THE SEROLOGY OF PNEUMOCOCCAL INFECTION. A STUDY OF THE LABORATORY DIAGNOSIS OF PNEUMOCOCCAL INFECTION AND THE DISTRIBUTION OF PNEUMOCOCCAL TYPES.

by

© Leslie E. Smart, F.I.M.L.S., M.I.Biol.

being a thesis submitted for the
Degree of
Doctor of Philosophy
in the
University of Glasgow

June 1987

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

This thesis is dedicated to the late Dr. John S. Stevenson, Consultant Bacteriologist, Stobhill Hospital, Glasgow, whose original ideas and guidance inspired this work.

CONTENTS

List of Tables

List of Figures

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my supervisors, Professor M.C. Timbury and Dr. D.J. Platt, for their excellent advice and constructive criticism throughout the period of the study.

I would also like to thank Drs. R.W.A. Girdwood and A.J. Dougall, Bacteriology Department, Stobhill Hospital for their encouragement and for allowing me to set up the pneumococcus laboratory.

I am indebted to Dr George Addis, Consultant Respiratory Physician and other staff in the clinical units concerned for access to their patients' records and for their co-operation throughout the clinical study.

My thanks also to Dr Jorgen Henrichsen, WHO Collaborating Centre for Reference and Research on Pneumococci , Statens Seruminstitut, Copenhagen, Denmark for invaluable discussions and hospitality when I visited his laboratory.

I greatly appreciate the co-operation of laboratory staff at Stobhill Hospital. In particular I wish to thank Mrs A. Blackwood and Mr T. Cowan for their expert technical assistance.

I am grateful to colleagues in many other laboratories who sent cultures and clinical material and Dr. Helena Hughes, Consultant Pathologist, Royal Infirmary, Glasgow, for teaching me respiratory cytology .

The support and patience of my wife, Joan, has been essential for the accomplishment of the result.

SUMMARY

The aim of this study was to assess the value of serology in the laboratory diagnosis of pneumococcal infection. This included the serotyping of pneumococci isolated on culture, detection of pneumococcal capsular polysaccharide antigen in body fluids and estimation of antibody responses.

The co-agglutination test which uses staphylococcal protein A, was found to correlate well with the standard capsular reaction test. However, since co-agglutination was more sensitive and therefore less specific, a number of bacteria which were not pneumococci reacted in the co-agglutination test. The detection of pneumococcal capsular polysaccharide antigen in clinical material has been widely used for the diagnosis of pneumococcal infection. Antigen detection in material obtained from patients who had been treated with antibiotics provided evidence of infection. Four methods for antigen detection were compared. These were counterimmuno-electrophoresis (CIE), co-agglutination (CoA), latex agglutination (LA) and enzyme-linked immunosorbent assay (ELISA). Their sensitivity was compared in tests carried out using purified pneumococcal capsular polysaccharide and with different kinds of pneumococcal antisera. ELISA was the most sensitive method but only with antisera which contained a limited number of pneumococcal antibody specificities. Using the same antisera, CoA and LA showed similar intermediate

sensitivity : CIE was the least sensitive method. However, in tests with polyvalent pneumococcal antisera CIE, CoA and LA were equally sensitive although pneumococcal antigen of types 7F, 14 and 33F could not be detected by CIE. The four methods were also evaluated to discover their suitability for the detection of pneumococcal antigen in body fluids and secretions. Co-agglutination with antiserum pools or pneumococcal typing sera was the most reliable method for the detection of pneumococcal antigen in respiratory secretions - where specificity but not sensitivity is important. Latex agglutination on the other hand was preferred for the examination of serum, urine and cerebrospinal fluid where sensitivity is the more important criterion. ELISA was a useful confirmatory test when carried out on serum or CSF which contained small amounts of pneumococcal antigen. On this basis a protocol was established and used in a comprehensive study of pneumococcal infection in hospitalised patients.

Patients with respiratory and non-respiratory pneumococcal infection were classified on the basis of clinical history and the severity of disease. The distribution of pneumococci and pneumococcal antigen in clinical material obtained from different body sites was found to differ in patients in the different clinical groups. The presence of pneumococcal antigen in serum and urine was associated with severe pneumococcal

disease.

Since the laboratory diagnosis of pneumococcal chest infection depends, to a large extent, on examination of expectorated sputum the relationship between the results of conventional examination for pneumococci and of tests for pneumococcal antigen was studied in detail. The presence of pneumococcal antigen in sputum correlated well with clinical evidence of infection. Antigen detection proved to be a more sensitive and reliable method for the diagnosis of pneumococcal pneumonia than were the traditional techniques of culture and Gram's stain. The value of serial sputum examination in different groups of patients was demonstrated : this was able to distinguish between relapse and new infection.

An indirect immunofluorescent assay (IFA) was developed for the detection of type-specific pneumococcal antibodies. Pre-absorption of the serum to remove antibody to pneumococcal C-polysaccharide improved the specificity of the assay which proved to be sufficiently sensitive to detect changes in antibody titre between acute and convalescent phases of infection. Moreover, for the majority of serotypes the minimal amount of antibody detectable by IFA was less than that currently recognised as protective against bacteraemic pneumococcal disease. Type-specific pneumococcal antibodies were often detected in acute phase sera especially in less severe

infections. The presence of pre-existing type-specific antibody in serum, however, did not prevent systemic pneumococcal disease due to homologous strains of Streptococcus pneumoniae.

The serotype distribution of 874 strains of S.pneumoniae was determined in relation to patients' age and to the frequency of isolation from systemic disease. Types 14 and 18 in pre-school children, and types 1,4,7,8 and 12 in patients over 5 years of age were significantly associated with systemic disease. The serotype distribution of pneumococci or pneumococcal antigen in the sputum of 1,682 patients with pneumococcal chest infection was determined. Types 3 and 8 were significantly associated with pneumonia whereas types 6 and 17 were associated with the milder clinical diagnosis of chest infection. Type 1 was common in patients with pneumonia but was rarely isolated from the sputum of other infected patients.

The results of this study confirmed that serotyping of pneumococci and the detection of pneumococcal antigen in clinical material was a valuable laboratory tool for the diagnosis and assessment and also for management and prognosis of pneumococcal infection. However, interactions in vivo between pneumococcal antigen and antibody are complex. Preliminary results suggest that antibody determination may contribute to clinical assessment but further studies will be required to establish this unequivocally.

ABBREVIATIONS

CIE	counterimmunoelectrophoresis
CoA	co-agglutination
CoA(K)	co-agglutination using a 10% suspension of staphylococci (Kronvall, 1973)
CoA(T)	co-agglutination using a 2% suspension of staphylococci (Trollfors et al., 1983)
COAD	chronic obstructive airways disease
CRT	capsular reaction test
CSF	cerebrospinal fluid
CRP	C-reactive protein
DIC	disseminated intravascular coagulation
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
IFA	indirect immunofluorescent assay
IHA	indirect haemagglutination assay
LA	latex agglutination
PMN	polymorphonuclear neutrophil
RIA	radioimmunoassay

CHAPTER 1

INTRODUCTION

1.1.HISTORY OF PNEUMONIA

"Pneumonia occupies a place of its own both from strictly local diseases and from those which affect the system generally. Differing from either, it has something in common with both. Besides the local injury, its general phenomena exhibit over a wide area, involving every body function, are so fixed as to time and order of succession as to resemble a specific fever than pyrexia of a merely local inflammation"

Sturges, 1876

Pneumonia, formerly known as peripneumony after the nomenclature of Hippocrates, has been regarded from earliest times as one of the internal affections most readily recognised in man. Heffron (1939) cited the work of Ruffer who found evidence of pneumonia in Egyptian mummies which date from 1250 to 1000 B.C.

Peripneumony has been known to man for centuries although its early history is obscure since accounts by early workers were not sufficiently precise to make it certain that successive authors were describing the same disease. Hippocrates (around 400 B.C.) and Aretaeus (around 100 A.D.) described an affection characterised by fever, chest pain and coloured sputa, but failed to distinguish peripneumony from acute pleurisy. The distinction between the two was made during the last century when Laennec introduced percussion to the investigation of chest diseases.

Traces of a disease resembling pneumonia can be found

in the medical history of Europe. The plague of Athens in 430 B.C. has been termed "Gangerous Pneumonia", and the main symptom of the 14th century "Black Death" was a cough and blood-stained sputum. During the 16th and 17th centuries epidemics of a disease were reported which had the characteristics of pneumonia. These epidemics were associated with poor or crowded living conditions and cold north winds and rain. Pneumonia was usually preceded by catarrhal infection, followed by severe cough and fever, pain in the side and difficult respiration : blood stained sputum occurred on the third day and death in five to eight days. The usual outcome of epidemic pneumonia in Europe was death, but it is likely that this was caused by other diseases e.g. cholera, dysentery or measles. In England pneumonia exhibited a "catarrhal" form and was a feature of the influenza epidemics of 1755 and 1762.

In the 18th century it was realised that certain groups of patients were more prone to the disease, particularly individuals with "thick blood, flat breath, the elderly and those who drank strong viscid liquor". It was also believed that pneumonia was caused by "obstructive perspiration" during cold weather, wet clothes or by an increased circulation of blood brought about by violent exercise and as a consequence of other respiratory diseases. Eighteenth century physicians adopted the same methods of treatment as had been proposed by Hippocrates. The main treatment was

phlebotomy with the withdrawal of up to one pint of blood in the first 12 to 18 hours and repetition of the procedure over the following 3 days as seemed necessary. This depended on the age, and condition of the patient and on the season of the year. Other treatments designed to promote discharges from the bowels and skin were used and the appearance of the sputum and urine were observed to indicate management while awaiting the development of a "crisis".

By the 19th century it was realised that pneumonia had a mortality rate exceeding all other fevers, and was termed " captain of the men of death " by Sir William Osler in 1901. During the last half of the century the bacterial agents responsible for the disease were identified and the pneumococcus was found to be the most frequent cause of bacterial pneumonia.

The initial isolations of the pneumococcus were made from carriers rather than from patients with pneumococcal disease. For example, Pasteur isolated the pneumococcus in December 1880 from the saliva of an infant dying of hydrophobia as did Sternberg in April 1881 from samples of his own saliva.

In 1882 Friedlander described organisms which were probably pneumococci in sections of lung tissue from eight patients who died of pneumonia. However in 1883 he also reported the isolation of a Gram negative bacillus, which subsequently became known as Friedlander's bacillus. Frankel in 1884 described the isolation of an

organism from a fatal case of pneumonia which differed from Friedlander's isolate and there followed a period of controversy over the agent responsible for pneumonia. Weichselbaum resolved the issue in 1886 when he published the results of an extensive study on the bacteriology of pneumonia in which he defined the relative roles of both Friedlander's bacillus and the pneumococcus as causes of pneumonia.

Pneumococcal pneumonia, therefore, is not a new disease and was the subject of many studies carried out in the early part of the 20th century. Many of these studies have been collated in two monographs, "The Biology of the Pneumococcus" (White,1938) and "Pneumonia with Special Reference to Pneumococcus Lobar Pneumonia" (Heffron, 1939).

Recently, in his book " A Life with the Pneumococcus " Austrian (1985) has brought together papers which deal with historical aspects and which consider the developments which have resulted in the re-introduction of pneumococcal vaccines.

1.2.THE PNEUMOCOCCUS

1.2.1. Morphology

The pneumococcus is a Gram positive lanceolate or oval coccus which measures between 1.2 to 1.8 um (long axis) and 0.5 to 1.0um (short axis) - although these measurements vary with the conditions of growth. The bacteria are arranged in pairs (diplococci) or in short chains: when in pairs the opposite ends are more pointed. The pneumococcus is non-motile, multiplies by transverse fission and most strains have a capsule which envelops the paired or chained cocci. The size of the capsule varies with the serotype, and from strain to strain within a given serotype, under standard growth conditions.

Although the pneumococcus was classified in the genus Diplococcus by American taxonomists, with the discovery of filamentous variants of pneumococci (Austrian, 1953) and the genetic relationship between pneumococci and alpha-haemolytic streptococci in transformation reactions (Yurchak and Austrian, 1966), the organism was designated Streptococcus pneumoniae.

1.2.2. Antigenic structure

Pneumococci are normally capsulate and most interactions between a host and the pneumococcus involve the capsular polysaccharide. Two different kinds of somatic antigens have been described.

A. Cell wall antigen (C-polysaccharide)

The cell wall of the pneumococcus is typical of that of Gram positive bacteria and is composed of peptidoglycan and teichoic acid. Tillett, Goebel and Avery (1930) isolated a carbohydrate from pneumococcal cells which, unlike capsular polysaccharides, contained phosphate residues and was specific for the species rather than the type. This cell wall antigen, referred to as pneumococcal C-substance, appeared to be (or at least resembled chemically) the substance which reacts with C-reactive protein - an abnormal beta-globulin which is detected in human serum during the acute phase of certain illnesses. Tomasz (1967) showed that pneumococci incorporate choline into their teichoic acid and Volanakis and Kaplan (1971) later found that the interaction between C-reactive protein and C-polysaccharide was due to binding of the protein to phosphorylcholine. These results suggested that the phosphorylcholine component of teichoic acid was analogous to C-substance. This was subsequently confirmed by Jennings, Lugowski and Young (1980) who found that the teichoic acid moeity of C-substance consisted of glucose, 2-acetamido-4-amino-2,4,6,trideoxy-galactose (AATGal), ribitol phosphate and phosphorylcholine. Moreover, Jennings et al. (1980) proposed a structure for C-polysaccharide and showed that this structure was common to the cell walls of pneumococcal types 1,18C and 23F.

Recently 6 of the 83 serotypes of S.pneumoniae have been found to contain phosphorylcholine in their capsular polysaccharides (Sorensen et al., 1984). This is consistent with other reports in which components of the pneumococcal cell wall polysaccharide have been identified as constituents of one or another of the pneumococcal capsular polysaccharides (cited by Austrian, 1981). Moreover, Bornstein et al. (1968) recovered after transformation with exogenous DNA, a mutant strain of S.pneumoniae (designated C_s) in which C-substance extended beyond the cell wall and formed capsular material consisting solely of C-substance. These observations suggest that pneumococcal capsular polysaccharides may have evolved from cell wall polysaccharide (Austrian, 1981).

Sorensen and Henrichsen (personal communication) have shown that rabbit antiserum against pneumococcal C-polysaccharide can be used to differentiate pneumococci from other alpha-haemolytic streptococci.

B. R-antigens (synonymous with M-proteins)

These were originally extracted from non-capsulate pneumococci and were described by Heidelberger and Kabat (1938). R-antigens are believed to be proteins at or near the surface of the cell. Antiserum prepared against a non-capsulate variant of a particular capsular type was found to agglutinate, to a higher titre, non-capsulate pneumococci derived from a homologous capsulate type than a non-capsulate variant derived from

a heterologous type. This difference was due to **M-protein.** Austrian and MacLeod (1949) extracted, from both capsulate and non-capsulate pneumococci, a series of type-specific proteins which resembled the M-proteins of S.pyogenes. They found that antisera to M-protein agglutinated non-capsulate, but not capsulate variants of the homologous strain - suggesting that the M-antigen is situated on the surface of the cell wall beneath the capsule. They found that members of the same capsular type could have different M-antigens. However, they found that pneumococcal M-antigens differed from the M-antigens of Group A streptococci in that they did not determine virulence.

C.Capsular antigen

The capsular polysaccharide antigens are the major antigenic component of the pneumococcal cell. Differences in the composition and structure of the capsular material have allowed 83 serotypes to be recognised (Lund and Henrichsen, 1978). Pneumococcal capsules are composed of large polysaccharide molecules which form hydrophilic gels on the surface of the organism and for a given capsular type the amount of capsular material produced seems related to pathogenicity in mice (MacLeod and Krauss, 1950). The chemical composition and structure of a number of pneumococcal capsular polysaccharides are known. The capsular material and the cell wall are quite distinct although the capsule is held to the cell wall by salt-

like linkage between cell wall proteins and uronic acid and hexosamines in the capsular material (Jacox, 1947). Capsules may be permeated by other components which originate in the cell wall.

1.2.3. Development of the pneumococcal typing system

Sixteen years after the initial isolations of the pneumococcus by Pasteur (1881) and by Sternberg(1881), Bezancon and Griffon (1897) demonstrated that the convalescent serum of patients with pneumococcal pneumonia in some cases reacted only with the strain isolated from the patient in question, but not with other pneumococcal strains. This indicated the existence of more than one type of pneumococcus. Although other workers around the turn of the century noticed differences between individual pneumococcal strains, Neufeld and Handel (1910) demonstrated serological differences between strains using the Quellung (literally "swelling") reaction.

The first report concerning the serotypes of pneumococci from clinical infection (Dochez and Gillespie, 1913) was published in September 1913. These authors had studied strains isolated from patients with lobar pneumonia, in New YorK, USA and found that pneumococcal strains could be differentiated into three distinct types i.e. 1,11,111 and a large heterogeneous group 1V. In December of the same year Lister (1913), in a study of strains isolated from cases of pneumonia

amongst gold miners in South Africa, also divided pneumococcal strains into four groups, and indicated the existence of yet further types. In the 20 years that followed these initial studies, the number of pneumococcal groups identified in South Africa increased to 21 and these were designated by capital letters. During this period the group 1V strains, originally classified by Dochez and Gillespie, were examined in a number of laboratories in Europe and North America culminating in the report by Cooper et al. (1932) which divided group 1V into 29 distinct serotypes (i.e. 1 to XXX11). Arabic numerals gradually replaced Roman for designating pneumococcal types and Cooper's thirty-two types were reduced to thirty, as types 26 and 30 were considered identical to types 6 and 15 (Morch, 1943).*

In 1938 Ordman compared the pneumococcal types in South Africa with Cooper's system of classification and found that, with the exception of types 31 and 32, all the known types were demonstrated : there being two additional pneumococcal types, designated T and V, that differed from Cooper's types.

During the 1930s a number of studies showed that Cooper's scheme could be applied successfully. However, most workers reported strains which were either not-typable or reacted with more than one antiserum. A third group of strains were recognised which although reacting with a single antiserum differed morphologically or in the strength of the reaction

obtained when compared to the type strain. Later, a number of these strains were included in reports by Kauffmann, Morch and Schmith (1940) and Morch (1942) which brought the total to 68.

Two systems of nomenclature had arisen based firstly on the work of Cooper and then by Eddy in the United States and secondly by Lund (nee. Morch) in Denmark. According to the American system new types were given consecutive numbers i.e. in the order the types were detected. However, the Danish system was similar to the Kauffmann-White scheme for salmonella antigens and was based on cross-reactions between different types. Consequently the American scheme continued to recognise types 26 and 30, which in the Danish system were 6B and 15A respectively. In 1944 Eddy reported that the South African Group T strains were identical with the American type 72 and six years later Lund published 4 new types (Lund, 1950a) and included types 45 (American type 72) and 46(American type 73). Lund added a further 8 types in 1960 and with Kauffmann and Eddy systematically compared the American (81 types) and Danish (80 types) designations.**

Since then, types 48 (Lund, 1962), 12A (Lund and Munksgaard,1967) and 47A (Lund, Munksgaard and Stewart, 1972) have been included in the current Danish nomenclature, which is now universally accepted. It recognises 83 serotypes of S.pneumoniae (Lund and Henrichsen, 1978).

New types continue to be found however, and two have been reported recently i.e. type 25A by Henrichsen (cited in Robbins et al., 1983) and type 16A (Austrian et al.,1985).

The chronology of pneumococcal type identification and a comparison of the Danish and American designations is shown in Table 1.

* = types 26 and 30 are not represented in the Danish nomenclature

** = the discrepancy is due to Danish type 35A being designated as 47 and 62 in the American system.

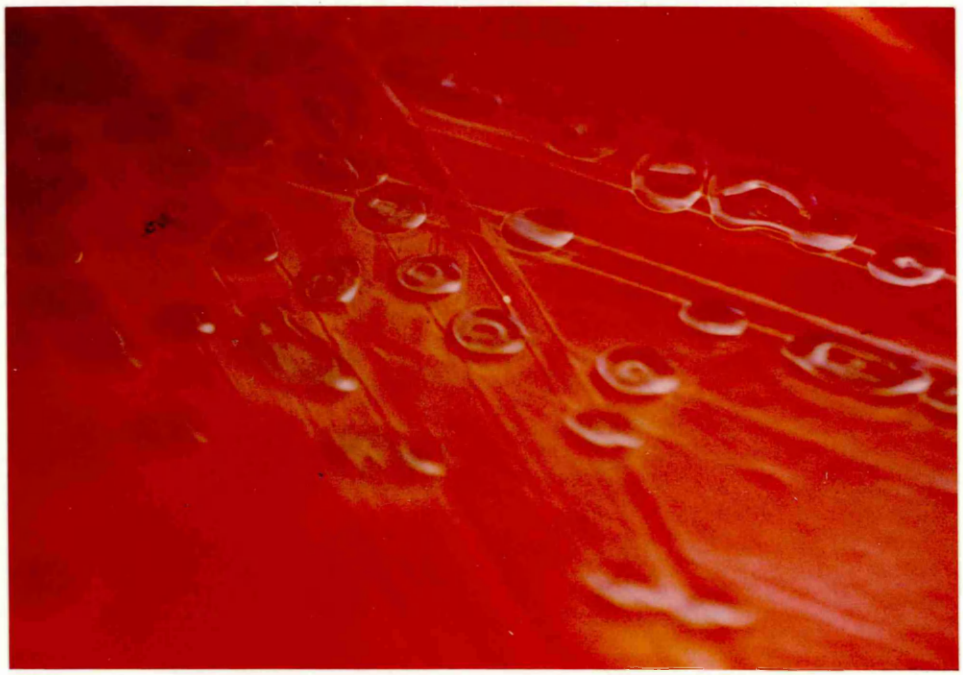
1.2.4. Biological characteristics

Cultural characteristics

Pneumococci grow well on blood agar and produce colonies approximately 1.0mm in diameter after 18 hours incubation : usually surrounded by alpha-haemolysis of the blood beneath and around the colony (Fig. I). Colony size varies with the amount of capsular material produced, for example a type 3 colony may reach 3mm in diameter and have a watery or mucoid surface whereas non-capsulate strains form colonies with a finely granular surface and are more compact. Some strains (approximately 8%) are carbon dioxide dependent (Austrian and Collins, 1966) and anaerobic culture with 5-10% carbon dioxide improves the isolation rate from clinical material (Howden, 1976).

Figure I:

Culture of Streptococcus pneumoniae on blood agar



Courtesy of Mr. W. McCormack

Table 1:
Comparison of the Danish and American designations for pneumococcal type : chronology of their identification.

Cooper (1932)	Kauffmann, Morch and Schmith (1940)	Morch (1942)	Lund (1950)	Lund (1960)
1 (1)				
2 (2)				
3 (3)				
4 (4)				
5 (5)				
6A (6)	6B(26)			
7A (7)	7F(51),7B(48),7C(50)			
8 (8)				
9N (9)	9V(68),9L(49)	9A(33)		
10F (10)	10A(34)			
11F (11)	11A(43)	11B(76)		11C(53)
12F (12)				
13 (13)				
14 (14)				
15F (15)	15A(30),15B(54)			15C(77)
16F (16)				
17F (17)				
18F (18)		18A(44),18B(55),18C(56)		
19F (19)	19A(57)	19B(58),19C(59)		
20 (20)				
21 (21)				
22F (22)		22A(63)		
23F (23)	23A(46)	23B(64)		
24F (24)		24A(65)		24B(?60)
25F (25)				
27 (27)				
28F (28)				28A(79)
29 (29)				
31 (31)				
32F (32)		32A(67)		
	33F(70),33A(40)	33B(42),33C(39)		
	34(41)			
	35F(35),35A(47,62)	35B(66)		35C(61)
	36 (36)			
	37 (37)			
	38 (71)			
		39(69)		
		40(45)		
		41(38)	41A(74)	
		42(80)		
			43(75)	
				44(81)
			45(72)	
			46(73)	
				47F(52)

type 48 (82) Lund (1962) : type 12A(83) Lund and Munksgaard (1967)
type 47A(84) Lund, Munksgaard and Stewart (1972)
NB. Current Danish designation used, ( )= American designation

In serum broth, capsulate pneumococci give a diffuse turbidity with no deposit whereas non-capsulate strains produce a deposit with a clear supernate. Pneumococci grow best at pH 7.6 and when broth cultures are in log phase the bacteria are Gram positive and the capsular material is well developed. However as the culture reaches late log phase, many bacteria become Gram negative due to the action of autolytic enzymes and in stationary phase the bacteria autolyse. Therefore, when preparing pneumococcal suspensions for use in serological procedures, the growth must be checked regularly by Gram's stain and the capsular reaction test and stopped by the addition of 2% (v/v) formaldehyde when in log to late log phase. The heating of formolinised bacteria at 75 °C for 30 minutes inactivates the cell's autolytic enzyme and allows the bacteria to be resuspended in a fluid medium which lacks formaldehyde. Pneumococcal cells treated in this way retain all their serological properties (Dubos, 1938).

Bile solubility

The changes which occur in broth cultures are due to the action of an autolytic enzyme and autolysis is stimulated by surface-active agents such as bile salts or sodium deoxycholate (Mair, 1929). The autolytic enzyme activated by sodium deoxycholate is an L-alanine-muramyl amidase which acts on the muramic acid of the cell wall, only when the normal choline-containing teichoic acid is present; pneumococci in which the

choline has been replaced by ethanolamine in the teichoic acid are resistant to bile and do not undergo autolysis.

The lytic effect of bile on the pneumococcus was reported by Neufeld (1900). He tested ox bile against a wide range of organisms, and found that only "Frankel's diplococcus" was dissolved. Consequently, the bile solubility test was used to differentiate pneumococci from other morphologically similar alpha-haemolytic streptococci. Although the bile solubility test was reliable when performed on capsulate pneumococci, it was found that non-capsulate pneumococci were more resistant to the action of bile (Reimann, 1925).

Bile solubility tests are normally carried out on cultures of pneumococci in serum broth. However, Howden (1976) observed that pneumococci, when grown in an anaerobic atmosphere containing carbon dioxide, produced sufficiently large colonies which were suitable for use in a rapid bile solubility test (Howden, 1979).

Biochemical characteristics

Pneumococci are facultatively anaerobic and with other streptococci are classified as lactic acid bacteria. Pneumococci ferment lactose, sucrose, usually trehalose and raffinose. Inulin fermentation was considered useful as a diagnostic test for pneumococci but many strains fail in this respect and inulin is metabolised by other streptococci e.g.S.salivarius.

Optochin (ethylhydrocuprein) susceptibility

Optochin susceptibility is currently the most widely used test for the presumptive identification of S.pneumoniae. Morgenroth and collaborators (1911) demonstrated that optochin (a derivative of quinine) protected mice against infection by pneumococci and Moore (1915) suggested that optochin could be used to identify pneumococci. Pneumococci are killed by optochin at a concentration of 2-10 ug/ml whereas streptococci are affected by concentrations of 200 ug/ml and above (Morch, 1943).

The optochin susceptibility test was re-introduced, in the form of impregnated paper strips, by Bowers and Jeffries (1955) when typing serum, which was a by-product of therapeutic serum, became scarce. Unlike the bile solubility test, both capsulate and non-capsulate strains of S.pneumoniae are inhibited by optochin. Some strains of alpha-haemolytic streptococci, which are insoluble in bile, are partially inhibited by optochin (Lund, 1959). Optochin susceptibility has an accuracy of 90-95% for S.pneumoniae when the test is carefully standardised (Austrian, 1975a).

Antibiotic sensitivity and resistance

The pneumococcus was amongst the first bacterial species recognised as being capable of developing resistance to an antimicrobial agent (Austrian, 1975a). Morgenroth and Kaufmann (1912) reported that pneumococci developed resistance to optochin in vivo and Tugendreich and Russo (1913) described optochin

resistant mutants in a culture of pneumococcus. Moore and Chesney (1917) demonstrated a 20 fold increase in resistance of a strain of pneumococcus isolated from a patient treated with optochin. Recently strains of S.pneumoniae have been found which have developed resistance to a number of antibiotics used to treat pneumococcal infection.

Sulfapyridine was introduced in the 1930s and was used alone or in combination with pneumococcal serum therapy. Pneumococci resistant to sulfonamide were reported in 1943. Tetracycline resistance was first described by Evans and Hansman (1963) and thereafter was frequently reported by other workers. Since tetracycline resistant pneumococccci were isolated more frequently from male patients, over 50 years of age, with a history of recent hospitalisation and treatment with tetracycline, it was postulated that resistant strains developed in elderly males with chronic bronchitis who had received tetracycline therapy.

Penicillin was introduced in 1940 and became the antibiotic of choice for pneumococcal infections. S.pneumoniae strains were uniformly sensitive to penicillin (M I C <0.05 ug/ml) and this greatly simplified the treatment of pneumococcal infection. However with the appearance of penicillin resistant pneumococci, it has become necessary to test strains of S.pneumoniae for penicillin susceptibility and to ascertain susceptibility patterns for alternative

antimicrobial agents.

Although penicillin-resistant mutants had been induced *in vitro*, the first strain from clinical material was reported in Sydney, Australia (Hansman and Bullen, 1967). Thereafter, strains with increased resistance to penicillin (M I C 0.1 to 1.0 ug/ml) were reported from a number of centres throughout the world. In almost all cases, the strains were isolated from patients who had received prolonged courses of penicillin. These strains could be effectively treated by increasing the penicillin dosage or by using other related antibiotics e.g. ampicillin, to which the organism remained sensitive. These strains were termed intermediate penicillin resistant (IPR).

In contrast to the IPR strains, multiple antibiotic resistant pneumococci were isolated in South Africa (Jacobs *et al.*, 1978). The multiple resistant strains were moderately resistant to penicillin (M I C 4 to 8 ug/ml) and had similar levels of resistance to other beta-lactam antibiotics. Furthermore, these strains were invariably resistant to other antibiotics e.g. chloramphenicol, tetracycline. Zighelboim and Tomasz (1981) investigated the biological mechanisms which determined resistance in the South African strains and found that these differed from sensitive strains by having different patterns of penicillin binding proteins.

The PHLS (CDR 86/07) has reported an increase in the number of penicillin resistant strains in England.

Whereas in 1982-84 only one or two strains were reported, the number reported in 1985 increased to 9 and in the first six weeks of 1986, 6 strains were reported. These strains have all been IPR and are serotype 23F. The IPR strains give a reduced zone of inhibition with penicillin discs (1.2 units) and can easily be mistaken as fully sensitive (Rees and Waterworth, 1980). The National Committee for Clinical Laboratory Standards (NCCLS) has recommended that pneumococci be screened for penicillin resistance with an oxacillin(1 ug) disc.

The occurrence of penicillin resistant pneumococci highlights the need for the re-introduction of serotyping of S.pneumoniae strains. Pneumococcal vaccines may in selected groups prevent the development of systemic pneumococcal infection by penicillin resistant strains. Currently 15 of the 22 different serotypes, in which penicillin resistance has been demonstrated, are represented in the 23 valent pneumococcal vaccine formulation and a further 4 serotypes are antigenically related to serotypes included in the vaccine. Serotyping is also necessary as a control of measures designed to limit the spread of penicillin resistant strains in the community.

1.2.5.Pathogenic mechanisms

The way in which the pneumococcus damages the host it invades is not known. Since death from pneumococcal

pneumonia can occur days after antibiotic therapy has eliminated viable S.pneumoniae from the tissues or blood, it is presumed that death results from irreversible injury resulting from the infection. Although the precise nature of the injury is not known, S.pneumoniae produces a number of toxins which may be involved in pathogenesis. Furthermore, the pneumococcal capsule and cell wall have been shown to contribute to the pathology of pneumococcal infection.

Pneumococcal toxins

Pneumococci produce a haemolytic toxin (Pneumolysin) which is a sulphydrl-activated cytolytic toxin which binds to cholesterol in the host cell membrane. It is immunologically related to the oxygen labile "O" haemolysin of haemolytic streptococci, C.tetani and C.perfringens and has similar properties e.g. depression of chemotaxis and killing of polymorphonuclear leucocytes (Johnson, Boese-Marrazzo and Pierce, 1981). However pneumolysin is cell-associated (found in both capsulate and non-capsulate strains) whereas the others are exotoxins. Pneumolysin is inhibited by normal serum whereas the others are not. Paton and collaborators (1983) have shown that pneumolysin can affect the immune response by :

1. Preventing the establishment of an adequate antibody response by binding to lymphocytes.
2. Activating complement by the classical pathway and therefore reducing the opsonic activity of serum.

They also showed that mice immunised with inactivated pneumolysin were protected when challenged with virulent pneumococci and concluded that the incorporation of inactivated pneumolysin into the pneumococcal polysaccharide vaccine may improve its efficiency in preventing invasive pneumococcal disease. Antibody to pneumolysin has been found in the serum of a proportion of patients recovering from pneumococcal pneumonia (Kalin et al., 1987).

Neuraminidase is produced by capsulate strains of S.pneumoniae, but does not correlate with virulence (Kelly, Grieff and Farmer, 1966) and a purpura-producing principle, which causes dermal and internal haemorrhages when injected into rabbits has been identified.

There is no conclusive evidence that pneumolysin, neuraminidase or purpura-producing principle play a role in the pathology of pneumococcal infection (Johnston, 1981).

The role of the pneumococcal capsule and cell wall in pneumococcal infection

Pasteur (1881) in his description of the pneumococcus made note of what is now known as the pneumococcus capsule. Frankel (1885) found that rabbits, which had recovered from pneumococcal infection, were immune to challenge by viable pneumococci and this was confirmed by the Klemperers (1891) who used immune serum in patients. Since then the pneumococcus capsule has been

the subject of detailed examination.

Studies by Griffith (1928) demonstrated the importance of the pneumococcus capsule in infection. He showed that when avirulent non-capsulate pneumococci were mixed with heat-killed capsulate pneumococci and injected subcutaneously into mice, the mice succumbed to pneumococcal infection yielding capsulate pneumococci of the capsular type of the heat-killed suspension. This phenomenon, which has become known as transformation, was shown by others to occur *in vitro* and the report by Avery, MacLeod and McCarty (1944) which established deoxyribonucleic acid as the active principle of transformation provided a foundation for the science of molecular genetics.

The pneumococcus capsule is antiphagocytic and permits the establishment and progression of infection in the non-immune host. Non-capsulate strains of pneumococci are susceptible to phagocytosis and are rarely isolated from systemic disease. Antibody to the capsular material, which alters the surface properties of the cell, promotes phagocytosis and recovery from infection. The different pneumococcal serotypes are not equally invasive and both the chemical composition and quantity of capsular material produced appear to contribute to virulence (Austrian, 1981).

Dochez and Avery (1917) reported the presence of Specific Soluble Substance (SSS) in the blood and urine of patients with lobar pneumonia and showed that it was

produced by pneumococci during growth. The SSS, which later became known as capsular polysaccharide (CPS) was present in the urine of a large percentage of patients at some stage of the infection, but only occurred in the serum of patients with severe disease. Subsequent studies (Blake,1918; Bullowa, Bukantz and de Gara, 1941; Bukantz, de Gara and Bullowa, 1942) demonstrated that capsular polysaccharide was more often present in the serum of patients with bacteraemic pneumococcal pneumonia and was associated with mortality. Because circulating capsular polysaccharide interfered with the host defences (Felton and Bailey, 1926; Sickles, 1927), Bullowa and collaborators (1941) proposed that anti-pneumococcal serum should be given to patients, with capsular polysaccharide in their circulation, to neutralise its effect.

Since a toxin has not been implicated in pneumococcal disease and as a result of observations which suggest a relationship between the presence of capsular polysaccharide in serum and mortality, Johnston (1981) has suggested that the toxaemia and tissue damage which results from pneumococcal infection is mediated by host defence mechanisms directed against the capsular polysaccharide material or pneumococcal cell wall.

Recent studies in rabbits (Tuomanen et al., 1985) have shown that the pneumococcus cell wall is the most potent activator of meningeal inflammation. They have demonstrated that any of the structural components of

the cell wall - peptidoglycan and teichoic acid - could induce inflammation but capsular polysaccharide had no effect, at concentrations likely to be produced as a result of infection. They also found that killed encapsulate pneumococci had no effect and argued that the capsular polysaccharide had masked the effect of cell wall material and suggested that during active infection capsulate pneumococci expose or release underlying cell wall components which cause the inflammatory response.

1.3.LABORATORY INVESTIGATION OF PNEUMOCOCCI

1.3.1. Direct smears

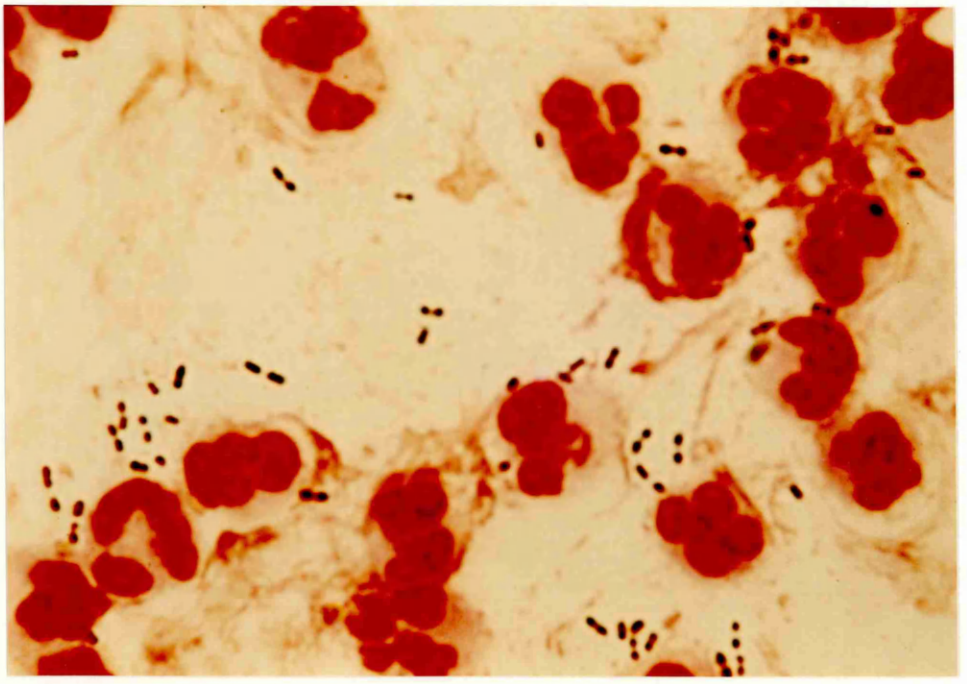
Pneumococci were probably first seen by Klebs in 1875, in the bronchial contents of patients dying from pneumonia. Dr.Christian Gram, who had devised a technique for staining bacteria in tissue sections, reported that he had seen bacteria which retained gentian violet in 19 of 20 lung sections from fatal cases of lobar pneumonia, prepared by Friedlander. However, in the remaining section he found that the bacteria were decolourised and noted that it was from this case that Friedlander had obtained most of the cultures of the organism which he had identified as the causative agent of pneumonia (Austrian, 1975a). Neither Gram nor Friedlander appreciated the significance of Gram's observation and the importance of Gram's stain was not recognised until Weichselbaum (1886) described his findings. He demonstrated pneumococci in 94 of 129 cases of fatal pneumonia and isolated pneumococci on 54 occasions.

