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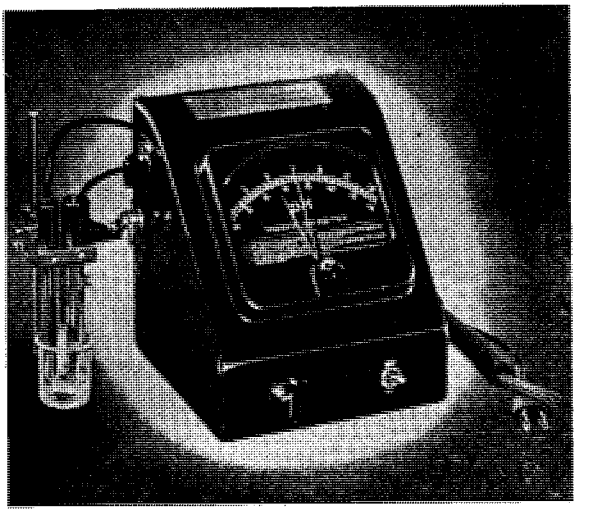

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A HISTORY OF THE CLASSIFICATION OF THE STREPTOCOCCI

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From ancient writings it is evident that diseases caused by Streptococci, particularly erysipelas have long been known, but it was not until the middle of the last century that they were recognised as infectious diseases. As early as 1874, Billroth published a considerable volume in which he attempted a morphological classification of the bacteria found in the putrefying tissues and in discharges from the animal body. It was by him that the term Streptococcus was introduced. He classified the coccal forms as Monococcos, Diplococcos, Streptococcos and Gliacoccos and noted the presence of "Streptococcos" forms in 50 per cent of the cases of erysipelas he examined, but argued at length to show that there was no satisfactory evidence for supposing that the inflammatory conditions examined were due to the bacteria present.

From further writings and discussions at the Academie de Medecine (1879), it is evident that the great Pasteur was convinced that a "micrococcus sous forme de chapelets" played an important part in the causation of puerperal fever. The first experimental demonstration, however, was carried out in 1878 by Koch, who succeeded in obtaining pure streptococcal infections characterised by spreading tissue necrosis or cellulitis and multiplication of chain cocci in the tissues of field mice. The streptococci are clearly figured in his illustrations.

The first complete proof, however, of the connection of cocci wtih disease in man we owe to Ogston, who from 1880 to 1883, demonstrated in a large series of cases the presence of chain cocci in human lesions and reproduced similar lesions in experimental

animals, both with injections of pus and with cultures of cocci obtained in sterile eggs. Meanwhile, Fehleisen in 1882, using Koch's new methods for obtaining pure cultures, put the connection of streptococci with erysipelas beyond all possible doubt. Fehleisen's work included the production of erysipelas in a woman suffering from sarcoma by injection of a streptococcal culture.

The next step was carried out by Rosenbach (1884) who introduced the term Streptococcus pyogenes to indicate a form responsible for suppurations, but distinct from the streptococcus of erysipelas. The criteria by which he distinguished these two forms, however—appearances on gelatin and agar slopes—were not found satisfactory by subsequent workers. The importance of Rosenbach's work, however lies in the fact that it gave birth to the idea, that streptococci producing diseases which are markedly different clinically, must differ in other respects. This idea was followed by subsequent workers.

As might be anticipated, other attempts to separate the Streptococci into definite groups were based purely upon morphological grounds. Von Lingelsheim (1891) noted that Streptococci isolated from infected tissues usually appeared as long chains in fluid media, whilst the saprophytic types almost invariably occurred as short chains of not more than six or eight individual cells and he postulated two groups, Streptococcus longus and Streptococcus brevis. The wide distribution of the latter group and its apparent lack of significance in human pathology focussed attention on the "longus" type and attempts were made, incorporating Rosenbach's idea, to classify the Streptococci according to the diseases from which they were derived. In this way Streptococcus erysipelatis, Streptococcus pyogenes and Streptococcus scarlatinae, etc., originated.

The fallacy of this classification was soon shown by the work of Koch and Petruschky, whose experiments proved that any given strain of streptococcus could give rise to different diseases in rabbits according to its degree of virulence. Similarly it was shown that the same strain in human subjects may give rise, first to erysipelas, then sepsis or purulent inflammation, pericarditis, etc., and when transferred to other individuals may give rise to still other pathological conditions.

Marked variations were found to occur in the nature of growth in nutrient broth, serum and ascitic fluid. According to the degree of alkalinity or the peptone content, the same streptococcus gave rise in broth to a turbidity, precipitate, flocculence or pellicle. The forms taken by the chains elongated closely or slightly coiled, ball-like or loose, depended to a great extent on the nature of the nutrient medium and was therefore not a characteristic which could be utilised in the definition of varieties.

Consequently the next phase of the streptococcus question concerned itself with biological characters and as the study of

bacterial action upon carbohydrate or "sugar" media had proved valuable in the differentiation of intestinal bacteria, attempts were made to classify the Streptococci on these lines. Gordon, (1904-5) first applied fermentative and other biochemical tests as differential criteria. He used the clotting of milk in three days, the reduction of neutral red in broth cultures within 48 hours under anaerobic conditions, and the fermentation of sugars. The results of these tests, however, led to the differentiation of a bewildering number of types. Andrews and Horder (1906) then took up the problem and using Gordon's tests and examining large numbers of streptococci, indicated the existence of at least half a dozen groups.

1. Streptococcus equinus.—Found in horse dung and characterised by inability to ferment lactose or mannitol or clot milk.

2. Streptococcus pyogenes.—Common in suppurative conditions and characterised by haemolytic activity and inability to reduce neutral red.

3. Streptococcus mitis.—Common in mouth and faeces and characterised by good growth in gelatin at 20° C, and reduction of neutral red.

4. Streptococcus salivarius.—Common mouth streptococcus characterised by clotting of milk, reduction of neutral red and frequently by fermentation of raffinose.

