are forming a ring mid-way between margin and centre. (The central ovoid shadow is due to the inoculation point having cracked the surface of the medium.
Compare fig.32. x 9 diameters.

Fig.48.
Gonococcus (KS) 10 days' growth.
Naked eye this colony appeared rather opaque and possessed no central granulation. Towards X o'clock position well marked super-growth granulations have appeared. This genuine strain may be contrasted with figs.45 and 46. x 9 diameters.

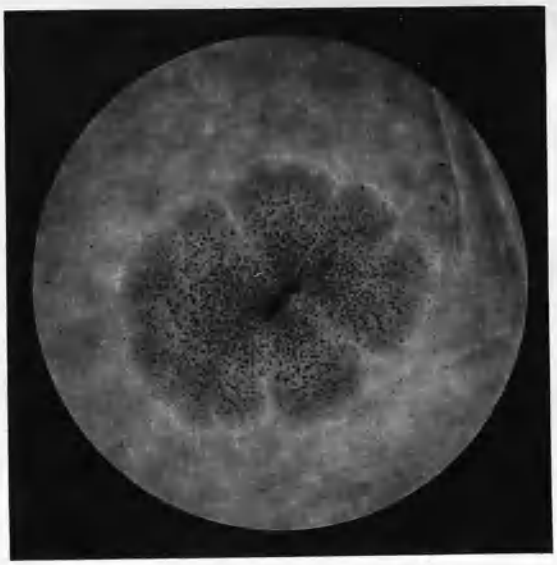


Fig. 43

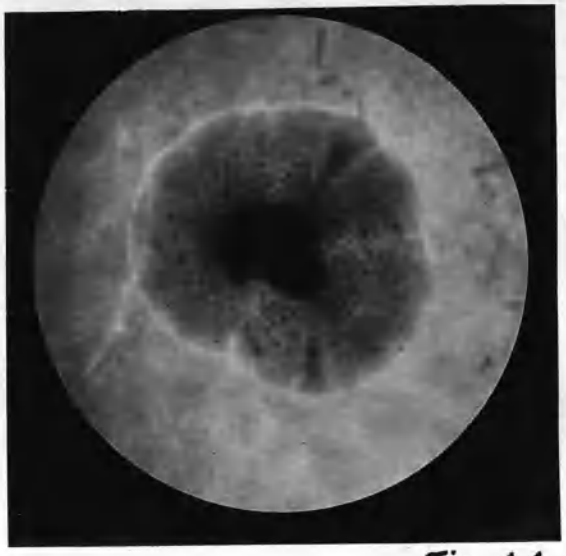


Fig. 44

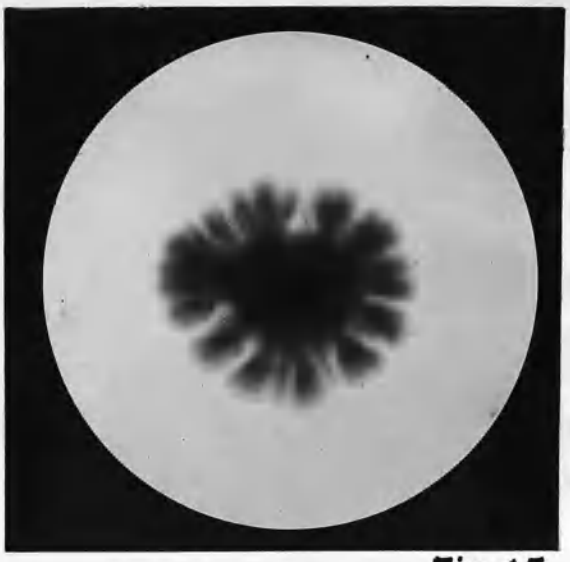


Fig. 45

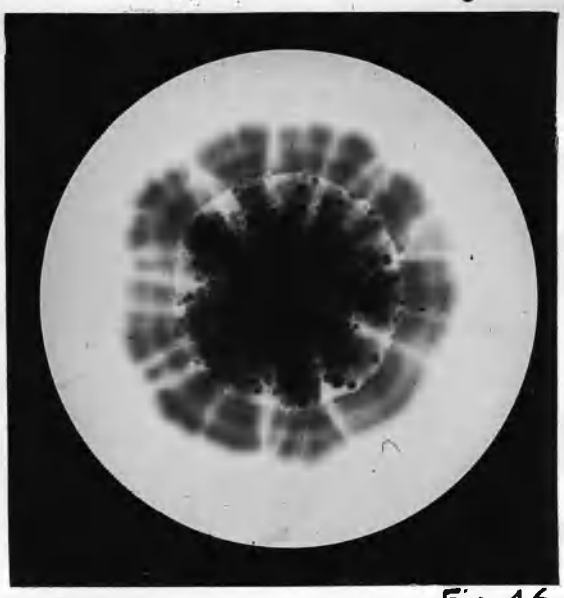


Fig. 46

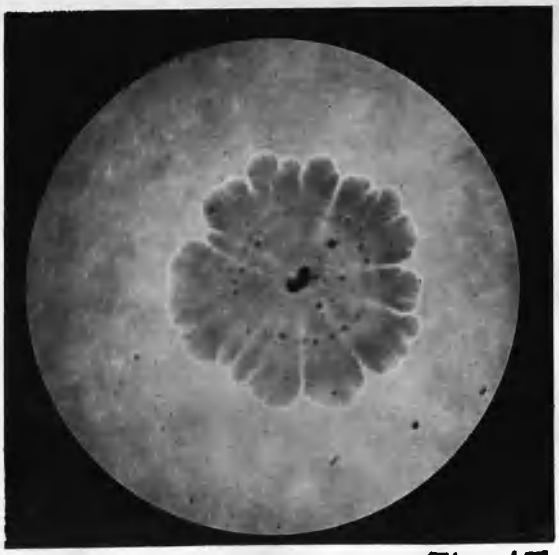


Fig. 47

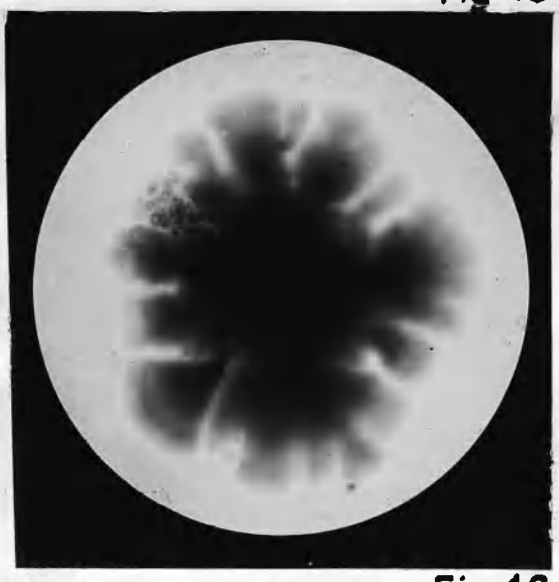


Fig. 48

Plate 9.

Fig.49.
Gonococcus (W) 3 days' growth.
The colony is extremely small and poorly differentiated. Growth had probably ceased before this period. x 9 diameters.

Fig.50.
Gonococcus (W) 9 days' growth.
The colony was very opaque naked eye. The character of the margin is merely indicated in the photograph. x 9 diameters.

Fig.51.
Gonococcus (W) 10 days' growth.
This is the same strain as the colonies shown in figs. 49 and 50 were obtained with. The appearance approaches very closely some of the more typical colonies already shown in the preceding plates. (The super-growths are out of focus). x 9 diameters.

Fig.52.
Gonococcus (C) 6 days' growth.
The very dark oval shadow in the centre is due to the inoculation point having fractured the medium. x 9 diameters.

Fig.53.
Micrococcus catarrhalis 3 days' growth.
The general opacity of the colony and the coarse granulation of the centre are shown, but the naked eye appearance is much more characteristic. x 9 diameters.

Fig.54.
Micrococcus catarrhalis 1 week's growth.
Note the extremely crenated margin and the very opaque centre.
x 9 diameters.