"The methods of investigation of bacteria", which was published in 1885, may have been the first text in which Gram's stain was suggested for the identification of bacteria (Austrian, 1985).

Figure II shows the Gram's stain of cerebrospinal fluid obtained from a patient with pneumococcal

Figure II:

Gram's stained smear of cerebrospinal fluid showing Gram positive lanceolate diplococci



Courtesy of the slide collection, Bacteriology Department, Royal Infirmary, Glasgow.

meningitis. However, pneumococcal morphology present in respiratory secretions or body fluids from patients may not be typical (i.e. Gram-positive lanceolate diplococci) and therefore Gram's stain alone cannot be used to distinguish pneumococci from other species of streptococci (Austrian, 1974).

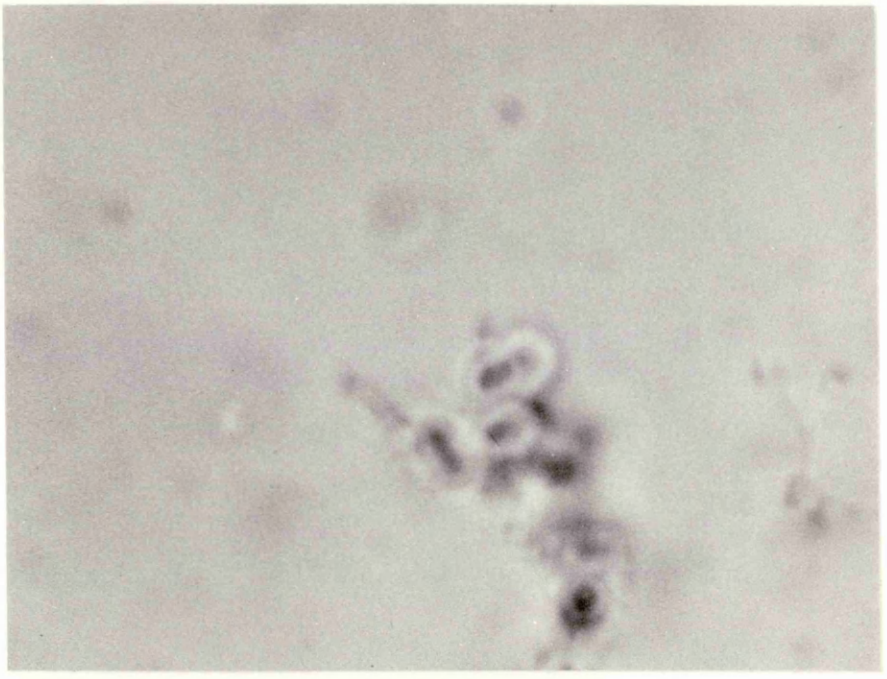
Capsular reaction test (Quellung reaction)

Although other bacteria give the capsular reaction with appropriate antisera e.g. Klebsiella , E.coli etc., the test was originally described for pneumococci. Before the introduction of antibiotics to treat pneumococcal infections, pneumococci were routinely serotyped by the capsular reaction test performed on clinical material. This enabled physicians to treat patients promptly with the appropriate pneumococcal antiserum (Austrian, 1975a). Recent reports have re-established the value of the capsular reaction test for the rapid identification of pneumococci in blood cultures (Morello and York, 1982). The capsular reaction test can be performed when sufficient pneumococci are seen in a Gram-stained smear (Fig.III). Consequently the value of the capsular reaction test is limited by the sensitivity of microscopic examination. Although the sensitivity of a Gram's stain has not been established for pneumococci, studies on positive blood cultures showed that pneumococci were detectable when the bacterial concentration was more than 10^4 cfu/ml (McCarthy and Senne, 1980). Immunofluorescence

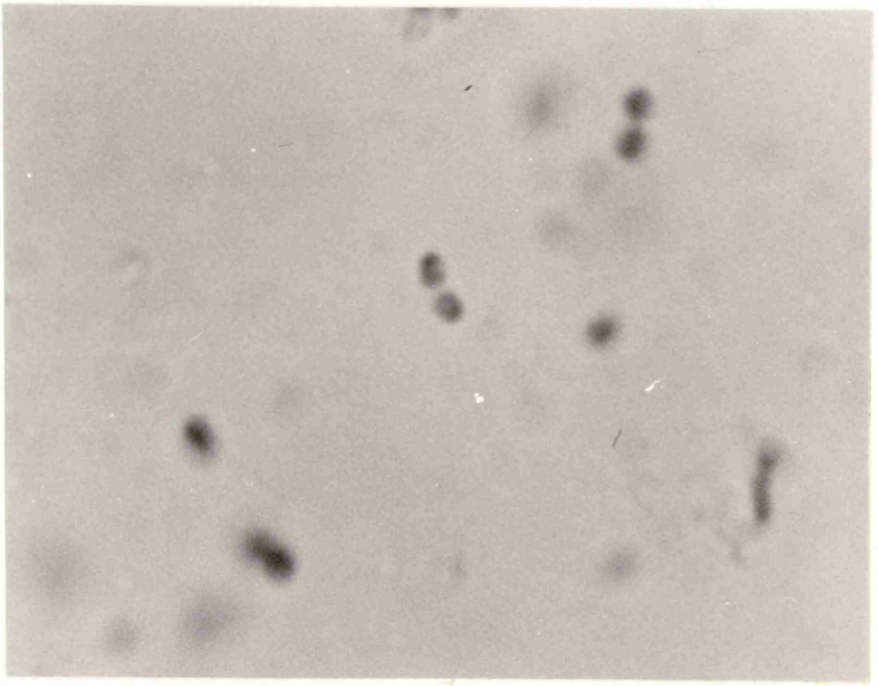
Figure III:

Capsular reaction test (CRT) carried out on Streptococcus pneumoniae in blood culture broth

A. Tested with homologous pneumococcal antiserum- CRT positive



B. Tested with heterologous pneumococcal antiserum - CRT negative



increases the sensitivity of a direct smear for the diagnosis of bacterial meningitis (Olcen, 1978).

1.3.2. Culture

S.pneumoniae grows on unenriched nutrient media prepared from fresh beef infusion broth. The initiation of growth of small numbers of bacteria is inhibited in infusion broth, exposed to air, and therefore, a reducing agent such as cysteine is incorporated to prevent this. Serum at a concentration of 10% (v/v) improves growth by acting as a buffer and serum can be replaced with sodium carbonate (Holt, 1962). Although pneumococci grow less well on media prepared from dehydrated ingredients, tryptone soya agar with 5% horse blood prepared in this way is satisfactory for routine laboratory work (Austrian, 1974).

Identification

Optochin susceptibility and, to a lesser extent, bile solubility are the tests most often performed in routine laboratories for the presumptive identification of S.pneumoniae. Most strains of S.pneumoniae are susceptible to optochin whereas most alpha-haemolytic streptococci are not. Tests for optochin susceptibility require careful standardisation since the size of the inhibition zone around an optochin disc is smaller when the pneumococcus is grown in an atmosphere containing carbon dioxide (Ragsdale and Sanford, 1971). Furthermore, optochin susceptibility does not distinguish

between capsulate and non-capsulate strains. Bile solubility characterises most strains of capsulate pneumococci and the bile solubility test can be performed on primary culture plates which have been incubated anaerobically - when incubation is carried out aerobically, colony size is reduced and interpretation is more difficult (Howden, 1979). Since occasional strains of alpha-haemolytic streptococci and non-haemolytic streptococci may also be lysed, the bile solubility test cannot be regarded as definitive.

Serological identification

Serological tests are the simplest, most rapid and most accurate way to identify pneumococcal cultures. Omni serum (Lund and Rasmussen, 1966) available from the Statens Seruminstitut, Copenhagen, reacts with all 83 serotypes of S.pneumoniae in the capsular reaction test. Recently Omni serum has been used as the source of pneumococcal antibody in other serological tests such as counterimmunoelectrophoresis (Sottile and Rytel, 1975), co-agglutination (Burdash and West, 1982) immuno-fluorescence (Wicher et al.,1982) and latex agglutination (Smith and Washington, 1984). Sottile and Rytel (1975) and Burdash and West (1982) found that an occasional strain of S.pneumoniae was optochin-resistant and a strain of S.sanguis was optochin-sensitive. However, of greater importance was the finding, that in all of these studies, some strains of alpha-haemolytic streptococci reacted in tests with

pneumococcal antisera. Holmberg et al. (1985) have shown that 68% of strains of alpha-haemolytic streptococci they examined reacted with Omni serum when used in latex agglutination, immunofluorescence and counter-immunoelecrophoresis, but they did not carry out the capsular reaction test. It is well known that some strains of respiratory streptococci react with pneumococcal antisera in the capsular reaction test and these have been studied (Lund, 1950b ; Yurchak and Austrian, 1966; Austrian, Buettger and Dole, 1972). However the capsular reaction of these cross-reacting strains is less pronounced than the capsular reaction of capsulate strains of S.pneumoniae.

1.3.3.Serotyping methods

Cultures of pneumococci

The first attempt at pneumococcus typing was by Ungermann (1910) who used a mouse protection test. In this test, immune serum, obtained from a patient recovering from pneumococcal infection, was injected into the peritoneum of a mouse. After a few hours, infected sputum was injected and the peritoneal exudate examined after 3 hours. Phagocytosis of the injected pneumococci proved that the pneumococcus and the immune serum were homologous. Early workers involved in the classification of pneumococcal types mostly used agglutination tests. As a result of the work by Neufeld and Etinger-

Tulczynska (1931) the capsular reaction test became the recognised method for typing pneumococci. Although it remains the method of choice, other methods for typing pneumococci have been reported which reduce the amount of serum, and in some methods, the time required for typing compared with the capsular reaction test. Micro-agglutination (Kirkman, Fisher and Pagano, 1970), co-agglutination (Kronvall, 1973), counterimmunoelectro-phoresis (Sottile and Rytel, 1975) and capillary precipitation (Russell et al., 1978.) have been used. A recent study by Henrichsen, Berntsson and Kaijser (1980) has shown that serotypes 7F,7A,14,33F,33A and 37 cannot be detected by counterimmunoelectrophoresis when performed in conventional buffers and that serotypes within serogroups cannot be distinguished, since many of the factor sera used for typing do not contain sufficient amounts of precipitating antibody. Recently Smart (1986) has shown that the results of serotyping S.pneumoniae strains by co-agglutination agree with those obtained by the capsular reaction test.

Pneumococci in clinical material

Early workers identified pneumococci serologically by performing agglutination or precipitation tests on the peritoneal exudate resulting from injecting mice with infected material (Avery, 1915 ; Blake, 1917). Avery (1918) showed that a 4 to 5 hour broth culture of the infected material was suitable for agglutination tests, and, after treatment with ox bile, could be used in

precipitation tests. Armstrong (1931) reported that the capsular reaction test could be performed on peritoneal exudates and the following year Armstrong (1932) and Logan and Smeall (1932) found that the capsular reaction test could be used directly on sputum to type pneumococci. Recently Merrill et al. (1973) have shown that the capsular reaction test is more accurate than Gram's stain for the identification of pneumococci in sputum and Perlino (1984) has shown its value in the diagnosis of pneumococcal pneumonia.

1.3.4. Detection of pneumococcal antigen in clinical material

Dochez and Avery (1917), used a precipitation technique to demonstrate that pneumococcal antigen was present in the serum and urine of patients with pneumonia and Blake (1918) found that antigenaemia in bacteraemic pneumococcal pneumonia was more often associated with fatality than when bacteraemia occurred without antigenaemia.

Mitchell and Muns (1917) typed the infecting pneumococcus by employing a direct precipitation test on sputum and this technique was developed by Krumwiede and collaborators (1918). They carried out antigen precipitation tests after the sputum was treated with antiformin and later showed that pneumococcal antigen could be detected in a saline homogenate of sputum which had been coagulated by heat.

Amoss (1930) typed pneumococcal antigen in serum and Bukantz et al. (1942) demonstrated that the incidence of pneumococcal antigenaemia varied with the type of the infecting pneumococcus.

During the 1940s, penicillin replaced serum therapy for the treatment of pneumococcal pneumonia and significantly reduced the mortality rate. Consequently typing of pneumococcal strains and therefore the capacity to detect pneumococcal antigen in clinical material, became less important.

Current interest in the detection of pneumococcal antigen in clinical material stems from the work by Dorff, Coonrod and Rytel (1971). They used counter-immunoelectrophoresis (CIE), and pneumococcal Omni serum, to detect antigen in the serum of patients with pneumococcal pneumonia and Coonrod and Rytel (1972) used CIE to determine the aetiology of bacterial meningitis. Kenny et al.(1972) increased the sensitivity of CIE by using specific pneumococcal antisera, instead of Omni serum, and demonstrated that 60% of patients with bacteraemic pneumococcal pneumonia had pneumococcal antigen in their serum (antigenaemia). Coonrod and Rytel (1973) showed that antigenaemia without bacteraemia was rare but they found pneumococcal antigen in concentrated urine from 37% of patients with pneumococcal pneumonia who did not have bacteraemia or antigenaemia. Fossieck, Craig and Paterson (1973) used CIE and Omni serum to detect pneumococcal antigen in cerebrospinal fluid but

were unable to detect pneumococcal antigen in the CSF from one patient from which S.pneumoniae was isolated.

Latex particles sensitised with Omni serum were used to detect bacterial antigen in CSF (Whittle et al., 1974) and in serum (Coonrod and Rylko-Bauer, 1976). These workers found that the latex agglutination test was more convenient, detected types 7 and 14, which were not detected by CIE(Kenny et al.,1972) and was more sensitive than CIE in tests with purified pneumococcal capsular polysaccharide. However they found that the latex agglutination test was less sensitive than CIE when used to detect pneumococcal antigen in CSF and serum.

Tugwell and Greenwood (1975) established the value of CIE for the diagnosis of pneumococcal pneumonia. They were able to demonstrate pneumococcal antigen in serum, urine, pleural fluid and sputum and observed that patients who developed complications, such as jaundice and pleural effusion, as a result of pneumococcal pneumonia often had pneumococcal antigen in their serum. This association was confirmed by other workers (Rytel et al., 1974; Coonrod and Leach, 1976; Coonrod and Drennan, 1976) and they suggested that the complications which developed as a result of pneumococcal antigenaemia contributed to the high mortality rates found in these patients.

Dirks-Go and Zanen (1978) and Olcen (1978) found that co-agglutination was more sensitive than CIE for the

diagnosis of pneumococcal meningitis. Similarly, Edwards and Coonrod (1980) found co-agglutination superior to CIE when they examined the sputum of patients who had received antibiotic therapy. Mayer, Jan Geiseler and Harris (1983) used co-agglutination to serotype pneumococcal antigen in sputum, and detected antigen in serum and urine.

Kaldor, Asznowicz and Buist (1977) improved the efficiency of latex preparations for the detection of pneumococcal antigen and studies which used latex particles sensitised with pneumococcal antisera, other than Omni serum, have shown that latex agglutination and co-agglutination are more sensitive than CIE (Dirks-Go and Zanen, 1978; Leinonen, 1980). Cerosaletti, Roghmann and Bentley, (1985) found that a commercial latex suspension prepared from Omni serum detected pneumococcal antigen in the serum and urine or both of 46% of elderly patients with pneumococcal pneumonia compared with 15% detected by CIE.

CIE (Thompson and Wise, 1982) and latex agglutination (Thompson and Wise, 1983; Browne, Miegel and Stottmeier, 1984) were effective for the rapid identification of pneumococci in blood cultures.

However recent studies have confirmed that cerebrospinal fluid infected with S.pneumoniae may not contain sufficient antigen to be detected by co-agglutination, latex agglutination or CIE (Tompkins, 1983; Ingram, Pearson and Occhiuti, 1983; Tilton, Dias

and Ryan, 1984).

Enzyme-linked immunosorbent assay (ELISA) has been shown to be 25 times more sensitive than CIE by Harding et al. (1979) and they demonstrated pneumococcal antigen in all CSF specimens obtained from experimentally infected animals. However, ELISA tests for pneumococcal antigen performed in a clinical laboratory were not successful (Sippel et al.,1984).

The sensitivity of antigen detection techniques varies with the quality of antiserum used (Leinonen, 1980). Although Omni serum is the most widely used source of pneumococcal antibody for the detection of pneumococcal antigen, its value has been questioned (Tugwell and Greenwood, 1975; Kenny et al., 1972; Miller et al.,1978, Harding et al.,1979; Leinonen,1980).

Holmberg et al. (1985) have developed an ELISA for the detection of C-polysaccharide antigen in sputum rather than type-specific antigen. They found that 82% of sputa from which S.pneumoniae was isolated were positive by ELISA.

1.3.5 Examination of human serum for pneumococcal antibody

Pneumococcal antibodies were first demonstrated by G and F Klemperer (1891). They showed that the serum from patients who had recovered from pneumococcal disease protected rabbits against infection with the pneumococcus. Neufeld and Handel (1910) showed that mice

were similarly protected and demonstrated that pneumococcal antibodies were type-specific. In the 35 years that followed, and before the introduction of penicillin for the treatment of pneumococcal disease, many workers used the mouse protection test as a guide to the *in vivo* activity of pneumococcal antibodies, but attempted to determine antibody activity *in vitro* by agglutination (Finland and Sutliff, 1932), precipitation (Heidelberger, Sia and Kendall, 1930) and opsonic index (Robertson *et al.*, 1930). Chickering (1914) found pneumococcal agglutinins in the blood of most patients with lobar pneumonia - at some stage of the disease. Blake (1918) studied the development of pneumococcal agglutinins and precipitins in the blood of patients with lobar pneumonia. Subsequent studies (Francis, 1932; Heidelberger and Kabat, 1936) showed that the agglutinating and precipitating antibody in horse anti-pneumococcus serum was the same antibody and that this reacted with the pneumococcal capsular polysaccharide. Heidelberger and co-workers improved the method for performing the precipitation test which allowed a quantitative estimation of antibody (Heidelberger and Kendall, 1929) and increased the sensitivity (Heidelberger and Kendal, 1935) and specificity (Heidelberger and Anderson, 1944) of the reaction. They increased the specificity of the reaction by removing anti-C polysaccharide antibody, present in almost all human sera, by absorption with pneumococcal C-

polysaccharide before testing for type-specific precipitins.

Morch (1943) found that the pneumococcal capsular reaction and agglutination titre of serum from patients with pneumococcal disease was the same. Moreover, she used the capsular reaction to examine patients' sera against bacterial suspensions of all pneumococcal types, in cases where bacteriological diagnosis had not been made.

Most strains of pneumococci produce, in broth culture, a haemolysin which is antigenic and species-specific (Morch, 1946). However, anti-pneumolysin titres were not found to differ significantly in patients with pneumococcal pneumonia when compared to patients without pneumococcal pneumonia (Cole, 1914).

An indirect haemagglutination technique (Baker, Stashak and Prescott, 1969) was used by Ammann and Pelger (1972) to determine antibody responses in patients who had been immunised with pneumococcal vaccine. Sloyer et al. (1974) found an excellent correlation between the indirect haemagglutination test and the indirect immunofluorescence test for pneumococcal antibody in the serum of children with acute otitis media.

Schiffman and Austrian (1971) developed a radio-immunoassay (RIA) based on the Farr technique and found that RIA was 1000 times more sensitive than the quantitative preciptiation test for pneumococcal

antibody. Furthermore, Schiffman et al. (1980) found a good correlation between RIA and indirect haemagglutination. Consequently RIA has been used extensively for the determination of pneumococcal antibodies in patients immmunised with pneumococcal vaccines (Kass, 1981). However, RIA requires reagents and equipment which are generally not available in diagnostic laboratories and enzyme-linked immunosorbent assay (ELISA) has been suggested as an acceptable alternative (Berntsson, Broholm and Kaijser, 1978). Recent studies have shown that antibody assays by ELISA correlate well with the results obtained by RIA (Koskela and Leinonen, 1981; Pedersen, Henrichsen and Schiffman, 1983).

Antibody responses after infection or vaccination render the host immune to subsequent infection by a homologous type providing that sufficient antibody is available to promote phagocytosis by polymorphonuclear neutrophil leucocytes. Phagocytosis requires opsonisation by serum antibody and complement. Consequently an antibody assay which measures the opsonic activity of immune serum relates more closely to innate resistance of an individual to systemic pneumococcal infection (Giebink et al., 1980). A few studies have compared antibody responses with enhancement of type-specific opsonisation after vaccination and found that the tests were comparable for

some types but not for others (Giebink et al.,1980; Bortolussi et al., 1981; Simberkoff et al., 1980; Johansen and Pedersen, 1982).

Pneumococci possess, in addition to type-specific capsular polysaccharide, a C-polysaccharide antigen which is species-specific. Antibody to pneumococcal C-polysaccharide is known to exist in most human sera (Heidelberger and Anderson, 1944). Recently anti-C-polysaccharide antibody in serum has been determined by ELISA (Pedersen et al., 1982; Gray, Dillon and Briles, 1983; Holmberg, Krook and Sjogren,1985).

1.3.6. Examination of sputum

The ability of an individual to produce a sputum implies some abnormal process of the bronchopulmonary system and the cellular and non-cellular components are a reflection of the pathological process (Chodosh, 1970). Unless samples are taken directly from the bronchi or lungs patients with a lower respiratory tract infection are investigated by the examination of expectorated sputum - a heterogeneous material which consists of an exudate from the lower respiratory tract "contaminated" to a variable extent by upper respiratory and oral secretions. It has been suggested that the results of sputum examination are of little value in the management of a patient with chronic bronchitis (May, 1952 ; Lees and McNaught, 1959a) and that sputum cultures are of no value in the diagnosis of

pneumococcal pneumonia (Barrett-Connor,1971).

Macroscopic examination

The appearance of expectorated sputum varies with the pathological process. Patients with pulmonary disease e.g. tuberculosis, carcinoma or pulmonary embolus, produce blood stained sputum and patients with pulmonary oedema produce copious frothy sputum which is white or pink. Rusty sputum which is viscous and sticks to the specimen container is characteristic of lobar pneumonia, whereas a patient with chronic bronchitis will produce a mucoid sputum. Purulent sputum which is thick and yellow but is not viscous characterises bacterial infection. However, macroscopic purulence is due not only to intact pus cells but also to deoxyribonucleo-protein fibres which are left when they lyse (Elmes and White, 1953). Macroscopic assessment is misleading as an index of short term changes in bronchial inflammation. Furthermore Miller (1963) found that 2 observers obtained only 88% agreement using a scale 1 to 5 for purulence, and suggested that a microscopic examination for pus would be more accurate.

Microscopic examination

Chodosh (1970) examined the cellular components of sputum microscopically and identified cells according to four broad categories.

1. Exfoliated cells from the bronchial epithelium
2. Cells arising from pulmonary tissue
3. Cells originating from the blood stream

4. Exfoliated cells from the mouth and nasopharynx

The numbers, type and cytological condition of the bronchial epithelial cells reflect the extent of abnormality of the bronchial mucosa and they are differentiated into basal, intermediate, ciliate and goblet cells. Bronchial epithelial cells which are metaplastic may be indistinguishable from squamous epithelial cells from the buccal mucosa (Irwin et al., 1980). Furthermore the majority of bronchial epithelial cells cannot be distinguished from cells from the nasopharyx. Alveolar macrophages (dust cells) in sputum are the best assurance that the material has arisen from the lower respiratory tract. However during infection macrophages are replaced by pus cells which in turn are replaced by macrophages when the infection resolves.

Numbers of squamous epithelial cells in sputum are used to indicate the level of salivary contamination which has occurred during expectoration and heavily contaminated specimens are not suitable for bacteriological examination (Bartlett, 1974; Murray and Washington, 1975; Heineman, Chawla and Lofton, 1977).

Gram-stained smear

Gram-stained smears of sputum can provide an early indication of bacterial infection, and pneumococci can be distinguished from other streptococci by the capsular reaction test (Merrill et al., 1973). However, the accuracy of a Gram's stain for identifying

pneumococci in sputum can be improved by adopting criteria for a positive smear (Rein et al., 1978). These worker considered the Gram stain positive for pneumococci when pneumococci predominate or more than 10 Gram-positive lanceolate diplococci were seen per oil immersion field (X1000).

Culture of pneumococci

S.pneumoniae is irregularly distributed throughout the expectorated sputum of patients with chronic bronchitis and the distribution is not necessarily associated with purulent areas (May, 1953). May recommended that 5 areas should be sampled to achieve optimum recovery of the organism. Rawlins (1953) recognised that multiple sampling was unsuitable for routine laboratories and demonstrated that a single culture from a homogenised sputum gave equivalent results. These findings were not supported by other workers who suggested that homogenisation was unnecessary providing that the culture was taken from a purulent area or mucoid portion of the sputum (Lees and McNaught, 1959b) and that a sufficiently large area was sampled (Allibone, Allison and Zinnemann 1956). Massaro, Fedorko and Katz (1964) argued that homogenisation of the whole sample would distribute upper respiratory bacteria evenly throughout the specimen and since S.pneumoniae is a commensal of the nasopharynx in 30-70% of humans, depending on the season of the year (Austrian, 1974) it would seem likely that false positive cultures for S.pneumoniae

might follow as a result of homogenisation. Brumfitt, Willoughby and Bromley (1957) isolated S.pneumoniae from the sputum and throat swab of 14 of 42 patients whose companion bronchial swab was sterile and confirmed that sputum is often contaminated from the nasopharynx by organisms falsely regarded as bronchial pathogens.

Methods for reducing the amount of oropharyngeal contamination have been suggested and include culture of bronchial secretions aspirated at bronchoscopy (Brown et al., 1954), bronchial swabs (Brumfitt et al., 1957; Lees and McNaught, 1959b; Laurenzi, Potter and Kass, 1961), transtracheal aspiration (Pecora and Yegian, 1958 ; Reis, Levison and Kaye, 1974) and bronchoscopic protected alveolar brush (Wimberley, Faling and Bartlett, 1979).

An alternative approach is to modify the way the sputum is processed to take account of likely oropharyngeal contamination. Mulder (1956) suggested that washing sputum with three successive changes of saline produced sputum culture results which were as reliable as cultures from bronchoscopy specimens, however Laurenzi et al. (1961) found that 5 to 9 serial washings were necessary to eliminate a pharyngeal marker which was introduced in to the nasopharynx of patients with chronic bronchitis or bronchopneumonia. Bartlett and Finegold (1978) developed a washing technique which used a continuous stream of water but suggested that the method was unrealistic for use in clinical laboratories.

Quantitative culture was suggested by Dixon and Miller (1965) and is based on the hypothesis that an organism causing inflammation of the bronchi or lungs will be present in sputum in greater numbers than will organisms which have superficially contaminated the sputum during passage through the pharynx. It is generally accepted that more than 10^7 cfu/ml of a potential respiratory pathogen are significant (Monroe et al., 1969; Wilson and Martin, 1972).

Pneumococcal antigen in sputum

Spencer and Savage (1976) examined the sputum from patients with acute respiratory tract infections. They found that pneumococcal antigen was always present in sputum when S.pneumoniae was isolated by culture. Conversely, pneumococcal antigen was not detected in upper respiratory secretions of 22 pneumococcal carriers with colds (Miller et al., 1978). Congeni and Nankervis (1978) found antigen determination on nasopharyngeal secretions distinguished patients with pneumonia from those who were carriers. Numerous reports have confirmed that the detection of pneumococcal antigen in sputum was a more reliable index of pneumococcal infection than was culture of S.pneumoniae. However they also found that pneumococcal antigen was present in the sputum of patients without pneumonia (Spencer and Savage, 1976; Miller et al., 1978; Downes and Ellner, 1979). Consequently it was realised that pneumococcal antigen in sputum was not specific for pneumonia. However, El-

Refaie and Dulake (1975) observed that antibiotic therapy cleared pneumococcal antigen from the sputum of patients with chronic bronchitis more effectively than from the sputum of patients with pneumonia. They reasoned that infected lung fluid was the source of the persisting antigen. Moreover they were able to demonstrate pneumococcal antigen in lung tissue, taken at post-mortem, which did not yield pneumococci on culture despite histological evidence of pneumonia (El-Refaie et al., 1976).

1.4.PNEUMOCOCCAL INFECTION

1.4.1 Habitat

Pneumococci are common in the upper respiratory tract of humans and between 40 and 70 per cent of adults carry one or more serological types of pneumococci in their nasopharynx (Austrian, 1974). In children, pneumococci are more often isolated from cultures from the nasal passages than from throat swabs, while adults generally carry pneumococci in their throat (Webster and Hughes, 1931). Rates of pneumococcal carriage are highest in pre-school children, and adult carriers are more often found in households with pre-school children (Hendley et al., 1975; Klein, 1981). Carriage of pneumococci in both adults and children is lower during the summer months and the seasonal incidence of pneumococcal carriage parallels that of other respiratory tract infections (Webster and Hughes, 1931). Since pneumococci form part of the upper respiratory tract flora of a significant proportion of healthy people, the presence of pneumococci in the respiratory secretions of ill patients cannot per se be used to indicate an association between pneumococci and lower respiratory tract infection.

1.4.2. Upper respiratory tract infection

Pneumococci from the nasopharynx can invade the middle ear and sinuses and give rise to acute otitis media,

sinusitis and purulent conjunctivitis. Otitis media is the commonest pneumococcal infection in children and 71% of all children have at least one episode of acute otitis media before they reach the age of three (Klein, 1981). The pneumococcal types associated with this disease are the same as the types most commonly found in the upper respiratory tract of carriers in this age group (Gray, Converse and Dillon, 1979). Furthermore the type involved in the infection may have been carried for some time before, and continued to be carried after the episode of otitis media (Austrian, Howie and Ploussard, 1977).

Pneumococcal conjunctivitis is seen in adults and children but is rare in neonates (Brook, 1980; Brook et al., 1979). Pneumococci, isolated from eye swabs are often nontypable because they lack a type-specific capsular polysaccharide (Shayegani et al., 1982).

1.4.3. Lower respiratory tract infection

Infection of the lower respiratory tract usually develops as a result of viral infection of the upper respiratory tract. An increase in mucous secretions in the nose and pharynx enhance the risk of aspiration of pneumococci from the upper respiratory tract. In addition, anaesthetics, alcohol intoxication and body chilling slow the epiglottal reflex and consequently promote aspiration. Pulmonary oedema fluid is a suitable "culture medium" for aspirated pneumococci and

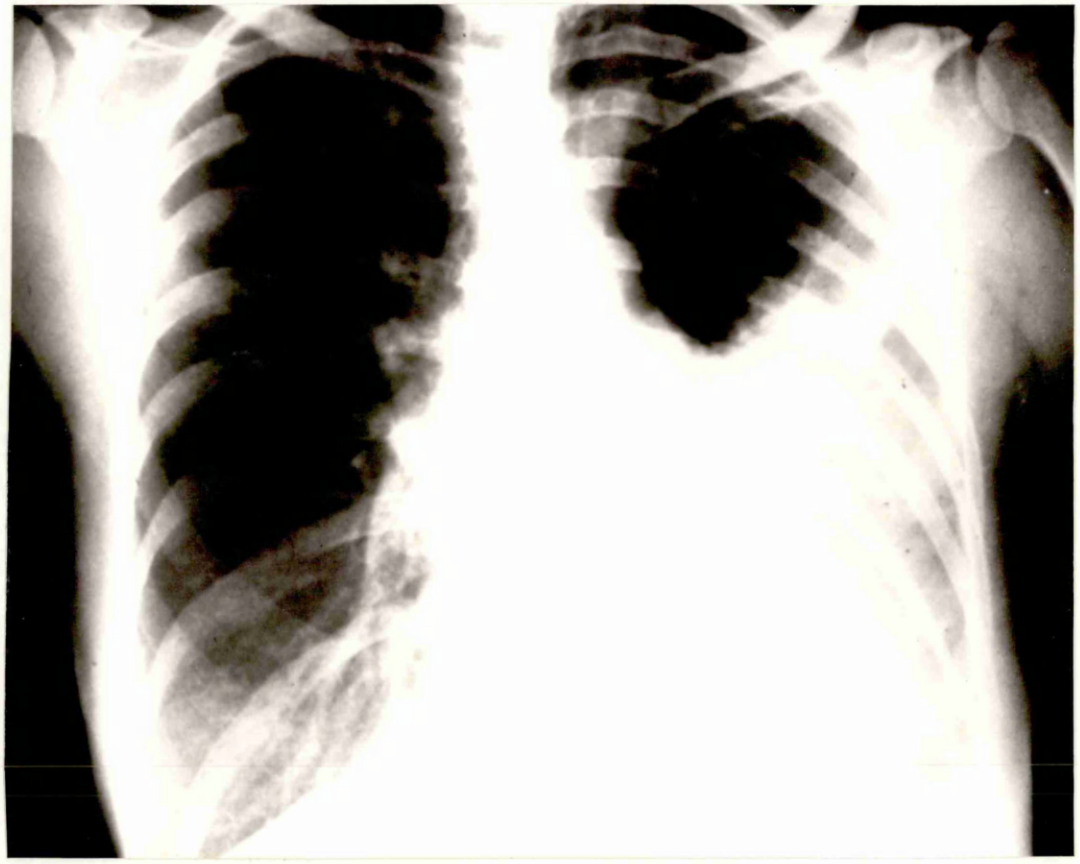
prevents phagocytosis of bacteria by pulmonary macrophages. Inhalation of irritant chemicals, trauma to the thorax, cardiac failure, viral infections of the lungs and pulmonary stasis all produce local and generalised pulmonary oedema. Consequently the pneumococcus can set up infection in a range of pathological processes which involve the lower respiratory tract. These processes can be classified according to the specific anatomical site initially involved.

Pneumonia

Pneumonia is defined as an acute inflammation of the alveolar spaces of the lung. Primary lobar pneumonia is due to pathogenic micro-organisms that reach the lower respiratory tract via the airways and initiate infection in a previously healthy individual. The pneumococcus, particularly the lower numbered types, account for 80-90% of primary lobar pneumonia. Studies of the evolution of the lung lesion in human pneumococcal pneumonia and in experimental infection in rats have shown that the infection process can be divided into three phases. The alveoli become filled with acellular serous fluid which serves as a culture medium for the organisms and allows the pneumococci to spread to adjacent areas of the lung. Polymorphonuclear neutrophil leucocytes and a few erythrocytes begin to accumulate and eventually fill the alveoli : this results in consolidation (Fig. IV). Once the polymorphs have completed phagocytosis of the

Figure IV:

X-ray of left lower lobe consolidation



Courtesy of the slide collection, Department of Bacteriology, Royal Infirmary, Glasgow.

pneumococci, alveolar macrophages, which remove cellular debris, replace the polymorphs and the lesion resolves. When the process involves alveoli under the pleura, pleurisy develops and the pleural cavity often becomes infected. If unchecked by the body cellular and humoral defences, empyema can result and the adjacent pericardium may also be affected.

Bronchopneumonia is a patchy consolidation of the lungs as opposed to the consolidation of a whole lobe characteristic of lobar pneumonia. The inflammation may affect the walls of the bronchi and bronchioles leading to inflammation of the supporting interstitium of the lung (i.e. interstitial pneumonia). Bronchopneumonia is usually secondary to bronchitis or chronic illnesses such as diabetes, anaemia or carcinoma. Post-operative and post-traumatic pneumonias are of this type. The distribution of pneumococcal types involved in bronchopneumonia resembles the type distribution found in healthy carriers, rather than the virulent types which are associated with lobar pneumonia.

Exacerbation of chronic obstructive airways disease (COAD)

COAD is a progressive disorder of the lower respiratory tract characterised anatomically by ever-increasing inflammatory destructive changes of the bronchial mucosa and dissolution of alveolar septae. Acute exacerbations of chronic bronchitis are characterised by fever, increased breathlessness and the

production of a purulent sputum. Infection may occur spontaneously or following a viral upper respiratory tract infection. Pneumococci and Haemophilus influenzae are the commonest bacteria isolated from the sputum in acute exacerbation of COAD. Calder and Schonell (1971) found that no particular type of pneumococcus predominated in patients with chronic bronchitis and that a large percentage of patients carried the same type for long periods despite antibiotic treatment.

1.4.4. Pneumococcal infection of other body sites

Meningitis

Unlike meningococcal meningitis, which is a sequel of colonisation of the upper respiratory tract and of bacteraemia, pneumococcal meningitis mainly arises in two different ways. In some patients it appears as a complication of pneumococcal pneumonia and bacteraemia, but in others, meningitis results from a progressive infection originating in the paranasal sinuses, middle ear or after fracture of the skull. In a 10 year study involving more than 600 cases, Davey et al. (1982) found that meningococci and H.influenzae were more common than pneumococci in meningitis in patients less than 25 years of age, but pneumococci predominated in older patients. They also found that pneumococcal meningitis had a high mortality rate (30%).

Peritonitis

Primary pneumococcal peritonitis, or peritonitis

following bacteraemia were amongst the most serious acute abdominal emergencies in children in the pre-antibiotic era. McCartney and Fraser (1921) reported that primary pneumococcal peritonitis was only found in female children, whereas peritonitis in male and some female children, was secondary to infection in another body site such as the lungs or pleura. They showed that infection of the peritoneal cavity in female children originates in the genital tract. Pneumococci have been found in acute appendicitis complicated by secondary peritonitis (Heltberg, Korner and Schouenborg, 1984). Bergqvist and Troyik (1985) have reported 3 cases of neonatal pneumococcal infection (i.e. two septicaemia and one wound infection) in which cervical colonisation was the likely source of the pneumococcus in 2 of the cases.