5. Streptococcus anginosus.—Not distinguished from S. pyogenes, except by reduction of neutral red and clotting of milk.

6. Streptococcus faecalis.—Common bowel streptococcus, which is characterised by H_2S production and by the activity of its fermentations.

Their chief results may be summed up as follows:

Previously, in 1903, Schottmuller had published his classification of the streptococci based on colonial appearance on blood agar. This classical work may be said to be the basis of our modern classification. Schottmuller differentiated three main types according to the changes they produced on blood agar.

1. Streptococcus longus haemolyticus.—Including all highly pathogenic strains and characterised by the development of clear colourless zones of haemolysis around the colonies.

2. Streptococcus mitior (viridans).—Including mainly strains derived from the respiratory or digestive tracts and of low pathogenicity. These are distinguished by their tendency to produce a green discolouration of the blood agar in their immediate vicinity.

3. Streptococcus mucosus.—Of much rarer occurrence and distinguished by its abundant mucoid colonies and by the well defined capsule seen in microscopic preparations, even from cultures on solid media. This group was later identified with the Pneumococcus.

Schottmuller at the time, however, failed to differentiate the

Pneumococcus from either types 2 or 3 and his scheme was therefore severely criticised.

A good deal of criticism was brought forward in regard to both schemes of classifications. In Germany the view was expressed repeatedly that haemolytic streptococci can be changed during sub-culture to green forming strains and vice versa, and hence that there is really only one form of streptococcus subject to variations according to conditions of preservation and culture. Some writers actually extended this idea of ready conversions of streptococcal types to include the Pneumococcus. Schottmuller in 1924, however, stoutly maintained his position and pointed out the absurdity of a contention for the unity of streptococci when anaerobic forms producing foul smelling gases are well recognised and when viridans strains are readily distinguished from haemolytic strains by their marked sensitiveness to the bactericidal action of defibrinated blood.

The criticism raised against the classification of Gordon Andrews and Horder however, was more justified. Many workers contended that the sugar fermentation and other tests which they employed were not given constantly enough by any one strain of streptococcus to serve as a basis for differentiations and showed that it was only necessary to give a streptococcus a more favourable medium in order to enable it to ferment some sugars which it had not previously fermented. For some time the value of fermentation tests was a controversial point. Little interest was taken in the subject in Germany and France, except by Maas, who, in 1913, expressed the opinion that there was no correspondence between groupings by sugar fermentation and source or pathogenic importance of streptococci.

In North America, however, a large amount of work was devoted to the subject. Holman (1916) most definitely asserted the validity of fermentation reactions and using lactose, mannitol and salicin in combination with ability to produce a haemolysin, arrived at 8 haemolytic and 8 non haemolytic types.

			Haemolytic	Non haemolytic
Lactose +	Mannite +	Salicin +	S. infrequens	S. faecalis
		Salicin —	S. haemolyticus 1.	S. non haemolyticus 1.
Lactose +	Mannite —	Salicin +	S. pyogenes	S. mitis
		Salicin —	S. angiosus	S. salivarius
Lactose —	Mannite +	Salicin +	S. haemolyticus 2.	S. non haemolyticus 2.
		Salicin —	S. haemolyticus 3.	S. non haemolyticus 3.
Lactose —	Mannite —	Salicin +	S. equi	S. equinus
		Salicin —	S. subacidus	S. ignavus

S. infrequens
Frequent in throat in scarlet fever.
S. pyogenes.
Largest group common in streptococcal pyogenic conditions.
S. angiosus.
Common in throat tonsils and nose, occurs in endocarditis.
S. haemolyticus, 1, 2, 3.
All rare.
S. equi.
Rare in man. Present in "strangles" in horses.
S. subacidus.
Found in throat, abscesses, blood infections and endocarditis.
S. faecalis.
Streptococcus of human faeces, also occurring in blood endocarditis and wounds.
S. non haemolyticus, 1, 2, 3.
All rare.
S. mitis.
Commonest of non-haemolytic strains found in throat and in endocarditis.
S. salivarius.
Common in throat and occasionally in endocarditis.
S. equinus.
In horse and human faeces.
S. ignavus.
The most inactive type.

Much literature was published in the succeeding ten years, upholding this "sugar" classification but a greater volume was of a more critical nature and the general consensus of opinion became firstly, that fermentative reactions alone are not a safe guide to the pathological significance of a streptococcus, and secondly, that the majority of those who investigated the problem, failed to establish for the streptococci, any such close relationship between antigenic properties and fermentative activities, as exists in the typhoid—paratyphoid group of bacteria. There is no doubt, however, that used by Horder and Andrews as they were, and together with other characteristics to establish the existence of different types of streptococci predominant in different areas of the body, they have served their purpose of developing our knowledge of the bacterial flora of the alimentary tract, as well as that of the streptococcal group.

The definition of Streptococcus faecalis as a special type distinct from the mouth streptococcus, appears more justifiable than the separation of the bowel streptococci as distinct bacteria under the name of "Enterococcus" as done by the French.

The monograph published by Brown (1919) discussed in detail the haemolytic activity of the streptococci and contained an admirable review of the literature dealing with the classification of

streptococci up to that time. In this monograph, great emphasis is laid on the importance of employing a uniform and standard technique and in particular on the superiority of poured plates and observation of deep colonies over plates which have been inoculated by surface spreading only. The medium recommended consists of veal peptone agar containing 5 per cent of horse blood. The agar is stored in tubes of 12 c.c. amounts. When required for use, a tube is melted and cooled to 45° C. 0.66 c.c. of horse blood is added and evenly mixed with the agar, the medium is inoculated with a loopful or two of a 24 hours' broth culture and poured into a petri dish, 9 cm in diameter, thus giving a layer about 2 mm. thick. The plates are examined after 24 and 48 hours' incubation at 37° C. and finally after a further 24 hours in the ice chest

Brown recorded four different types of reaction in blood agar plates which he designated as follows:—

α A somewhat greenish discolouration and partial haemolysis of the blood corpuscles immediately surrounding the colony, forming a rather indefinitely bounded zone of 1-2 mm. in diameter outside of which is a second, narrow, clearer, not discoloured zone. Under the microscope many corpuscles are seen in the inner zone and these are obviously discoloured. Very few corpuscles remain in the outer clearer zone and these are never discoloured. These typical appearances may fail to appear after 24 hours or even 48 hours incubation, at the end of which time the narrow outer zone of haemolysis may not have developed. In such cases this zone makes its appearance during the subsequent 24 hours in the ice chest. If a plate which has developed the typical appearances is reincubated for a further 24 hours and then placed in the ice chest for a further 24 hours, a double series of rings will frequently develop.