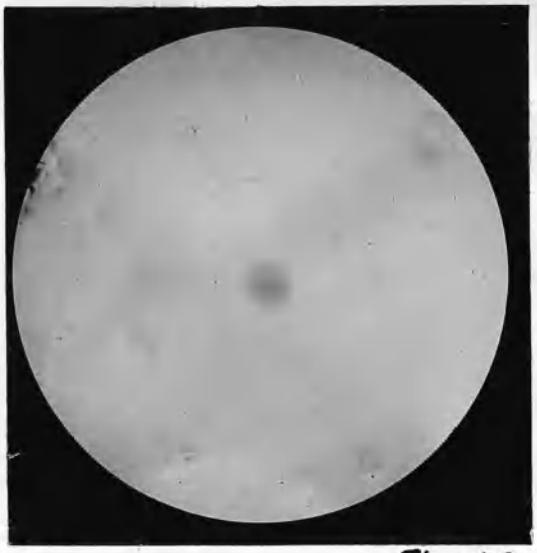


Fig. 49

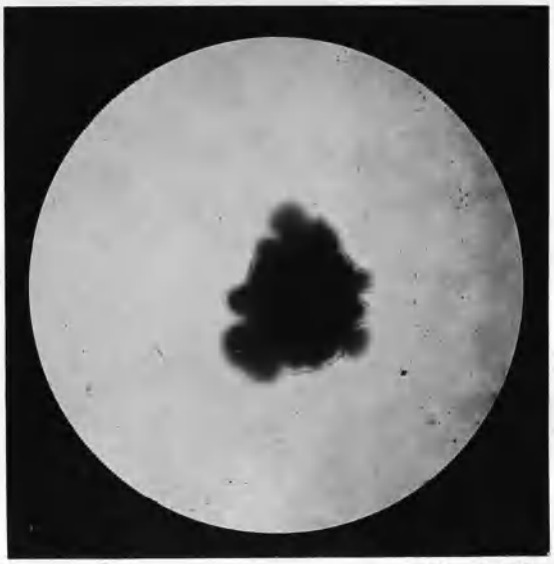


Fig. 50

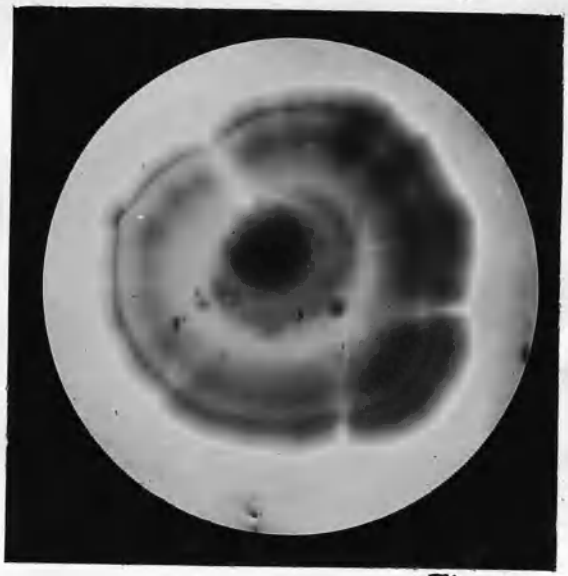


Fig. 51

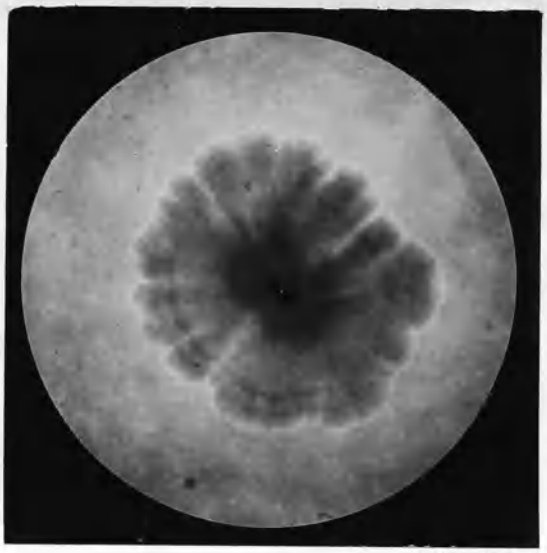


Fig. 52

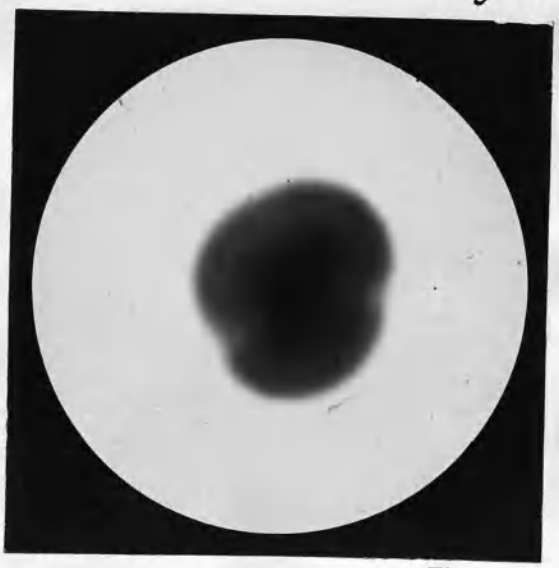


Fig. 53

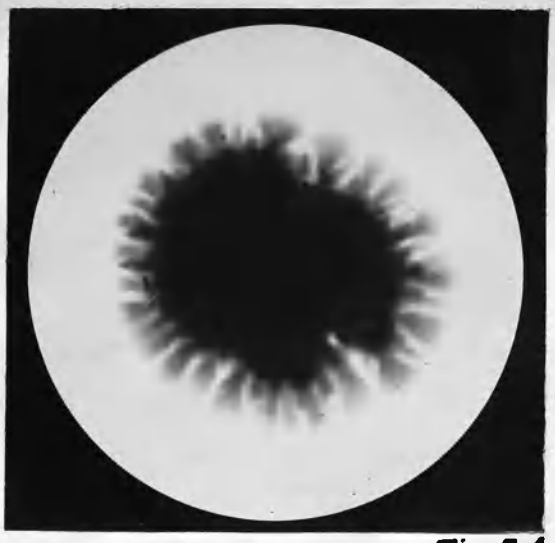


Fig. 54

Plate 10.

Fig.55.

Chromogenic catarrhalis-like organism (K) 5 days' growth. The appearance presented is a rare one. It is not unlike that of a Gonococcus, but naked eye the greenish yellow colour made confusion impossible. This organism fermented levulose and maltose. x 9 diameters.

Fig.56.

Chromogenic catarrhalis-like organism (S) 5 days' growth. An indication of the thick raised centre is seen in the photograph. The coarse granulation visible to the naked eye is not discernible. This organism fermented glucose, levulose, maltose and saccharose. It was a light yellow colour. x 9 diameters.

Fig.57.

Catarrhalis-like organism (B) 5 days' growth. This colony is so thick that it is impossible to have it perfectly focussed. The lighter shadows are due to surface irregularities. This organism was extremely tenacious in culture, practically colourless and fermented glucose, levulose, maltose and saccharose. x 9 diameters.

Fig.58.

Gonorrhoeal arthritis film from exudate. Numerous gonococci are seen mostly intra-cellular. This is a rare picture. Contrast with Plate 1, fig.4. Gram & Carbol fuchsin x 1000 diameters.

Fig.59.

Photograph of a drawing of Gonococcus colonies. A light and shade stereoscopic effect impossible with direct photography of the cultures is obtained.

1 Gonococcus (C) 20 days' growth, showing abundant super-growth.
2 Gonococcus (KS) 20 days' growth, showing large super-growths.
3 Gonococcus (T) 6 days' growth. A very highly characteristic Gonococcus is shown.
4 Gonococcus (V) 23 days' growth.

Fig.60.