Peritonitis during pregnancy has been described (Nuckols and Hertig, 1938) and recently Gruer, Collingham and Edwards (1983) have described pneumococcal peritonitis associated with intra-uterine contraceptive device (IUCD).

Bacteraemia

Early workers realised the significance of bacteraemia in pneumococcal pneumonia. Cole (1902) found that 8 patients with bacteraemic pneumonia died compared with 4 of 21 patients without bacteraemia. Tilghman and Finland (1937) found that for each type of pneumococcus, the death rate in cases with bacteraemia was twice or more

times as high as in cases without bacteraemia.

Austrian (1981) has suggested that pneumococcal bacteraemia develops in one of three ways:

1)Pneumococci within the pulmonary parenchyma, or at the mediastinal lymph nodes can pass into the circulation via the thoracic duct and the left subclavian vein - bacteraemia as a sequel to lobar pneumonia may spread to the endocardium, pericardium, meninges, peritoneum or joint cavities.

2) Invasion of the subarachnoid space may be followed by passage of pneumococci through the arachnoid villi into the cerebral venous sinuses. Austrian (1964) has shown that bacteraemic meningitis was often fatal, whereas no fatalities were found in a group of patient with meningitis without bacteraemia. In addition he found that meningitis as a sequel to pneumococcal pneumonia had a high fatality rate.

3) Pneumococci can enter the circulation in the same way as other capsulated bacteria via the cervical lymphatics and thoracic duct as a sequel to colonisation, but in the absence of apparent upper respiratory tract infection. Bacteraemia without an obvious focus of infection is common in children and has also been described in adult patients. Furthermore it seems that pneumococcal infections of joints, in patients with no pulmonary infection, develop as a result of transient pneumococcal bacteraemia and that these patients lack antibody to the individual pneumococcus type responsible

for the infection (Austrian, 1981). In this respect these patients resemble the children in which this type of bacteraemia was first recognised.

1.4.5. Host defence mechanisms

Pneumococci, which are present in the healthy nasopharnyx, presumably cause disease at other body sites of the host as a result of a defect in the host's defence mechanism. The nature of the defect is one factor which determines the site and the severity of the infection.

Mechanical barriers

The respiratory mucociliary system is the main way in which the respiratory tract prevents invasion by pneumococci resident in the nasopharynx. Infections of the respiratory tract are probably the result of a physiological defect of the mucociliary system which allows access to pneumococci which have colonised the nasopharynx. The ability of some strains of pneumococci to adhere to epithelial cells (Andersson, Svanborg-Eden and Hanson, 1982) and produce extracellular IgA protease (Mulks, Kornfeld and Plaut, 1980) may also help these organisms to resist elimination.

Phagocytosis

Phagocytosis by macrophages and polymorphonuclear neutrophil (PMN) leucocytes however is the primary host defence mechanism against pneumococci.

Alveolar macrophages maintain sterility of the

alveolar spaces by phagocytosis of bacteria that escape the respiratory mucociliary system (Green and Kass, 1964). However, encapsulated pneumococci resist ingestion by alveolar macrophages and induce a rapid infiltration of PMN leucocytes into the alveoli (Vial, Toews and Pierce, 1984). In vitro studies have shown that fixation of C3b to pneumococci is required for effective phagocytosis by PMN leucocytes (Johnston et al., 1969). The importance of active PMN leucocytes in host defence is supported by studies carried out in the pre-antibiotic era which showed that pneumococcal bacteraemia was common in neutropenic patients (quoted by Howard, Strauss and Johnston , 1977). However, provided that pneumococci are efficiently opsonised they can be killed by defective neutrophils such as those from patients with chronic granulomatous disease which fail to kill catalase positive bacteria such as staphylococci and Gram-negative enteric bacteria (Mandell and Hook, 1969).

C-reactive protein

Tillett and Francis (1930) found that the serum obtained from patients during the acute phase of lobar pneumonia precipitated a dilute solution of pneumococcus C-polysaccharide and, when serial samples were tested from the patient, the precipitation reaction closely paralleled the clinical course of infection. They also found that the serum from patients with other infections such as rheumatic fever, infective

endocarditis and staphylococcal osteomyelitis, had the same property. Abernethy and Avery (1940) found that the reactive component was contained in the albumin fraction of serum and called it C-reactive protein (CRP).

It is now known that CRP is a beta-globulin which is synthesised by hepatocytes (Hurlimann, Thorbecke and Hochwald, 1966) and binds to a wide range of cellular products such as lipids, phospholipids, polycations and polyanions. These products probably originate from damaged tissue and are removed from the circulation by binding to CRP.

The complex formed by C-reactive protein with pneumococcal C-polysaccharide can fix the first component of the complement sequence (Kaplan and Volanakis, 1974) and initiate phagocytosis of the pneumococci. This may then cause activation of the classical complement pathway, in the pre-antibody phase of pneumococcal infection (Johnston, 1981).

Complement

The initial studies on the role of complement as an opsonin in pneumococcal infection were carried out by Ward and Enders (1933). Although Gram-positive bacteria are not susceptible to the lytic action of complement, pneumococci can activate complement directly and indirectly to produce opsonically-active C3b. C3b is necessary for the attachment of pneumococci to PMN leucocytes and non-activated macrophages, whereas

antibody (IgG) is responsible for ingestion (Mantovani, 1975). Recent studies have shown that C5 and C5a play an important role in producing pulmonary inflammation in mice (Toews and Vial, 1984) and cause accumulation of PMN leucocytes in the cerebrospinal fluid of rabbits infected with pneumococci. (Ernst et al.,1984).

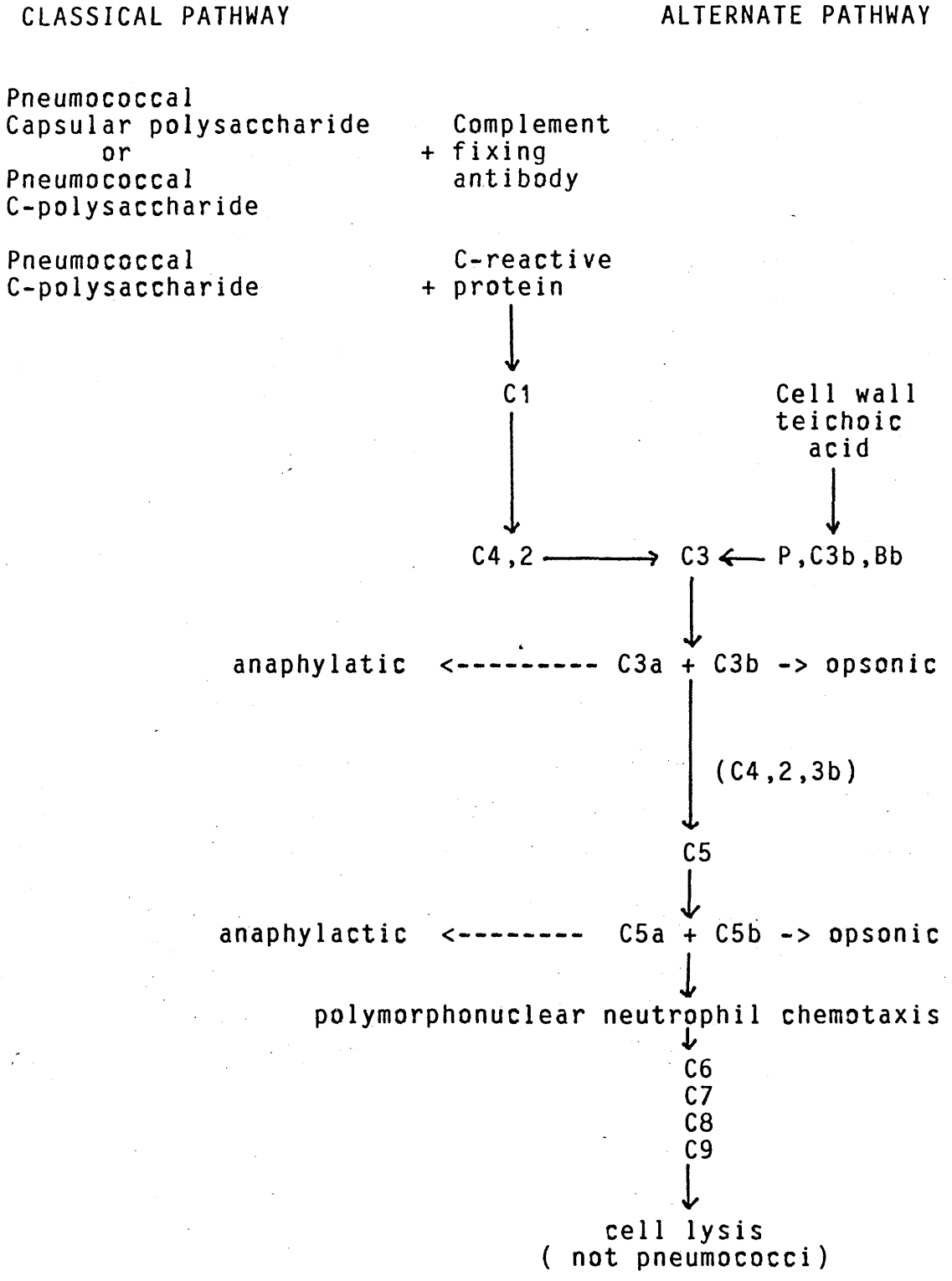
The classical pathway may be activated by the interaction of C-reactive protein with cell wall C-polysaccharide or, in the immune host, by the interaction of pneumococcal antibody with the capsular polysaccharide. However, Coonrod and Rylko-Bauer(1977) found in most instances that human pneumococcal anti-capsular antibody failed to fix complement. They suggested that not all classes or subclasses of antibody - produced in response to pneumococcal capsular polysaccharide - fix complement. This has been found with polysaccharides of both H. influenzae type b and Neisseria meningitidis. It is known that human IgG antibody to polysaccharides is predominately IgG2 (Siber et al., 1980) and antibodies of this subclass do not fix complement as efficiently as antibody of subclass IgG1 or IgG3 (Spiegelberg, 1974). Pneumococci activate the alternate complement pathway in the absence of antibody - a fact that may be important in natural immunity. The cell wall teichoic acid appears to play the major role in activation of the alternate pathway (Winkelstein and Tomasz, 1978). A number of studies have shown that individual types of S.pneumoniae

differ in their ability to activate the alternate complement pathway (Fine, 1975; Stephens, Williams and Reed, 1977; Winkelstein, Abramovitz and Tomasz, 1980). Fine (1975) found that, in general, the more pathogenic types, such as type 1,3,4 and 8, failed to activate the alternate complement pathway and suggested that this may contribute to their virulence. Stephens et al. (1977) concluded that the reaction depended on the presence of Fc receptors on the capsular polysaccharide (Stephens et al., 1974). Winkelstein, Bocchini and Schiffman (1976) showed that capsular polysaccharide was not required since activation of the alternate pathway was initiated by non-capsulate pneumococcal strains. Later they showed (Winkelstein and Tomasz, 1977; 1978) that the alternate pathway was activated by pneumococcal cell walls and in particular the cell wall teichoic acid. The same workers (Winkelstein et al., 1980) suggested that since not all pneumococcal serotypes activate the alternate complement pathway, their capsular material may in some way interfere with the activation or fixation of C3b to the cell wall and therefore prevent opsonisation. The importance of the complement system to opsonisation is evident by the life-threatening pneumococcal infection so often seen in patients with congenital C3 deficiency or C3 deficiency due to a lack of C3b activator.

The different ways that pneumococci activate complement are shown in figure V.

Figure V:

Activation of complement by pneumococci



The role of the liver and spleen

Both the liver and spleen can remove pneumococci from the circulation by phagocytosis. Experimental infection in animals has shown that the fixed macrophages in the liver clear bacteria from the blood when antibody is present, whereas the spleen is responsible for the removal of small numbers of bacteria in the non-immune animal (Schulkind, Ellis and Smith, 1967). The lack of receptor sites for C3b on splenic macrophages may explain the differing role of the spleen and liver in phagocytosis (Wara, 1981). In addition to its major role as a phagocytic organ, the spleen produces specific antibody during the early stages of infection when the antigen is in particulate form contained in bacterial cells. It also produces components necessary for the activation of the alternate complement pathway (Wara,1981).

The susceptibility of individuals, who are functionally or anatomically asplenic, to pneumococcal infection is well known. The risk of infection is significantly greater in patients with an underlying disease involving the reticulo-endothelial system and the young, than in patients who undergo splenectomy because of trauma (Wara,1981). Therefore splenectomy or functional asplenia leaves the host without an efficient phagocytic mass and predisposes to bacteraemia. Patients with sickle cell disease are also particularly susceptible to pneumococcal septicaemia and meningitis.

These patients in addition to having a functional asplenia also exhibit defective opsonistion by the alternate complement pathway (Winkelstein and Drachman, 1968). Phagocytosis by the liver is defective in infants or immunocompromised patients unable to form specific antibody.

The presence of specific antibody in humans, which can be induced by subcutaneous injection of pneumococcal antigen when the spleen has been removed or is not functional (Ammann et al., 1977) can compensate for the lack of functional splenic tissue by allowing phagocytosis by the liver.

1.4.6. Host response to pneumococci

Early studies

Pneumococcal antibodies, in the serum of patients with pneumococcal pneumonia, were first demonstrated by Klemperer and Klemperer (1891). They found that the serum from patients with pneumococcal pneumonia, after the crisis, cured experimental pneumococcal infection in rabbits. Bezancon and Griffon (1900) noted the presence of agglutinins in the blood of patients suffering from or convalescent from lobar pneumonia. Neufeld (1902) found agglutinins in the serum of some patients recovering from pneumonia and demonstrated both agglutinins and precipitins in convalescent sera. His experiments indicated that both reactions were due to the same substance which differed only in the way it was

detected. Neufeld and Handel (1910) demonstrated that immune sera and normal human serum protected mice from pneumococcal infection and they believed that the crisis in lobar pneumonia depended on the formation of specific antibodies. Dochez (1912a) found that protective antibody was not present in the blood before the crisis and suggested that it played a part in the patient's recovery from infection. Furthermore he found that, in some cases, protective antibody became evident only some time after the crisis i.e. it could not be demonstrated at any time during the disease. Eggers (1912) showed that the serum of pneumonia patients at or shortly after the crisis was bactericidal for the pneumococcus. Clough (1913) demonstrated protective antibodies, which were specific for the infecting strain of pneumococcus, in the serum of the majority of patients after the crisis and showed that phagocytic activity corresponded with the ability of a serum to protect mice inoculated with the same strain of pneumococcus.

These early studies were fundamental to the clinical studies which followed.

Immune response in patients with lobar pneumonia

The appearance of agglutinins in the blood of patients during the course of lobar pneumonia was also studied by Chickering (1914). He found agglutinins in 73.8% of patients infected with Groups 1,11 and 1V pneumococci but not in severe and fatal cases or in

patients with type 111 infection. He suggested that the absence of agglutinins during the later stages of the disease may have unfavourable prognostic significance. Clough (1919) confirmed Chickering's findings and found that patients who failed to develop agglutinins in their blood succumbed to the infection. Blake (1918) investigated the antigen-antibody balance in patients with lobar pneumonia. On a day-to-day basis, he examined blood cultures, determined the concentration of "pneumococcus soluble susbtance" in serum and urine and carried out tests for precipitating and agglutinating antibody on the patients' serum. He noted that before or at the time agglutinins appeared in the serum, pneumococci disappeared from the blood. Patients who developed an excess of precipitating or agglutinating antibody relative to antigen in their serum, invariably recovered : those who showed a progressive increase in antigen concentration without the development of demonstrable antibodies in the blood invariably died. He also established a relationship between the presence of antigen in the urine and the presence of precipitating antibody in the blood : all patients who failed to excrete antigen in their urine developed precipitating antibody in their blood at or about the time of the crisis ; conversely all patients who excreted antigen during the disease failed to develop precipitating antibody in their blood. Clough (1919) examined sera, from patients with lobar pneumonia, for

agglutinating antibody and tested them for phagocytic activity. He found that 85% of the sera had phagocytic activity and demonstrated agglutinins in 79%. Later, Lord and Nesche (1929) found that the results of the agglutination test were not always in accord with the results of mouse protection tests. Baldwin and Rhoades (1925) assayed protective antibody in the sera of patients recovering from pneumococcal pneumonia. They noted that protective antibody in serum usually showed an inverse relationship to the presence of pneumococcal bacteraemia and was an important factor in overcoming the infection. However, the presence of protective antibody did not always prevent toxaemia or the development of complications. Lord and Persons (1931) on the other hand reported cases in which the patient's recovery preceded the development of protective antibodies in the blood.

Pneumococcal antibodies in normal human sera

Although pneumococcal antibodies were often detected, by agglutination and precipitation tests, in the serum of patients recovering from pneumococcal pneumonia, these techniques failed to detect pneumococcal antibodies in normal human sera (Neufeld, 1902; Wadsworth, 1903). However, Clough (1924) confirmed earlier findings of Neufeld and Handel (1910) that normal human sera may contain substances capable of protecting mice against infection with specific types of pneumococci. Later studies showed that the

pneumococcicidal activity was more sensitive than other tests such as agglutination and mouse protection in the detection of immune substance in normal human blood (Sutliff and Finland, 1932). The level of natural immunity to pneumococcus types 1,11 and 111 was investigated by Ward (1930) and Robertson and Cornwell (1930). They found that normal humans possessed a degree of natural immunity to pneumococci but individuals vary in the amounts of pneumococcal bactericidal substance demonstrable in their blood for the different types of pneumococci. Furthermore Sutliff and Finland (1932) reported that the incidence of pneumococcicidal activity in human serum varied with the age of the subject. They summarised their findings on the bactericidal activity of normal human blood for pneumococci of types 1,11 and 111 as follows;

In the first 10 days after birth, the pneumococcicidal activity of the blood resembles that of the mother. Infants from 3 weeks to 15 months of age have little or no pneumococcicidal activity in their blood whereas, among children 2 to 11 years, type-specific antibodies appear with considerable frequency and may be the result of chance exposure to pneumococci. The highest incidence of pneumococcicidal antibodies was found in individuals 19 to 39 years of age and the incidence among adults 39 to 56 years of age was less than in young adults. They suggested that, in whole blood, differences in the incidence of the bactericidal activity for pneumococci

in infancy and old age may be related to clinical variations in pneumococcal pneumonia, in different age groups. Robertson et al. (1930) examined the relationship of natural humoral anti-pneumococcal immunity to the inception of lobar pneumonia using the pneumococcicidal test. Contrary to earlier findings (Dochez, 1912a) they demonstrated that, in the majority of patients, the initial blood specimens (taken 4 to 48 hours after onset of disease) possessed pneumococcicidal activity and that there was a relationship between the level of activity and the presence of pneumococci in the blood. However they found no correlation between the presence or level of blood pneumococcicidal activity and the extent of the lung lesion and concluded that circulating antibody merely prevented blood invasion by destroying pneumococci at the periphery of the lung lesion.

Recent studies

Gwaltney et al. (1975) used RIA to measure pneumococcal antibodies in normal individuals who had S.pneumoniae type 19 or 23 in their nasopharynx. They showed that the level of homologous antibody to the type carried in the nasopharynx, was high in adults, whereas children had low or undetectable levels. Riley and Douglas (1981) determined, by RIA, the antibody response in patients with bacteraemic pneumococcal pneumonia. They found that antibody was present in acute phase sera of many of the patients but more than half failed to

show an increase in antibody level during convalescence. The results of antibody determination in pneumococcal pneumonia by ELISA have differed in two reports. Berntsson et al. (1978) detected a significant antibody response in 16 of 17 patients, whereas Kalin and Lindberg (1985) detected a significant increase between acute and convalescent sera in only 50% of patients.

Antagonistic action of pneumococcal capsular polysaccharide

Although pneumococcal capsular polysaccharide is non-toxic it can enhance the invasive power of the infecting pneumococci by interfering with host defence mechanisms or by blocking the reaction between the capsule of the pneumococcus and the homologous antibody to prevent phagocytosis (Felton and Bailey, 1926). Blake (1918) did not detect circulating antibody in patients with capsular polysaccharide in their serum nor in patients who excreted a large amount of antigen in their urine. Coonrod and Drennan (1976) found that antigenaemia was associated with bacteraemia in pneumococcal pneumonia and with delayed appearance of detectable antibody. Onyemelukwe et al. (1985) reported that some patients, who had recently recovered from a severe pneumococcal infection, failed to produce pneumococcal antibodies when given pneumococcal vaccine - possibly indicating a suppressive effect of the infection.

Dochez (1912b) demonstrated that the blood of patients

in the acute phase of lobar pneumonia failed to coagulate normally. Recently, disseminated intravascular coagulation (DIC) has been recognised as a complication in patients with fulminant pneumococcal bacteraemia and antigenaemia (Rytel et al., 1974; Coonrod and Leach, 1976).

DIC and immune complex formation

Pneumococci and their capsular polysaccharides exert several effects on clotting parameters in vitro (Guckian, 1975). Pneumococcal antigen-antibody complexes induce the release of serotonin from human platelets which may play a role in the thrombocytopenia and/or thromo-haemorrhagic complications accompanying pneumococcal infection (Zimmerman and Spiegelberg, 1975). Cronberg and Nilsson (1970) reported a case of pneumococcal sepsis with generalised Shwartzman reaction.

Complement provides the link between pneumococci (and pneumococcal antigen-antibody complexes) and defects in the coagulation mechanism. Activation of the alternate pathway by pneumococcal cells (Winkelstein and Tomasz, 1978) or of the classical pathway by complexes consisting of pneumococcal C-polysaccharide and C-reactive protein (Kaplan and Volanakis, 1974) generate C3a and C5a. These peptides can stimulate mast cells to release histamine and serotonin. This aspect of pneumococcal activity has been reviewed by Johnston (1981).

1.4.7. Treatment of pneumococcal infection

Before the pneumococcus was recognised as a common aetiological agent in pneumonia, the standard treatment for patients with the disease consisted of frequent bleeding and purging and the administration of chemicals, such as boric acid. Digitalis, strychnine, alcohol and adrenalin were given to prevent cardiac failure due to the toxaemia which frequently occurred as a consequence of infection. Specific treatments for pneumococcal pneumonia were first developed during the early part of the twentieth century.

Chemotherapy

Optochin. Morgenroth and Levy (1911) found that ethylhydrocuprein (optochin), which is a derivative of quinine, protected mice from experimental pneumococcal infection. A number of studies in which optochin was used to treat patients with pneumococcal pneumonia were reported during the 25 years which followed the initial observation. However Moore and Chesney (1917) reviewed the early literature in which optochin was used to treat 787 patients and found that 4.5% of patients developed temporary deafness and blindness. In some cases optochin treatment was associated with permanent blindness and they demonstrated a 20 fold increase in resistance of a strain of pneumococcus isolated from a patient treated with optochin. Later Moore and Chesney (1918) suggested that optochin did not prevent or cure pneumococcal

bacteraemia.

Sulfonamides. Sulfanilamide was the first therapeutically effective derivative to achieve widespread use (Finland, 1979). Although it was highly effective against haemolytic streptococcal infection, it was not as successful in pneumococcal pneumonia. Sulfapyridine and sulfathiazole were found to be more effective and, although initially used in combination with therapeutic pneumococcal antiserum, they eventually replaced specific serum therapy for the treatment of pneumococcal pneumonia (Finland 1979). Kneeland and Mulliken (1940), in two papers, compared the effect of these sulfonamides on the development of pneumococcal precipitating and agglutinating antibodies in patients with pneumococcal pneumonia. They found that 80% of patients treated with sulfapyridine recovered without the appearance of antibodies in their serum whereas in those treated with sulfathiazole, 80% had antibodies present in their serum at the time of crisis. They concluded that sulfapyridine was a more powerful anti-pneumococcal agent than sulfathiazole. Treatment with sulfonamides was often associated with side-effects such as fever, rashes and blood dyscrasias. Furthermore, cases in which there was a recurrence of pneumonia due to the same type of pneumococcus and increased resistance to the drug were reported (Lowell, Strauss and Finland, 1940).

Penicillin first became available in 1940 and was

shown to be very effective for the treatment of pneumococcal pneumonia caused by all types of pneumococci. Clinical improvement was rapid and side-effects and resistance were not reported. Moreover, pneumococci were eliminated from the sputum in 50% of patients within 48 hours (Meads et al., 1945) in contrast to the results of Goodwin, Wilcox and Finland (1945) who showed that sulfonamide did not readily clear pneumococci from sputum. Penicillin is still the antibiotic of choice in patients with pneumococcal pneumonia and it is unlikely that another agent will be discovered to which the pneumococcus is as sensitive (Austrian, 1981). However, an increase in the number of pneumococcus strains which are moderately resistant to penicillin has been reported in the United Kingdom (CDS 86/07) although the incidence of penicillin resistance among strains of pneumococci associated with infection is very low (Gransden, Eykyn and Phillips, 1985).

Tetracycline and Erythromycin are as effective as penicillin against pneumococci although resistance and side-effects are more common. Erythromycin can be used as an alternative to penicillin in patients who are allergic to the drug.

Treatment of Chronic bronchitis. Antibiotics, given in either long or short courses, do not alter the rate of deterioration of respiratory function or the frequency of exacerbation of bronchitis (Calder, Lutz and Schonell, 1968). However, they shorten the duration and

severity of exacerbation. Since exacerbations are commonly associated with H. influenzae - with or without a pneumococcus - ampicillin, amoxycillin or tetracycline are the drugs most often used for treating patients with chronic bronchitis. (Pickering, 1975).

Serum treatment

Although Klemperer and Klemperer (1891) gave small subcutaneous doses of immune rabbit serum to patients with pneumonia, further developments in this field were not made until Neufeld and Handel (1910) described multiple types of pneumococci. Cole (1913) classified pneumococci into types 1, 2, 3 and group 4 and produced antiserum to types 1 and 2 in horses. By 1929 Cole had treated 371 patients and reduced the fatality rate, from an expected 25%, for type 1 to 10.5%. Finland (1930), in a five year study, found that serum therapy reduced the mortality rate in patients with type 1 and group 4 pneumonia but the effect of type 2 and type 3 antisera was less marked. Other workers produced therapeutic pneumococcal antisera but did not achieve the same degree of success. Felton, in a series of reports (cited in White, 1938; Heffron, 1939) demonstrated that therapeutic antisera could be concentrated so as to retain all the antibody but eliminate most of the proteins responsible for hypersensitivity. He made and supplied antisera to types 1 and 2 and these were used in clinical trials in the United States and the United Kingdom. Sutliff and

Finland (1931) in the USA treated adult patients with type 1 and Armstrong and Johnson (1932) in the UK patients with types 1 and 2 pneumococcal pneumonia. The results of these studies showed that, in patients treated with serum, crisis occurred earlier and the disease was less severe provided that treatment was given on or before the 4th or 5th day. The difference in mortality rates in patients treated with serum compared to those not given serum was greatest for patients treated within the first 3 days after the onset of symptoms (Sutliff and Finland, 1931). Concentrated serum was prepared in horses, and later in rabbits, for other types commonly found in pneumococcal pneumonia. Finland and Brown (1939) examined the effect of antisera to types 1,2,5 and 7 on mortality rates in pneumococcal pneumonia. They showed that mortality rates were reduced to one half of those amongst untreated cases for both bacteraemic and non-bacteraemic patients. In spite of these favourable results, serum therapy for pneumococcal pneumonia was not universally accepted. Difficulties in making an early bacteriological diagnosis, cost of the serum, lack of familiarity with the technique of giving serum, undue fear of reactions and uncertainty as to the value of the procedure contributed to the general reluctance of physicians to use serum therapy.

Attempts to treat patients with pneumococcal pneumonia with the blood or serum obtained from patients convalescent from pneumococcal pneumonia of homologous

type (Beebe and Sutliff, 1930) or from normal persons following their immunisation with pneumococcal vaccine (Barach, 1931) have not been successful. However, Siber et al. (1984) have prepared human hyperimmune globulin, to Haemophilus influenzae type b, Streptococcus pneumoniae and Neisseria meninigitidis which cause serious infection in children. They suggested serum prophylaxsis as an alternative to vaccination in children, under 18 months old, at risk from infection and noted that it may be useful in treating overwhelming pneumococcal infection.

1.4.8. Incidence of pneumococcal types

Pneumococcal antisera were the only agents available for the treatment of serious pneumococcal infection before the introduction of modern antibacterial therapy. Their successful use clearly depended on the availability of a simple and rapid method to determine the type of the infecting pneumococcus. Serotyping also provided valuable epidemiological information which established that pneumococcal lobar pneumonia was a communicable disease whereas pneumococcal bronchopneumonia was essentially an autogenous infection. Furthermore, serotyping was of practical importance since knowledge of the infecting pneumococcus type was of prognostic value and allowed clinicians to differentiate those patients who had a recurrence of infection from those who were re-infected.

With the introduction of penicillin and other antibacterial agents which were highly active against pneumococci irrespective of type, there was no longer a need for typing pneumococci on purely clinical grounds. Recently, there has been a re-newed interest in the use of pneumococcal vaccines to prevent serious infection and consequently typing has been re-introduced in a number of bacteriology laboratories.

The results of typing pneumococcal strains over the past three-quarters of a century , although limited by the range of typing sera available at any one time, have shown that the incidence of different disease-producing types varies with time, geographical area and with the age of the patients studied.

Incidence of types 1,2,3 & group 4

The incidence of pneumococcus types 1,2,3 and group 4 reported from many parts of the world from 1913 to 1935 was analysed by Heffron (1939). In 16,813 cases of lobar pneumonia, pneumococcus type 1 was isolated in 35.8%, type 2 in 20.6%, type 3 in 10.8% : pneumococci listed as group 4 were found in 35.8% of cases. He noted that the incidence of type 2 varied with the country of origin and at different times in the same country, whereas the incidence of type 1, which was usually the predominant type in all studies , remained essentially unchanged. The incidence of type 3 also varied geographically and studies in Glasgow by Grant (1922) showed that the incidence of type 3 in lobar pneumonia was 5.3%. A low

incidence of type 3 in lobar pneumonia was reported by other workers in Great Britain and in Scandinavia. Christie (1934) compared the incidence of pneumococcal types in lobar pneumonia during two epidemic periods of pneumonia and influenza in Glasgow. In the lobar pneumonia period (February to June, 1932) he found that the incidence of type 1 was 42%, type 2 was 36.6%, type 3 was 1.8% and group 4 was 9.6%. However, during the influenza period (from December 1932 to January 1933) the frequency of pneumococcal types in lobar pneumonia changed so that the incidence of type 1 was 23.3%, type 2 was 53.3%, type 3 was 11.7% and group 4 also 11.7%.

Incidence of types 4 to 32

Typing sera for pneumococcal types 4 to 32 became generally available after 1932. Heffron (1939) combined the results of three American series to show the approximate incidence of higher types in lobar pneumonia in adults. In a total of 3,713 cases he found that types 1, 2 and 3 were still the most common and accounted for 53.5% of cases. Types 5, 7 and 8 were next in order of frequency and when combined with the first three types accounted for 75.7% of the total number of cases. Types 4,14,6 and 18 in that order were the next most frequent and accounted for 9.8% of cases. He concluded that types 1 to 8 were by far the most common types. They were responsible for 81% of the 3,713 cases whereas the remaining twenty-three types accounted for only 19%. Christie (1934) in Glasgow found that 53% of a small

series of 53 strains which belonged to group 4 could be typed with antisera to types 4 to 32. However, he included strains isolated from children, adults convalescent from pneumonia and healthy carriers. Studies which reported the incidence of pneumococcal types in patients with bronchopneumonia were analysed by Heffron (1939). He found a marked difference in the reported figures of the combined incidence of types 1 and 2 (10.6%) in bronchopneumonia compared to their incidence in lobar pneumonia (over 50%). In addition, he noted that the frequency with which the various types of pneumococci are found in bronchopneumonia broadly parallels the frequency with which they are carried in the mouths of normal persons. Hence in bronchopneumonia, types 3,8,10,20,18 and 7 in that order were the most common types and accounted for 49.6% of cases.

In Heffron's analysis of 2,745 cases of pneumococcal lobar pneumonia in infants and children, he found that types 14,1,6,19,5,4,3 and 7 in descending order of frequency accounted for 46.3% of the total cases. He found that the occurrence of different types varied with the age of the patient. In infants and young children under 2 or 3 years of age, type 14 predominated, whereas in older children type 1 was the most common and types 5, 7 and 14 were also frequent. He concluded that as the age increases, the incidence of type 14 decreases while the incidence of type 1 increases. As can be seen from the figures quoted above type 2 was a relatively rare

cause of lobar pneumonia in children.

Heffron (1939) summarised these early studies of the distribution of pneumococcal types in adults and children and this is reproduced in Table 2.

Changes in the distribution of different pneumococcus types over a period of some 40 years have been studied by Finland and Barnes (1977) and Lund (1970). Both studies noted a shift away in recent years from the predominance of types 1, 2 and 3 infection and a sharp decline in the frequency of types 2 and 5. Lund (1970) noted a decline in type 1 infection in Denmark after 1955 and in type 2 infection as early as 1937. Finland and Barnes (1977) noted that type 2 was not isolated after 1953 and similarly type 5 markedly decreased after 1967. However Mufson et al. (1974) noted an outbreak of type 5 infection in 1974 during an epidemic of influenza A.

In the 1930s, types 1, 2 and 3 caused two-thirds of serious infection and no other type accounted for more than 8% of the isolates (Tilghman and Finland, 1937). Twenty years later Austrian and Gold (1964) found that types 1 and 3 formed only a quarter of the total whereas types 4,7,8 and 14 accounted for one third of isolates. Recent reports (Broome et al., 1980 ; Colman and Hallas, 1983) confirmed that the pneumococcal types isolated from serious infection were more evenly distributed in that there was less tendency for predominance of only a few types. They also demonstrated

Table 2:

Pneumococcus lobar pneumonia and bronchopneumonia in infants and children and in adults. Types of pneumococci most commonly found

	types most commonly found in order of approximate frequency
Lobar pneumonia	
Infants and children	14,1,6,5,7
Adults	1,3,2,5,8,7
Bronchopneumonia	
Infants and children	6,19,18
Adults	3,8,10,20,18,7

Reproduced and adapted from Heffron (1939)

a difference - noted in earlier years - in the types causing infection in children and adults.

Pneumococcal vaccines

Austrian and Gold (1964) analysed the factors which determined the outcome in 455 patients with bacteraemic pneumococcal pneumonia. They found a mortality rate of 17% among 437 patients who had received antimicrobial therapy. Furthermore they showed that the fatality rate in patients over 50 years of age was 28% : in patients with a variety of chronic systemic illness 27% died. Although untreated patients with bacteraemic pneumococcal pneumonia died more than 5 days after the onset of symptoms, 60% of the deaths in patients receiving antibiotics took place in this five-day period. Austrian and Gold (1964) also found that the mortality rate of type 1 bacteraemic pneumococcal pneumonia declined from 86%, in patients who did not receive penicillin, to 7% in those treated with penicillin whereas the mortality rate in type 3 bacteraemic pneumococcal pneumonia fell from 98% to only 48%.

As a result of these findings steps were taken to re-develop a polyvalent pneumococcal vaccine composed of capsular polysaccharides. Other studies had also identified groups of patients who might benefit from vaccination such as patients with sickle cell anaemia. A vaccine composed of capsular polysaccharides of pneumococcus types 1,2,5 and 7 had been shown previously

to be effective in preventing pneumonia caused by these types in a military population (MacLeod et al., 1945). Austrian et al. (1976) reported the results of an eight year study of the pneumococcal types isolated from blood cultures of 3,644 patients of which 14 types accounted for 82.5%. The purified polysaccharides of each of these types were prepared and combined to produce a polyvalent vaccine which was licensed in the United States in 1977. The vaccine consisted of the following types (Danish nomenclature) : 1,2,3,4,6A,7F,8,9N,12F,14,18C,19F,23F and 25. The types within groups 6,7,12,18,19 and 23 were chosen on the basis that these were the most commonly occurring types world-wide and it was assumed that immunisation with one type in a group would confer a degree of protection for the other types within the group. Schiffman (1981) determined the extent of cross-reaction between different types within groups. He found that the protective effect of the vaccine would be improved if type 6B replaced type 6A but showed that the types within groups 18 and 23 showed no appreciable degree of cross-reactivity. Thus, the inclusion of one type from each of the groups 18 and 23 would have to be based on studies of prevalence. The cross-reactivity of types within groups 9 and between types 19F and 19A was investigated in clinical studies (Szu et al., 1982 ; Penn et al., 1982). On this basis types 9N and 9V were chosen from group 9, and from group 19, types 19F and 19A, should be included in the vaccine. In 1985, the 14

valent pneumococcal vaccine was withdrawn and replaced by a new 23 valent formulation. This was devised on the basis of both additional information on the distribution of pneumococcal types and the results of studies on the immunological relationships between different capsular types : the subject has been reviewed by Robbins et al. (1983).

AIMS OF THE PRESENT STUDY

This investigation sets out to :

1. Assess critically the methods currently used in the diagnosis of pneumococcal infection.
2. Develop techniques for the detection of pneumococcal antigen.
3. Compare the sensitivity and specificity of these techniques.
4. Apply these techniques in the investigation of pneumococcal respiratory disease.
5. Extend the investigation to include antigen detection in blood, urine, CSF and tissue.
6. Evaluate these findings for the prognostic evaluation and management of patients.
7. Use the same techniques to determine the prevalence of pneumococcal serotypes to establish an epidemiological base.
8. Correlate the observations on the distribution of the organism and its antigens with studies on the antibody response to further understanding of the pathogenic mechanisms of pneumococcal disease,

CHAPTER 2

MATERIALS AND METHODS

2.1.LABORATORY MATERIALS

2.1.1. Buffers

Phosphate buffered saline pH 7.2 (PBS)

Anhydrous disodium hydrogen phosphate (Na_2HPO_4) :1.07g

Sodium dihydrogen phosphate($NaH_2PO_4.2H_2O$) :0.39g

Sodium chloride :8.5g

Distilled water :1000ml

Barbitone buffer pH 8.2 (ionic strength 0.05 at 25°C)

Barbituric acid :3.44g

Sodium barbitone :7.57g

Sodium azide :1g

Distilled water :1000ml

Barbitone buffer pH 6.6 (ionic strength 0.05 at 25°C)

Solution A = 0.2 molar sodium barbitone (41.2g/l)

Solution B = 0.2 molar hydrochloric acid (7.3ml/l)

37.5ml of solution A was added to approximately 60ml. of solution B and the pH was adjusted to 6.6 before making up to 100ml with distilled water.