β The colonies are surrounded by sharply defined clear, colourless zones of haemolysis 2-4 mm. in diameter. Under the microscope no corpuscles can be seen within this zone. The *β* haemolysis develop more rapidly than those of the *α* type. They are often well developed after 18 hours' incubation.

α' (*α* prime). The colonies are surrounded by a zone of haemolysis which is slightly hazy and less sharply limited than in the case of true *α* haemolysis. The colony itself is not sharply defined and examination with the microscope shows that the haemolysed zone contains throughout a moderate number of unaltered corpuscles which are most numerous in the immediate neighbourhood of the colony. There is no discolouration. It is noted that some strains which produce *α*'haemolysis on horse blood agar, may produce typical *χ* haemolysis on rabbit blood agar.

γ The colonies develop on blood agar without any change in the surrounding medium.

These haemolytic strains of Brown correspond to the Streptococcus haemolyticus of Schottmuller and the α strains to his viridans. The significance of the α' type of haemolysis is not clear, and it seems to be of infrequent occurrence. The terms α and β haemolysis have attained general usage in bacteriological literature and serve a useful purpose. By "haemolytic" streptococcus is usually meant one producing β haemolysis, but a "non haemolytic" streptococcus may be one producing α haemolysis or no change at all in the medium.

Some workers have preferred to confine the term "haemolytic" streptococcus to strains that in addition to producing β haemolysis on blood agar, can be shown to have a soluble haemolysin. Much experimentation has been carried out on Streptococcal haemolysin, and it seems probable that two types of haemolysin are produced—a filterable and non-filterable. A broth culture is prepared and filtered and to this is added fresh red blood cells. If a filterable haemolysin is present, the cells will be haemolysed in about two hours at 37° C. The type of media in which the organism is grown is found to have a marked effect on the production of haemolysin. A medium containing yeast extract, glucose, sodium bicarbonate and sodium phosphate and sterilised by filtration instead of autoclaving, was found by Todd and Hewitt (1932), to be particularly useful.

Again, the present age of the culture has a marked effect on the amount of haemolysin present. The optimum time appears to be about 8 hours. After 14 hours, much of it has disappeared. The haemolysin is extremely sensitive to oxygen, heat and acid.

Heating at 55° C. for 30 minutes destroys it completely. It has also been noticed (Fry 1933) that certain strains producing α haemolysis aerobically produce β haemolysis when grown anaerobically. From these facts it is doubtful whether there are two types of haemolysin, as the so-called non-filterable may simply have been oxidised during the investigation.

With regard to the mechanism of the production of α haemolysis, even less is known. Several workers have described a soluble haemolysin produced by the Pneumococcus of the oxygen heat sensitive type and undergoing reversible oxidation at low temperatures.

At one time the most widely accepted view in regard to the green colouration associated with α haemolysis was that due to the formation of methaemoglobin or of some closely allied substance. Many workers, however, maintained this theory was untenable, and in 1933 and 1934 Hart and Anderson, working with the Pneumococcus, found that when small quantities of laked blood were added to broth cultures in the presence of an alkaline buffer solution, an olive green precipitate was formed. This could be separated, washed and dissolved in dilute alkali to give a green solution. Crystalline haemoglobin and methaemoglobin when in-

cubated under suitable conditions, gave the same green pigment. Spectroscopic and chemical analysis of this pigment suggest that it is an iron containing derivative of haemoglobin. An identical or very similar pigment can be obtained by incubating laked blood haemoglobin or methaemoglobin with various chemical reducing systems such as ascorbic acid or cysteine-glucose. From the results obtained with autolysed bacterial cells, washed bacteria and bacterial extracts, it would seem that the green pigment results from the activity of a bacterial oxidation reduction system, one component of which is intra-cellular. This system is not peculiar to the Pneumococcus and is shared not only by the streptococci giving α haemolysis, but those giving β haemolysis and the enterococci which produce no change at all in blood agar. The production of the green pigmentation by some species and not others seems to be due, not so much to the presence or absence of the necessary enzyme system as to secondary factors determined by the metabolism of the organisms concerned, which sometimes permit this system to function and sometimes suppress it.

Thus summarising Brown's observations in conjunction with the possession of a filterable haemolysin, the streptococci can be divided into three or possibly four groups.

1. Haemolytic streptococci—producing β haemolysis.
   (a) Producing a filterable haemolysin.
   (b) Producing no demonstrable haemolysin.
2. Streptococci giving α haemolysis.
3. Streptococci which have no action on blood media under the usual conditions of testing.

THE BOWEL STREPTOCOCCI

With regard to the streptococci of faeces, little is known about their discovery which appears to belong to the dark ages of bacteriology. Hirsch and Libman (1897), drew attention to the presence of streptococci in stools of patients suffering from summer diarrhoea and called the organism S. enteritis.

About the same time, Thiercelin (1899), in France, described a short-chained or diplococcal bowel streptococcus under the name of Enterococcus. He recognised it as a normal inhabitant of the bowel and differentiated it from the other streptococci, mainly on morphological grounds. The same or an indistinguishable organism had previously been noted by Escherish (1886) in the stools of young infants and designated Micrococcus ovalis.