5 Gonococcus (KC) 2 days' growth.
6 Gonococcus (KC) 6 days' growth.
7 Gonococcus (KC) 12 days' growth. (discrete.
8 Gonococcus (T) 2 days' growth. Stroke, showing tendency to remain
9 Gonococcus (T) 12 days' growth. Stroke, showing super-growths.
10 Gonococcus (KC) 12 days' growth. Stroke, super-growths few and large.

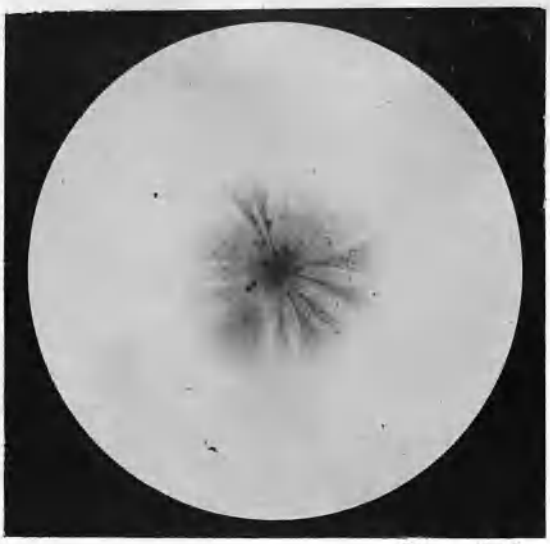


Fig. 55

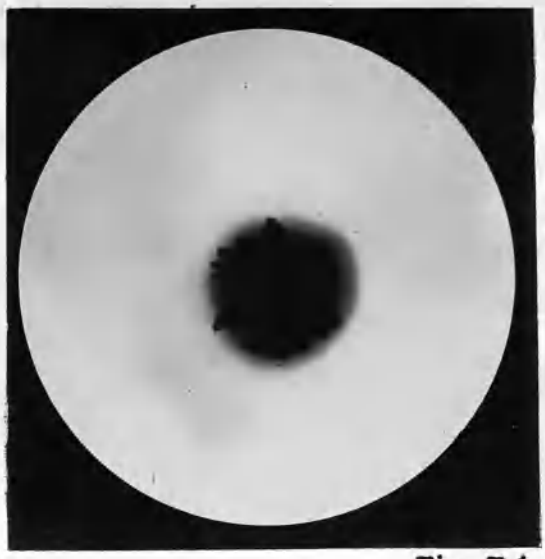


Fig. 56

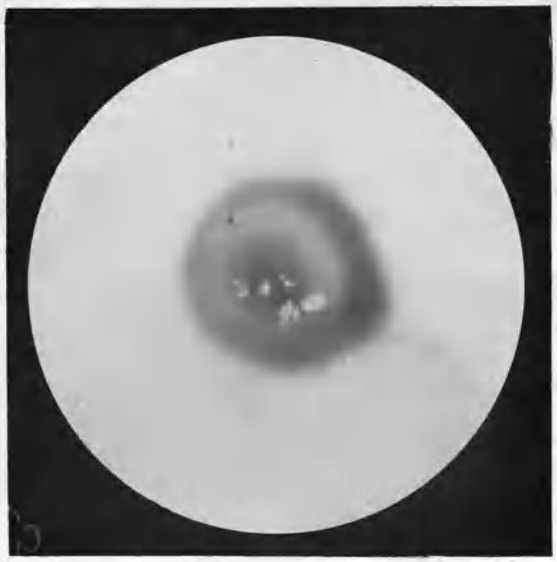


Fig. 57

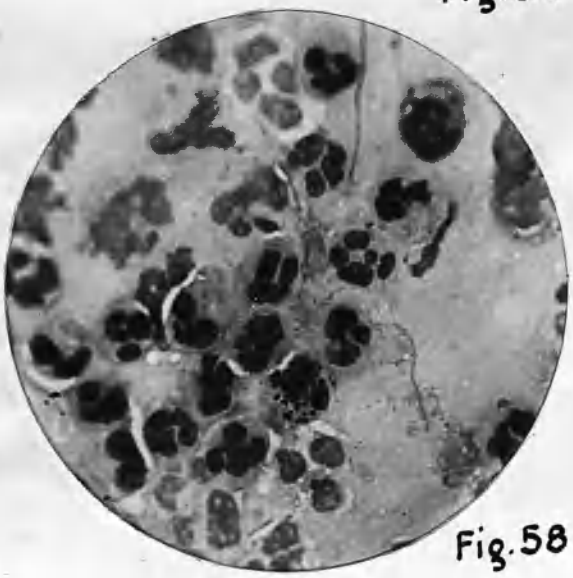


Fig. 58

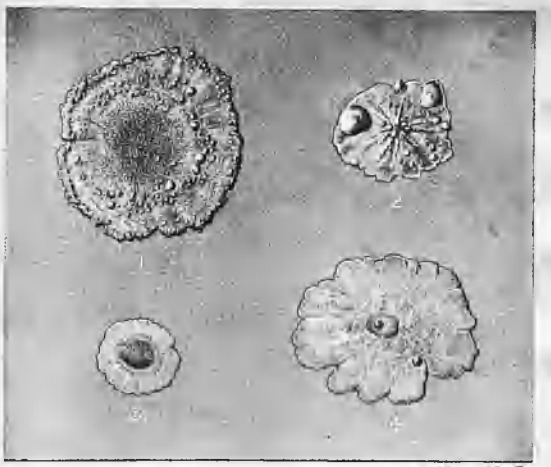


Fig. 59

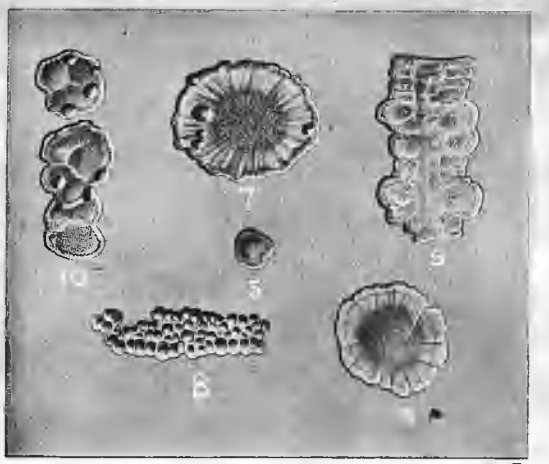


Fig. 60

Plate II.

Chart of case of Cerebro-spinal Fever referred to on page 27.
It shows all the details of pulse, temperature, respirations, leucocyte counts treatment and the lumbar puncture dates.

Notes on the Cerebro-spinal Fluid:-

1st Decr. slightly turbid, rich polymorpho-nuclear leucocyte sediment, no tubercle bacilli in films but a few diplococci intra- and extra-cellular. Numerous colonies obtained in plate culture.

2nd Decr. slightly turbid as on 1st Decr. organisms all appeared intra-cell.

3rd Decr. distinctly less turbid, very few organisms in films. Plate culture gave only a few colonies in contrast to 1st Decr. As regards the vitality of the polymorpho-nuclear cells of the exudate, using them as phagocytes in an opsonic determination with a normal serum and a staph. aureus emulsion, about 30% were actively phagocytic.

9th Decr. slightly turbid, only slight deposit on centrifugalising. Organisms very scanty in films. Only one or two colonies in plates from 10 c.c.fluid.

10th. Decr. slightly turbid, deposit 1-5th of that of 1st Decr., fluid spontaneously coagulated still. Organisms scanty, mostly degenerate. No growth in plates even from the deposit of 20 c.cs. of fluid.

28th Decr. slightly opalescent, fair deposit, fluid spontaneoulsy coagulated. Organisms in films scanty and only intra-cellular ones seen. Plate cultures gave 50 colonies from 20 c.cs. of fluid.

The fluids obtained on 29th and 31st Decr. were admixed with blood and showed an increased sediment. A few colonies only grew.