For use the buffer was diluted 1:4 (0.05M) with distilled water.

Glycine buffer saline pH 8.2 (GBS)

Glycine :7.3g

Sodium chloride :10g

Distilled water :900ml

The pH was adjusted to 8.2 using 1M sodium hydroxide and the volume was made up to 1 litre.

Acetate buffer pH 4.0

Solution A = Glacial acetic acid :57ml/l

Solution B = Anhydrous sodium acetate :82g/l

To 100ml of solution A approximately 20ml of solution B was added and the pH was adjusted to 4.0. For use 6ml was diluted to 100 ml.

Carbonate buffer pH 9.6

Disodium carbonate : 1.50g

Sodium bicarbonate : 2.93g

Sodium azide : 0.2g

Distilled water : 1000ml

Wash buffer

Phosphate buffer saline pH7.2 containing 0.05% (v/v) tween 20.

Dilution buffer

Wash buffer containing 0.25% (w/v) bovine serum albumin and 0.01% (w/v) sodium azide.

Bacto FA buffer (Difco laboratories)

Dried FA buffer(10g) was dissolved in 1000ml distilled water.

Mounting fluid (buffered glycerol)

10ml of 0.5M carbonate : bicarbonate buffer pH 9.0

90ml glycerol (reagent grade)

2.1.2. Bacterial strains

Lyophilised cultures of Streptococcus pneumoniae types 3,6A,7F,8,9L,11A,13,14,18C,19F,34,41A and 42 were kindly provided by Miss C. Fraser, Streptococcus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9. and types 7A,7B,7C,9A,11F,11C,12A, 16A,17A,19B,19C,22A,23A,23B,33A,33B,33C and C-mutant strain (CSR,SC S-2 clone 1) were kindly provided by Dr. J. Henrichsen, WHO Collaborating Centre for Reference and Research on Pneumococci, Statens Seruminstitut, Copenhagen, Denmark.

S.pneumoniae types 1,2,4,5,20,21,25,27,29,31,36,37,38, 48 and strains belonging to multitype groups 24,32 and 35 were isolated from clinical material and were typed by the capsular reaction test at the Bacteriology Department, City Hospital, Edinburgh, and types 6B,9N,9V,10F,10A,11B,12F,15F,15A,15B,15C,16F,17F,18F,18A, 18B,19A,22F,23F,28A and 33F, also isolated from clinical material were typed by the capsular reaction test at the Statens Seruminstitut, Copenhagen.

2.1.3. Purified pneumococcal capsular polysaccharide

10mg of types 1,2,3,4,5,6A,6B,7F,8,9N,9V,10A,11A,12F,14, 15B,17F,18C,19F,19A,20,22F,23F,25 and 33F (Merck,Sharp and Dohme Ltd.) were stored at a concentration of 2mg/ml in phosphate buffered saline pH 7.2 at -20^{o} C.

2.1.4. Diagnostic pneumococcal antisera and reference serum

Five kinds of rabbit pneumococcal antisera were used.

A. Omni serum, which reacts with all 83* known pneumococcal types.

B. Nine pooled sera (pools A to I), each reacting with between 7 and 11 types and together included all 83* types.

C. Antisera to types 2,5,39,43,44,45 and 46 and to groups 6,7,9,10,11,12,15,17,18,19,22,23,24,28,32,35 and 47.

Antisera A to C were obtained from the Statens Seruminstitut, Copenhagen.

D. Antisera to types 1,3,4,6A,6B,7F,7A,7B,7C,8,9N,9A, 9V,9L,10F,10A,11A,11B,12F,12A,13,14,15B,16F,16A,17F,17A, 18F,18A,18B,18C,19F,19A,19C,20,21,22F,22A,23F,23A,23B, 25,27,28A,29,31,33F,33A,33B,33C,34,37,38,41A,42,48 and pneumococcal C-polysaccharide were raised in rabbits at the Bacteriology Department, Stobhill Hospital, Glasgow as described on pages 117-119.

E) Factor sera 9g,10b,10c,11b,11c,11f,11g,15b,15c,15e, 15h,17b,17c,19f and 33b were kindly provided by Dr. J. Henrichsen.

Factor sera 6b,6c,7b,7c,7e,7f,9b,9c,9d,9e,9g,10b,10c, 11b,11c,11f,12b,12c,15b,16b,16c,17b,17c,18c,18d,18e,18f, 19b,19c,7h,19f,22b,22c,23b,23c, 33b,20b,33c and 33f were prepared here from type antisera by absorption.

A polyvalent serum, reacting with pneumococcal types represented in the 23 valent pneumococcal vaccine and antiserum to pneumococcal C-polysaccharide antigen were kindly provided by Dr. J. Henrichsen.

* During the term of this study Austrian (1985) described the isolation of a new serotype (Type 16A), bringing the number of described types to 84.

Human reference sera

Pooled human reference serum for the standardisation of antibody determination was kindly provided by Dr.E.A.Fitzgerald, Division of Product Quality Control, Office of Biologics Research and Review Centre for Drugs and Biologics, Food and Drug Administration, Bethesda, Maryland, U.S.A.

2.1.5. Culture media

Tryptone Soya Agar (Oxoid CM131) containing 5% horse blood (Gibco Europe) was used as the basal medium throughout this study for growth unless otherwise specified.

Blood Culture Bottles

1) Casteneda bottles (Difco Laboratories) with tryptone soya agar as the solid phase and tryptone soya broth as the liquid phase.

2) "Bactec" bottles 6B (Johnston Laboratories, Becton Dickinson) for use in the "Bactec" (model 460)

instrument. Both bottles, containing 30 ml of broth, were inoculated with 5ml of whole blood.

Tryptone soya broth (Oxoid CM 131) containing 15% glycerol (BDH) was prepared in 1ml amounts in glass vials (Laboratory Sales (UK) Ltd. code VV2)

Todd-Hewitt broth (Difco 0492-01) containing 5% horse serum (Gibco Europe 034-6050) was prepared in 500 ml amounts.

CCY Medium (Woodin modification)

Solution A

casein hydrolysate :200g

yeast extract(Difco) :50g

sodium glycerophosphate :100g

50% sodium lactate :50ml

anhydrous disodium hydrogen phosphate (Na_2HPO_4) :4g

Potassium dihydrogen phosphate(KH_2PO_4) :2g

ammonium sulphate :5g

The reagents were dissolved in 3 litres of warm distilled water.

Solution B

DL-tryptophane :0.4g

L-cysteine hydrochloride :0.5g

The reagents were dissolved in 300ml of warm distilled water and solution A mixed with solution B. Distilled water (1.7 litres) and Davis agar(100g) were added before heating to dissolve solids. After distribution in 500 ml amounts the medium was autoclaved at 121 ^{o}C for 30mins.

Vitamin Stock Solution

thiamin :0.2g/l

nicotinic acid :0.4g/l

Trace Element Stock Solution

magnesium sulphate($MgSO_4.7H_2O$) :2g/l

manganese sulphate($MnSO_4.4H_2O$) :1g/l

ferrous sulphate($FeSO_4.7H_2O$) :0.6g/l

citric acid :0.3g

Both solutions were sterilised by autoclaving and 5ml of each was added to 500ml of the sterile basal medium. 20ml of the complete medium was distributed into 100mm by 18mm petri dishes (Sterlin).

2.1.6.Preparation of Stabilised Staphylococci(Protein A)

Staph.aureus strain Cowan 1 (NCTC 8530) was grown on plates of CCY agar, overlaid with dialysis membrane (Technicon part number 933-0225-01), and incubated at 37 °C overnight. The bacteria were harvested from the membranes by agitation in PBS, the suspension washed twice in PBS, and suspended in 0.5% formaldehyde in PBS for two hours at room temperature. The formolinised suspension was washed twice in PBS, heated at 80 °C for one hour, washed a further three times in PBS, and resuspended at a concentration of 10% (v/v) in PBS containing 0.1% (w/v) sodium azide.

2.1.7. Additional materials and reagents

Sterile disposable plastic loops 1ul and 10ul (Exogen Ltd.).

Glass slides 76mm X 26mm and 76mm X 51mm (Chance propper Ltd.) and PTFE coated 76mm X 26mm (Hendley-Essex).

Sputolysin (dithiothreitol, Behring Diagnostics) was prepared by diluting 10ml of concentrate in 90ml of sterile distilled water.

Loffler's methylene blue was prepared by mixing 300ml of a saturated solution of methylene blue in ethanol and 1000ml of 0.01% potassium hydroxide.

Aqueous crystal violet solution (0.5%)

Polystyrene monodispersed latex 0.17u diameter (cat. number 7304 Polysciences, Inc.).

Bovine serum albumin, 22% protein concentration (Ortho diagnostic systems).

Fluorescein labelled anti-human immunoglobulin (Wellcome reagents).

Urease conjugated sheep anti-rabbit IgG (CSL) and Urease substrate solution (CSL) (Sera-Lab).

Wellcogen S.pneumoniae latex test (Wellcome diagnostics).

Phadebact pneumococcus test (Pharmacia).

API 20 Strep (API Systems SA).

0.45 um filter units, Millex-HA, cat. number SLHA 025BS. (Millipore (UK) Ltd.

Dialysis membrane 8/32" (The Scientific Instrument

Centre Ltd.).

Microelisa plates 129A (Dynatech Microelisa systems).

Limbro plate sealers 33.3 cm X 8.3 cm (Flow Laboratories).

Streptex kit for the identification of streptococci (Lancefield groups A,B,C,D,F and G) and antisera for Lancefield groups K and H were obtained from Wellcome Diagnostics.

2.2. CLINICAL MATERIAL AND PATIENTS

2.2.1 Preliminary studies

Clinical material used in preliminary studies was obtained from patients admitted to hospital between January 1982 and June 1984.

Sputum specimens

Three hundred and eleven samples were studied from adult patients with acute community-acquired pneumonia admitted to hospital during a prospective study conducted in 27 hospitals throughout the UK between November 1982 and December 1983. The specimens were originally sent to the Bacteriology Department, City Hospital, Nottingham where, after homogenisation with N-acetyl-l-cysteine they were tested for pneumococcal antigen using CIE and Omni serum. They were frozen at -20^{o}C and sent to me at the Bacteriology Department, Stobhill Hospital, Glasgow in June 1984 where they were also stored at -20^{o}C. Batches of 20 samples were allowed to thaw at room temperature before additional tests for pneumococcal antigen were carried out.

Sputum specimens sent to the Bacteriology Department, Stobhill Hospital, for routine examination and culture were processed and tested for pneumococcal antigen on the same day as they were obtained from the patient. A total of 1,056 samples was investigated using different methods for the detection of pneumococcal antigen to determine the correlation between these

results and the quality of sputum, Gram's stain and pneumococcal culture with clinical infection.

Quantitative culture of pneumococci was carried out on 291 specimens which contained pneumococcal antigen or had a preponderance of Gram positive lanceolate diplococci in a Gram's stained smear. The results were then correlated with the presence or absence of clinical signs of a chest infection.

Four hundred and seven specimens were investigated to determine the correlation between macroscopic and microscopic assessments of cellular material in sputum and to investigate the correlation between predominant pneumococci seen in a Gram's stained smear and their isolation on culture.

Direct and semi-quantitative culture ($> 10^6$ cfu/ml) was compared in 623 specimens.

Specimens other than sputum

Post-mortem lung tissue (24), pleural fluid (8), cerebrospinal fluid (28) and knee aspirates (2) were obtained from patients with pneumococcal infection.

Acute phase serum was obtained from 24 patients with pneumococcal pneumonia.

2.2.2. Patients

The main clinical study

Most of the patients studied were admitted to Stobhill Hospital, Glasgow during a four year period from February 1982 to February 1986. A few patients with

severe pneumococcal disease had been admitted to other hospitals in the Glasgow area during the same period. In addition to material obtained from the primary focus of infection in these patients, blood culture, serum or urine was obtained during the acute or convalescent phase of the infection. A clinical summary and details of antimicrobial therapy at the time the specimens were taken were available for these patients. Patients were assigned to one of three clinico-pathological groups.

Group "P" (pneumococcal pneumonia) consisted of 122 patients with no previous history of respiratory disease and severe pneumonia was the reason for their admission to hospital.

Group "C" (chronic bronchitics) contained 101 patients with pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease (COAD). In addition to S.pneumoniae or pneumococcal antigen , Haemophilus influenzae was often isolated from the sputum of these patients. However, for the purpose of this study the role of H.influenzae in the infection was not considered.

Group "A" (acute chest infection) consisted of 163 patients who had a productive cough and sputum on admission to hospital, without definite clinical or radiographic evidence of pneumonia, or developed a lower respiratory tract infection secondary to cardio-respiratory disease, post-operatively or during hospitalisation for treatment of diseases not associated

with the respiratory tract.

Group "S" (systemic disease) comprised 28 patients with non-respiratory pneumococcal infection.

Other clinical studies

Serial sputum samples (i.e. two or more specimens) obtained from 398 patients whose original specimen contained pneumococci or pneumococcal antigen were examined during the whole period of the study. A clinical summary and details of antimicrobial therapy at the time each specimen was taken were available. Specimens examined on days 1 to 7 after the initial specimen were considered part of the same episode whereas those examined after 7 days were considered to represent a new episode.

Nasopharyngeal swabs from 94 patients admitted to hospital (February 1983 to April 1983) for elective surgery and who subsequently developed a pneumococcal chest infection were matched with sputum samples obtained post-operatively.

A prospective study of pneumococcal infection was carried out on 30 patients with chronic bronchitis, centred at the Royal Infirmary , Glasgow (January 1981 to December 1982). Specimens of sputum for bacteriological assessment were obtained before and at the time of exacerbation. Further samples taken at 3, 11 and 38 days after treatment were available for some patients.

2.2.3. Bacterial strains

Clinical isolates of Streptococcus pneumoniae

A total of 99 strains, most of which were isolated from blood and cerebrospinal fluid were referred to me by staff in other laboratories in the West of Scotland (January 1981 to December 1985). These were serotyped and the results later confirmed by Dr. J. Henrichsen, Statens Seruminstitut, Copenhagen, Denmark.

Serotyping was also carried out on 1,682 pneumococci or sputa containing pneumococcal antigen from patients admitted to Stobhill Hospital, Glasgow, during the period February 1982 to February 1986. Patients were assigned to one of three clinico-pathological groups. Group "P"(128 patients), Group "C" (618 patients) and Group "A" (938 patients).

Eight hundred and seventy four strains of Streptococcus pneumoniae isolated in 15 bacteriology laboratories throughout Scotland (between January 1982 and December 1985) were referred to my laboratory for serotyping. Patient identification, the source of the isolate and the clinical diagnosis accompanied each strain. Strains which were isolated from blood, cerebrospinal fluid, post-mortem lung tissue and other normally sterile body cavities were considered to be responsible for systemic disease. Strains isolated from the nasopharynx were designated upper respiratory flora as were isolates from non-purulent sputa with obvious

oropharyngeal contamination. The latter strains conformed to each of the following criteria.

1. Isolation with mixed respiratory tract flora in numbers below 10^5 cfu/ml of homogenised sputum.

2. Isolation from sputum in which no pneumococcal antigen was detected (Miller et al., 1978).

3. Isolation in absence of signs and symptoms of clinical infection.

Bacterial strains which cross-reacted with pneumococcal antisera

1. A collection of 72 strains of Streptococcus milleri, isolated from a variety of anatomical sites were accumulated during the period January 1985 to January 1986 by Mr. Grant Mackenzie, Bacteriology Department, Stobhill Hospital, Glasgow.

2. Thirty five strains of alpha and non-haemolytic respiratory streptococci and 25 strains of enterobacteriaceae which cross-reacted with pneumococcal antisera were isolated from sputum cultures.

2.3. METHODS

2.3.1. Stock cultures

Type strains were preserved by resuspending 5-10 colonies from a blood agar plate (incubated anaerobically with 5-10% carbon dioxide for 18 hours) in 1ml tryptone soya broth with 15% (v/v) glycerol. Strains were stored at -70°C.

2.3.2 Preparation of stock suspensions of pneumococci

The growth from two blood agar plates was inoculated into 500ml of Todd-Hewitt broth containing 5% horse serum. The broth was incubated at 37°C for 4 to 7 hours aerobically until growth was in late log phase. Subculture was carried out to confirm purity, and 2% (v/v) formaldehyde was added. The suspensions were left overnight at room temperature. The bacterial cells were harvested the next morning by centrifugation (3,500 rpm for 30 minutes), resuspended in phosphate buffered saline (PBS pH 7.2) and after heating at 75°C for 20 minutes, washed with PBS. 1ul of concentrated suspension was diluted 1:1000 in PBS and examined by Gram film and for the capsular reaction with Omni serum. Stock pneumococcal suspensions were stored at 4°C.

2.3.3 Production of antisera

Vaccines for antisera production were prepared by dilution of stock pneumococcal concentrated suspensions in PBS to a concentration of 1-2 X 10^9 diplococci per ml.

Immunisation procedure

Lop ear rabbits approximately 2-3 kilogrammes in weight were used for antiserum production. Injections were given intravenously on alternate days for five weeks using the following protocol:

First week: 0.25ml,0.5ml,0.75ml

Second week: 1ml,1ml,1ml

Third week: 1.5ml,1.5ml,1.5ml

Fourth week: 2.0ml,2.0ml,2.0ml

Fifth week: 2.0ml,2.0ml,2.0ml

The rabbits were bled after 3 and 6 weeks and the antibody titre of the sera tested by the capsular reaction test : titres of 1:16 or greater were accepted as adequate. The rabbits were bled terminally by cardiac puncture under intravenous anaesthetic (Nembutal 0.5ml/kg body weight). The blood was allowed to clot at $37^{o}C$ for 30 minutes and kept at $4^{o}C$ overnight. After centrifugation, 0.1% (w/v) sodium azide was added to the serum : storage was at $-20^{o}C$ in 5ml aliquots.

The antibody titre of each serum was tested by the capsular reaction test against a suspension of homologous vaccine. Specificity was checked against heterogenous vaccines with the capsular reaction test. The results of the tests were recorded and used to characterise the antisera for use in experiments.

Capsular reaction test (Quellung reaction)

A 10ul loopful of antiserum was mixed with 1ul loopful of a suspension of the test organism on a 76mm

X 26mm glass slide. A 1ul loopful of Loffler's methylene blue was added and a coverslip placed over the preparation for examination using oblique light under an oil immersion lens.

The pneumococcal suspension was used at a concentration of less than 50-100 bacteria/field to avoid prozone effect.

2.3.4 Production of factor sera

Antisera to types 6A,6B,7F,7A,7B,7C,9N,9A,9V,9L,10F, 10A,11A,11B,12F,12A,15B,16F,16A,17F,17A,18F,18A,18B,18C, 19F,19A,19C,22F,22A,23F,23A,33F,33A,33B and 33C were absorbed with suspensions of the appropriate heterologous strains to remove common group antibodies.

500ul concentrated suspension was centrifuged at 3,500 rpm for 30 minutes and the supernatant removed. 250ul antiserum was absorbed by resuspension of the bacterial deposit in the antiserum at room temperature for 15-30 minutes. A second absorption was carried out in the same way with 500ul of the same suspension. 50ul of the twice absorbed serum was then mixed with 500ul of staphylococcal protein A and tested against suspensions of the appropriate pneumococcal types to confirm that the common antibody had been completely removed. Two absorptions were generally sufficient to remove all common antibody, but some high titred antisera required further absorption.

2.3.5.Production of absorbed group antisera

Pneumococcal antisera prepared at Statens Seruminstitut, Copenhagen against groups : 6,7,9,10,11, 12,15,17,18,19,22,23 and 33 were used.

500ul of a concentrated suspension of pneumococci of each type within a group was centrifuged and the supernatant removed. 250ul group antiserum was mixed with each bacterial deposit for 15-30 minutes at room temperature and centrifuged. A second absorption was carried out using 500ul of the same suspension. 50ul of the absorbed serum was then mixed with 500ul of staphylococcal protein A and tested against a suspension of the type used for absorption to confirm that homologous antibody had been completely removed. Two absorptions were generally sufficient but occasionally a third absorption was necessary to remove all traces of homologous antibody.

2.3.6. Isolation and identification of S.pneumoniae strains

Culture for S.pneumoniae was carried out on blood agar plates after anaerobic incubation for 18 hours with 5-10% carbon dioxide. An optochin disc on the inoculation area allowed presumptive identification of S.pneumoniae on primary isolation.

Blood cultures from patients suspected of having pneumococcal infection were subcultured after a minimum

of 18 hour incubation, a smear of the blood broth was examined by Gram's stain and approximately 1ml of the blood culture broth examined for pneumococcal antigen. Blood cultures, showing no growth at this time, were incubated and examined by naked eye twice daily for evidence of growth and where indicated subcultured and examined as above.

Tissue. Approximately 1 gram of tissue was suspended in 1ml of PBS, homogenised in a Griffith's tube, and after centrifugation (2,500 rpm) the supernatant removed for pneumococcal antigen examination. Culture for S.pneumoniae and a smear for Gram's stain were made from the deposit.

Pathological body fluids eg. pleural fluid, cerebrospinal fluid, aspirates and pus were centrifuged (2,500 rpm) immediately on receipt in the laboratory. The supernatant was retained for pneumococcal antigen examination and the deposit cultured for S.pneumoniae and stained by Gram's method.

Swabs were plated onto blood agar with an optochin disc on the inoculation area.

Sputa, bronchial and tracheal aspirates were homogenised in an equal volume of "Sputolysin" (dithiothreitol, Behring). Culture for S.pneumoniae, Gram's stain and tests for pneumococcal antigen were carried out on uncentrifuged homogenates. Additional culture plates were inoculated with 10ul and 1ul loopfuls of a 1 in 1000 dilution of homogenised sputum,

for semi-quantitative estimate of the numbers of S.pneumoniae present. Culture plates were examined after incubation and colonies which resembled S.pneumoniae were tested for pneumococcal antigen by the co-agglutination test.

Typing of S.pneumoniae strains by Kronvall's co-agglutination test. (CoA(K))

Four different kinds of pneumococcal antisera were used to prepare co-agglutination typing reagents. These were:

a) Three polyvalent antisera prepared from pools A to I
   polyvalent 1 = 1ml of pools A,B and C mixed together
   polyvalent 2 = 1ml of pools D,E and F mixed together
   polyvalent 3 = 1ml of pools G,H and I mixed together
b) Nine pooled sera (A to I).
c) Type sera, reacting with a single type, and group sera, reacting with all the types within one group.
d) Factor sera and absorbed group antisera used for the differentiation of types within a group (available from January 1985).

50ul of each antiserum, (i.e. a to d above) were added to 500ul of 10% (v/v) suspension of heat-treated and formolinised staphylococci.

Serotyping

Ten to 20 colonies of S.pneumoniae from a blood agar plate were suspended in 0.5ml PBS. Tests were carried out on 76 mm X 26 mm glass slides by mixing 1ul of

bacterial suspension with 1ul of each of the three polyvalent reagents and followed by the appropriate antiserum pools. The type or group was then determined by testing with single type or group reagents appropriate to the pool which gave the positive reaction. A positive co-agglutination reaction was taken as visible clumping after rocking the slide to and fro for two minutes. Only strong reactions which appeared within two minutes were considered specific for pneumococcal capsular polysaccharide. In most instances positive reactions were detected in a few seconds.

From January 1985 typing within the thirteen serogroups (6,7,9,10,11,12,15,17,18,19,22,23 and 33) represented in the 23 valent pneumococcal vaccine was carried out with reagents prepared from factor sera and absorbed group antisera. The latter were prepared by absorption of group antisera with a strain of a type within the group. The type was determined by the results of the co-agglutination test on a strain tested with each factor serum or absorbed group serum for the group according to the typing scheme of Lund and Henrichsen (1978).

Other alpha-haemolytic streptococci

Optochin-sensitive (i.e. with a zone diameter greater than 18mm) alpha-haemolytic streptococci, which did not react in the co-agglutination test with polyvalent antisera, were tested with a co-agglutination reagent prepared from antiserum to pneumococcal C-polysaccharide.

Optochin-resistant (i.e. zone diameter less than 14mm) alpha- haemolytic streptococci, which reacted in the co-agglutination test with one of the polyvalent antisera, were tested in three ways.

1) for type-specific pneumococcal polysaccharide antigen by the co-agglutination test.

2) for streptococcal antigen of groups A,B,C,D,F and G (Streptex, Wellcome Diagnostics). Latex suspensions for streptococcal groups K and H were prepared from antisera from Wellcome Diagnostics in the laboratory in the same way as for pneumococcal latex preparation (see below, pages 128-129).

3) for pneumococcal C-polysaccharide antigen by the co-agglutination test.

The organisms were then identified biochemically using the API"Strep" system.

2.3.7. Methods for the detection of pneumococcal antigen in clinical material

1. Preparation of specimens

Specimens were first deproteinised by heating and centrifugation (2,500 rpm) : the supernatant fluid was then tested for pneumococcal antigen.

Blood culture broths

Approximately 1ml of broth, incubated for a minimum of 18 hours, was removed asceptically from the blood culture bottles and centrifuged after heating in a boiling water bath for 3-5 minutes.

Pathological body fluids

Pleural fluid, cerebrospinal fluid, aspirates and pus were centrifuged (2,500 rpm) for 10-15 minutes immediately after receipt in the laboratory. The supernatant was removed, heated in a boiling water bath for 3-5 minutes and clarified by centrifugation before use.

Tissue

The supernatant from tissue preparation was heated in a boiling water bath for 3-5 minutes before centrifugation for clarification.

Respiratory secretions -

Sputa, bronchial and tracheal aspirates were homogenised in an equal volume of "Sputolysin" (dithiothreitol, Behring). Approximately 1 ml of homogenate was heated in a boiling water bath for 3-5 minutes.

Urine

Specimens, deproteinised by heating in a boiling water bath for 3-5 minutes were tested before and after concentration with 95% ethanol as follows:

15ml of 95% ethanol, stored at -20 ^{0}C, was added to 5ml of centrifuged urine. The precipitate obtained by chilling for 2 hours at 4 ^{0}C, was removed by centrifugation and dissolved in 0.25 ml of PBS.

Serum

Samples were diluted with an equal volume of PBS and heated over a bunsen flame to coagulate the proteins.

After centrifugation, the supernatant fluid was tested.

2. Tests for pneumococcal antigen

A. Kronvall's co-agglutination test (CoA(K))

Specimens (with the exception of serum and urine) were tested with co-agglutination reagents prepared for typing S.pneumoniae strains (See pages 122-123). Tests were performed the same day as cultures were set up and results reported separately.

B. Counterimmunoelectrophoresis (CIE)

Two sets of glass slides (76mm X 51 mm) were coated with 8ml of agarose (Miles Laboratories) in barbitone buffer at either pH 8.2 or pH6.6. Slides were stored at 4^{o}C in a moist chamber and used within one week of preparation.

Parallel wells 3mm in diameter were cut 3mm apart; a 40 well pattern with 2 rows of 10 pairs of parallel wells was cut on each slide. 10ul of antigen was placed in each of the cathode wells and 10ul of pneumococcal antiserum was placed in each anode well. The slides were attached by filter paper wicks (Whatman No1) to the tank reservoir containing 200ml of barbitone buffer (pH 8.2 ionic strength 0.05). The tank was connected to a Shandon power pack and a constant current of 30 milliamps applied across the slides for 1 hour at room temperature. The slides were cooled at 4^{o}C before examination for precipitin bands using indirect

illumination.

Respiratory secretions , cerebrospinal fluid and serum were tested by CIE using type antisera. The individual antiserum used was determined from the results of co-agglutination tests carried out on the same material (or on different specimens taken at or about the same time) from the same patient.

Quantitation of pneumococcal antigen

Doubling dilutions of the material from 1:2 to 1:512 in PBS were tested with type-specific antisera used in CoA(K) and CIE to determine the titre of antigen present.

Other tests for the detection of pneumococcal antigen in serum, urine and cerebrospinal fluid

Four different preparations of pneumococcal antisera were used in tests for pneumococcal antigen in serum, urine (before and after concentration) and cerebrospinal fluid. These specimens were tested by Trollfor's co-agglutination (CoA(T)) and latex agglutination (LA). The pneumococcal antisera used were:

a) Omni serum.

b) Three polyvalent antisera prepared from pools A to I as previously described (page 122) and a polyvalent antiserum to the 23 types contained in the current pneumococcal vaccine.

c) Pools A to H.

d) Antisera to types 1,3,4,8,14,20 and to groups 6,7,9,10,11,12,15,17,18,19,22,23,33 and pneumococcal C-

polysaccharide.

C. Trollfor's co-agglutination test (CoA(T))

50ul of pneumococcal antiserum was added to 500ul of 10%(v/v) suspension of staphylococcal protein A. After mixing for one hour and centrifugation at 3,500 rpm for 10 minutes the supernatant was discarded. 500ul of filtered 2% solution of methylene blue was added to the antibody-coated staphylococci. After further mixing and centrifugation again, the staphylococci were resuspended in 2.5ml of PBS. 40ul of heat treated specimen was mixed with 40ul of reagent on disposable cards from Phadebact kits (Pharmacia Diagnositics). The agglutination reaction was read after 60 s.

The IgG fraction of pneumococcal antisera (i.e. a to d described on page 122) were prepared and used in the LA and antigen capture enzyme-linked immunosorbent assay (AC-ELISA).

Preparation of the IgG fraction of pneumococcal antisera

1ml of antiserum was mixed with 2ml of dilute acetate buffer pH 4.0 and repeatedly agitated at room temperature for 30 minutes. Octanoic acid (0.1 ml per ml of serum) was added drop by drop with thorough mixing. After 30 minutes at room temperature, the mixture was centrifuged (3,500 rpm for 15 minutes) and the supernatant retained. The supernatant fluid, containing the IgG fraction, was clarified by passing through a

0.45 um Millex filter unit (Millipore) and dialysed for 24 hours against two changes of PBS (2 litres each).

The titre of type-specific antibody in the IgG preparation was determined by the capsular reaction test against an appropriate suspension of S.pneumoniae.

D.Latex agglutination

The IgG fraction was diluted in glycine buffer saline (GBS pH 8.2) containing 0.2% (w/v) bovine serum albumin (BSA, Dade Diagnostics, Inc.) and 0.1% (w/v) sodium azide to the optimum dilution. The optimum dilution was established from the antibody titre in the capsular reaction test. Diluted IgG was mixed with an equal volume of 1% latex particles (0.17u diameter Polysciences Ltd) and incubated at 37 ^{0}C for 2 hours. The sensitised latex was centrifuged (3,500 rpm) for 15 minutes, the supernatant fluid removed and the latex resuspended in an equal volume of GBS containing 0.2% (w/v) BSA and 0.1% (w/v) sodium azide. 40ul of heat-treated specimen was mixed with 40ul of latex reagent on tiles supplied in the Wellcome Streptex kits (Wellcome Diagnostics). The agglutination reactions were read after 30 to 60 s.

Specimens were tested first with polyvalent reagents, then with appropriate pools and precise typing was carried out using the limited range of type antisera. However, when the type of S.pneumoniae had been recently established for a given patient either by culture of the specimen being examined or from other specimens taken at

or about the same time, the specimen was tested with the appropriate type reagent.

E. Antigen capture enzyme-linked immunosorbent assay (AC-ELISA)

Antigen detection, in serum by AC-ELISA, was carried out when S.pneumoniae or pneumococcal antigen had been serotyped from other clinical material. The IgG fraction of rabbit type or group pneumococcal antiserum was used to capture and detect specific pneumococcal antigen according to the method of De Jong (1983).

Flat bottom micro-elisa plates (Dynatech) were coated with the IgG fraction from pneumococcal antisera (diluted 1:1000 in 0.1 M sodium carbonate buffer pH 9.6) overnight at 4°C. The wells were rinsed three times in PBS containing 0.1% Tween 20 (wash buffer), 50ul of heated serum was added to the wells and the plate incubated at 37°C for 2 hours. Plates were washed 3 times in wash buffer and 50ul of antibody-enzyme conjugate, specific for the antigen being detected, were added: the plate was incubated at 37°C for 30 minutes. Antibody-enzyme conjugates comprised of IgG diluted 1:400 and urease conjugated anti-rabbit IgG diluted 1:50 in PBS. After incubation at 37°C for 90 minutes, an equal volume of rabbit IgG to Haemophilus influenzae type b diluted 1:20 in PBS was added to the mixture and incubated for a further 30 minutes. Plates were washed three times in wash buffer and three times with distilled water. 50ul of urease substrate was added to

each well and the plate sealed before incubation at 37°C for 2 hours. Plates were examined visually and a positive test was indicated when the substrate changed from yellow to purple.

Quantitation of pneumococcal antigen

Doubling dilutions of antigen positive specimens from 1:2 to 1:256 were prepared in PBS and tested by CoA(T), LA or AC-ELISA with reagents prepared from type antisera. The antigen concentration was determined from tests carried out in parallel using doubling dilutions of homologous pneumococcal capsular polysaccharide, at a concentration of 1 ug/ml and 100ng/ml, diluted in PBS from 1:2 to 1:32. The test procedures and the pneumococcal antisera used for the examination of different kinds of clinical material are summarised in Table 3.

2.3.8. Pneumococcal antibody determination

Antibody to pneumococcal C-polysaccharide, present in most human serum, was removed by absorption with a heavy suspension of the C-mutant strain.

Immunofluorescence assay (IFA)

Pneumococci used as antigen in IFA tests were prepared by resuspending 1ul of stock pneumococcal suspension in 500ul of FA buffer. 1ul loopfuls were placed in the wells of a PTFE coated glass slide (Hendley-Essex) and allowed to dry at room temperature. The bacterial cells were fixed to the glass slide by heating. 1ul loopfuls

Table3: Type of test and pneumococcal antisera used for the detection of pneumococcal antigen in body fluids, tissue and secretions.

Test/ Antisera	Blood culture	Respiratory secretion	Tissue	Fluids & Pus	CSF	Urine	Serum
Kronvall's co-agglutination (CoA(K))							
polyvalent antisera□	yes	yes	yes	yes	yes	no	no
pools (A to I)	yes	yes	yes	yes	yes	no	no
type/group	yes	yes	yes	yes	yes	no	no
factor sera	yes	yes	yes	yes	yes	no	no
Trollfor's co-agglutination (CoA(T)							
polyvalent antisera■					yes	yes	yes
pools (A to H)					yes	yes	yes
type/group▼					yes	yes	yes
Counterimmunoelectophoresis (CIE)							
type/group		yes			yes		yes
Latex agglutination (LA)							
polyvalent antisera■					yes	yes	yes
pools (A to H)					yes	yes	yes
type/group▼					yes	yes	yes
Antigen-capture enzyme-linked immunosorbent assay (AC-ELISA)							
type/group							yes

□= polyvalent antisera 1,2, and 3 from pools A to I.
■= polyvalent antisera 1,2, and 3 from pools A to I and antiserum reacting with the 23 types represented in the pneumococcal vaccine formulation.
▼= type antisera reacting with one of the 23 types in the pneumococcal vaccine formulation.

of patients' serum were overlaid onto the heat fixed bacteria and the slides incubated at 37°C for 30 minutes in a humid chamber. After rinsing in distilled water, the slide was washed for 10 minutes in two changes of FA buffer. The preparation was allowed to dry and 1ul loopful of a working dilution of fluorescein-conjugated anti-human immunoglobulin in FA buffer containing 2% tween 80 added. The slide was replaced in the humid chamber and incubated at 37°C for 30 minutes. After rinsing in distilled water the slide was washed in carbonate buffer for 10 minutes, allowed to dry and mounted in buffered glycerol. Slides were examined using a Leitz Dialux 20 microscope fitted with a 20 watt tungsten halogen lamp.

Determination of antibody concentration

The IFA was standardised using human reference serum. The level of antibody to type-specific pneumococcal capsular polysaccharides in the reference serum (pre-determined by G.Schiffman by standard radioimmunoassay) were recorded as nanograms of antibody nitrogen per ml serum (ng Ab N/ml serum). Antibody concentrations in patients' sera were determined from the results of antibody titration on doubling dilutions (1:2 to 1:64) of patients' sera compared to those of the reference serum tested simultaneously.

2.3.9. Examination of sputum samples

Specimens were assigned one of four broad groups on

the basis of macroscopic examination before homogenisation. The four groups were :

1. Purulent (P): pus amounting to more than two-thirds of the specimen.
2. Mucopurulent (MP): ranging from mucus with a trace of pus to specimens where pus amounted to one third of the sample.
3. Mucoid (M): mucus with neither pus or saliva.
4. Saliva (S): thin fluid specimens (but including those with a trace of mucus).

Microscopic assessment

10ul of homogenised sputum was mixed with 10ul of a solution of crystal violet (0.05%) on a glass slide. A coverslip was placed over the preparation which was examined microscopically (X 400 magnification) for the presence of macrophages, broncho-epithelial cells, pus cells and squamous epithelial cells. A semi-quantitative estimate of their numbers was recorded (+,++,+++).

Gram's stain

Smears were prepared from homogenised sputum and examined microscopically (X 1000 magnification) for Gram-positive lanceolate diplococci and evidence of oro-pharyngeal organisms. Smears were assessed as described below :

1. Positive : more than 10 pairs of Gram-positive lanceolate diplococci per oil immersion field with little or no evidence of oropharyngeal organisms.
2. Equivocal : equal numbers of Gram-positive lanceolate

diplococci (but only in numbers greater than 10 pairs per oil immersion field) and oropharyngeal organisms.

3. Negative : oropharyngeal organisms only or no organisms seen.

Quantitative culture

In addition to semi-quantitative culture and culture of undiluted sputa, quantitative culture was carried out on specimens which had either a positive Gram smear alone or a positive Gram smear together with a positive reaction with one of the three polyvalent pneumococcal co-agglutination reagents.