Tissier (1900) studied the enterococcus fully and differentiated it from S. enteritis upon morphological grounds; its greater hardiness in culture and its more rapid action in clotting milk. He considered the enterococcus identical with the M. ovalis of Escherich. The organism became generally recognised in French

bacteriological literature and the name Enterococcus was adopted and has persisted ever since.

The recognition of a faecal group of streptococci on other than morphological grounds came as the result of Gordon's sugar fermentation tests. As stated above, Horder and Andrews introduced later the group S. faecalis and according to them, it had the following characteristics:—

"A short chained organism rendering broth uniformly turbid, growing readily at 20° C. and forming sulphuretted hydrogen in broth cultures. It has no haemolytic power and little virulence, but clots milk, reduces neutral red and ferments saccharose, lactose, salicin, coniferin and mannite. The mannite reaction is specially characteristic of this intestinal type." It is interesting to note that Andrews and Horder did not admit the existence of a S. faecalis which failed to ferment mannite, this being the critical test which they established for the group.

In view of the confused state of the literature, Dibble (1921) undertook an extended study of the streptococci to be found in the faeces, utilising both normal and pathological stools. The general conclusions which emerged were that faeces contained both long and short chained varieties, the former of the S. enteritis type which were largely, if not entirely surviving, salivary organisms having features closely akin to those of the mouth and throat streptococci. The short chained type agreed in most particulars with the enterococcus of French workers. From time to time, rigid criteria have been laid down for the faecal streptococci.

Weissenbach (1918) considered that the enterococcus could be differentiated from the pathogenic streptococci by its ability to grow in bile glucose-peptone broth which inhibited the latter; Meyer and Schoefeld require that it shall split aesculin and Dibble utilised the heat resisting powers of the organism as an essential criterion. The latter writer, however was careful to point out that exceptions to these tests do occur and that it is necessary to recognise the existence of varieties. In general, however, these characters mentioned above occur positively and what is of great practical moment, that they are characters which are rare in other streptococci, and are scarcely ever met with in combination one with another.

LACTIC STREPTOCOCCI

Gunther and Thierfelder (1895) described the occurrence in milk of an organism which was responsible for spontaneous clotting and souring. They described the organism as a short bacillus, but Heinemann (1906), when investigating the bacterial flora of milk some ten years later, pointed out that a particular streptococcus, which was almost always present in fresh milk was probably identical with the organism of Gunther and Thierfelder.

Baehr (1910) confirmed the frequent presence of this streptococcus and noted that it produced a large amount of acid and rapid clotting. Sherman and Albus (1918) in a careful comparative study of strains of this organism noted several characteristics. The lactic acid streptococcus grew predominately as diplococci or short chains: it clotted milk within 24 hours, produced high acidity in milk (0.75 per cent or more measured as lactic acid), grew poorly at 43° C. but well at 10° C., and rapidly reduced methylene blue, litmus, indigo, carmine and neutral red. This particular streptococcus for a long time enjoyed specific rank under the title S. Lactis, but it was early noted that many of its most striking characteristics were shared by the enterococcus.

Avers and Johnson (1924) carried out a careful comparative study of these two types, testing them as regards their reaction on blood agar, their morphology, their ability to withstand heating at 60° C., their reaction to litmus milk and Janus green medium, their fermentation reactions, and the final pH obtained in broth. They were unable to find any differences in behaviour except that enterococci appeared to form acid less vigorously than S. lactis. Other workers however, consider that differentiation is possible, and recently Lancefield has shown the two organisms to be quite different antigenically.

ANAEROBIC STREPTOCOCCI

Our knowledge of the anaerobic streptococci dates from the work of Veillon (1893), who described under the name of Micrococcus foetidus a strictly anaerobic coccus which he isolated from cases of Ludwig's angina and perinephric abscesses. This organism grew in the form of diplococci and in short chains and produced gas and a foetid odour in culture. Later similar organisms were isolated by Rist (1898) from cases of otitis media and by Halle (1898) and Guillernot (1898) from cases of gangrene of the genital and pulmonary systems respectively.

Meanwhile in Germany, Kronig (1895) and Menge (1895), had independently described a strictly anaerobic streptococcus morphologically indistinguishable from S. pyogenes, which they found in the vagina in pregnancy and later in collaboration they isolated several strains from the vagina and lochia in infected puerperal cases as well as from parametrial suppurations and peritonitis. The observations of these workers served to demonstrate clearly the existence of streptococci of strictly anaerobic character, although they made little attempt to classify them beyond calling attention to one group which produced gas and foul-smelling decomposition products in artificial media. Following upon the objection of Koblenck (1899) and others who still denied the existence of strictly anaerobic streptococci, Natwig (1905) confirmed the work of Kronig and Menge in describing six

strains, but he included them all in a single group which he called S. anaerobius.

Lewkowicz (1901), however, had previously isolated an extremely minute streptococcus growing only under strictly anaerobic conditions and producing neither gas nor odour in culture media. This organism, which he called S. anaerobius micros, was obviously very different from those already described by Veillon and Kronig, so that Natwig's conclusions were clearly unjustified.

From this time until 1910, strains of anaerobic streptococci isolated from various suppurative or gangrenous conditions were identified by their discoverers with the varieties named up to that time. In this year, Schottmuller described a strictly anaerobic streptococcus isolated chiefly from cases of puerperal sepsis, both from the local lesion and sometimes from the blood during life. This organism produced gas and odour only in media containing blood, remaining inactive in ordinary media. He called it S. putridus, believing that it determined the putrid character of the infection in many cases. Later this organism was found to be present in the normal vagina in 40 per cent of cases.

Schottmuller's work has been extended and confirmed by many subsequent workers, and it would seem that these anaerobic streptococci form part of the normal flora of the female genital tract, and that this is their principle normal habitat. It is certain that they comprise many different groups species or types, but the data available is as yet far too scanty to permit any systematic classification. It is noted as above, that many are extremely small in size, but they vary in subculture. Many, but not all strains, produce abundant gas in fluid culture, thus differing sharply from the aerobic and facultatively anaerobic types. Again, many but not all, produce an extremely foul odour.