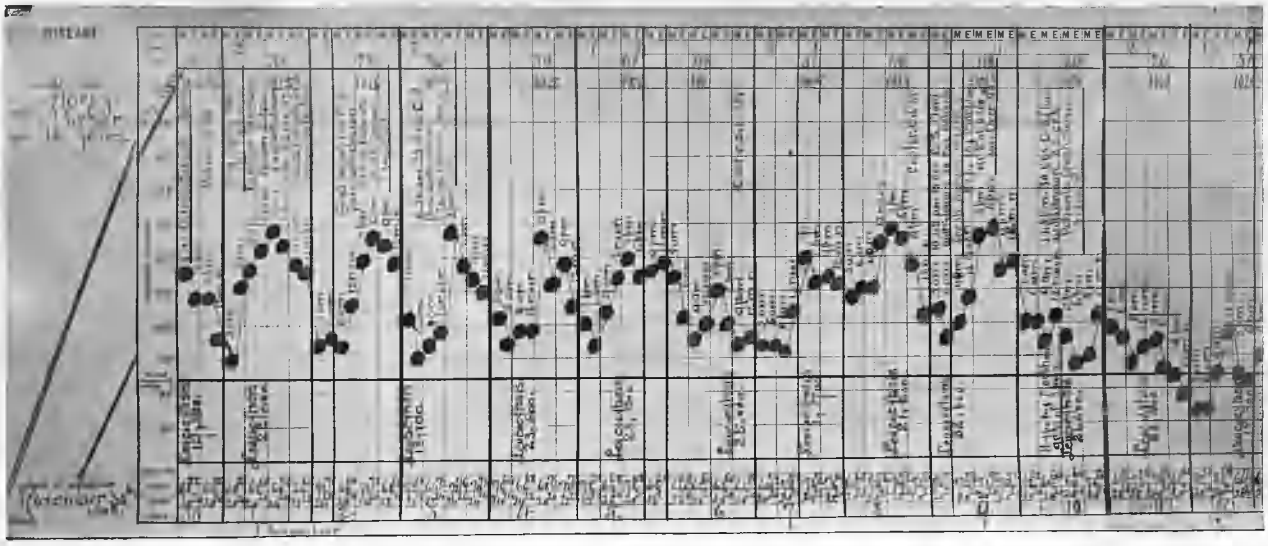
2nd Jany. slight sediment, slight admixture with blood, no organisms seen in films but they were obtainable by culture.

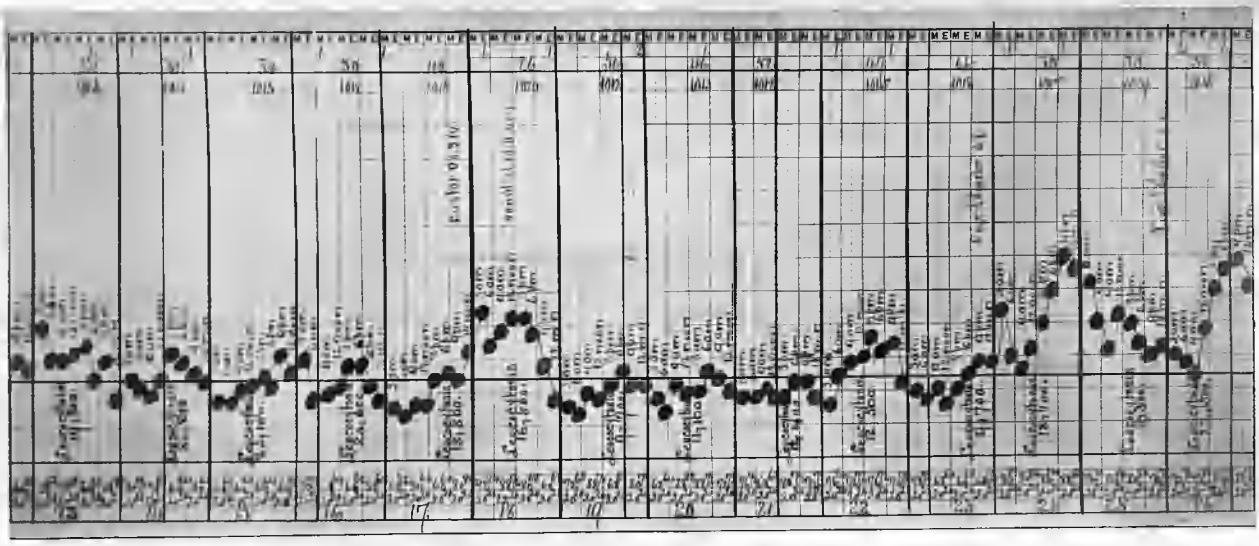
5th Jany. although free from blood, the fluid was serum coloured and it gave as copious a deposit as on any previous occasion. In films organisms were found to be more numerous than ever they had been before, but they all appeared intra-cellular; plate cultures showed about 1200 colonies from 10 c.cs. of fluid.