Ten-fold dilutions (10^{-1} to 10^{-6}) of homogenised sputum in sterile PBS, were plated onto blood agar and incubated anaerobically with 5-10% carbon dioxide for 18 hours at 37^{o}C. The bacterial count was determined from the highest dilution of homogenised sputum which showed growth of S.pneumoniae : the count was described as "pure" or "mixed" depending on the presence of any accompanying growth of recognised respiratory tract commensals (eg. alpha and non-haemolytic streptococci, coagulase negative staphylococci and diphthroid bacilli).

Inhibitory substances and beta-lactamase

Inhibitory substances were detected by pipetting 100ul of homogenised sputum into a well cut in a blood agar plate seeded with the Oxford Staphylococcus - after overnight incubation at 37^{o}C, a zone of no growth around the well indicated the presence of an inhibitory

substance in the sputum. Beta-lactamase was detected by placing a 25ug ampicillin disc approximately 20mm from the well and noting the growth of the Staphylococcus between the well and the ampicillin disc after overnight incubation at 37 ^{o}C.

Clinical correlation

The results of clinical examination of the patients chest and chest x-ray were noted at the time the sample was taken : information was obtained either from the case record or by consultation with the clinician responsible for the patient.

CHAPTER 3

RESULTS

3.1.TECHNIQUES FOR THE LABORATORY INVESTIGATION OF PNEUMOCOCCI AND PNEUMOCOCCAL INFECTION.

3.1.1.Serotyping:

Co-agglutination test for serotyping pneumococci

Pneumococcal antisera prepared in rabbits were examined for type-specific and cross-reacting pneumococcal antibodies. Tests were carried out using a suspension of pneumococci, diluted 1:100, which had been treated with formaldehyde and heat.

Antibody titres

A representative number of rabbit pneumococcal antisera were titrated by the standard capsular reaction and co-agglutination test on suspensions of homologous pneumococci. The results for types other than type 3, were 4 to 8 fold higher by the co-agglutination test (Table 4). Factor sera were prepared by absorption of type antisera with concentrated suspensions of appropriate heterologous types. Table 5 shows the results of the capsular reaction and co-agglutination test with factor sera carried out on standard strains of pneumococci. The reactions agreed with those reported in the typing scheme set out by Lund and Henrichsen (1978). The antibody titres of the prepared factor sera were 4 to 8 fold higher by the co-agglutination test than the capsular reaction test (Table 6).

Table 4:

Titres of pneumococcal antisera by capsular reaction and co-agglutination tests.

Antiserum	Titre* against homologous type	
	Capsular reaction test	Co-agglutination test
1	16	64
3	32	64
4	512	>2000
6A	8	32
6B	64	512
7F	64	512
7A	32	1024
7B	256	>2000
7C	64	512
8	32	256
9N	32	256
9A	128	>2000
9V	32	512
9L	16	256
12F	64	512
12A	128	>2000
14	128	1024
17F	128	1024
17A	128	>2000
18F	128	>2000
18A	32	512
18B	64	512
18C	64	1024
19F	4	32
19A	16	64
19C	8	128
20	8	128
23F	128	>2000
23A	32	512

* Titres expressed as the reciprocal of the highest dilution giving a positive reaction.

Table 5 :

Factor sera* tested with standard strains by capsular reaction and co-agglutination.

Antiserum	absorbed with type	factor	Standard types			
				6A	6B	
6A	6B	6b		+	-	
6B	6A	6c		-	+	
			7F	7A	7B	7C
7F	7B	7b	+	+	-	-
7A	7F	7c	-	+	-	-
7B	7C	7e	-	-	+	-
7C	7B	7f	-	-	-	+
			9N	9A	9V	9L
9L	9A	9b	+	-	-	+
9A	9N	9c	-	+	+	+
9A	9L	9d	-	+	+	-
9N	9L	9e	+	-	-	-
9V	9A	9g	-	-	+	-
				12F	12A	
12F	12A	12b		+	-	
12A	12F	12c		-	+	
				17F	17A	
17F	17A	17b		+	-	
17A	17F	17c		-	+	
			18F	18A	18B	18C
18C	18B	18c	+	-	-	+
18A	18B	18d	-	+	-	-
18C	18F	18e	-	-	+	+
18F	18C	18f	+	-	-	-
			19F	19A	19B	19C
19F	19A	19b	+	-	-	-
19A	19F	19c	-	+	+	+
7B	-	7h	-	-	+	+
19C	19B	19f	-	-	-	+
			23F	23A	23B	
23F	23A	23b	+	-	+	
23A	23F	23c	-	+	-	
28	-	28	-	-	+	

Letters in upper case are used to indicate a pneumococcal type while those in lower case are used to indicate a factorial component of the antigenic structure according to the conventional nomenclature (Lund and Henrichsen, 1978).

* prepared by absorption from type antisera.

Table 6:

Titres of factor sera, prepared from type antisera, tested by capsular reaction and co-agglutination tests.

Factor serum	serotype of test suspension	Titre* Capsular reaction test	Co-agglutination test
6b	6A	2	8
6c	6B	undiluted	8
7b	7F	64	512
7b	7A	2	16
7c	7A	4	32
7e	7B	32	256
7f	7C	64	256
9b	9N	8	64
9b	9L	8	64
9c	9A	128	1024
9c	9V	128	1024
9c	9L	2	16
9d	9A	64	1024
9d	9V	128	1024
9e	9N	undiluted	8
9g	9V	undiluted	16
12b	12F	4	32
12c	12A	4	32
17b	17F	4	32
17c	17A	32	128
18c	18F	128	1024
18c	18C	128	1024
18d	18A	8	64
18e	18B	4	16
18e	18C	8	64
18f	18F	4	16
19b	19F	undiluted	8
19c	19A	4	16
19c	19B	nil	undiluted
19c	19C	nil	undiluted
19f	19C	32	128
23b	23F	16	128
23b	23B	16	128
23c	23A	16	128

* Titres are expressed as the reciprocal of the highest dilution giving a positive reaction.

note: factor sera were prepared for use in the co-agglutination test.

Cross-reactions of type antisera in co-agglutination tests with standard strains of pneumococci

A total of 36 type antisera were tested by co-agglutination against each of 68 standard strains of pneumococci to check for possible cross-reactions. Most antisera in fact showed these and the titre of reaction with homologous and cross-reacting serotype(s) are recorded in Table 7. With the exception of antisera to types 1,3,4 and 8, the remaining type sera showed cross-reactions with one or more heterologous types. However, in most cases the cross-reactions were restricted to types which shared an antigen with the type used to raise the antiserum and to types known to cross-react although no common antigen has been demonstrated. The cross-reactions observed with antisera to types 7C,9V,17A,18C,22F,22A,23F and 23A, which are underlined in Table 7, have not previously been reported. They were investigated by titration of each antiserum, after absorption, with homologous and heterologous types and the results are presented in detail in Table 8.

Table 7:

Cross-reactions of type antisera in co-agglutination tests with standard strains of pneumococci.

Antiserum(a)	Cross-reacting type (titre of reaction)
1 (32)	none
3 (64)	none
4 (1024)	none
6A (32)	6B(16)
6B (512)	6A(128)
7F (512)	7A(16),7B(2),7C(2)
7A (1024)	7F(512),7B(32),7C(4)
7B (256)	7F(16),7A(8),7C(64): 18F(4),18A(2),20(2),24F(4),33A(2)
7C (512)	7F(64),7A(32),7B(512), : 20(128),24F(8), 31(128),33F(128),33A(64),42(256)
8 (256)	none
9N (256)	9A(64),9V(16),9L(128)
9A (1024)	9N(256),9V(1024),9L(256)
9V (512)	9N(16),9A(256),9L(32):8(16)
9L (256)	9N(128),9A(64),9V(64)
10F (128)	10A(16)
10A (512)	10F(32): 21(32)
11A (512)	11F(32),11B(32),11C(64): 16F(64),16A(32)
11B (1024)	11F(256),11A(64),11C(1024):16A(4)
12F (512)	12A(512)
12A (1024)	12F(1024)
14 (1024)	15F(64)

15B (1024)	15F(32),15A(16),15C(256): 23A(32)
17F (1024)	17A(1024)
17A (>2000)	17F(1024) : 23A(16),41A(128)
18F (>2000)	18A(128),18B(128),18C(512): 7C(4),20(4),23F(4),23B(2)
18A (512)	18F(128),18B(64),18C(32) : 23F(4),23B(4),31(2)
18B (512)	18F(512),18B(512),18C(512) : 23F(8),23B(8),8(2),11A(2), 15A(2)16F(2),19A(2),21(2),22F(2)
18C (1024)	18F(1024),18A(256),18B(128) : 7C(32),9L(4)11A(8),11C(8),16F(8), 16A(8),20(16),21(4),22F(8), 33A(32),42(8)
19F (32)	19A(16),19B(4),19C(4)
19A (64)	19F(16),19B(8),19C(8)
19C (128)	19F(2),19A(8),19B(64): 7B(32),7C(4)20(32),21(32), 22F(32),24F(32),33A(16)
20(32)	2(4),31(2),33A(2),36(2),42(2)
22F (256)	22A(128) : 20(16),31(128),36(8)
22A (64)	22F(32) : 17F(4),31(16)
23F (>2000)	23A(128),23B(128) : 24A(16),32(64),41A(32)
23A (512)	23F(64), 23B(8) : 15F(4),15A(16),15B(32),15C(32), 22A(4),32(4),42(8)

(a)= titre of reaction with homologous type.

Types underlined indicate a cross-reaction not previously reported (Morch, 1943).

Table 8:

Investigation by absorption of cross-reactions not previously reported.

Antiserum	absorbed wth type	Test suspensions : titre of coagglutination reaction							
		7B	7C	20	24F	31	33F	33A	42
7C	C-mutant	512	512	128	8	128	128	64	256
7C	20	64	256	-	-	-	2	-	32
7C	24F	256	512	64	-	64	4	32	128
7C	31	128	256	32	4	-	4	-	64
7C	33F	128	512	32	-	64	-	16	64
7C	33A	128	512	16	4	4	4	-	64
7C	42	64	256	32	-	-	-	-	-
7C	7C	-	-	-	-	-	-	-	-

Antiserum	absorbed wth type	9V	8
9V	C-mutant	512	16
9V	9A(factor g)	16	-
9V	8	512	-
9V	9V	-	-

Antiserum	absorbed wth type	17F	17A	23A	41A
17A	C-mutant	512	1024	16	8
17A	23A	256	256	-	8
17A	41A	256	512	16	-
17A	17F (factor c)	-	128	-	4
17A	17A	-	-	-	-

		22F	22A	20	31	36
22F	C-mutant	64	64	8	32	-
22F	20	8	8	-	16	-
22F	31	16	16	-	-	-
22F	36	16	16	-	-	-
22F	22A(factor b)	8	-	-	8	-
22F	22F	-	-	-	-	-

		22F	22A	31	17F
22A	C-mutant	32	64	16	-
22A	31	16	16	-	-
22A	22F(factor c)	-	8	-	-
22A	22A	-	-	-	-

		23F	23A	23B	24A	32	41A
23F	C-mutant	1024	128	32	16	8	32
23F	24A	256	64	32	-	-	-
23F	32	1024	128	64	-	-	-
23F	41A	1024	128	32	8	16	-
23F	23A(factor b)	128	-	16	8	16	-
23F	23F	-	-	-	-	-	-

		23F	23A	23B	22A	32	42
23A	C-mutant	64	256	8	4	2	4
23A	22A	32	128	-	-	-	-
23A	32	32	128	-	-	-	-
23A	42	32	128	4	4	4	-
23A	23F	-	128	-	-	-	8
23A	23A	-	-	-	-	-	-

		18F	18A	18B	18C	7C	9L	11A
18C	C-mutant	512	32	64	256	16	4	4
18C	7C	512	32	16	256	-	-	-
18C	9L	512	32	4	256	8	-	-
18C	11A	256	16	8	128	8	-	-
18C	11C	256	16	16	256	8	-	-
18C	16F	128	16	16	256	8	-	-
18C	16A	256	16	16	256	8	-	-
18C	20	16	16	16	32	-	-	-
18C	21	256	64	16	512	8	-	2
18C	22F	256	64	64	512	8	-	-
18C	33A	256	64	64	512	8	-	-
18C	42	256	32	16	256	8		2
18C	18B(factor c)	1024	-	-	1024	32	-	-
18C	18F(factor e)	-	-	-	64	-	-	-
18C	18C	-	-	-	-	-	-	-

		11C	16F	16A	20	21	22F	33A	42
18C	C-mutant	4	4	4	32	4	4	32	8
18C	7C	-	4	-	32	-	-	4	-
18C	9L	4	8	-	32	-	-	16	-
18C	11A	-	-	-	32	4	-	8	2
18C	11C	-	4	-	32	4	-	8	4
18C	16F	-	-	-	32	4	-	8	2
18C	16A	-	-	-	32	4	-	8	2
18C	20	-	-	-	-	-	-	-	-
18C	21	2	16	2	32	-	-	8	2
18C	22F	-	-	-	32	-	-	-	-
18C	33A	4	8	-	32	4	-	-	-
18C	42	4	8	-	32	4	-	8	-
18C	18B(factor c)	-	-	-	-	-	-	32	16
18C	18F(factor e)	-	-	-	-	-	-	-	-
18C	18C	-	-	-	-	-	-	-	-

Serotyping of clinical isolates

The results of co-agglutination and capsular reaction test on 99 clinical isolates of S.pneumoniae are shown in Table 9. These strains, which depend on factor sera for differentiation, belonged to groups which are represented in the 23 valent pneumococcal vaccine. The capsular reaction test was carried out independently by Dr. J. Hendrichsen, using factor sera, at the Statens Seruminstitut, Copenhagen, Denmark. The co-agglutination test was carried out using factor sera prepared by absorption of type antisera (method 1) and group antisera after absorption with different types within the group (method 2). The results (Table 9) show a concordance with the capsular reaction of 100% for method 1 and 94% for method 2. However, group 9 antiserum (method 2) failed to distiniguish types 9A and 9V by co-agglutination.

Table 9:

Concordance of co-agglutination and capsular reaction in typing clinical isolates of *Streptococcus pneumoniae*.

TYPE	number of strains typed by co-agglutination test		number of strains confirmed by capsular reaction
	Method 1.	Method 2.	
6A	4	4	4
6B	12	12	12
7F	8	8	8
7C	2	2	2
9N	5	5	5
9A	2	unable to differentiate	2
9V	4	types 9A and 9V	4
10F	not done	2	2
10A	not done	1	1
11A	not done	2	2
11B	not done	2	2
12F	6	6	6
15F	not done	1	1
15A	not done	2	2
15B	not done	2	2
15C	not done	1	1
18F	1	1	1
18A	1	1	1
18C	6	6	6
19F	8	8	8
19A	6	6	6
19C	1	1	1
22F	not done	4	4
22A	not done	1	1
23F	9	9	9
23A	4	4	4
33F	not done	2	2
Total	79	93	99

Method 1=factor sera prepared from type-specific antisera

Method 2=factors remaining in group antisera after absorption with different types within a group

Analysis of the antigenic structure of selected strains of Streptococcus pneumoniae using the co-agglutination test.

The co-agglutination reactions given by certain selected strains of S.pneumoniae, tested with reagents prepared from group antisera after absorption with different types within the group, are shown in Table 10. These formed the typing scheme used to identify clinical isolates by co-agglutination method 2 (Table 9) and showed some unexpected and previously unreported results. For example, co-agglutination, carried out with group 7 antiserum absorbed with type 7A and tested against type 7F, showed evidence of an antigenic structure different from that reported by Lund and Henrichsen (1978). If the accepted antigenic formulae of types 7F (7a,7b) and 7A (7a,7b,7c) were correct, absorption of group 7 antiserum with type 7A should have depleted the serum of antibody which reacts with type 7F : however it did not do so. This anomaly was investigated, by co-agglutination, in tests with types 7F and 7A antisera. The results are recorded in detail in Table 11 :

Table 11a shows that absorption of type 7F antiserum with types 7A,7B or 7C did not completely deplete type 7F antiserum of homologous antibody. The type 7F antiserum absorbed with type 7B and type 7F antiserum absorbed with type 7C were further absorbed with type 7A and re-tested against types 7F and 7A (Table 11b).

These sera were now specific for type 7F and therefore confirmed that types 7F and 7A have a common antigen. Finally type 7A antiserum was tested against types 7F,7A,7B and 7C after absorption with each of the types (Table 11c). The results confirm that type 7A antiserum contained the expected factors (7a,7b and 7c) and therefore that those were contained on the type 7A strain used to prepare the antiserum. Table 10 also shows that individual serotypes within a serogroup are antigenically distinct. Thus, when a group antiserum, which is prepared in rabbits with a vaccine composed of all types within a group, is absorbed with a particular type, in most cases antibody remains which reacts in co-agglutination tests with the other types.

Table 10:

Co-agglutination reaction of group antiserum absorbed with individual types of pneumococci contained within the group (Smart and Henrichsen,1986).

Antiserum group	Serotype used for absorption	Co-agglutination reaction with standard types			
		6A	6B		
6	6A	-	+		
6	6B	+	-		
		7F	7A	7B	7C
7	7F	-	+	+	+
7	7A	+	-	+	+
7	7B	+	+	-	+
7	7C	+	+	+	-
		9N	9A	9V	9L
9	9N	-	+	+	+
9	9A	+	-	-	+
9	9V	+	-	-	+
9	9L	-	+	+	-
		10F	10A		
10	10F	-	+		
10	10A	+	-		
		11F	11A	11B	11C
11	11F	-	+	+	+
11	11A	+	-	+	+
11	11B	+	+	-	+
11	11C	+	+	+	-
		12F	12A		
12	12F	-	+		
12	12A	-	-		
		15F	15A	15B	15C
15	15F	-	+	+	+
15	15A	+	-	+	+
15	15B	+	+	-	+
15	15C	+	+	+	-
		17F	17A		
17	17F	-	-		
17	17A	+	-		

		18F	18A	18B	18C
18	18F	-	+	+	+
18	18A	+	-	+	+
18	18B	+	+	-	+
18	18C	+	+	-	-

		19F	19A	19B	19C
19	19F	-	+	+	+
19	19A	+	-	+	+
19	19B	+	+	-	+
19	19C	+	+	+	-

		22F	22A
22	22F	-	+
22	22A	+	-

		23F	23A	23B
23	23F	-	+	+
23	23A	+	-	+
23	23B	+	+	-

		33F	33A	33B	33C
33	33F	-	+	+	+
33	33A	-	-	+	+
33	33B	+	+	-	+
33	33C	+	+	+	-

Table 11:

Analysis of the antigenic structure of *Streptococcus pneumoniae* type 7F using the co-agglutination test.

11a Antiserum to type 7F (factors 7a,7b,)

Absorbed with type	factors	Standard strains 7F	7A	7B	7C
7F	nil	-	-	-	-
7A	nil	+	-	-	-
7B	7b	+	+	-	-
7C	7b	+	+	-	-

11b Antiserum to type 7F (factors 7a,7b,)

Absorbed with types	factors	Standard strains 7F	7A
7B and 7A	nil	+	-
7C and 7A	nil	+	-

11c Antiserum to type 7A(factors 7a,7b,7c)

Absorbed with type	factors	Standard strains 7F	7A	7B	7C
7F	7c	-	+	-	-
7A	nil	-	-	-	-
7B	7b,7c	+	+	-	-
7C	7b,7c	+	+	-	-

Antigenic formulae (Lund and Henrichsen,1978)

type 7F = 7a,7b
type 7A = 7a,7b,7c
type 7B = 7a,7d,7e,7h
type 7C = 7a,7d,7f,7g

Cross-reactions of pneumococcal antisera in co-agglutination tests with bacteria other than pneumococci

A number of different bacteria were found to cross-react in Kronvall co-agglutination tests with pneumococcal antisera. Table 12 shows the results of co-agglutination with pneumococcal typing sera carried out on 72 strains of Streptococcus milleri which contained different Lancefield group polysaccharide. The 42 strains which did not react with pneumococcal antisera were predominately Lancefield group F : conversely, 23 of 30 cross-reacting strains were also Lancefield group F. However, 14 of the 23 strains of Lancefield groups C or K reacted with pnemococcal antisera. Cross-reactions, were limited to 6 groups (25 strains) of pneumococcal typing sera and of 5 strains to antiserum to pneumococcal C-polysaccharide. Five of the 9 strains of S.milleri which reacted with group 7 antiserum also reacted with factor sera to give the reactions of pneumococcus type 7A - a rare serotype of S.pneumoniae. The other 4 of the 9 strains reacted with group 22 antiserum as well as group 7 antiserum but the cross-reaction was not specific for any of the types within groups 7 or 22. All the streptococcal strains which reacted with group 7 antiserum were Lancefield group F. Conversely, 14 strains which reacted in the co-agglutination test with pneumococcus group 10 antiserum, and including those which reacted with groups 10 and 35 antiserum, reacted with streptococcus

group K antiserum in latex agglutination tests, although 9 also reacted with streptococcus group F antiserum.

Other bacteria which cross-reacted in co-agglutination tests with pneumococcal antisera are shown in Tables 13 and 14. These were detected in the course of testing sputum cultures for pneumococci by co-agglutination. Table 13 records the details of cross-reactions with some strains of optochin-resistant alpha and non-haemolytic streptococci. The results show that 4 of 7 strains of S.mitis reacted in tests with antiserum to pneumococcal C-polysaccharide but not with any of the typing sera. All 6 strains of S.salivarius reacted with group 19 pneumococcal antiserum, whereas the strains of S.sanguis detected gave a range of cross-reactions with a variety of pneumococcal antisera. Table 14 details the cross-reactions with some strains of enterobacteriaceae isolated from sputa and shows that, with them too, many reacted with several pneumococcal antisera.

Table 12:

Pneumococcal cross-reactions with strains of *Streptococcus milleri* tested by co-agglutination.

Pneumococcal Cross-reaction	Lancefield group A	C	F	C&F	K	F&K	no group
not detected	1		36				5
type 7A			5				
group 7 & 22			4				
group 10					4	6	
group 10 & 35					1	3	
type 27		1	1				
C-polysaccharide		1		3		1	

Table 13:

Pneumococcal cross-reactions with some isolates of alpha and non-haemolytic respiratory streptococci by co-agglutination.

Pneumococcal cross-reaction	organism identification a	b	c	d	e
C-polysaccharide	4				
type 2			1		1
type 5	1				1
group 7			1		
group 10					1
type 16			2		
group 18			1		
group 19	1	6	1	2	1
type 21			2	1	
group 24			2		
group 35			2		
groups 10&35			2		
type 36	1				
group 41					1
group 42				2	
pool E *		1	1		

a= S.mitis : b= S.salivarius :c = S.sanguis

d= Lancefield Group D streptococci

e= Not identified by API profile

* These strains reacted in the co-agglutination test with Pool E (Statens Seruminstitut) but not in tests with appropriate type antisera.

Table 14:

Pneumococcal cross-reactions with some isolates of enterobacteriaceae in the co-agglutination test.

Pneumococcal cross-reaction	organism identification				
	a	b	c	d	e
type 1	1				
type 2	5	1			
type 3	3				
type 4			1		
group 9	3				
group 10					1
group 22			6	1	
group 32			1		
type 36	1				
group 41	1				

a = *E.coli*

b = *Klebsiella pneumoniae*

c = *Klebsiella aerogenes*

d = *Klebsiella oxytoca*

e = *Hafnia alvei*

3.1.2. Methods for the detection of pneumococcal capsular polysaccharide

Quantitative determination of purified pneumococcal capsular polysaccharide

Counterimmunoelectrophoresis (CIE), co-agglutination (CoA), latex agglutination (LA) and enzyme-linked immunosorbent assay (ELISA) were compared in assays of purified pneumococcal capsular polysaccharides. The sensitivity of each method was compared with different pneumococcal antisera and compared in tests with 21 pneumococcal capsular polysaccharides. The results are shown in Tables 15 to 20.

The results of CIE are shown in Table 15. Capsular polysaccharides of types 7F,14 and 33F could not be detected by this method but the other polysaccharides tested reacted at concentrations between 60 and 300 ng/ml. The sensitivity of the assay was the same regardless of the kind of antiserum used. Thus, Omni serum, which reacts with all 83 serotypes of S.pneumoniae, was as sensitive as type-specific antisera in CIE tests. The results of CoA(K), with a 10% staphylococcal suspension (Kronvall, 1973), are shown in Table 16. All polysaccharides tested were detected by this method at concentrations between 100 and 2,000 ng/ml. Omni serum was less sensitive than other kinds of antiserum in the CoA(K) test. For example, only four (types 1,2,3, and 8) were detected at a concentration of

300 ng/ml or less, whereas polyvalent, pools and type antisera detected totals of 10,18 and 17 polysaccharides respectively. However, the CoA(T) test which used a 2% staphylococcal suspension (Trollfors et al., 1983) was more sensitive (Table 17) and detected polysaccharides at concentrations between 20 and 500 ng/ml. The results of LA, which are shown in Table 18, indicate that LA has a similar level of sensitivity to CoA(T). A summary to compare the sensitivity of the three methods (CIE, CoA and LA) together with the different kinds of antiserum used are shown in Table 19. The results are expressed as the number of different polysaccharides detected by each method at different threshold concentrations i.e. 300, 100 and 20 ng/ml. It can be seen that the sensitivity of methods for the detection of pneumococcal capsular polysaccharide varied with the different antisera and the way in which they were used. LA and CoA(T) (i.e. with a 2% staphylococcal suspension) were more sensitive than CIE: CoA(K) (using a 10% staphylococcal suspension) on the other hand, was the least sensitive method. Omni serum and polyvalent serum were as sensitive as pools and type-specific antisera in CIE, CoA(T) and LA tests when the concentration of polysaccharides was greater than 100 ng/ml : Types 7F,14 and 33F were not detected by CIE. However pools and type-specific antisera detected a larger number of polysaccharides when the concentration of polysaccharide was less than 100 ng/ml.

Table 15:

Sensitivity of counterimmunoelectrophoresis (CIE).

Detection of purified pneumococcal capsular polysaccharide by different antisera.

Polysaccharide concentration ng/ml	Antiserum			
	Omni	polyvalent	pools	type
	serotype of capsular polysaccharide			
60			3	3
80	1,3,	1,3,	1	5
100	2,5,6B,8, 10A,15B,18C, 22F,23F	2,5,6B,8, 15B,18C	2,5,6B,8, 15B,18C	1,2,6B,8 11A,15B, 18C,20
200	4,9N,11A, 12F,17F, 19F,20	4,9N,10A, 17F,19F 20,22F,23F	4,9N,10A, 12F,19F, 20,22F,23F	4,9N,10A, 12F,19F, 22F,23F
300		11A,12F	11A,17F	17F
not detectable at 1000 ng/ml	7F,14,33F	7F,14,33F	7F,14,33F	7F,14,33F

Omni serum (Statens Seruminstitut, Copenhagen) reacts with 83 serotypes of S.pneumoniae.

Polyvalent serum reacts with 23 serotypes of S.pneumoniae represented in the 23 valent pneumococcal vaccine.

Pools The different polysaccharides were tested in groups against the appropriate pool as shown below :

Types 1,2,4,5,18C - pool A.
Types 3,6B,8,19F - pool B.
Types 7F,20 - pool C.
Types 9N,11A - pool D.
Types 10A,12F,33F - pool E.
Types 17F,22F - pool F.
Types 14,15B,23F - pool H.

Type was homologous type-specific antiserum.

Table 16:

Sensitivity of co-agglutination (Kronvall,1973)- CoA(K).

Detection of purified pneumococcal capsular polysaccharide by different antisera.

Polysaccharide concentration	Antiserum*			
ng/ml	Omni	polyvalent	pools	type
	serotype of capsular polysaccharide			
100		2,5,18C	1,2,3,4, 5,8,18C	1,2,3,5, 6B,8
200	1,2,3	1,3,6B,8, 19F	6B,7F,12F, 15B,19F,20, 22F,23F	12F,15B, 20, 22F, 33F
300	8	4,14	9N,14,23F	4,11A, 17F,18C, 19F,23F
400	5,20	12F,20	11A	7F,9N,14
500	6B,7F,11A, 15B,18C, 19F,22F	15B,22F		
1000	4,9N,10A, 14,23F,33F	7F,9N,10A, 11A,33F	10A	10A
2000	12F,17F	17F,23F	17F	

* used as in Table 15.

Table 17:

Sensitivity of co-agglutination (Trollfors et al., 1983)-CoA(T).

Detection of purified pneumococcal capsular polysaccharide by different antisera.

Polysaccharide concentration	Antiserum*			
ng/ml	Omni	polyvalent	pools	type
	serotype of capsular polysaccharide			
20		2,3	1,2,3,6B 12F,18C	2,3,12F
40	3	1,5,8	4,5,7F, 8,14,19F, 22F,33F	5,6B,7F, 8,11A,14, 20,22F,23F
60	1,2,5, 18C,15B	4,6B,7F, 14,15B,19F	9N,11A, 15B,20	1,4,15B, 17F,18C,19F
80	11A,20	12F,18C	23F	9N,33F
100	4,8,14, 19F	11A,20,33F		
200	6B,7F,9N, 10A,22F, 23F,33F	9N,10A,22F	10A,17F	10A
300	12F			
400	17F			
500		23F,17F		

* used as in Table 15.

Table 18:

Sensitivity of latex agglutination (LA).

Detection of purified pneumococcal capsular polysaccharide by different antisera.

Polysaccharide concentration	Antiserum*			
ng/ml	Omni	polyvalent	pools	type
	serotype of capsular polysaccharide			
20		2,3,18C,20	2,3,6B,9N, 12F,18C, 19F	2,3,6B, 12F,14, 23F
40	2,3,20, 22F,23F	1,4,5,7F, 8,14,17F, 19F,22F, 23F	1,4,5,7F 8,11A,14, 15B,17F, 20,22F, 23F,33F	4,5,7F,8 9N,11A,, 15B,17F, 19F,22F, 33F
60	1,5,15B	15B		1,18C,20
80	11A,18C	11A,12F		
100	4,8,19F	6B,9N		
200	6B,7F, 10A,14, 17F	10A,33F	10A	10A
300	9N,12F, 33F			

* used as in Table 15.

Table 19:

Summary of results in Tables 15-18:

Comparative sensitivity of counterimmunoelectrophoresis (CIE), co-agglutination (CoA) and latex agglutination (LA) in tests with different kinds of pneumococcal antisera.

Total number of capsular polysaccharides detected at a concentration of 300 ng/ml

	Antiserum*			
	Omni	polyvalent	pools	type
CIE	18	18	18	18
CoA(K)	4	10	18	17
CoA(T)	19	19	21	21
LA	21	21	21	21

Total number of capsular polysaccharides detected at a concentration of 100 ng/ml

	Antiserum*			
	Omni	polyvalent	pools	type
CIE	11	8	8	10
CoA(K)	nil	3	7	6
CoA(T)	12	16	19	20
LA	13	19	20	20

Total number of capsular polysaccharides detected at a concentration of 20 ng/ml

	Antiserum*			
	Omni	polyvalent	pools	type
CIE	nil	nil	nil	nil
CoA(K)	nil	nil	nil	nil
CoA(T)	nil	2	6	3
LA	nil	4	7	6

* used as in Table 15.

The results of ELISA tests are shown in Table 20. Omni serum and the polyvalent serum did not react in ELISA tests with concentrations of pneumococcal capsular polysaccharides less than 50 ng/ml. Antiserum pools detected concentrations of 20 ng/ml or less with 18 of 21 polysaccharides and 14 of these were detected at a concentration of 10 ng/ml or less. Similarly, type-specific antisera detected 17 of 18 polysaccharides at 20 ng/ml and in the case of 15 of these, at a concentration of 10 ng/ml or less.

Table 20:

Sensitivity of enzyme-linked immunosorbent assay.

Detection of purified pneumococcal capsular polysaccharide by different antisera.

Polysaccharide concentration	Antiserum*		
ng/ml	Omni	polyvalent pools	type
	serotype of capsular polysaccharide		
1-5		1,2,3,4,5 6B,12F,15B, 18C	1,3,4,6B,7F 8,15B,18C, 19F 20,23F
5-10		7F,9N,11A, 14,18C,19F	12F,14,17F, 22F,33F
10-20		8,17F,20,22F	9N,11A
20-50		10A,23F,33F	10A
	NR	NR	types 2&5 not tested

NR= no reaction at a concentration of less than 50 ng/ml

* used as in Table 15.

Quantitation of pneumococcal capsular polysaccharide and viable bacteria in broth cultures of Streptococcus pneumoniae.

Strains of S.pneumoniae, which represented 8 different types, were inoculated into blood culture bottles (Bactec 6B). Five ml of pneumococci diluted, in defibrinated sheep blood, to a concentration of 20 cfu/ml was used as the inoculum. Broths were incubated at 37°C on a metabolic shaker and sampled each hour for ten hours. Samples were tested for total viable count (Miles and Misra) and by latex agglutination for pneumococcal capsular antigen. Antigen concentration was determined from the results of latex agglutination tests carried out on doubling dilutions of the broth supernatant and 1 ug/ml concentrations of purified pneumococcal capsular polysaccharide, of the same type, tested in parallel. Table 21 shows the number of viable pneumococci when the concentration of antigen in the broth was 1 ug/ml. The results show that between 10^5 and 10^9 cfu/ml of viable pneumococci were required to produce 1 ug/ml of free capsular polysaccharide in the growth medium and that this depended on the capsular type of the pneumococcus.

Table 21:

Quantitation of pneumococcal capsular polysaccharide in relation to the number of viable bacteria in broth cultures of selected strains of Streptococcus pneumoniae.

Pneumococcal type	Numbers of viable bacteria (cfu/ml) in broth cultures containing 1ug/ml of pneumococcal polysaccharide*
1	7×10^6 cfu/ml
3	5×10^5 cfu/ml
4	1×10^7 cfu/ml
5	4.5×10^8 cfu/ml
6B	5×10^8 cfu/ml
7F	2.5×10^7 cfu/ml
8	1×10^8 cfu/ml
10A	7×10^8 cfu/ml

* Determined by Miles and Misra method.

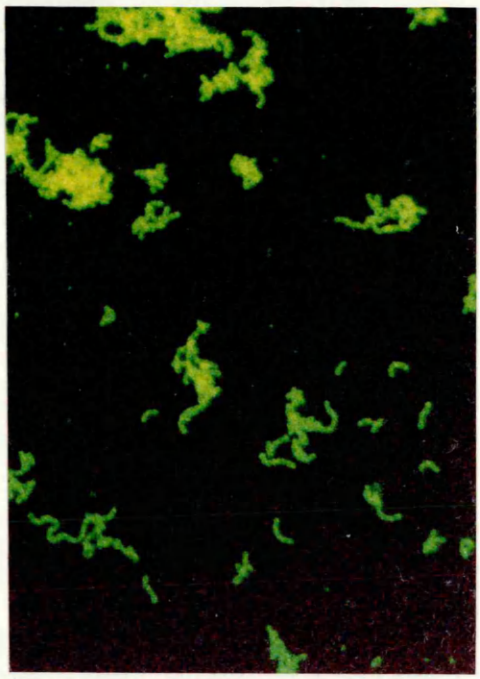
3.1.3. Determination of pneumococcal antibodies by indirect immunofluorescence assay

Indirect immunofluorescence assay (IFA) was used to measure pneumococcal antibodies in human sera. Preliminary studies, showed that most human sera contained antibody to pneumococcal C-polysaccharide. Figure VI shows the extent of fluorescence obtained when pooled normal human serum, diluted 1:2, 1:8, 1:32 and 1:128 was tested with a suspension of a mutant strain of S.pneumoniae (CSR,SC S-2 clone 1) that produces a capsule composed of C-polysaccharide. Figure VII shows the degree of fluorescence obtained in equivalent tests with a suspension of S.pneumoniae type 8. However, after the serum was absorbed with a suspension of the C-mutant strain, the level of fluorescence with the type 8 strain was reduced (Fig. VIII). Differences in the extent of fluorescence obtained with pooled human reference serum, undiluted and after dilution 1:2, 1:4 and 1:8 in tests with S.pneumoniae types 9N and 8 are shown in Figures IX and X respectively. These tests were carried out after the serum had been absorbed with the C-mutant strain and show that the intensity of peripheral fluorescence of the bacteria was proportional to the antibody content of the serum. This demonstrates that the IFA test can distinguish between antibody to pneumococcal C-polysaccharide and antibody to pneumococcal capsular

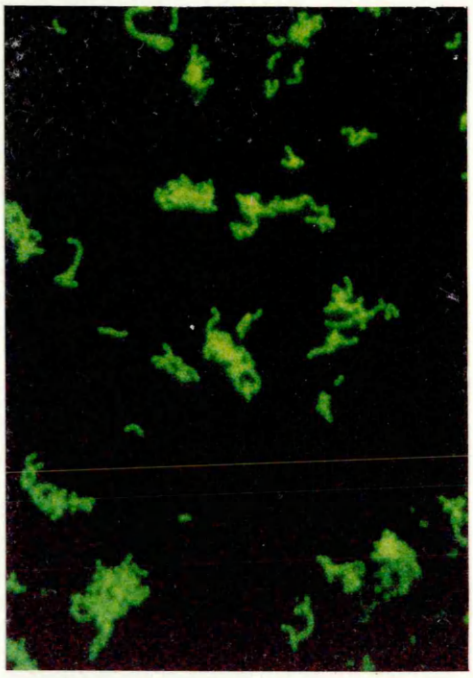
Figure VI:

Fluorescence obtained with normal pooled human serum tested with a suspension of a mutant strain of Streptococcus pneumoniae (CSR,SC S-2 clone 1) that produces a capsule composed of C-polysaccharide.