THE PNEUMOCOCCUS

The history of this organism is intimately bound up with the study of pneumonia. The occurrence of micro-organisms in this disease was first noted by Klebs (1875) and Eberth (1881). In the latter year, Koch published photographs of diplococci in sections of the lung and kidney of a case of pneumonia complicating relapsing fever. Leyden (1882), found similar cocci during life in blood obtained from pulmonic puncture in a case of pneumonia.

Friedlander (1882) again found cocci in pneumonic exudate in eight cases, and the modern ideas on the bacteriology of pneumonia date from the publication of his paper in 1883. In one case he successfully isolated the organism in culture and described its characters. He was of the opinion that the coccus was distinguishable from other cocci in three ways. (1) By its possession of a capsule; (2) by the characteristic appearance of the cultiva-

tion in gelatin and (3) by its pathogenic properties of which its failure to produce disease in rabbits was particularly emphasised.

In a series of 25 cases in the Hotel Dieu, Paris, Talamon found repeatedly an elongated ellipsoidal coccus, the shape of which he compared to a lancet. He grew it in meat extract and found that the cultures were pathogenic for 16 out of 20 rabbits. He clearly expressed the view that pneumonia was an infective disease caused by a special microbe.

In the year 1884, Albert Fraenkel's classical works on the pneumonia cocci, began to appear. He found that they agreed with the description given by Talamon rather than with that of Friedlander, obtaining cultures quite unlike the "nail" cultures described as characteristic by Freidlander and he also found the organism to be highly pathogenic for rabbits.

A short period of controversy followed, during which several workers confirmed Friedlander's work and others, Talamon and Fraenkel's work. Finally it was realised that two entirely different organisms were being investigated and Fraenkel himself showed that his organism was Gram positive, while that described by Friedlander was Gram negative. Weichelbaum (1886) in an exhaustive study of the bacteriology of no fewer than 129 cases of pneumonia of different types showed that by far the most common organism was the pneumococcus of Fraenkel, but he also showed that at least three other organisms may cause pneumonia. The organism described by Friedlander, now bears his name and is known as Bacillus Friedlander.

The committee of the Society of American Bacteriologists separated the pneumococcus from the main streptococcal group by forming a genus Diplococcus with Diplococcus pneumoniae as the type species. British bacteriologists, however, consider this undesirable for several reasons, and retain the name Streptococcus pneumoniae.

The organism is oval or oatshaped and usually occurs in pairs, especially in body fluid or in culture media containing serum. In cultures, however, in fluid media there is a tendency to form chains. The possession of a capsule, however, is one of the most important features and several methods of staining have been evolved in order to demonstrate these capsules. As a rule a single oval capsule surrounds that lancet shaped diplococcus. On blood agar a zone of typical α haemolysis is usually seen around the colony. As stated above, the Streptococcus mucosus of Schottmuller is now recognised as a variety of pneumococcus.

Neufeld (1900) making observations with pneumococcus on the lines of Koch's work on immunisation against "rinderpest" found that bile had a specific bacteriolytic effect on the pneumococcus. This observation formed the basis of the now common bile solubility test for distinguishing the pneumococci from other streptococci of the viridans group. Neufeld originally maintained

that only freshly isolated and virulent strains showed this reaction, but it is now used as a definite means of identification of all strains. The pneumococcus is an organism that readily undergoes autolysis in culture and Avery and Cullen (1923) showed that extracts of washed pneumococci contain an enzyme that lyses the bacterial cells. There seems little doubt that the action of bile is simply to accelerate this process. Mair (1917) showed that the most actively lytic bile constituent is sodium deoxycholate, a pure solution of which forms the most satisfactory reagent.

THE ANTIGENIC CLASSIFICATION OF THE PNEUMOCOCCI

Neufeld and Handel (1909) first demonstrated the existence of antigenically different types of pneumococci. They studied the protective effect of different anti-pneumococcal sera in mice and found that a given serum would protect against a homologous strain of pneumococcus, but not against the heterologous strains. Dochez, Avery and other workers later studied the antigenic relationships of a large collection of pneumococci, using the methods of direct agglutination and agglutinin absorption. They confined their attention for the most part to strains isolated from cases of lobar pneumonia in man and were able to recognise three well differentiated types (1, 2 and 3) leaving a large heterogeneous group unclassified. Thus a reasonably adequate picture of the distribution of these three types in cases of pneumonia and other pneumococcal infections in healthy contacts and in the population at large was obtained. For many years no attempt was made to analyse the considerable number of strains comprising the unclassified heterogeneous group. Since 1929, however, Cooper and her colleagues have carried out a detailed study of this group and have identified 29 new antigenic types, making 32 in all. Most of these types are sharply differentiated from one another and can be identified by direct agglutination.

It was long recognised that the antigenic behaviour of intact pneumococci in the normal smooth state was probably determined by the nature of the capsules surrounding the bacterial cells. Neufeld (1902) noted that the capsules of pneumococci when acted upon by a specific anti-serum became greatly swollen and later this phenomenon was used in the identification of pneumococcal types. A great advance was made in the study of antigenic structure in general when Avery and Heidelberger attacked the problem from the chemical side. By suitable methods of extraction, followed by fractional precipitation, it was found possible to separate the capsular components that determine type specificity in a state of chemical purity. These compounds were found to be complex polysaccharides and some of the physical and chemical properties of the three capsular components of the classi-

cal types have been determined. Solutions of these polysaccharides give specific precipitation in high dilution when mixed with coresponding anti-sera. As yet, the work is incomplete but it is safe to assume that each pneumococcal type is characterised by a specific capsular polysaccharide that determined its anti-genic behaviour.

Another polysaccharide has been isolated from the pneumococcal cell by Tillett, Graebel and Avery (1930). It differs from the capsular polysaccharide in containing phosphoric acid and is not type specific, but appears to characterise the pneumococcus as a whole. There is also a nucleo-protein antigenic component precipitable from extracts of acetic acid. It is probably situated deeply within the intact bacterial cell. It is shared by all pneumococci, and by many other bacteria, including all those species of streptococci that have been examined.