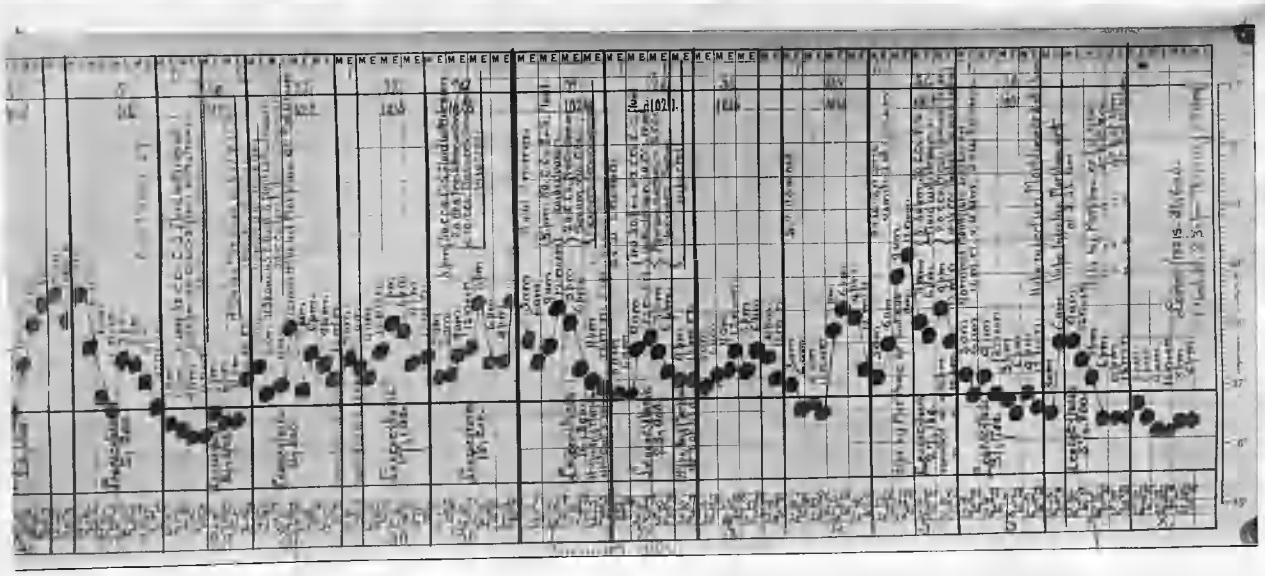
On all these occasions the cultures obtained were pure ones of typical Meningococci, which was proved by extensive cultural study and fermentation reactions. The organisms were also tested in a bactericidal experiment against normal and the patient's own serum and a distinct killing off was observed with the higher proportions of serum.

The case is reported at length in Dr.Gemmell's Ward Journal (Ward 31, Vol.51 p.270, 30th.Novr.1908) & in the Journal of the Pathological Department P.M.7844.

PLATE 11







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Much of the work has only been possible through the munificence of the Carnegie Trustees for the Universities of Scotland.

The low power photographs were taken in the Photographical Department of the University with Mr Fingland's co-operation, and the high power ones in the Pathological Department with the assistance of the Attendant, Mr. J. Kirkpatrick.

The Chart (Plate 11) is the work of Sister Lyle, Western Infirmary.

Figs.59 and 60 are from drawings executed by Mr A.K.Maxwell.