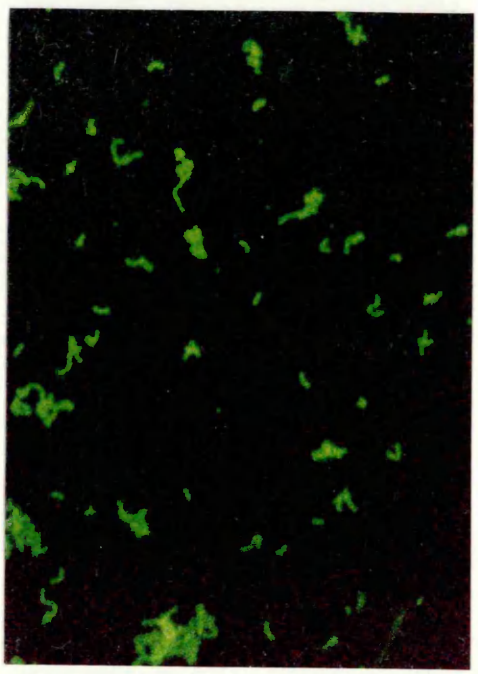
Diluted 1:2



Diluted 1:8



Diluted 1:32



Diluted 1:128

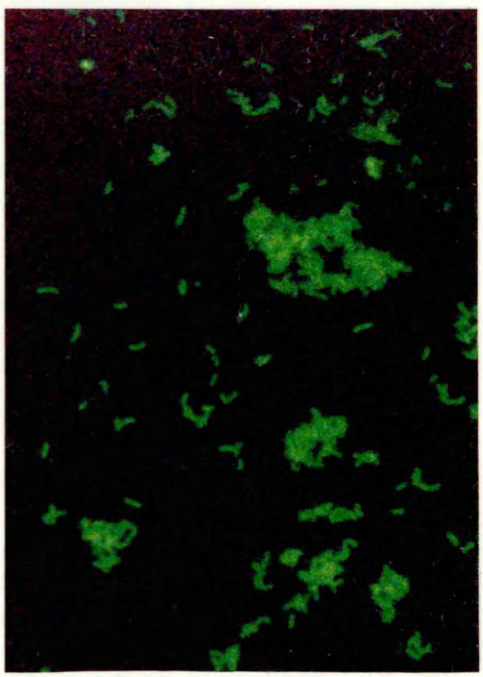
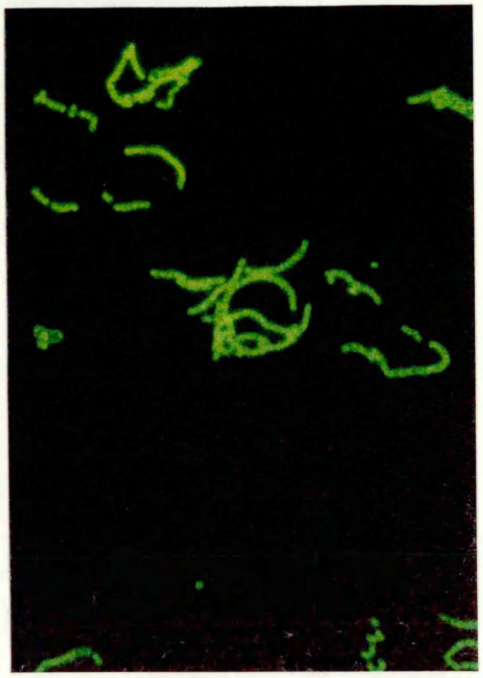


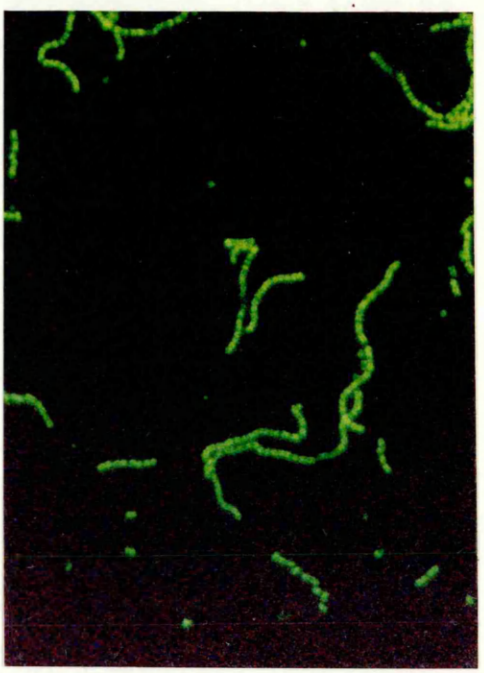
Figure VII:

Fluorescence obtained with normal pooled human serum tested with a suspension of Streptococcus pneumoniae type 8.

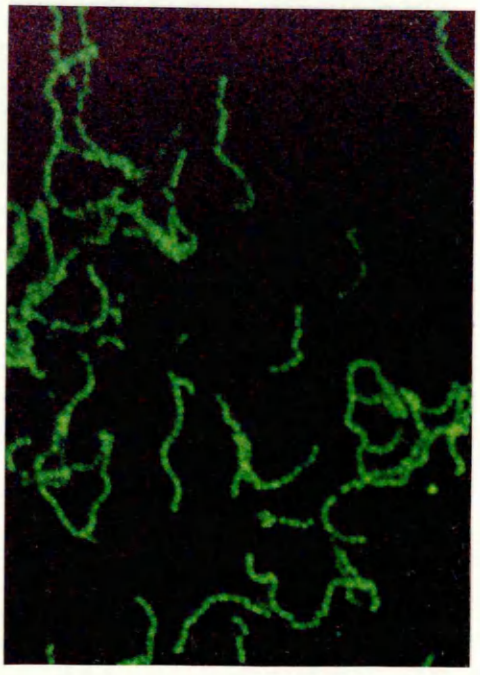
Diluted 1:2



Diluted 1:8



Diluted 1:32



Diluted 1:128

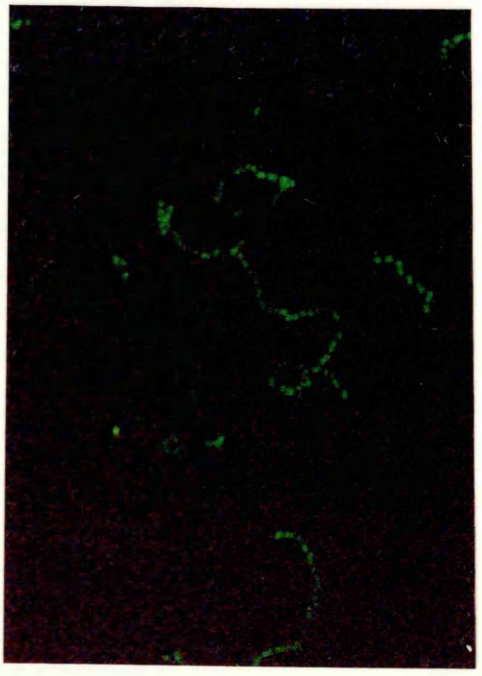
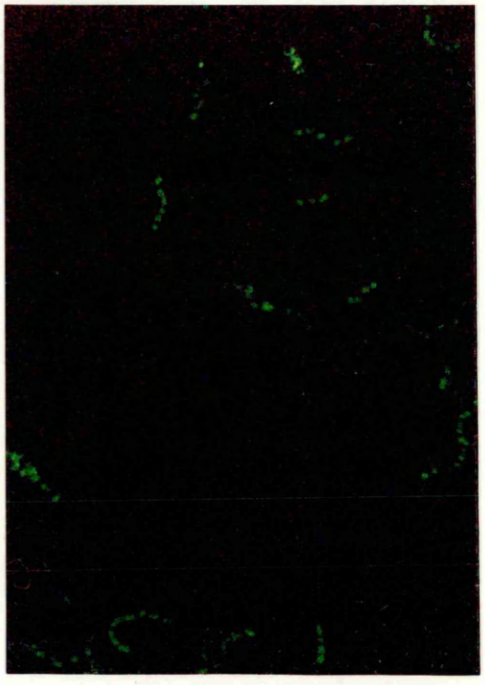


Figure VIII:

Fluorescence obtained with normal pooled human serum tested with a suspension of Streptococcus pneumoniae type 8 after absorption with a suspension of the C-mutant strain.

Diluted 1:2



Diluted 1:8

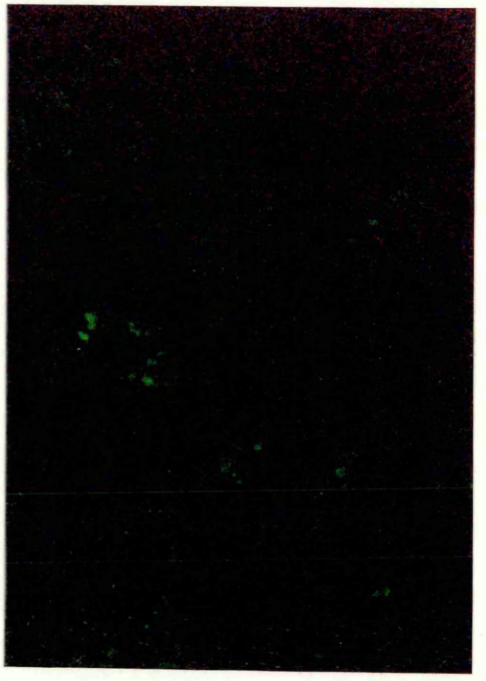
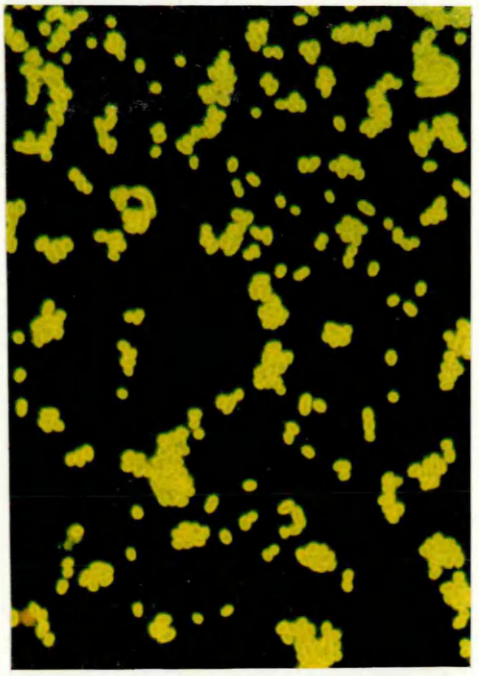


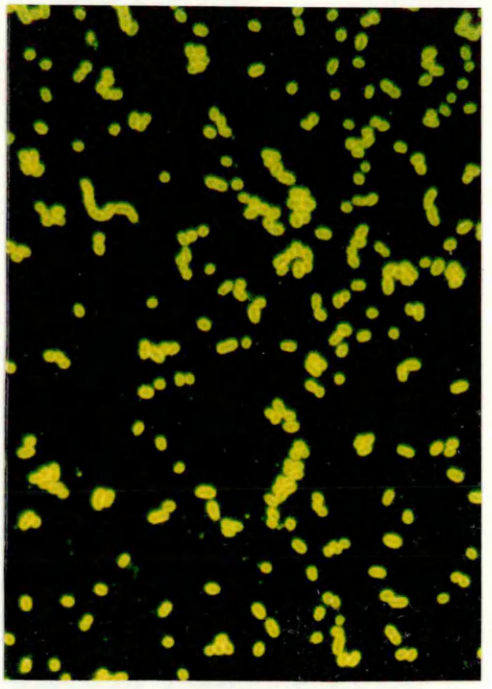
Figure IX:

Fluorescence obtained with pooled human reference serum tested with a suspension of Streptococcus pneumoniae type 9N after absorption with a suspension of the C-mutant strain.

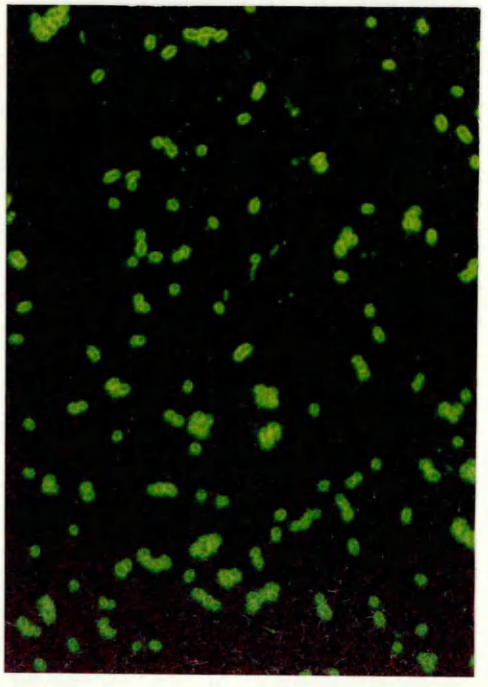
Undiluted



Diluted 1:2



Diluted 1:4



Diluted 1:8

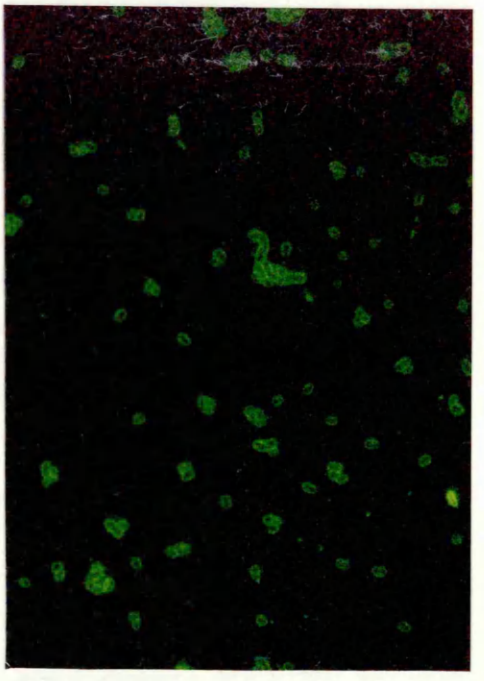
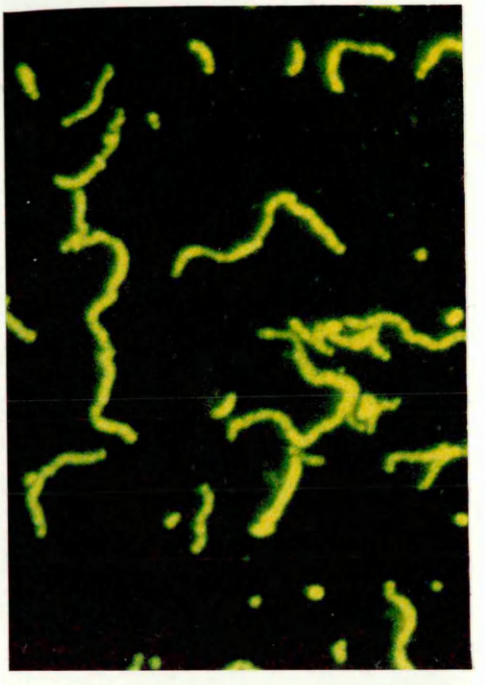


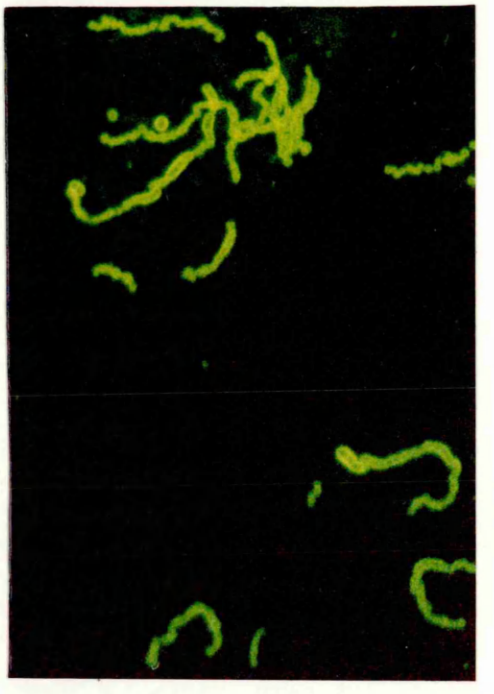
Figure X:

Fluorescence obtained with pooled human reference serum tested with a suspension of Streptococcus pneumoniae type 8 after absorption with a suspension of the C-mutant strain.

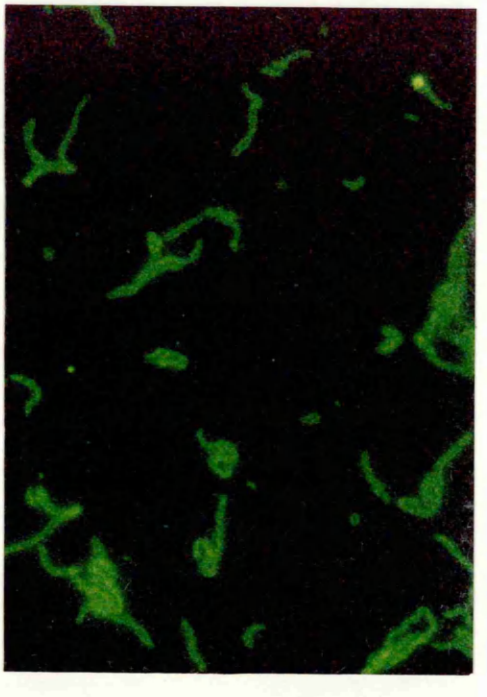
Undiluted



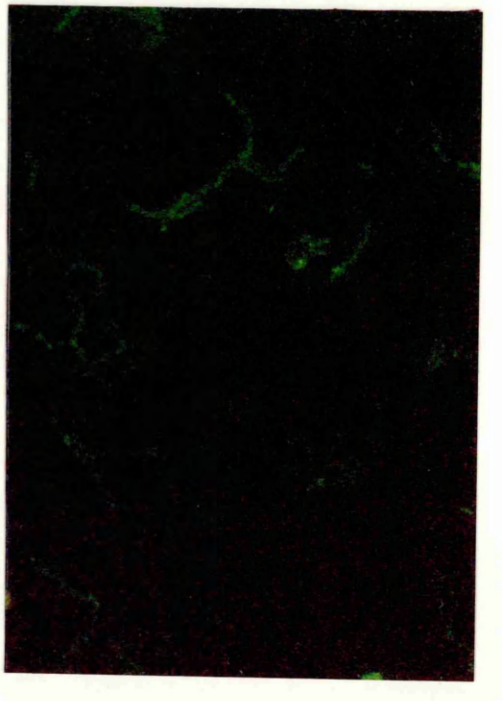
Diluted 1:2



Diluted 1:4



Diluted 1:8



polysaccharide. Thus, type-specific antibody reacts with the capsular polysaccharide material with fluorescence located at the periphery of the bacterial cell. In contrast antibody to pneumococcal C-polysaccharide, which is species-specific rather than type-specific, is located on the cell wall and results in staining of the whole bacterium. Although IFA distinguished between type-specific antibody and antibody to pneumococcal C-polysaccharide, the extent to which pneumococcal C-polysaccharide antibody affects the detection of low levels of type-specific antibody could not be predicted. Therefore, in clinical studies, all sera were absorbed with the C-mutant strain before titration of type-specific pneumococcal antibody.

A representative number of rabbit pneumococcal antisera were titrated against suspensions of homologous pneumococci by the standard capsular reaction test and immunofluorescence. Antibody titres by the immunofluorescence assay ranged from 100 to 2000 times greater than by the capsular reaction test (Table 22).

In tests carried out on human pneumococcal reference serum, the antibody titres by immunofluorescence, for types other than 1 and 12F, correlated with the results of radioimmunoassay (Table 23).

Table 22:

Comparison of titres of pneumococcal antisera by capsular reaction test (CRT) and indirect immunofluorescence assay. (IFA)

Antiserum	CRT	IFA
	Titre* against homologous type	
1	16	32,000
3	32	32,000
4	512	64,000
6B	64	64,000
7F	64	>64,000
8	32	8,000
9N	32	2,000
9V	32	16,000
10	64	64,000
11A	64	16,000
12F	64	>64,000
14	128	32,000
15B	128	16,000
17F	128	32,000
18C	64	16,000
19F	4	4,000
19A	16	8,000
20	8	2,000
22F	16	4,000
23F	128	32,000
33F	32	16,000

* Titres expressed as the reciprocal of the highest dilution giving a positive reaction with a suspension of homologous type which had been treated with formaldehyde and heat.

Table 23:

Antibody levels of human pneumococcal reference serum carried out on five occasions : Comparison of radioimmunoassay (RIA) and indirect immunofluoresence assay (IFA).

Pneumococcal type	RIA* (ng Ab N/ml serum)	Geometric mean IFA titre$ (range)
1	1472	4.6 (4-8)
2	1170	13.9 (8-16)
3	461	2.0 (undiluted-4)
4	2405	13.9 (8-32)
5	2873	48.4 (32-64)
6B	1338	16.0
7F	512	4.6 (4-8)
8	1419	8.0
9N	973	9.3 (4-16)
9V	1160	7.0 (4-8)
10A	2715	27.8 (16-32)
11A	2152	10.5 (8-16)
12F	2544	3.0 (2-4)
14	1144	10.5 (4-16)
15B	1960	27.5 (16-32)
17F	1848	32
18C	1944	18.4 (8-32)
19F	429	4
19A	1831	21.0 (16-32)
20	1527	27.8 (16-32)
22F	2421	32
23F	1038	5.3 (4-8)
33F	2352	16.0 (8-32)

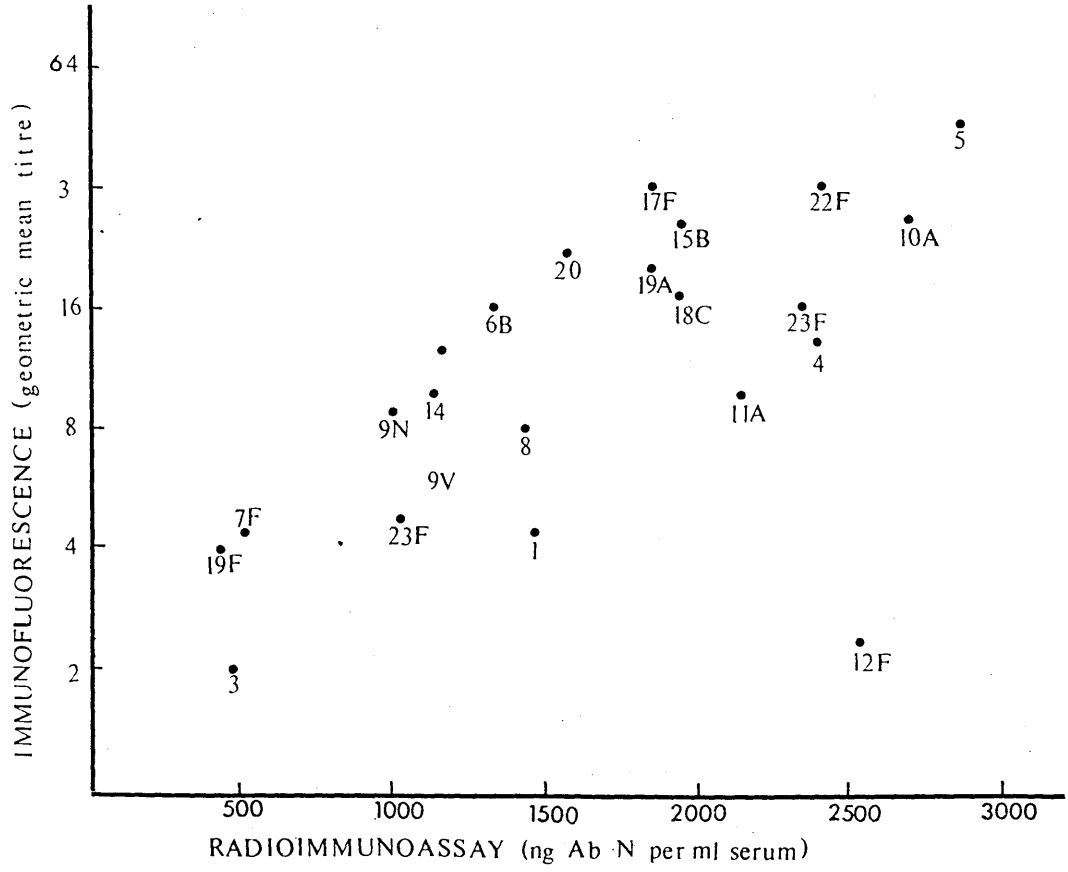
* Radioimmunoassay performed by the laboratory of G. Schiffman 25/3/85.

$ Immunofluorescence titre is the reciprocal of the highest dilution of the serum giving a one plus peripheral fluorescence with the homologous pneumococcal type which had been treated with formaldehyde and heat.

The scatter diagram (Figure XI) confirms the correlation between IFA and RIA and that the IFA showed a dose-response curve. For example, an IFA titre of 1:4 represented approximately 500 ng Ab N/ml, whereas titres between 1:4 and 1:16 corresponded to levels of 1,000 to 1,500 ng Ab N/ml. Titres above 1:16 generally indicated an antibody level greater than 1,500 ng Ab N/ml. However, the sensitivity of the IFA varied with serotype.

Figure XI:

Scatter diagram showing the correlation between immunofluorescent assay and radioimmunoassay.



3.2. METHODS FOR THE LABORATORY EXAMINATION OF SPUTUM.

3.2.1 Visual examination

Specimen evaluation

Cellular material in 407 specimens of sputum was assessed macroscopically and microscopically. The latter was carried out on films of homogenised sputum stained with 0.05% crystal violet and Gram's stain. The microscopic appearance of a number of different cell types which were stained with 0.05% crystal violet are illustrated in Figures XII to XVII. Cells which originated from the respiratory tract were stained with crystal violet and were identified by their morphological characteristics. However, pus cells and red blood cells were not stained by crystal violet at the concentration used. Thus, reaction with crystal violet stain distinguished broncho-pulmonary and pus cells. Specimens of sputum were categorised (groups 1 to 6) subject to the numbers and type of cells seen in films stained with crystal violet. The scatter diagrams (Figures XVIII and XIX) show the correlation between macroscopic and Gram film assessment compared with assessment of crystal violet stained films. These show the general trend but clearly demonstrate that within certain crystal violet groups there is poor correlation.

The details of subgroups are defined in Tables 24 and 25. Consequently, 91 specimens were purulent (groups 1 and 2), 188 contained a preponderance of broncho-

Figure XII:

A pulmonary macrophage stained with 0.05% crystal violet.

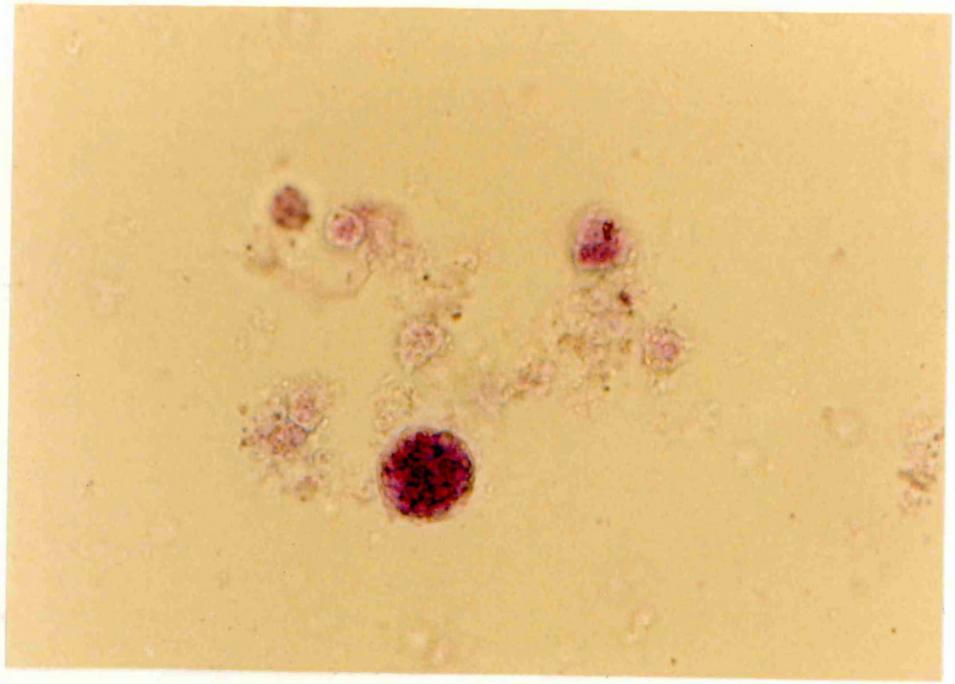


Figure XIII:

Squamous epithelial cells stained with 0.05% crystal violet.

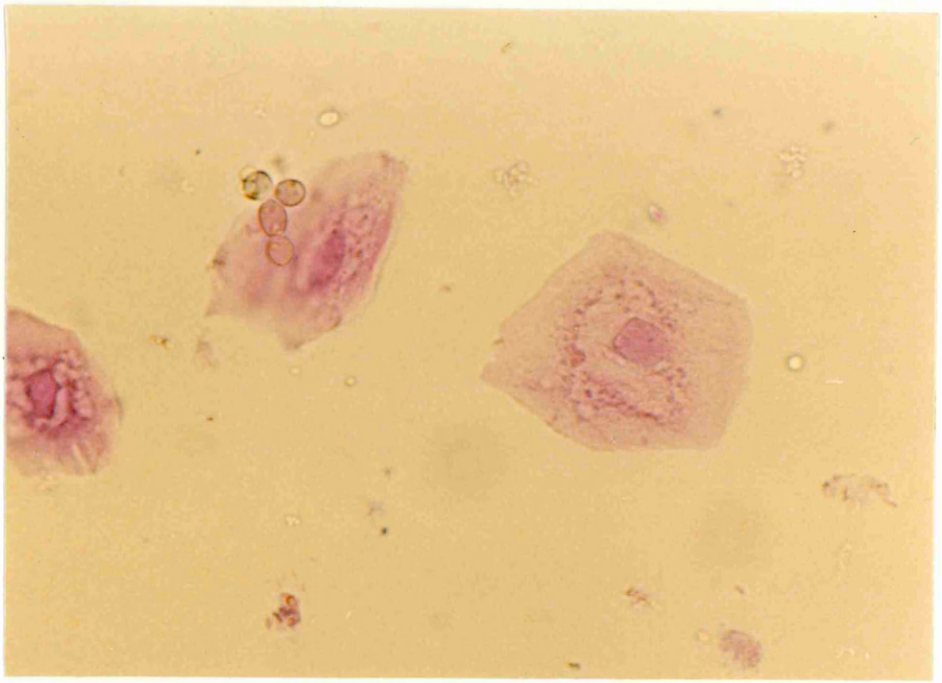


Figure XIV:

Bronchial epithelial cells stained with 0.05% crystal violet.

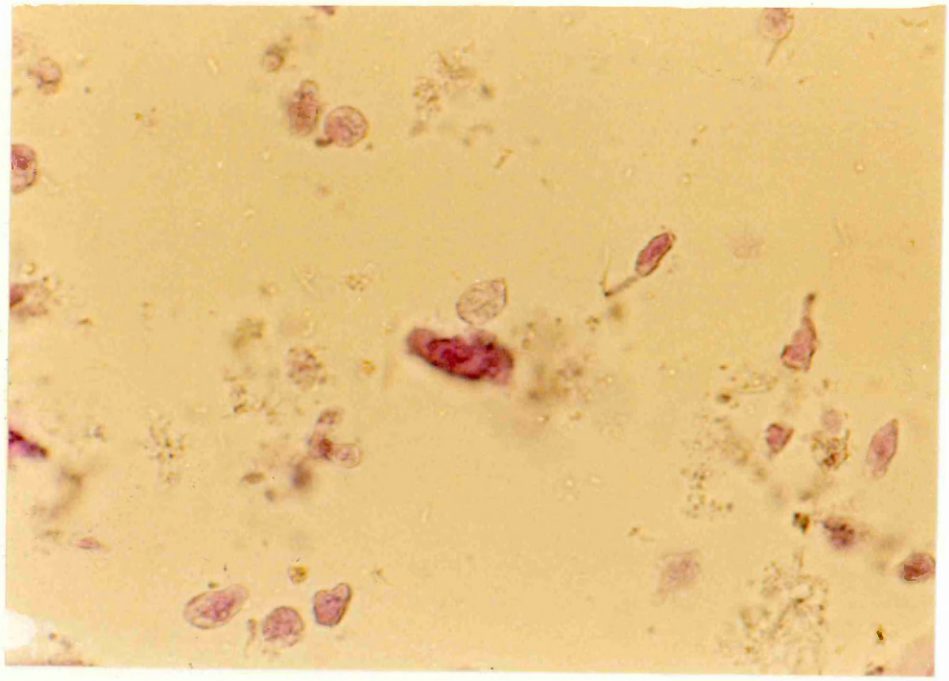


Figure XV:

Pus cells in sputum showing no reaction with 0.05% crystal violet.

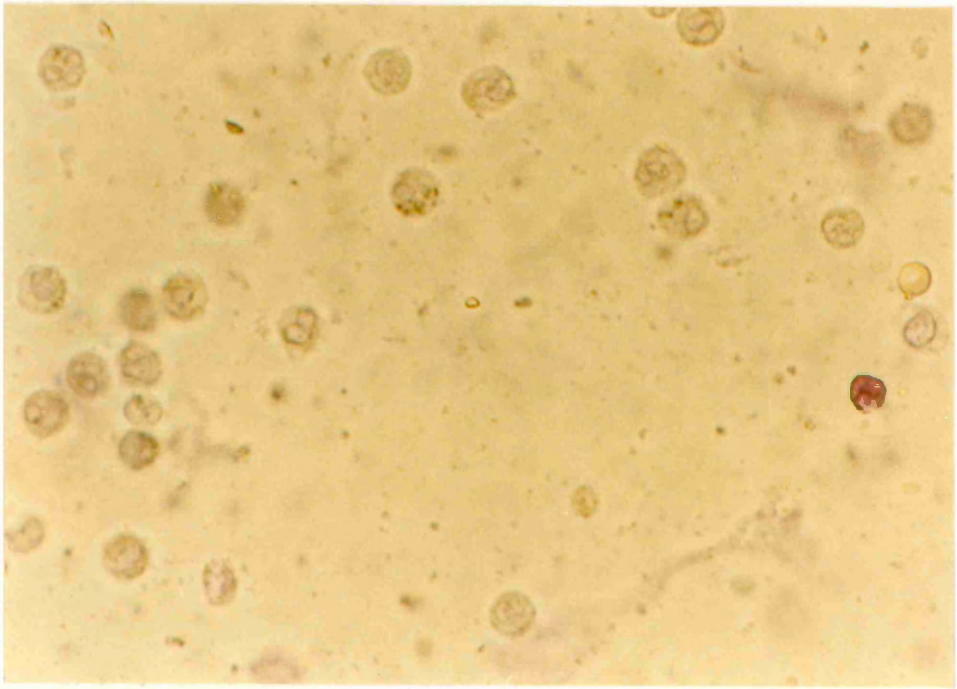


Figure XVI:

Differential staining of broncho-pulmonary cells and pus cells with 0.05% crystal violet.

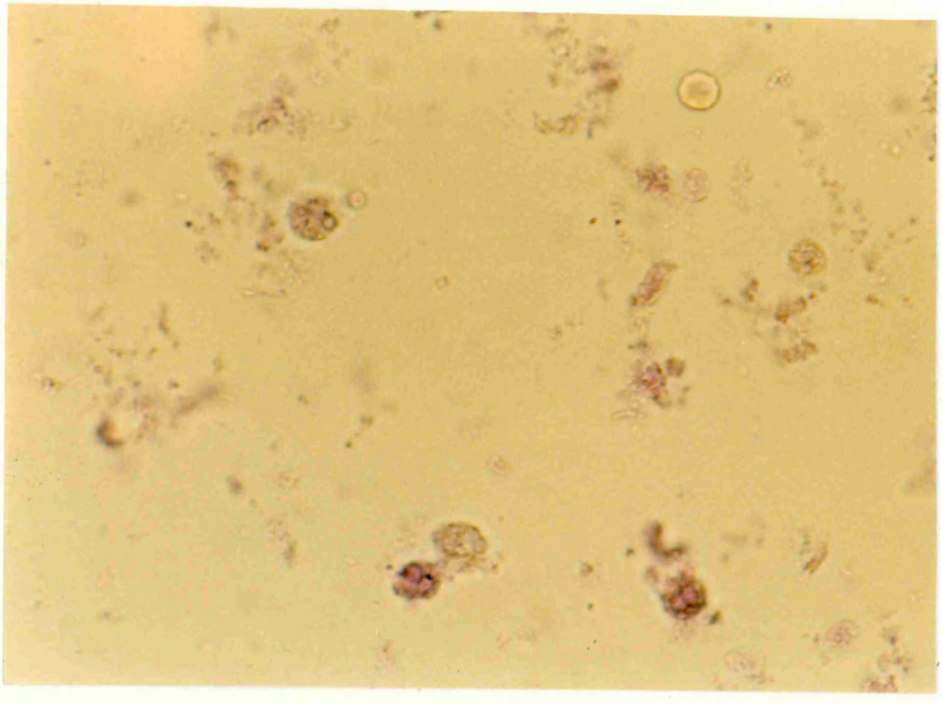


Figure XVII:

Crystal violet stain showing a sputum which is heavily contaminated with oropharyngeal material.

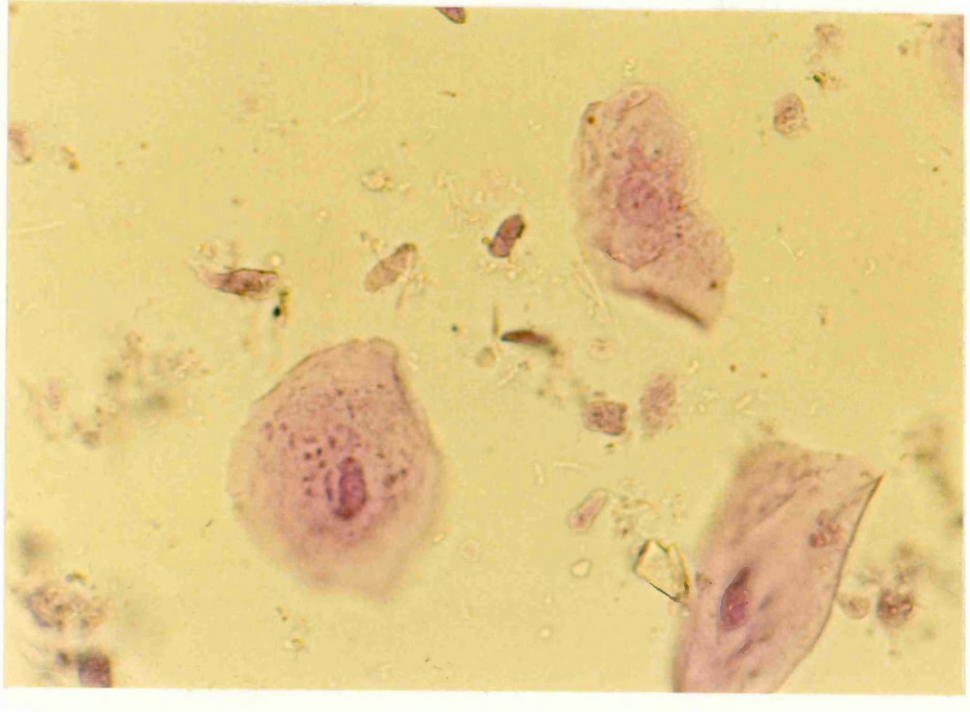


Figure XIX:

Correlation between Gram film and crystal violet microscopic assessments of sputum.