The antigenic picture of the species S. pneumoniae that emerges from these studies is that there is a central protoplasmic portion of the cell which, in its antigenic relationships is neither species nor type specific. Situated probably at the surface of the cell there is another component, mainly carbohydrate in nature, that is specific for the S. pneumoniae as a species. External to this in the smooth forms, there is a capsule composed of a polysaccharide that is specific for each pneumococcal type. It must be assumed that there are 32 different capsular polysaccharide. The virulence and antigenic behaviour of pneumococcal cells are determined by these antigenic components.

THE ANTIGENIC CLASSIFICATION OF THE HAEMOLYTIC STREPTOCOCCI

The antigenic structure of the Haemolytic streptococci is not nearly as straight forward as that of the pneumococci, since it is not easy to define exactly a haemolytic streptococcus and also since the technical difficulties of anti-genic analysis are much greater than in the case of the pneumococcus.

All the earlier attempts to define anti-genic relationships of haemolytic streptococci were carried out by medical bacteriologists, mainly with the object of determining what differences, if any, distinguished the strains causing such human infections as erysipelas, cellulitis, tonsillitis, scarlet fever, puerperal sepsis, etc. It happened, therefore, that all these studies were carried out, not on haemolytic streptococci as a group, but on collections of strains isolated from particular types of infection. The method mainly employed was that of agglutination and agglutinin absorption and it was beset with many difficulties. Many strains of haemolytic streptococci give a granular growth in broth and are autoagglutinable in saline, so that it may be difficult to obtain a satisfactory suspension for agglutination tests. Moreover, there is much

more cross-agglutination between different types of haemolytic streptococci than between different types of pneumococci, so that the absorption method has to be freely employed. These earlier attempts, although they gave definite indications of antigenic differentiation, left a confusing picture and it was not until certain observation on the aetiology of scarlet fever stimulated a renewed interest in this group that further progress was made. Griffiths from 1926-35, was the main contributor to the problem and differentiated and numbered 27 types of pathogenic haemolytic streptococci isolated from various lesions in man.

Lancefield, since 1928, has attacked the problem from another angle. Instead of employing the agglutination test, she prepared extracts from different strains of streptococci and tested them against homologous and heterologous antisera by means of the precipitin test. She has been able to differentiate three different antigenic components in her extracts. First, a non-specific nucleoprotein antigen shared by all haemolytic streptococci, pneumococci S. Viridans and by certain more distantly related organisms such as Staphylococcus aureus. Secondly, a carbohydrate complex which serves to divide the haemolytic streptococci into well defined groups with an apparent correlation between anti-genic type and habitat. Finally, Lancefield has demonstrated an acid soluble protein characteristic of each strain, and serving to divide them into antigenic types, just as pneumococci are divided by their capsular components.

Group A

The streptococci of Group A all share a common antigen and are the common pathogens of man. In addition to producing β haemolysis on blood agar, they appear without exception to form a soluble haemolysin. They produce a final pH of 5.0-5.6 in glucose broth; they do not hydrolyse sodium hippurate; they do not reduce methylene blue in milk; they almost always ferment salicin and lactose, but seldom mannitol raffinose or inulin. They also ferment trehalose but not sorbitol. They may or may not grow in 10 per cent bile agar, but seldom on 40 per cent bile agar.

Strains identified as belonging to this group have in the main been derived from infections in man such as tonsillitis, scarlet fever, erysipelas, cellulitis and puerperal fever. They have also been isolated from nose and throat of normal persons and occasionally from cases of mastitis in cattle, though in this latter case there have usually been grounds for suspecting a human source of infection.

Group B

The members of this group all share a common group specific antigen that is carbohydrate in nature and differs from the Group A specific antigen. Further division is possible into four main

antigenic types and a number of sub-types. These type specific antigens are apparently not acid soluble proteins, but complex carbohydrates of different structure to the carbohydrates that determine group specification. Haemolysis on blood agar plates is variable within the group. They differ from Group A strains in producing a low final pH (4.2-4.8) in glucose broth, hydrolyse sodium hippurate, and usually grow on 10 per cent and 40 per cent bile agar. They fail to reduce methylene blue however, and ferment trehalose, but not sorbitol. Other sugar reactions are variable. The majority of strains have been isolated from cases of mastitis in cattle. They have occasionally been isolated from the human throat, but are rarely pathogenic.

Group C

These strains share a common polysaccharide antigen, but the type specific antigen is of protein nature. A wide zone of β haemolysis is usually produced on blood agar plates. They produce in glucose broth a final pH intermediate between Group A and Group B. (It may vary between 4.5 and 5.4). No Group C strains, however, hydrolyse sodium hippurate. Many grow on 10 per cent bile agar, but few on 40 per cent bile agar. On the basis of their action on trehalose and sorbitol, three sub-groups can be differentiated.

1. Fermentation of neither trehalose or sorbitol.
2. Fermentation of sorbitol but not trehalose.
3. Fermentation of trehalose but not sorbitol.

It is interesting to note the correlation between fermentation reactions and habitat. Most of the strains belonging to the trehalose negative and sorbitol negative sub-group have been isolated from cases of strangles and clearly correspond to the S. equi of earlier writers. They ferment salacin but not lactose and do not reduce methylene blue in milk. The second sub-group have been isolated chiefly from lesions in horses and cattle, but there is no evidence to show their pathogenicity for man. The third sub-group fermenting trehalose, but not sorbitol have been isolated from animal and human sources, and it seems clear that at least some strains can be pathogenic to man.

Group D

Great confusion has existed in the past between haemolytic streptococci falling into Group D the enterococci (usually non-haemolytic) and the lactic streptococci (also non-haemolytic).