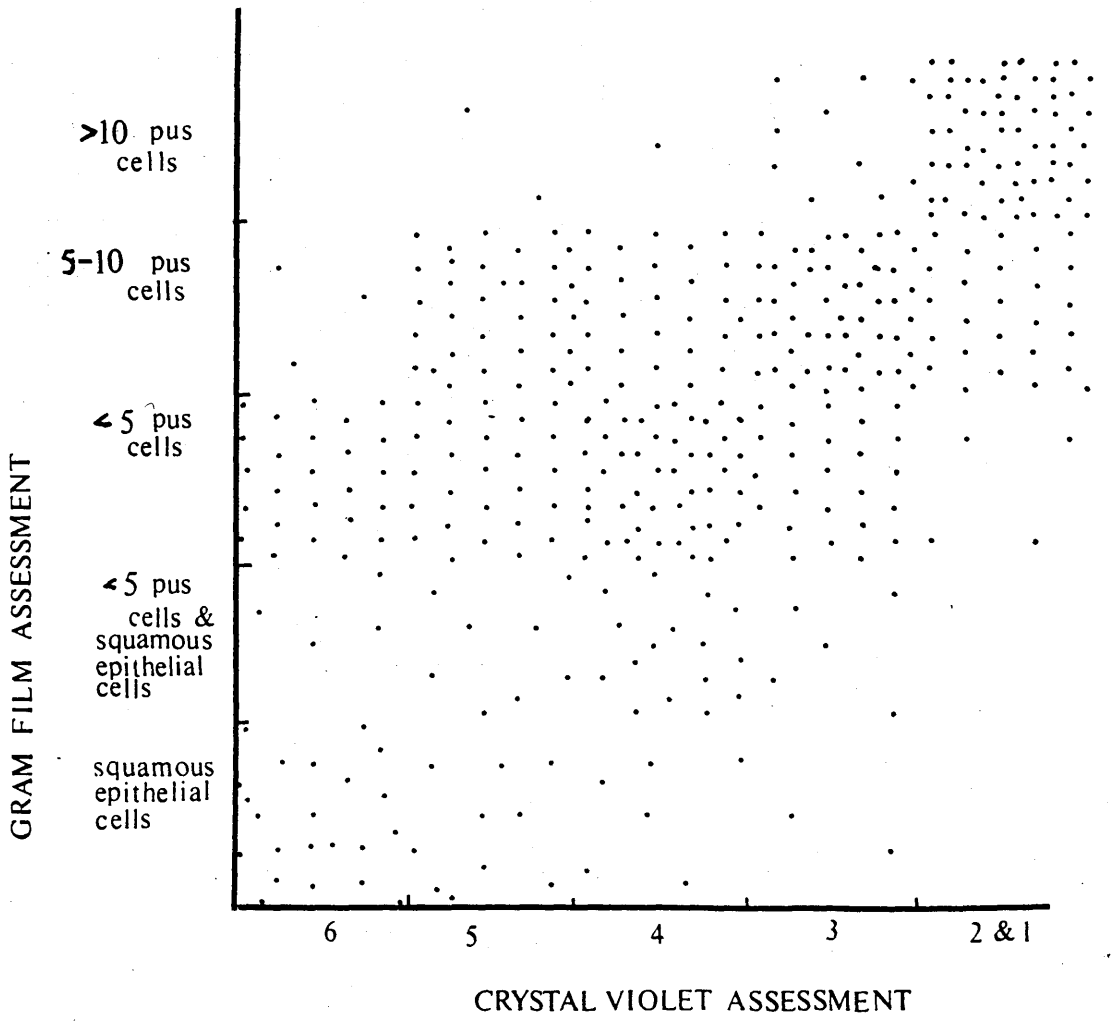
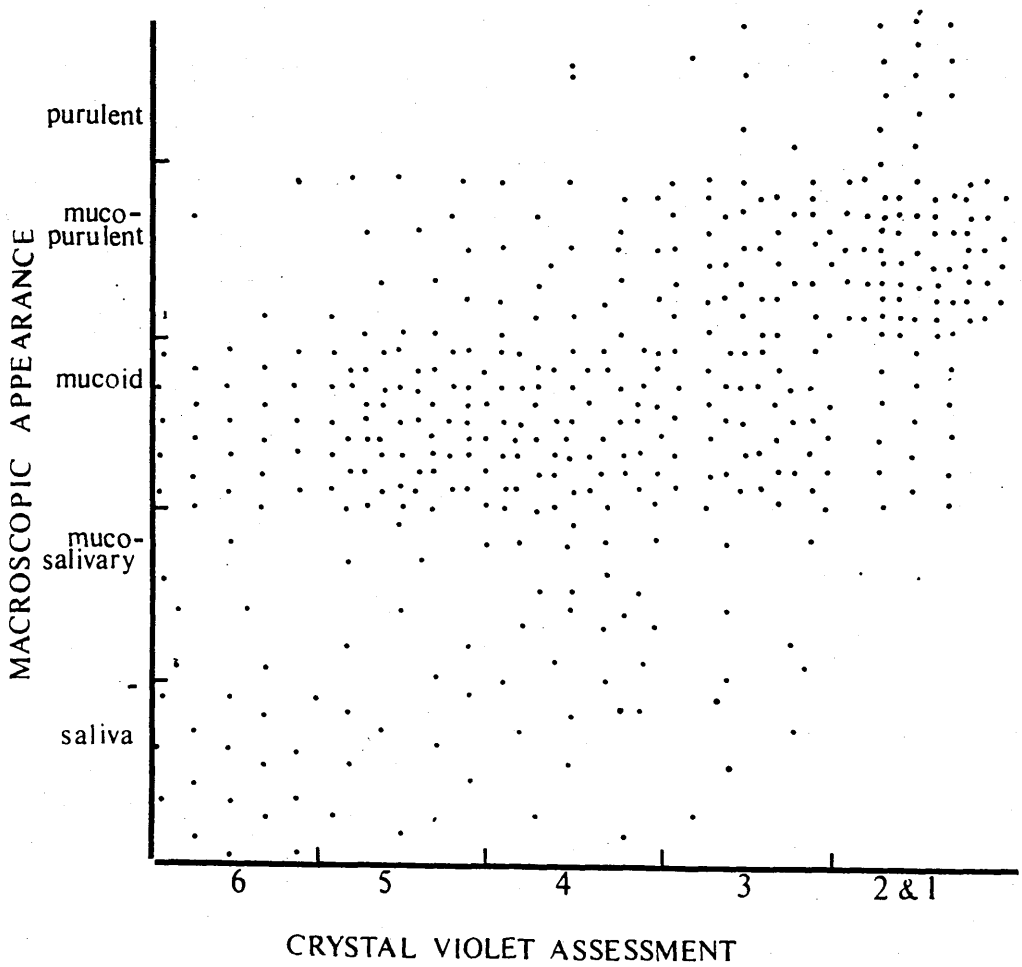


Figure XVIII:

Correlation between macroscopic appearance and crystal violet microscopic assessments of sputum.



pulmonary cells and were categorised as lower respiratory secretions (groups 3 and 4) and squamous epithelial cells predominated in 126 (Groups 5 and 6).

Numbers of specimens in each category which resulted from macroscopic examination were compared with crystal violet assessment in Table 24. The results showed that macroscopic and crystal violet assessments agreed in 211 of 407 specimens but 15 specimens, which were categorised as crystal violet groups 1 and 2, did not appear purulent when examined macroscopically. Macroscopic appearance did not indicate the presence of salivary material in 88 specimens which were classified in groups 5 and 6 by crystal violet microscopy. Gram's stain and crystal violet assessments agreed in 247 of 407 specimens (Table 25). Eighty six of 91(95%) purulent specimens (crystal violet groups 1 and 2) were identified by Gram's stain. However, 67 discrepancies between the two methods were observed; in 38 specimens (groups 5 and 6) a preponderance of broncho-pulmonary cells were recorded as pus cells in companion Gram's stained smears and a further 29 specimens (groups 3 and 4) were incorrectly assessed in Gram stained films because broncho-pulmonary cells were not distinguished from squamous epithelial cells in Gram films.

Table 24:

Comparison of macroscopic appearance with microscopic evaluation of homogenised sputum stained with 0.05% crystal violet.

Macroscopic appearance	Crystal violet assessment groups* 1	2	3	4	5	6
	numbers of specimens					
Purulent	12	1	5	1	nil	nil
mucopurulent	60	3	39	19	14	3
mucoid	14	1	40	55	46	25
mucoid/saliva	nil	nil	5	16	8	6
saliva	nil	nil	2	6	9	17

* Crystal violet assessment carried out with 0.05% crystal violet stain and films examined at X 400 magnification

Group 1 = more than 10 pus cells per high power field.

Group 2 = more than 10 pus cells per high power field with a ratio of greater than 1:10 squamous epithelial cells to pus cells.

Group 3 = less than 10 pus cells per high power field with a preponderance of broncho-pulmonary cells.

Group 4 = predominately broncho-pulmonary cells.

Group 5 = ratio of greater than 1:10 squamous epithelial cells to broncho-pulmonary cells.

Group 6 = predominately squamous epithelial cells.

Table 25:

Comparison of Gram's stain and 0.05% crystal violet for the microscopic examination of homogenised sputum.

	Crystal violet assessment groups *					
Gram film assessment. Cells per oil immersion field	1	2	3	4	5	6
	numbers of specimens					
>10 pus cells	57	3	11	1	2	nil
5-10 pus cells	25	1	49	26	33	3
< 5 pus cells	4	1	24	48	25	25
< 5 pus cells with squamous epithelial cells and/or oral bacteria	nil	nil	5	16	8	6
squamous epithelial cells and/or oral bacteria	nil	nil	2	6	9	17

* interpretation as in Table 24.

"Pus cells" was used as a collective term to describe or include polymorphs and cells of similar size and morphology.

Gram's stain for predominant bacteria

Table 26 details the results of crystal violet assessments, Gram's stain to identify predominant bacteria and bacteriological culture carried out on 363 homogenised sputa. Types of bacteria seen in Gram's stained smears and the results of culture are shown for each crystal violet assessment group. S.pneumoniae predominated in the Gram's stained smear and was cultured from 19 of 78 (24%) purulent specimens (crystal violet groups 1 and 2) and 4 of 55 (7%) lower respiratory secretions (crystal violet group 3).However, in the other groups (i.e. crystal violet groups 4,5 and 6) the Gram's stain and culture for S.pneumoniae did not always agree i.e. pneumococci were seen in 5 specimens but were isolated from 13. Oropharyngeal bacteria predominated in Gram's stained smears in 120 of 230 (52%) specimens which were in crystal violet groups 4,5, and 6, compared to 22 of 133 specimens in crystal violet groups 1,2 and 3. Oropharyngeal bacteria were often associated with squamous epithelial cells in Gram's stained films and confirmed the association between these bacteria and oropharyngeal contamination of sputum.

Table 26:

Comparison of Gram's stain for predominant bacteria and bacteriological culture in different crystal violet assessment groups.

	Crystal violet assessment groups*			
	1&2	3	4	5&6
Number of specimens	78	55	193	37
Predominant bacteria in Gram's stained film				
oropharyngeal bacteria	7	15	83	37
S.pneumoniae	19	4	5	nil
other respiratory pathogens$	22	7	10	3
Bacterial culture				
no respiratory pathogens isolated	34	39	15	33
S. pneumoniae	19	4	12	1
other respiratory pathogens$	25	12	31	3

* interpretation as in Table 24.

$ other respiratory pathogens included H.influenzae, Staph. aureus, B.cattarhalis.

3.2.2 Isolation of pneumococci from sputum cultures

Direct and quantitative culture of homogenised sputum

Six hundred and twenty three specimens of sputum were assessed microscopically using the crystal violet stain and cultured directly from the homogenised material and after dilution in sterile water (10^{-3}). Table 27 shows that S.pneumoniae was isolated by direct culture from 96 (15.4%) and by quantitative culture (more than 10^{6} cfu/ml) from 65 (10.4%). Colony counts of > 10^{6} cfu/ml of S.pneumoniae were isolated from 48 of 56 (86%) specimens in crystal violet groups 1,2 and 3 compared to 17 of 40 (42%) of specimens in groups 4,5 and 6. The numbers of specimens resulting in a "pure growth" of S.pneumoniae by direct culture and quantitative culture was 49 and 50 respectively.

Table 27:

Comparison of direct culture and quantitative culture of *Streptococcus pneumoniae* in homogenised sputum.

Growth of *S.pneumoniae*	Crystal violet assessment groups* 1	2	3	4	5	6
	numbers of specimens					
Direct culture						
pure growth	30	3	6	5	5	nil
mixed growth	9	6	2	6	20	4
Quantitative culture ($>10^6$ cfu/ml)						
pure growth	31	4	6	4	5	nil
mixed growth	3	3	1	1	7	nil

* interpretation as in Table 24.

3.3.METHODS FOR THE DETECTION OF PNEUMOCOCCAL ANTIGEN IN CLINICAL MATERIAL AND PRELIMINARY CLINICAL STUDIES

Purified pneumococcal capsular polysaccharide (pneumococcal antigen) can be detected by various methods and with different kinds of pneumococcal antisera (3.1.2). The studies that follow were carried out to find which method(s) were most suitable for the detection of pneumococcal antigen in clinical material.

3.3.1. Methods for the detection of pneumococcal antigen in sputum.

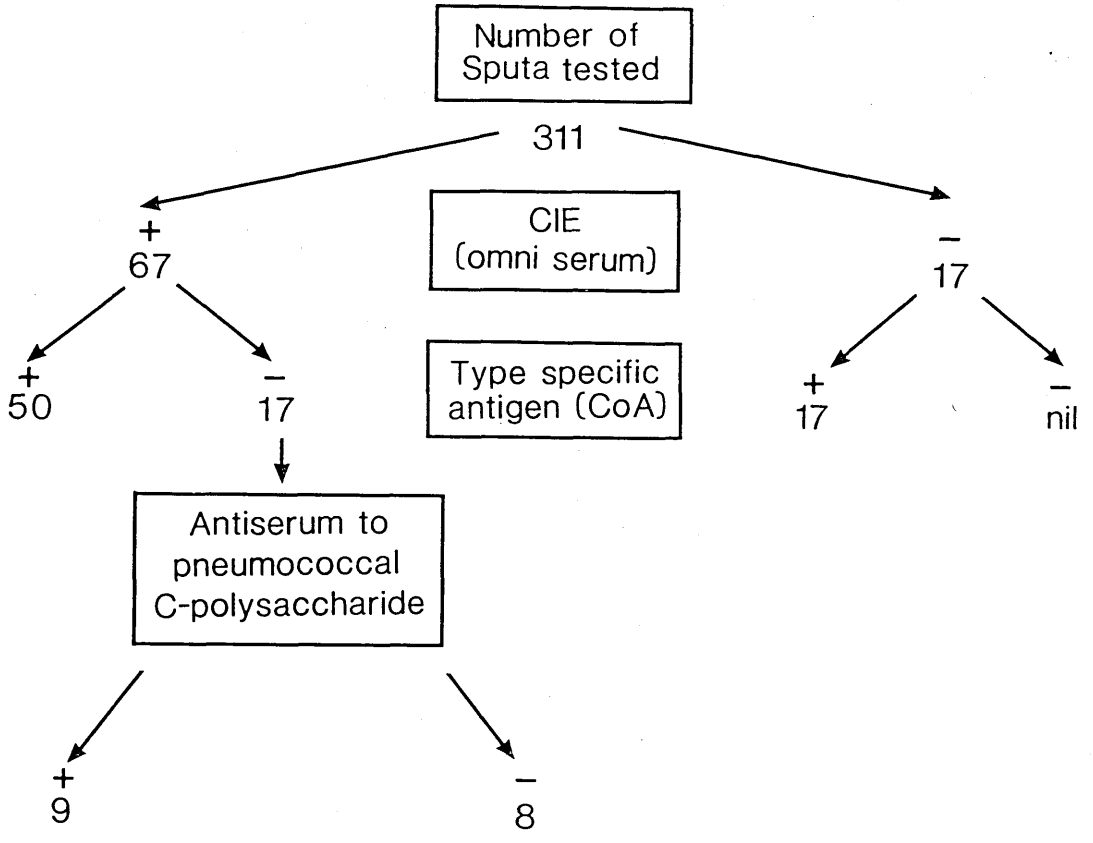
Pneumococcal antigens, which were detected in sputum by way of counterimmunoelectrophoresis (CIE), latex agglutination (LA) and Kronvall co-agglutination ($Co_A(K)$) in tests with Omni serum, were typed by co-agglutination.

Evaluation of CIE, using Omni serum, as a screening test for pneumococcal antigen in sputum

Specimens of sputum, sent to the Bacteriology Department, City Hospital, Nottingham, during a national survey of the aetiology of pneumonia were examined by CIE using Omni serum. These were then tested with nine CoA(K) reagents (Pools A to I, Statens Seruminstitut, Copenhagen). Pneumococcal antigens were typed by CoA(K) with antisera appropriate to the pool which gave the positive reaction. The results (Fig. XX) show that pneumococcal antigen was detected in 84 of 311 specimens

Figure XX:

Evaluation of counterimmunoelectrophoresis, using Omni serum, as a screening test for pneumococcal antigen in sputum.



by CIE or CoA(K). CIE was positive in 67 and negative in 17. The types detected, by CoA(K), in the latter group were type 7 (6), type 3 (2), type 48 (2) and one each of types 9,11,14,15,16,32 and 36. Of the 67 specimens which reacted in CIE tests with Omni serum, only 50 contained typable pneumococcal antigen. Type-specific pneumococcal antigen was not detected in 17 specimens and of these, 9 reacted in the CoA(K) with antiserum to pneumococcal C-polysaccharide. Pneumococcal antigen types 14 (3) and 7 (1) were detected in CIE tests with Omni serum at Nottingham.

These results show that CIE with Omni serum not only detects type-specific pneumococcal antigen but also pneumococcal C-polysaccharide antigen.

Evaluation of latex agglutination (LA) and Kronvall co-agglutination (CoA(K)) using Omni serum, as a screening test for pneumococcal antigen in sputum

A total of 1,056 specimens of sputum, sent to the Bacteriology Department, Stobhill General Hospital, Glasgow, for routine examination and culture, were tested for pneumococcal antigen with latex (Wellcogen, Wellcome Diagnostics Ltd.) and co-agglutination (Kronvall, 1973) reagents each sensitised with Omni serum (Statens Seruminstitut, Copenhagen). Specimens, which contained pneumococcal antigen or from which S.pneumoniae was isolated, were further tested by CoA(K) and CIE with pneumococcal typing sera. Positive LA and

CoA(K) tests with Omni serum were obtained in 154 and 88 specimens respectively (Table 28a). Of 139 sputa which contained type-specific pneumococcal antigen by CoA(K), 116 were positive with LA and 87 with CoA(K) reagents prepared with Omni serum. CIE, with the appropriate type-specific antiserum was positive in 107 of 139 specimens: types 7,14,33 and 37, which are known not to react in CIE tests, accounted for only 8 of 32 specimens which contained pneumococcal antigen and these were negative in CIE tests. The results were used to calculate the sensitivity, specificity, efficiency and predictive value of antigen tests in relation to a positive culture for S.pneumoniae (Table 28b).

LA was more sensitive than CoA(K) in tests with Omni serum, but CoA(K) was more specific. Consequently, the estimated efficiency and predictive value of LA were also lower. This was due to a number of false positive LA tests in specimens from which S.pneumoniae was not isolated (Figure XXI). CoA(K) with pools A to I was the most sensitive method for the detection of pneumococcal antigen in specimens of sputum and the estimated levels of specificity, efficiency and predictive value were comparable to those obtained with CoA(K) with Omni serum and CIE with specific antisera.

A detailed analysis of the numbers of specimens which were positive with each method is shown in Figures XXI and XXII. LA (Fig. XXI) was positive in 154 specimens, however, only 116 of these reacted in CoA(K) with

Figure XXI:

Evaluation of a commercial latex (Wellcogen) as a screening test for pneumococcal antigen in sputum.

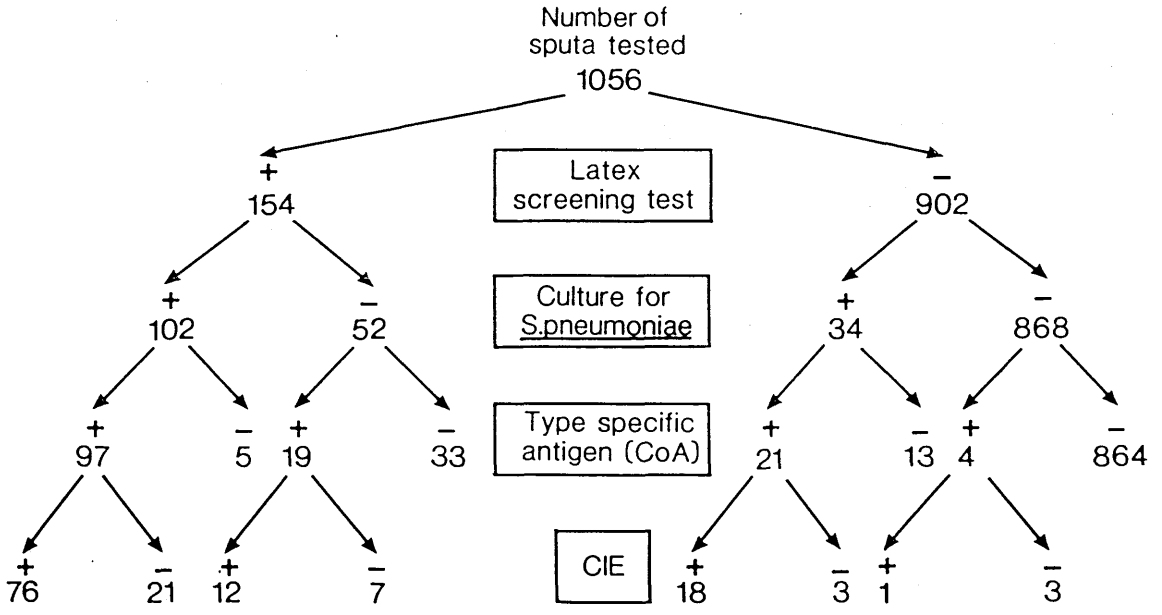


Figure XXII:

Evaluation of co-agglutination, using Omni serum, as a screening test for pneumococcal antigen in sputum.

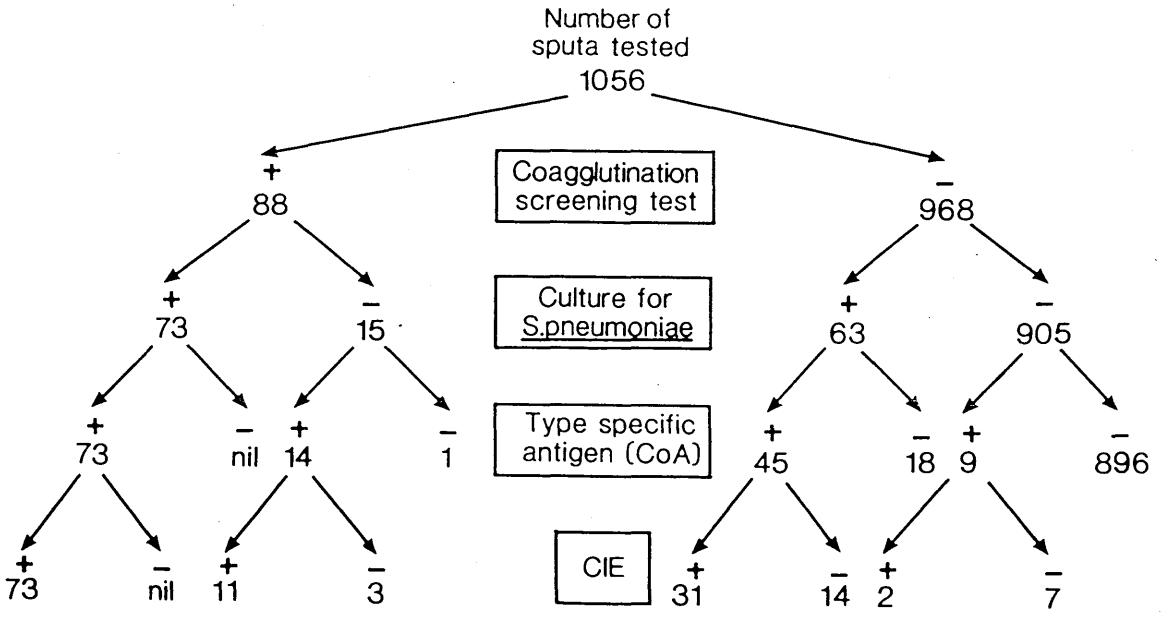


Table 28a:

Summary of results of different methods used to detect pneumococcal antigen in specimens of sputum: Comparison with the results of culture for Streptococcus pneumoniae.

	Numbers of positive specimens				
	Latex (Omni)	CoA(K) (Omni)	CoA(K) (type)	CIE (type)	Culture
Latex (Omni)	154	78	116	88	102
CoA(K)(Omni)		88	87	74	73
CoA(K)(type)			139	107	118
CIE (type)				107	94
Culture					136

Latex (Omni) : Latex suspension sensitised with Omni serum (Wellcogen, Wellcome Diagnostics)

CoA(K)(Omni) : co-agglutination reagent prepared from Omni serum according to the method of Kronvall (1973)

CoA(K)(type) : Specimens which were positive in latex agglutination or co-agglutination tests with Omni serum or from which S.pneumoniae was isolated were tested with co-agglutination reagents (CoA(K)) prepared from pools A to I (Statens Seruminstitut, Copenhagen) and then with type-specific antisera appropriate to the pool which gave the positive reaction.

CIE (type) : counterimmunoelectrophoresis carried out with type-specific antiserum.

Table 28b:

	Latex (Omni)	CoA(K) (Omni)	CoA(K) (type)	CIE (type)
Sensitivity(%)	75	54	87	69
Specificity(%)	94	98	98	99
Efficiency	92	95	96	95
Predicitive value of a positive result	60	83	85	87

Sensitivity is the percentage of specimens which contained pneumococcal antigen and from which S.pneumoniae was isolated by culture.

Specificity is the percentage of specimens which were negative with antigen tests and S.pneumoniae was not isolated by culture.

Efficiency is the percentage of antigen results which correlate with the culture results.

Predictive value of a positive test is the percentage of positive tests which correlate with culture.

pneumococcal typing antisera. S.pneumoniae was isolated from 5 specimens which did not contain type-specific pneumococcal antigen. Thus, 33 specimens, which did not contain type-specific pneumococcal antigen and from which S.pneumoniae was not isolated, reacted with latex particles sensitised with Omni serum. These specimens did not react with latex particles sensitised with normal rabbit immunoglobulin. However, 6 reacted in the Kronvall co-agglutination test with individual antiserum pools (A to I, Statens Seruminstitut, Copenhagen) but not with typing reagents. LA was negative on 23 sputa which contained type-specific pneumococcal antigen. S.pneumoniae types 6 (10 sputa), 19 (4 sputa), 28 (4 sputa) and one each of types 12,13 and 15 were isolated from 21 specimens, whereas pneumococcal antigen type 8 was detected in the remaining two.

The results of the CoA(K) test with Omni serum are shown in Figure XXII. With the exception of one specimen, all those positive in the CoA(K) test with Omni serum contained type-specific pneumococcal antigen and 84 of 88 were also positive in CIE tests. However, the number of specimens which contained pneumococcal antigen but which were negative in co-agglutination and latex tests with Omni serum was 54 and 25 respectively. S.pneumoniae was not isolated from 23 specimens which contained type-specific pneumococcal antigen: In tests with Omni serum, the LA was positive in 19 and CoA(K) was positive in 14. Conversely, 5 of 18 specimens which

were culture-positive and antigen-negative (type-specific co-agglutination) reacted in latex agglutination tests.

Table 29 compares the results of culture and methods for the detection of pneumococcal antigen when the sputa were categorised according to the results of crystal violet microscopic assessment. S.pneumoniae and or type-specific pneumococcal antigen was found in 122 specimens assigned to categories 1,2 and 3 : 111 of 122 were positive in latex agglutination tests. However 43 specimens in crystal violet assessment groups 4,5 and 6, which included non-purulent lower respiratory and oropharyngeal secretions, reacted in latex tests with Omni serum. S.pneumoniae was isolated from 23 and type-specific pneumococcal antigen was detected in only 17.

These results demonstrate differences in both the sensitivity and specificity of tests which use Omni serum for the detection of pneumococcal antigens in sputum and furthermore indicate that these parameters, for the methods, have different relationships with sputum purulence and the degree of oropharyngeal contamination. The importance of these differences in the choice of method for use in laboratory diagnosis will be further considered together with clinical findings.

Table 29:

Comparison of methods for the detection of pneumococcal antigen with culture in different crystal violet assessment groups.

	Numbers of specimens Crystal violet assessment groups$					
	1	2	3	4	5	6
* Latex (Omni)	63	3	45	11	23	9
CoA(K)(Omni)	49		27	3	9	0
CoA(K)(type)	73		49	5	12	0
CIE (type)	59		36	3	9	0
Culture positive	70	1	42	5	17	1

$ interpretation as in Table 24.

* used as in Table 28a.

Preliminary clinical studies : correlation of tests for pneumococcal antigen with Gram's stain and culture for the laboratory diagnosis of pneumococcal chest infection.

S.pneumoniae or type-specific pneumococcal antigen was detected in the sputum of 129 patients. Retrospective analysis of the clinical data showed that 106 were from infected patients and 23 were from patients with no clinical or radiological evidence of lower respiratory tract infection. A diagnosis of pneumonia, acute chest infection secondary to other pathology and acute exacerbation of chronic bronchitis was made in 10, 65 and 31 respectively. The results of different methods used to detect pneumococci in sputa and the relationship of these findings with clinical infection (and with each other) are summarised in Table 30a. From these data the sensitivity, specificity, efficiency and predictive value of a positive test were calculated and are summarised in Table 30b. The results show that type-specific pneumococcal antigen was detected and S.pneumoniae was isolated from 89 of 94 (95%) sputa in which pneumococci were seen in a Gram's stained smear and these findings correlated with infection : The calculated predictive value of a Gram's stained smear was 93% (Table 30b). However, pneumococcal antigen was not detected in a number of these specimens by LA and CoA(K) with Omni serum and CIE carried out with type-specific antiserum. Type-specific pneumococcal antigen was not found in 14 specimens from which

S.pneumoniae was isolated but the patients were not infected. Alternatively, type-specific pneumococcal antigen was detected, but S.pneumoniae not isolated, from the sputum of 16 infected patients who had received antibiotic therapy for a chest infection. The results (Table 30b) indicate that the detection of type-specific pneumococcal antigen is the most effective method for the laboratory diagnosis of a pneumococcal chest infection.

Table 30a

Comparison of tests for pneumococcal antigen with Gram's stain and culture of Streptococcus pneumoniae in the sputum of infected and non-infected patients.

	Gram's stain	Culture	Latex (Omni)	CoA(K) (Omni)	CoA(K) (type)	CIE (type)	Clinical critera Infected	Clinical critera Not infected
	Number of positive specimens							
Gram's stain■	94	89	76	56	89	71	87	7
Culture▼		107	81	56	93	72	88	19
* Latex (Omni)			101	65	97	71	89	12
CoA(K) (Omni)				70	70	60	64	6
CoA(K) (type)					115	85	104	11
CIE (type)						85	81	4
							106	23

■Gram's stain = preponderance of Gram positive diplococci

▼Culture = more than 10^5 cfu/ml of S.pneumoniae per ml of homogenised sputum.

* used as in Table 28a

Table 30b

Comparison of tests for pneumococcal antigen with Gram's stain and culture of *Streptococcus pneumoniae* in the sputum of infected and non-infected patients.

	Gram's stain	Culture	Latex (Omni)	CoA(K) (Omni)	CoA(K) (type)	CIE (type)
Sensitivity	82%	83%	84%	60%	98%	76%
Specificity	70%	17%	48%	74%	52%	82%
Efficiency	80%	71%	76%	63%	90%	78%
Predictive value of a positive test	93%	82%	88%	91%	90%	95%

Correlation of the co-agglutination test for pneumococcal antigen with quantitative culture of S.pneumoniae in the sputum of infected and non-infected patients.

Previous studies showed that CoA(K) with diagnostic pneumococcal antisera (Pools A to I, Statens Serum-institut, Copenhagen and type-specific antiserum) was more effective than LA, CoA(K) and CIE tests that used Omni serum. Furthermore, CoA(K) was more sensitive than CIE carried out with type-specific antiserum. Therefore, CoA(K) carried out with pools A to I was correlated with clinical status to evaluate pneumococcal antigen detection in sputum as an indicator of infection.

Quantitative culture and pneumococcal antigen determination by CoA(K) was undertaken on 291 specimens of sputum sent to the Bacteriology Department, Stobhill General Hospital, Glasgow, for routine examination and culture. Retrospective analysis of the clinical data showed that 225 were obtained from infected patients and 66 were from patients with no clinical or radiological evidence of lower respiratory tract infection. The bacterial counts of S.pneumoniae ranged from 10^2 cfu/ml from undiluted sputum to more than 10^8 cfu/ml in sputa diluted 10^{-6}.

Pneumococcal antigen was detected in 251 of 291 samples whereas S.pneumoniae was isolated from 250. Bacterial counts of S.pneumoniae of more than 10^7 cfu/ml were obtained from 155 specimens.

The results of quantitative culture and CoA(K) for

pneumococcal antigen carried out on 225 sputa from infected patients (Fig. XXIII) showed that antigen positivity was usually associated with the isolation of $>10^6$ cfu/ml of S.pneumoniae. However, the distribution was bimodal and the minor peak corresponded to specimens which contained pneumococcal antigen but from which S.pneumoniae was not isolated.

Similarly the results of quantitative culture and CoA(K) carried out on 66 sputa from patients who were not infected at the time the sputum was obtained are shown in Figure XXIV. Although the numbers of specimens examined in this group was small, 10^5 cfu/ ml of S.pneumoniae was isolated from the majority. However, only half of these contained detectable pneumococcal antigen, whereas in the infected group 97% of culture-positive specimens contained pneumococcal antigen.

The relationship between the presence of pneumococcal antigen, culture and quantitative culture of S.pneumoniae at a limit of $> 10^7$ cfu/ml in sputum was compared with the results of clinical and radiological examination of the lower respiratory tract at the time the sputum was collected (Table 31a). From these data the sensitivity, specificity, efficiency and predictive value of a positive test were calculated and are summarised in Table 31b. The results indicated that determination of pneumococcal antigen by co-agglutination was more sensitive than culture of S.pneumoniae from the sputum of infected patients and

Figure XXIII:

Comparison between pneumococcal antigen and quantitative culture of Streptococcus pneumoniae in the sputum of infected patients.

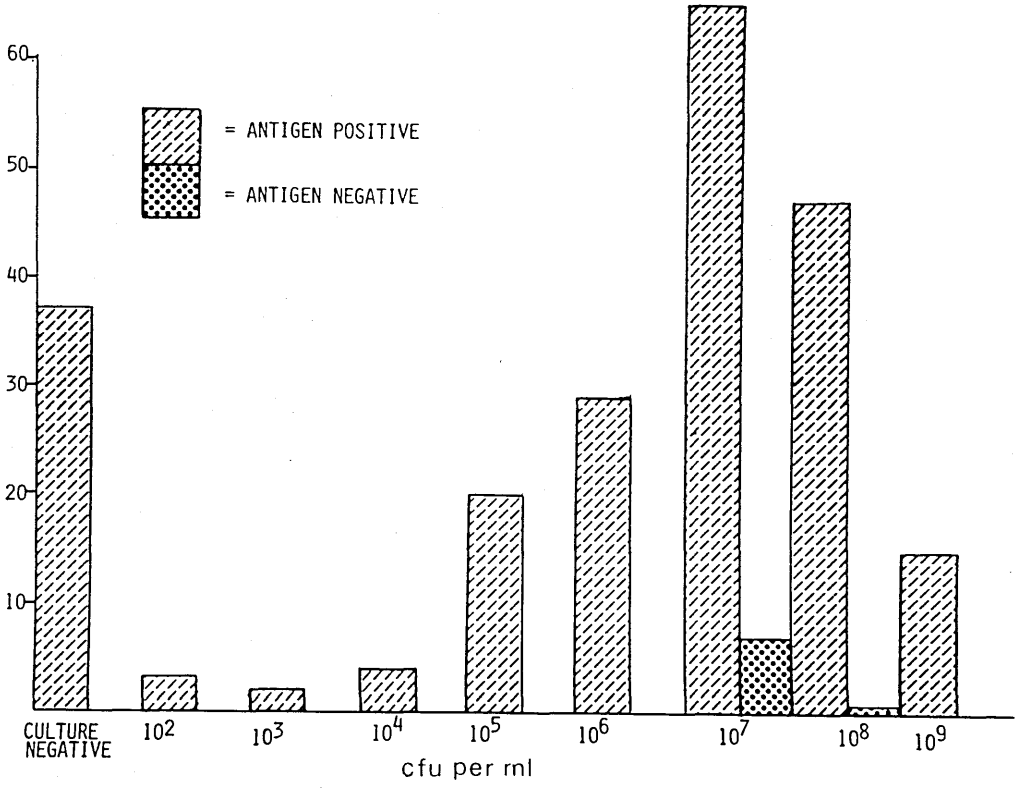
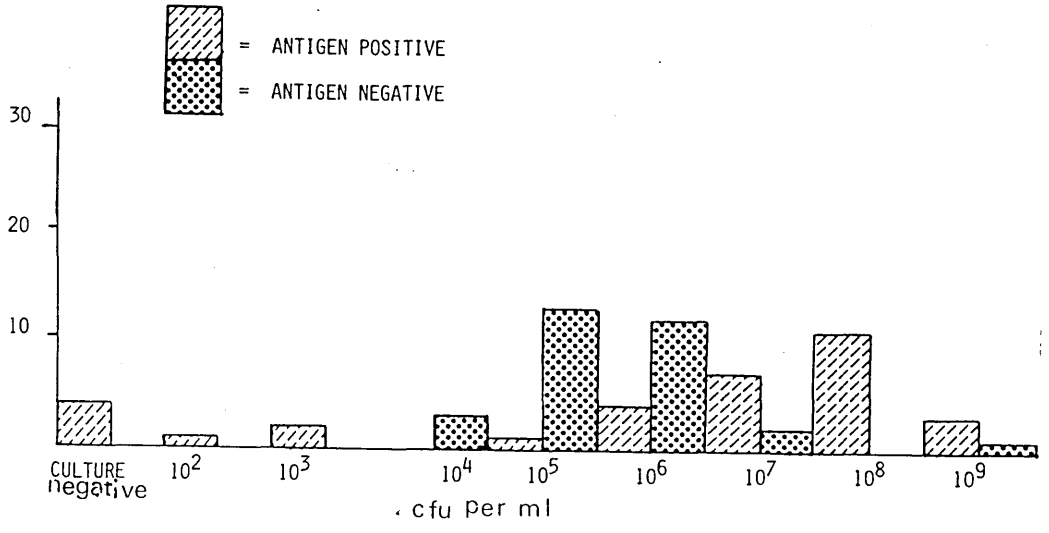


Figure XXIV:

Comparison between pneumococcal antigen and quantitative culture of Streptococcus pneumoniae in the sputum of patients with no evidence of clinical infection.



moreover in half of the cases distinguished between infected and uninfected patients when S.pneumoniae was isolated from sputum.