The organisms of the haemolytic streptococci have been isolated from cheese and human faeces. Morphologically, they tend to assume a diplococcal rather than a streptococcal formation. On horse blood agar they tend to give rise to β Haemolytic colonies. They give a final low pH of 4.0-4.8 in glucose broth, they grow on 10 per cent and 40 per cent bile agar and reduce methylene blue

in milk. They are heat resistant, withstanding a temperature of 60° C. for thirty minutes. They fail to hydrolyse sodium hippurate, but ferment salicin, lactose, nearly always mannitol and usually, but not always trehalose and sorbitol.

The enterococcus and lactic streptococci also give these reactions and as stated above, much argument has taken place as to whether or not they are one and the same organism.

Recently (1939-43), however, the position has been clarified by the work of several workers who have shown that the enterococcus possess the specific carbohydrate antigen of Group D haemolytic streptococci, but that the lactic streptococci do not. It would appear that the enterococci differ from Group D haemolytic streptococci only in their failure to produce characteristic β haemolysis on blood agar. In the second place, careful comparison between strains of enterococci and lactic streptococci have revealed differences. Sherman and Stark (1934) found that enterococci withstood exposure in sterile skim milk at a temperature of 65° C. for thirty minutes, grew vigorously at 45° C. and developed on lactose agar having a pH of 9.6, whereas lactose streptococci did none of these things.

Within the enerococcus group an attempt has been made to distinguish antigenic types, but so far, little progress has been made. Lancefield (1941) states that she has been able to define three types differing in the nature of their polysaccharide antigen.

Group E.

The few strains falling into this group were isolated from cow's milk by Lancefield (1933). As yet insufficient work has been done to give any idea of the character of the group.

Group F

This group possesses a characteristic group specific polysaccharide antigen and four antigenic types have been demonstrated. (Bliss 1937). As a rule growth is very slow on blood agar and minute pin point transparent colonies surrounded by a narrow zone of β haemolysis are produced. Strains of this group produce a final acidity in glucose broth of pH 4.8-5.2. They do not hydrolyse sodium hippurate or reduce methylene blue in milk, nor do they grow on 10 per cent or 40 per cent bile agar. Some ferment trehalose, but never sorbitol. The majority or strains have been isolated from the human throat.

Group G

Here again, the group specific antigen is of polysaccharide nature. It has been found (Hare 1935) that Group C antisera frequently give cross precipitation with extracts from Group G strain. This, according to Lancefield (1941) is due to the possession of a common protein antigen. Most strains have been isolated from

man and a few from the monkey and the dog. One of Griffith's types (Type 16) of human pathogenic streptococci falls into this group.

Of Groups H, K, L and M, little as yet is known except that anti-genically distinct organisms have been isolated.

Group H.

As already mentioned under Group O, the lactic streptococci differ antigenically from the enterococci and it has been suggested, in spite of the absence of haemolysis, to include them in the Lancefield series and assign them to Group N.

In conclusion it is interesting to trace the evolution of the classification of Streptococci and the failure of the various criteria laid down at various times to provide a complete and truly scientific classification.

The first characteristic, that of type of disease production and habital was obviously inadequate as shown by the very early experiments of Koch and Petruschky.

Likewise Von Lingelsheim's "longus" and "brevis" types and morphology in general have little application in modern classification except in a very general sense since they are subject to environmental conditions.

"Sugar" classification as stated above, was for many years the subject of much controversy, but to-day it still has its place though a minor one in classification. It is used in such places as the identification of the Enterococcus and Group D (in combination with other tests) and in the sub-division of Group C and may be regarded as an auxiliary rather than a fundamental criterion.

The classical work of Schottmuller has been confirmed and extended by Brown and other workers and for all practical purposes colonial appearance on blood agar is the modern everyday classification. This method, however, only serves as a basis for division into brood groups.

Serological typing was the first attempt at a truly scientific classification, but it broke down due to technical difficulties and under the weight of its own complexity.

Finally, the antigenic classification of Lancefield is one of the most important steps in the history of Bacteriology, for with improved technique it seems possible that the principles could be applied to any group of bacteria.

This the limitations of these earlier methods can clearly be seen in the light of the modern antigenic classification which after all, is a fundamental distinction concerned with the chemical make up of the organisms and not a characteristic of behaviour.

(This manuscript was submitted as an entry in the Prize Essay Competition, 1947, for Junior members).

BIOCHEMICAL METHODS, PART 2
URINE, FAECES AND GENERAL

J. B. BROWN

(From the Pathological Laboratory, Public Hospital Auckland)

A summary of methods used in the Auckland Hospital Laboratory. Note that M means minimum quantity of specimen, K stands for keeping properties of specimen, and N means normal.

A. URINE

Acetone and Aceto-acetic acid—Spot test Rothera—B.M.J. 1944. 1. 512. M. 5 drops. K. 24 hrs.

Aceto-acetic, acid Gerhardt—Harrison. Chem. Methods, Clin. Med. 2nd. ed. p. 18. M. 2ml. K. 8 hrs.

Alcohol as for blood.

Bilirubin Fouchet's. Harrison loc cit P. 18. M. 5ml. K. 24 hrs.

Diastase Wohlgemuth B.J. Exper. Path. 1922-3. 133.
N. under 30 units, M. 2ml. K. 18 hrs. in ice chest.

Ketosteroids (17) Callow et al. Biochem. J. 1938. 32. 1312 J. Endocrinology 1939. 1. 76. N. 3-15 mgms. in 24 hrs.
M. 100 ml. of well mixed 24 hr. specimen without preservative K. days.

Protein (Bence Jones) Jacobson and Milner. Am. J. Clin. Path. 1944. 14. 138. M. for complete testing 100 ml. K. 24 hrs. in ice chest.

Reducing substances, Harrison loc. cit. Chapt. 2.

Spectroscopic examination, do, Chapts. 10. 11.

Urea, Hypobromite method, do, p. 71 K. days M. 1ml.

Urobilin Schlesinger's test, do, p. 19, K. days.

Urobilinogen Ehrlich's aldehyde test (a) Qualitative Rec. Adv. in Med. 11th. ed. p. 149. M. 5ml. K. 3 hrs. (b) Quantitative Wallace and Diamond, Arch. Int. Med. 1925, 35, 698.

B FAECES

The constituents of biochemical significance are invariably stable.

Blood Gregerson's test. Harrison Loc. cit. p. 477.

Calcium for calcium balance. Shohl and Pedley. J. Biol. Chem. 1922. 50. 537.

Fat, Holt, Courtney and Fales. Harrison. Loc. cit. p. 465.

Fat Balance Cook et al. Quart. J. Med. 1946. 15. 141.

Stercobilin Spectroscopic Method. Harrison. Loc. cit. p. 481.

LABORATORY ROUTINE FOR THE FOLLOWING INVESTIGATIONS

Addison's disease. See Kepler and Wilson. Arch. Int. Med. 1941, 68. 979.

Plasma chloride: Serum sodium (gravimetric zinc uranyl acetate method); Robinson Power, Kepler or Mayo Clinic water, excretion test.

The following may give significant results and are performed if indicated. Cutler-Power-Wilder sodium withdrawal test; 17-ketosteroid excretion; insulin sensitivity test (see Fraser, Albright and Smith. J. Clin. End. 1941 1, 297).

Enzymes in Duodenal Fluid.

(a) Trypsin. Method of d'Este Emery. Harrison. loc. cit., p. 482.

(b) Lipase. Method of Carnot and Mauban. p. 273

Gastric Analysis—Fractional test meal. Harrison. p. 429.

Liver Function Tests

(a) Blood: bilirubin (Van den Bergh, qualitative and quantitative).

Urine: bilirubin and urobilin or urobilinogen.

Faeces: stercobilin.

(b) Serum: alkaline phosphatase.

(c) Serum protein.

(d) Prothrombin time before and after administration of Vitamin K.

(e) Quick's liver function (Hippuric acid) test. Weischselbaum and Probstein. J. Lab. Clin. Med. 1939. 24. 636.

(f) Empirical tests such as Takata-Ara which are performed only when requested.

Urinary Calculi

Micro methods of Winer and Mattic J. Lab. Clin. Med. 1943. 28. 898.

Renal Efficiency Tests

(a) Specific gravity and water elimination tests.

(b) Blood urea.

(c) Urea concentration test (Maclean).

(d) Urea clearance test (Van Slyke).

(e) Fowweather's test. This is a combination of 1 b, c. and d. The test is performed as follows: Blood is taken for urea estimation, and the bladder emptied, and 15 gms. of urea in a cup of water is ingested. Urine is collected and examined for volume and urea per cent at 1,2 and 3 hrs. after taking the urea, the time being carefully noted. A further blood is collected for urea estimation at 1¼ hrs. after ingestion of the urea. Using the second blood and the second urine specimen the urea clearance is calculated by applying Van Slyke's formula.

INDUSTRIAL MICROBIOLOGY

Dr. M. G. Somerville, (Part 2)

Beer is a complex substance containing 85-92% water, dextrose, maltose, dextrin, amino-acids, peptones, amides, resins, essential oils, tauric acid, acetic acid, higher alcohols, lactic acid, some vitamin B and of course some ethyl alcohol.

Ginger beer is produced by the fermentation of cane sugar by the ginger beer plant which contains the yeast Saccharomyces pyriformis and a bacterium Bacterium pyriforme entangled in its root. You no doubt have seen advertisements of this plant in the papers, but nowadays little ginger beer is produced by this method.

Before I close I must mention briefly firstly the Wine Industry for which Pasteur did so much in France last century. Red wine is simply made by fermenting grape juice, pips, skins and all by a yeast, Saccharomyces ellipsoideus Hansen, while white

wine is produced from the juice of the grapes only. Secondly in the distilling industries, rum is the distillate of the fermentation of sugar cane or molasses by S. cerevisiae, the distillation being carried out at a critical temperature, and caramel added for colouring. Whiskey is the distillate of the fermentation of various grains. In rye whiskey, rye malt and rye or rye malt and barley malt are the original substrate. In Bourbon, corn, barley and/or wheat malt are used. Scotch whiskey is usually made from wheat and barley and has been aged for not less than three years in charred oak barrels which give it its specific taste. Brandy is the distillate of fermented grape juice, Cognac being a blended brandy produced in Cognac in France. Gin is ethyl alcohol distilled over juniper berries, conander seeds, fennel seed, sweet orange, etc. Liqueurs are a redistillation of neutral spirits, brandy or gin over fruits, flowers, plants or fruit juices with added sugar after the distillation.

Ethyl alcohol production is carried out by the yeast Saccharomyces cerevisiae Hansen. This yeast ferments the sugar into carbon dioxide and alcohol. Molasses, starchy materials (corn), Jerusalem artichoke, wood, cellulose products, waste sulphite liquor, after suitable treatment to render their sugar available can be fermented by this yeast. In the process of manufacture there are three main steps. These are saccharification of the raw materials, fermentation and distillation of the final liquor. Before the last war absolute alcohol cost 20 cents per gallon to produce, and by the simple process of adding neutral sulphites or alkalis to the substrate, glycerol can be produced. The brewing industries are similar in many respects to the production of alcohol, except that there is no final distillation and hops have been added in the process. This industry can be divided into the Malt stage where the grain is allowed to sprout and is then heated thus producing the malt, which is darker the higher the temperature used. Mashing is the next stage, when the wort is produced from the hops and malt, which are then fermented by S. cerevisiae Hansen. The final stage is the finishing where the beer is clarified, carbon dioxide added and the beer bottled. Some firms pasteurize their product. Lager beer is usually made by bottom fermentation in which selected strains of the above yeast which grow in the depths of the fermentation tanks are used and little frothing occurs. Ale is made by top fermentation with vigorous carbon dioxide production. Stout is made in the same way as beer but the mash contains excessive hops, while porter is made from dark malt.

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