Table 31a:

Comparison of pneumococcal antigen determination and quantitative culture ($>10^7$ cfu/ ml) of pneumococci in infected and non-infected patients.

	Antigen Positive	Antigen negative	$<10^7$ cfu /ml	$>10^7$ cfu/ml
		Numbers of patients		
Infected	218	7	57	131
Not infected	33	33	38	24
TOTAL	251	40	95	155

Table 31b:

Comparison of pneumococcal antigen determination and quantitative culture (10^7 cfu/ml) of pneumococci in infected and non-infected patients.

	Antigen Positive	Culture Positive	Culture $>10^7$ cfu/ml
Sensitivity	97	84	58
Specificity	50	9	64
Efficiency	86	67	58
Predictive value	87	75	85

Sensitivity : the percentage of positive tests in the presence of clinical infection.

Specificity : the percentage of negative tests in the absence of clinical infection.

Efficiency : the percentage of test results which are true.

Predictive value of a positive test : the percentage of positive tests which are diagnostic.

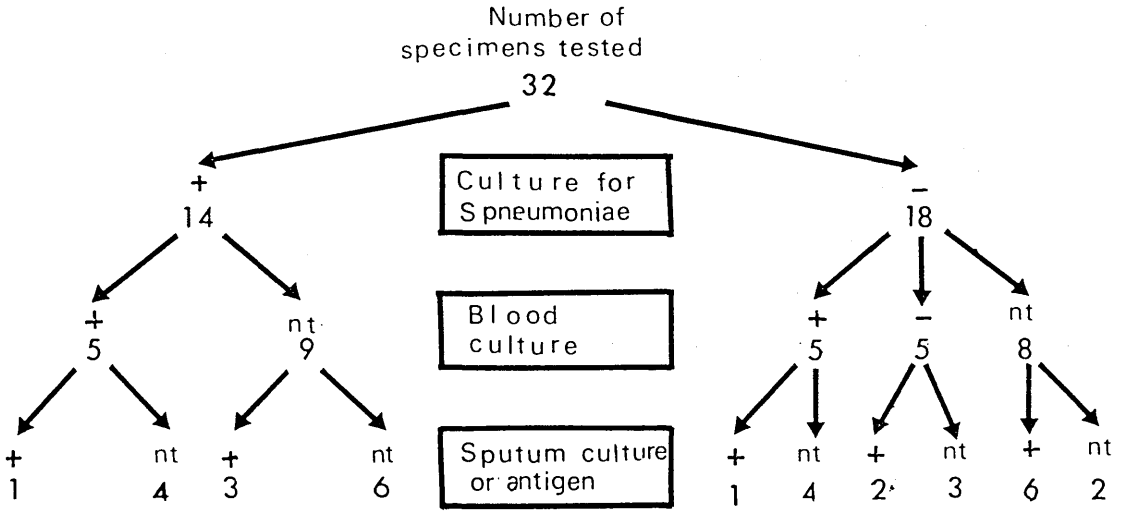
3.3.2. Detection of pneumococcal antigen by co-agglutination in clinical material other than sputum

Pneumococcal antigens, in clinical material other than sputum, were detected and typed by CoA(K). The results of antigen detection were compared with culture of S.pneumoniae carried out on the same specimen and, in some cases, with the results obtained from other material from the same patient, collected at or about the same time. In all cases studied, the type of pneumococcal antigen detected corresponded with the S.pneumoniae type isolated from the same specimen or with that in other material.

Figure XXV shows the results of culture for S.pneumoniae carried out on 24 specimens of post-mortem lung tissue and 8 pleural fluids which contained pneumococcal antigen. S.pneumoniae was isolated from 10 and 4 of these respectively. Blood culture confirmed the pneumococcal infection in 5 of 18 cases in which the organism was not isolated from the corresponding respiratory specimen. However, blood culture was not carried out or was negative for S.pneumoniae on the remaining 13. Sputum specimens were not examined (possibly not obtained) from 19 of these patients, although a severe respiratory infection was apparent. Furthermore, S.pneumoniae was isolated from only 6 of 13 sputum which contained the corresponding pneumococcal antigen. These results confirm that the co-agglutination test for pneumococcal antigen is a valuable tool in

Figure XXV:

Summary of evidence supporting the diagnosis of pneumococcal pneumonia on the basis of pneumococcal antigen in post-mortem lung tissue and pleural fluid.



nt: not tested

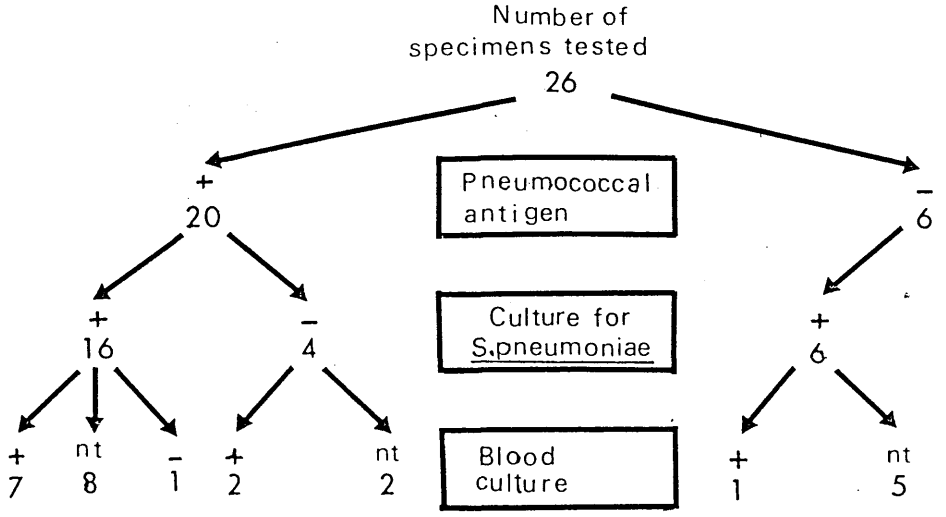
establishing the aetiology in patients with pneumonia and is particularly useful when pleural fluid is available for examination or for retrospective diagnosis using post-mortem lung tissue.

Figure XXVI shows the results of the CoA(K) test for pneumococcal antigen carried out on 26 samples of cerebrospinal fluid. S.pneumoniae was isolated from 22 and in 6 of these pneumococcal antigen was not detected by co-agglutination. However, pneumococcal antigen was detected in 4 specimens from which S.pneumoniae was not isolated. Blood culture confirmed the role of S.pneumoniae in 2 of these 4. It appears from these results that the CoA(K) test for pneumococcal antigen is less sensitive than culture for the diagnosis of pneumococcal meningitis. However, it should be stressed that most of these specimens were referred from laboratories where tests for pneumococcal antigen were not used. Consequently, an accurate assessment of the technique in the whole clinical spectrum has not been possible because the diagnosis was made using conventional culture and Gram's stain examination. Indeed, the 4 antigen-positive, culture-negative specimens were referred in an attempt to confirm other bacteriological evidence of pneumococcal infection.

S.pneumoniae and the corresponding pneumococcal antigen was also detected in the knee aspirates from two patients and S.pneumoniae was isolated from the blood of both.

Figure XXVI:

Summary of evidence supporting the diagnosis of pneumococcal meningitis on the basis of pneumococcal antigen in cerebrospinal fluid.



nt not tested

3.3.3. Comparison of methods for the detection of pneumococcal antigen in serum.

Co-agglutination, using a 10% suspension of staphylococci (CoA(K)) was the most reliable method for the detection and serotyping of pneumococcal antigens in clinical material such as sputum, pleural fluid and post-mortem lung tissue (3.3.1. and 3.3.2.). However, other methods were assessed for the detection of type-specific pneumococcal antigen in serum. These included counterimmunoelectrophoresis, co-agglutination using a 2% suspension of staphylococci, latex agglutination and enzyme-linked immunosorbent assay. The sensitivity of each method was compared in a small series of serum samples obtained from patients with pneumonia caused by S.pneumoniae that belonged to different serotypes.

The types chosen were representative of those most often associated with pneumococcal pneumonia. The results show that, in patients with pneumococcal antigenaemia, the concentration of pneumococcal antigen varies over a wide range (4 ng/ml to 4 ug/ml) in the same patient and also between different patients infected with the same pneumococcus type. A detailed analysis of antigen titres in serum, obtained with CIE, CoA(K), CoA(T), LA and ELISA is shown in Table 32a. Antigen concentrations were determined from the results of parallel assays carried out on pooled human serum, to which 1 ug/ml of appropriate purified pneumococcal capsular polysaccharide was added.

Table 32a:

Comparison of methods for the detection of type-specific pneumococcal antigen in the serum of patients with pneumococcal pneumonia.

Patient	Antigen type	Method of detecting antigen					Antigen level
		CIE	CoA(K)	CoA(T)	LA	ELISA	Ng/ml
		Antigen titre*					
RM	1	nil	nil	16	8	160	250
JF	1	nil	nil	nil	nil	16	25
RS	1	nil	nil	4	4	80	125
GH	1	8	8	64	64	1000	1000
AD	1	8	16	128	64	2000	2000
MK	3	nil	nil	nil	nil	8	8
JC	3	4	4	8	8	512	500
WH	3	nil	nil	nil	nil	4	4
JC	3	nil	nil	nil	4	16	60
EG	3	nil	nil	nil	nil	4	4
RS	3	nil	nil	8	8	512	500
G McK	3	2	2	32	32	1000	1000
G McK	3	nil	nil	4	4	256	250
CF	3	nil	nil	nil	4	32	60
MF	4	nil	nil	8	8	512	500
WB	4	nil	nil	nil	nil	4	12.5
MF	4	nil	nil	8	8	512	500
HM	4	nil	nil	nil	nil	4	12.5
MP	8	8	16	128	128	>2000	4000
HM	8	16	16	64	64	1000	2000
C McM	8	nil	nil	4	8	nil	250
WM	9	4	4	8	8	256	1000
RT	9	nil	nil	nil	nil	8	50
TJ	9	nil	nil	2	2	64	250

*= reciprocal of the highest dilution of serum which gave a positive reaction.

CIE= counterimmunoelectrophoresis

CoA(K)= co-agglutination using a 10% suspension of staphylcocci (Kronvall, 1973)

CoA(T)= co-agglutination using a 2% suspension of staphylococci (Trollfors et al., 1983)

LA = latex agglutination

ELISA= enzyme-linked immunosorbent assay

CIE and (CoA(K)) detected antigen concentrations in serum of more than than 500 ng/ml, whereas LA and CoA(T) detected concentrations of more than 100 ng/ml. ELISA was the most sensitive method for the detection of pneumococcal antigen in serum and detected as little as 4 ng/ml. Although the sensitivity of the ELISA for type 8 pneumococcal antigen was determined as 3ng/ml, one serum, which contained 250 ng/ml of type 8 pneumococcal antigen, was consistently negative when tested by ELISA.

The results (Table 32b) showed that CIE and CoA(K) detected 7 of 24 positive sera whereas CoA(T) and LA detected 15 and 17 respectively. ELISA was positive with 23 of 24 sera. Therefore , in the clinical studies that follow all sera which were negative in the Trollfor's co-agglutination and latex agglutination tests were also tested by ELISA.

table 32b:

Comparison of methods for the detection of type-specific pneumococcal antigen in the serum of patients with pneumococcal pneumonia.

*	CIE	CoA(K)	CoA(T)	LA	ELISA
	Number of positive sera				
CIE	7	7	7	7	7
CoA(K)		7	7	7	7
CoA(T)			15	15	14
LA				17	16
ELISA					23

* used as in Table 32a.

3.4. CLINICAL STUDIES

Results of laboratory tests on specimens from 426 cases, representing 414 patients with pneumococcal infection are shown in detail here. The cases were arranged on a clinical basis into four broad categories. These were:

1. Pneumococcal pneumonia [Group "P", (Table 33)].
2. Pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease [Group "C", (Table 40)].
3. Acute pneumococcal chest infection secondary to other pathology [Group "A", (Table 42)].
4. Non-respiratory pneumococcal infection [Group "S", (Table 44)].

3.4.1. Laboratory tests on patients with pneumococcal pneumonia

Table 33 shows the results on 123 cases of clinical pneumonia. Of 122 patients studied, 71 were male and there were 28 deaths. The mean age was 60 years (range 18-97) and almost three-quarters of patients were over 50 years of age. They had no previous history of respiratory disease and severe pneumonia was the reason for their admission to hospital. One patient had two episodes of pneumonia, due to different pneumococcal types, separated by a period of two weeks. The second episode, which proved fatal, took place while the patient was in hospital.

S.pneumoniae with or without pneumococcal antigen was detected in the acute phase blood in 76 (62%) of the patients and the same type of pneumococcus or pneumococcal antigen was also found in respiratory specimens from 53 of 56 patients. Respiratory specimens were not available from the remaining 20 patients with pneumococcal bacteraemia or antigenaemia.

With one exception, the type of S.pneumoniae or pneumococcal antigen detected in different specimens from the same patient taken at or about the same time was identical. The exception was case No 2, in whom S.pneumoniae type 3 was isolated from blood and a wound swab and pneumococcal antigen type 3 was present in serum and sputum. However, S.pneumoniae type 10 was

KEY TO TABLES 33,40,42,44 AND 49

★ acute phase taken as the date of admission or when lower respiratory infection was clinically diagnosed.

☆ convalescent phase was more than 5 days after the acute phase.

F= fatal, R= recovered, NK= outcome not known

nk not known

(-) patients treated with an antibiotic prior to sputum being sent for culture.

(+) pneumococcal antigen detected in blood culture broth, but S.pneumoniae was not isolated on culture.

■ further episode in the same patient.

▼ S.pneumoniae type 6 and Salm. typhimurium also isolated from P.M. lung and Salm.typhimurium was isolated from the blood culture.

Table 33:

Laboratory tests on patients with pneumococcal pneumonia

case No.	age	sex	type	clinical diagnosis	SPUTUM EXAMINATION microscopy pus	SPUTUM EXAMINATION microscopy DPC	SPUTUM EXAMINATION Pneumococcal Cult.	SPUTUM EXAMINATION Pneumococcal Ag.	OTHER SPECIMENS Pneumococcal Cult.	OTHER SPECIMENS Pneumococcal Ag.	ACUTE PHASE★ BLOOD Pneumococcal Cult.	ACUTE PHASE★ BLOOD Pneumococcal Ag. ng/ml.	ACUTE PHASE★ BLOOD Pneumococcal Ab.	CONVALESCENT☆ PHASE BLOOD Pneumococcal Ag. ng/ml.	CONVALESCENT☆ PHASE BLOOD Pneumococcal Ab	Outcome
P1	63	M	1	pneumonia	-	-	-	+			+	1000	-	500	16	R
P2	77	M	3	post-op. pneumonia	+	+	type 10	+	wound swab type 3		+	4000	-	125	8	R
P3	58	F	8	pneumonia & septicaemia	-	-	+	+			+	125	-	-	32	R
P4	78	F	1	pneumonia	+	-	(-)	+			+	125	-	500	-	R
P5	47	M	9	lobar pneumonia	+	+	(-)	+	Chest drain -	+	+	1000	-	1000	-	R
P6	76	F	1	bronchopneumonia	not available				P.M. lung -	+	+	2000	-	not done		F
P7	48	M	1	pneumonia	-	-	-	-			+	250	-	not done		R
P8	71	M	3	pneumonia & septicaemia	-	-	(-)	+			+	10,000	-	not done		NK
P9	27	F	3	pneumonia & meningitis	-	-	(-)	+			+	4000	-	not done		NK
P10	67	M	3	pneumonia	not available						+	4000	-	not done		NK

P11	67	M	3	pneumonia	not available						+	4000	-	not done		NK
P12	44	F	6	lobar pneumonia	not available				P.M. lung +	+	+	1000	-	not done		F
P13	54	F	6	pneumonia & lung abscess	not available						+	1000	-	not done		R
P14	68	F	7	bronchopneumonia	not available						+	50	-	not done		NK
P15	73	F	8	lobar pneumonia	not available				P.M. lung -	+	+	250	-	not done		F
P16	83	M	9	lobar pneumonia	not available						+	50	-	not done		NK
P17	82	F	9	pneumonia	not available						+	1000	-	not done		NK
P18	89	F	9	bronchopneumonia	not available				P.M. lung -	+	+	250	-	not done		F
P19	86	M	16	lobar pneumonia	+	-	+	-			+	+	-	not done		R
P20	72	M	16	bronchopneumonia	not available						+	+	-	not done		F
P21	nk	F	20	pneumonia	not available						+	2000	-	not done		NK
P22	71	F	23	pneumonia	not available						+	50	-	not done		NK
P23	49	M	29	lobar pneumonia	not available				P.M. lung +	+	+	+	8	not done		F
P24	76	M	29	bronchopneumonia	+	+	+	+	P.M. lung +	+	+	+	2	not done		F
P25	19	F	4	pneumonia	not available						+	100	4	not done		F
P26	59	M	8	pneumonia	-	-	+	+			+	500	4	not done		F
P27	18	M	1	lobar pneumonia	+	-	(-)	+			+	-	-	-	16	R

P28	76	M	4	pneumonia	not available						+	-	-	-	16	R
P29	71	F	5	pneumonia	-	-	-	+			+	-	-	-	32	R
P30	77	M	8	pneumonia	+	+	+	+			+	-	-	-	-	R
P31	50	M	1	pneumonia	not available						+	-	-	not done		R
P32	86	F	8	pneumonia	not available						+	-	-	not done		R
P33	36	M	8	lobar pneumonia	-	-	-	+			+	-	-	not done		R
P34	76	M	8	pneumonia	not available						+	-	-	not done		NK
P35	25	M	1	pneumonia	-	-	-	+	P. fluid +	+	+	-	2	-	64	R
P36	19	M	1	lobar pneumonia	not available						+	-	16	-	64	R
P37	64	M	14	lobar pneumonia	-	-	+	+			+	-	2	-	64	R
P38	66	F	4	bronchopneumonia	not available				P.M. lung +	+	+	-	8	not done		F
P39	45	M	8	atypical pneumonia	+	+	+	+			+	-	4	not done		R
P40	75	M	11	pneumonia	+	+	+	+			(+)	-	16	not done		F
P41	34	M	14	lobar pneumonia	+	+	+	+			+	-	8	not done		R
P42	84	M	14	bronchopneumonia	not available						+	-	8	not done		NK
P43	60	F	14	pneumonia	not available						+	-	2	not done		NK
P44	80	F	1	lobar pneumonia	not available				P.M. lung +	+	+	-	4	not done		F
P45	62	M	34	lobar pneumonia	not available						+	-	not done	not done		NK
P46	83	F	7	pneumonia	not available						+	not done		-	32	R

P47	50	M	1	lobar pneumonia	-	-	-	+			+	not done		not done			R
P48	44	F	11	bronchopneumonia	not available				P.M. lung +		+	not done		not done			F
P49	nk	F	19	bronchopneumonia	+	+	(-)	+			+	not done		not done			NK
P50	52	F	8	pneumonia & lung abscess	-	-	+	+	P.fluid -	+	-	10,000	-	2000	32		R
P51	63	M	8	lobar pneumonia	not available				Bronch.asp +	not done	-	1000	-	1000	-		R
P52	58	M	8	pneumonia	-	-	+	+	P.fluid -	+	-	500	-	-	-		R
P53	55	M	1	pneumonia	-	-	-	-			-	1000	-	not done			NK
P54	70	F	3	lobar pneumonia	-	-	(-)	+	P.M. lung -	+	-	2000	-	not done			F
P55	73	M	3	pneumonia	not available				P. fluid +	not done	-	2000	-	not done			NK
P56	30	M	4	pneumonia	+	+	+	+	knee aspirate +	+	-	2000	-	not done			F
P57	59	M	4	lobar pneumonia	+	+	+	+			-	4000	-	not done			NK
P58	67	F	7	pneumonia	not available				Bronch.asp. +	+	-	200	-	-	32		F
P59	26	F	18	pneumonia	not available				P.fluid -	not done	-	4000	-	not done			F
P60	41	M	19	lobar pneumonia	not available						-	1000	-	not done			NK

P61	42	M	1	lobar pneumonia	+	-	-	+			-	4000	16	2000	16	R
P62	56	F	3	pneumonia	-	-	+	not done			-	100	4	100	16	R
P63	51	M	3	pneumonia	-	-	(-)	+			-	30	2	15	4	R
P64	83	F	19	lobar pneumonia	not available						-	250	4	125	16	R
P65	nk	M	3	pneumonia	-	-	+	+			-	50	4	-	8	NK
P66	>70	M	3	lobar pneumonia	+	-	(-)	+	P.M. lung -	+	-	4	2	not done		F
P67	74	F	3	bronchopneumonia	not available				P.M.lung -	+	-	1000	8	not done		F
P68	78	F	3	pneumonia & septicaemia	-	-	(-)	+	P.M. lung -	+	-	8	2	not done		F
P69	nk	M	13	bronchopneumonia	+	+	+	+			-	+	8	not done		R
P70	nk	M	14	pneumonia	-	-	+	+			-	100	2	not done		NK
P71	47	F	3	pneumonia	+	-	+	+			not done	500	-	500	8	R
P72	66	M	8	pneumonia	+	-	+	+	P.fluid +	+	not done	500	-	not done		F
P73	18	M	8	lobar pneumonia	-	-	(-)	+			not done	100	-	-	-	R
P74	90	M	11	lobar pneumonia	+	+	+	+			not done	30	-	not done		R
P75	82	M	15	lobar pneumonia	+	-	(-)	+			not done	1000	not done	not done		F
P76	97	F	16	bronchopneumonia	+	+	+	+			not done	+	2	not done		R

P77	49	M	3	lobar pneumonia	-	-	-	+	throat swab type 3	-	-	-	100	-	R
P78	73	M	3	bronchopneumonia	-	-	+	+		-	-	-	not done		R
P79	86	M	3	bronchopneumonia	-	-	+	+		-	-	-	not done		R
P80	36	F	7	pneumonia	+	-	(-)	+		-	-	-	not done		NK
P81	55	F	9	lobar pneumonia	+	+	+	+		-	-	-	not done		R
P82	53	M	22	atypical pneumonia	-	-	+	+		-	-	-	not done		R
P83	25	F	1	pneumonia	-	-	+	+		-	-	8	-	8	R
P84	62	M	3	lobar pneumonia	+	+	+	+		-	-	4	-	8	R
P85	53	F	3	lobar pneumonia	-	-	(-)	+		-	-	4	-	16	R
P86	44	F	7	lobar pneumonia	-	-	+	+		-	-	16	-	128	R
P87	75	M	9	pneumonia	+	+	+	+		-	-	4	-	16	R
P88	72	M	9	bronchopneumonia	not available				P.M. lung - +	-	-	2	-	32	F
P89	nk	M	3	bronchopneumonia	+	+	+	+		-	-	2	not done		R
P90	66	F	3	lobar pneumonia	-	-	(-)	+	P.fluid - +	-	-	256	not done		R
P91	29	F	3	lobar pneumonia	+	-	+	+		-	-	16	not done		R
P92	46	F	4	bronchopneumonia	+	-	-	+		-	-	32	not done		R
P93	66	F	6	lobar pneumonia	+	-	+	+		-	-	32	not done		R

P94	75	F	7	lobar pneumonia	+	-	(-)	+			-	-	8	not done	R
P95	14	M	8	lobar pneumonia	-	-	+	+			-	-	16	not done	R
P96	90	M	8	lobar pneumonia	-	-	(-)	+			-	-	16	not done	F
P97	75	M	8	pneumonia	+	+	(-)	+			-	-	2	not done	F
P98	62	M	14	pneumonia	+	+	+	+			-	-	8	not done	R
P99	nk	M	15	pneumonia	not available				P.M. lung +	+	-	-	4	not done	F
P100	31	F	22	bronchopneumonia	+	-	(-)	+			-	-	32	not done	NK
P101	84	F	22	bronchopneumonia	+	-	(-)	+			-	-	64	not done	R
P102	27	M	33	lobar pneumonia	+	+	+	+			-	-	16	not done	R
P103	76	F	35	bronchopneumonia	+	+	+	+			-	-	4	not done	R
P104	43	M	41	bronchopneumonia	+	+	+	+			-	-	2	not done	R
P105	56	M	19	lobar pneumonia	not available				Bronch.asp -	+	-	-	not done	not done	F
P106	65	F	19	lobar pneumonia	+	-	-	+			-	-	not done	not done	R
P107	19	M	21	lobar pneumonia	-	-	+	+			-	-	not done	not done	NK
P108	23	F	8	lobar pneumonia	+	+	(-)	+			-	-	not done	not done	R
P109	77	M	3	pneumonia	not available				throat swab type 3		-	not done		-	16	R
P110	41	M	5	lobar pneumonia	not available				bronch.asp. +	not done	not done	-	-	-	512	R
P111	nk	F	23	bronchopneumonia	+	-	(-)	+			not done	-	-	-	-	R

P112	83	F	8	pneumonia	-	-	(-)	+			not done	-	-	not done	R
P113	53	M	3	pneumonia	not available				P.M. lung -	+	not done	-	2	not done	F
P114	64	M	3	pneumonia	-	-	(-)	+			not done	-	4	- 16	R
P115	82	M	6	lobar pneumonia	+	+	+	+			not done	-	8	not done	R
P116	38	F	18	pneumonia	+	+	+	+			not done	-	4	not done	R
P117	82	M	19	bronchopneumonia	+	+	+	+			not done	-	4	not done	R
P118	88	F	23	lobar pneumonia	+	+	(-)	+			not done	-	2	not done	NK
P119	69	M	23	bronchopneumonia	+	+	+	+			not done	-	8	not done	NK
P120	72	M	34	bronchopneumonia	+	+	+	+			not done	-	32	not done	R
P121	55	F	29	lobar pneumonia	+	+	+	+			not done	-	not done	not done	R
P122	50	M	34	pneumonia & lung abscess	+	+	-	+	P. fluid -	+	not done	-	not done	not done	NK
P123	61	M	34	pneumonia	+	+	+	+			not done		not done	- 32	R

isolated from his sputum.

The relationship between culture and the presence of pneumococcal antigen in acute phase blood and sputum of 83 cases of pneumococcal pneumonia is summarised in Table 34. Of 22 patients with pneumococcal bacteraemia from whom appropriate specimens were available, antigen was detected in the blood of 11 (50%) and the sputum of 20 (91%). However, S.pneumoniae was isolated from sputum in only 9 of 22 (41%) patients. Antibiotic therapy, before the sputum was taken, was the probable cause of failure to isolate the organism in 9 of 13 (70%) culture-negative patients who had antigen-positive sputa. Moreover, in 21 patients with pneumococcal antigenaemia but without bacteraemia, 90% of sputa were antigen positive compared with 57% positive on culture. S.pneumoniae was isolated from the same proportion (58%) of antigen-positive sputa from patients with pneumonia but who had neither pneumococcal bacteraemia or antigenaemia. These results show that tests for antigen in sputum are more likely to give a positive result in pneumococcal pneumonia than either sputum or blood culture.

Table 34:

Results of culture and antigen detection in sputum from patients with pneumococcal pneumonia in relation to bacteraemia.

	Number of cases	Antigen in serum	Sputum culture positive	Sputum antigen positive
Bacteraemia	22	11(50%)	9(41%)	20(91%)
No bacteraemia	61	21(34%)	35(57%)	59(97%)

Almost one third of patients in this group were unable to produce a specimen of sputum during the acute phase of infection. The correlation, in 39 patients, between culture and the presence of pneumococcal antigen in acute blood and respiratory specimens other than sputum, is summarised in Table 35. Post- mortem lung tissue(12), bronchial aspirate (4) pleural fluid (2) and a throat swab were the specimens obtained from 19 of the 39 patients. Blood alone was examined, during the acute phase, from the remainder. Pneumococcal antigen was present in serum of 15 of 27 (56%) patients with pneumococcal bacteraemia. Post-mortem lung tissue from 8 contained pneumococcal antigen and S.pneumoniae was isolated from five. Pneumococcal antigenaemia, without bacteraemia, was observed in 6 patients and of these S.pneumoniae was isolated from pleural fluid (1) and bronchial aspirate (2), whereas in one patient post-mortem lung tissue contained pneumococcal antigen. S.pneumoniae was not isolated from the latter nor from a pleural fluid - which was not tested for pneumococcal antigen. S.pneumoniae or pneumococcal antigen was detected in post-mortem lung tissue (3), bronchial aspirate (2) and a throat swab in the remaining 6 patients. They did not have bacteraemia or antigenaemia. These results show that tests for antigen in other respiratory material obtained from patients who were unable to produce a sputum, were important in establishing that S.pneumoniae was the cause of the pneumonia.

Table 35:

Results of culture compared to antigen detection in acute phase blood and other respiratory specimens of patients unable to produce a specimen of sputum

	No. of cases	Acute phase blood[$] culture positive	Acute phase blood[$] antigen positive	Other respiratory specimens[*] from 19 cases culture positive	Other respiratory specimens[*] from 19 cases antigen positive
		number of cases with a positive result(%)			
Bacteraemia	27	27	15(56)	5(62)	8(100)
Antigenaemia	6	6		3(60)	2(40)
No bacteraemia/ or antigenaemia	6			3(50)	4(66)

$ acute phase blood was the only specimen available from 20 patients

*
post-mortem lung	12
bronchial aspirate	4
pleural fluid	2
throat swab	1

Results of tests for type-specific antibody on 33 paired sera showed > 4 fold rise in 21 (63.6%) between acute and convalescent phases :in seven the antibody titre rose despite demonstrable pneumococcal antigen in the convalescent sample. Stationary levels of antibody were seen in 4 patients and in the remaining 8 no antibody was detected in either acute or convalescent sera.

Analysis of results of serological tests on acute phase blood in patients with pneumococcal pneumonia.

Results of blood culture and tests for pneumococcal antigen carried out on acute phase blood of 98 patients with pneumococcal pneumonia are compared in Table 36. There was no significant difference between the frequency of pneumococcal antigenaemia in patients with bacteraemia compared to those without bacteraemia ("p" >0.05).

The results of pneumococcal antibody tests carried out on 93 acute phase sera of pneumonia patients, with and without pneumococcal bacteraemia and antigenaemia are summarised in Table 37. Patients who had bacteraemia, antigenaemia or both together showed a significantly lower incidence of antibody (37%) than patients with neither bacteraemia nor antigenaemia (79%).

Table 36:

Pneumococcal antigen in acute serum of patients with pneumococcal pneumonia with and without bacteraemia.

	Pneumococcal antigen detected	Number of patients
Blood culture positive	26(59%)	44
Blood culture negative	21(40%)	52
Total		96

"p" >0.05 (X^2 =3.3, calculated from absolute values)

Table 37:

Pneumococcal antibody in acute serum of patients with pneumococcal pneumonia with and without pneumococcal bacteraemia and antigenaemia.

	Pneumococcal antibody detected	Number of patients
Bacteraemia or antigenaemia	24(36.9%)	65
No bacteraemia or antigenaemia	22(78.6%)	28
Total		93

"p" <0.001 (X^2 =13.6, calculated from absolute values)

The outcome in pneumonia patients, in relation to pneumococcal bacteraemia and antigenaemia is recorded in Table 38. Though the numbers of patients studied are small, the results show that the highest case fatality rate was in patients with antigenaemia. Thus death rate was significantly higher ($p<0.05$) in them than in those with either bacteraemia alone or in those who had neither antigenaemia nor bacteraemia. The outcome in pneumococcal pneumonia in relation to the presence of antibody in acute phase blood is shown in Table 39. There were no deaths in patients with bacteraemia alone or with neither bacteraemia nor antigenaemia provided circulating antibody was not present. Somewhat surprisingly all patients with circulating antibody and who had bacteraemia together with antigenaemia, died.

Table 38:

Outcome in patients with pneumococcal pneumonia in relation to bacteraemia and antigenaemia.

	Number of patients	Died (%)
Bacteraemia only	15	3 (20)
Antigenaemia only	15	7 (47)
Bacteraemia and antigenaemia	17	9 (53)
No bacteraemia or antigenaemia	26	5 (17)
Total	73	24 (33%)

Table 39:

Fatality rate in relation to the presence of antibody in acute phase blood.

	Number of patients	Antibody present	No antibody present
Bacteraemia only	15	38%	0%
Antigenaemia only	15	38%	57%
Bacteraemia + Antigenaemia	17	100%	38%
No bacteraemia/ No antigenaemia	26	19%	0%

3.4.2. Laboratory tests on patients with pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease (COAD)

Table 40 shows the results on 109 cases of pneumococcal infection following acute exacerbation of chronic obstructive airways disease and includes the results of two episodes, due to the same or different pneumococcal types, in eight patients. Of 101 patients, 67 were male and the mean age was 68 years (range 35-94). There was clinical evidence of pneumonia in 21 of 109 cases. The remainder had clinical signs consistent with a chest infection but no evidence of pneumonia. There were four deaths and bronchopneumonia was diagnosed in two of them.

Pneumococcal antigen was detected in 107 of 109 (98%) sputa, but S.pneumoniae was isolated from only 93 (85%). Antibiotics had been prescribed before the collection of the sputum sample in 15 of these 16 culture-negative, antigen-positive sputa. Blood cultures in 25 of the 109 cases were negative. However, antigen was detected in the acute phase blood of 6 cases in whom blood culture was negative and in a further 8 cases who did not have blood culture carried out.

Antibody was detected in only 3 of 13 (23%) sera which contained pneumococcal antigen. However 80 of 89 (90%) sera without pneumococcal antigen (Table 41) did contain antibody and this difference was statistically

Table 40:

Laboratory tests on patients with pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease.

case No.	age	sex	type	clinical diagnosis	SPUTUM EXAMINATION microscopy pus	SPUTUM EXAMINATION microscopy DPC	SPUTUM EXAMINATION pneumococcal Cult.	SPUTUM EXAMINATION pneumococcal Ag.	ACUTE PHASE★ BLOOD pneumococcal Cult.	ACUTE PHASE★ BLOOD pneumococcal Ag. ng/ml.	ACUTE PHASE★ BLOOD pneumococcal Ab.	CONVALESCENT☆ PHASE BLOOD pneumococcal Ag. ng/ml.	CONVALESCENT☆ PHASE BLOOD pneumococcal Ab.	OTHER SPECIMENS
C1	71	F	3	chest infection	+	-	+	+	-	60	-	not done		
C2	75	M	3	pneumonia	+	-	(-)	+	-	500	-	-	16	case No.C31
C3	94	F	9	bronchopneumonia	+	-	(-)	+	-	12	-	not done		
C4	72	F	4	bronchopneumonia	+	-	(-)	+	-	500	4	not done		
C5	nk	M	6	bronchopneumonia	+	-	(-)	+	-	25	4	not done		
C6	nk	M	3	pneumonia & septicaemia	+	-	(-)	+	-	2000	not done	not done		
C7	80	M	3	chest infection	+	-	(-)	+	-	-	2	not done		
C8	NK	M	3	lobar pneumonia	-	-	(-)	+	-	-	4	not done		type 3 in nose swab
C9	76	M	3	chest infection	-	-	(-)	+	-	-	4	not done		
C10	68	M	4	heart failure	+	+	+	+	-	-	8	not done		
C11	nk	F	6	chest infection	+	+	+	+	-	-	2	not done		
C12	64	M	6	chest infection	-	+	+	+	-	-	16	not done		
C13	81	M	6	bronchopneumonia	-	-	(-)	+	-	-	4	not done		fatal

C14	70	M	9	chest infection	+	+	+	+		-	-	2	not done	
C15	nk	M	11	chest infection	+	+	+	+		-	-	8	not done	
C16	63	F	19	chest infection	+	+	+	+		-	-	4	not done	case No.C98
C17	87	M	19	chest infection	+	+	+	+		-	-	2	not done	
C18	80	F	19	chest infection	+	+	+	-		-	-	8	not done	case No.C71
C19	85	M	4	chest infection	+	+	+	+		-	-	8	not done	case No.C109
C20	52	M	8	chest infection	+	+	+	+		-	-	8	not done	case No.C53
C21	64	M	9	chest infection	+	+	+	+		-	-	8	not done	
C22	62	F	14	respiratory arrest	+	+	+	+		-	-	16	not done	
C23	51	M	11	chest infection	+	-	(-)	+		-	-	-	not done	
C24	50	F	12	bronchopneumonia	+	+	+	+		-	-	-	not done	
C25	62	M	8	pneumonia	+	-	+	-		-	-	-	not done	
C26	63	M	3	lobar pneumonia	+	+	+	+	not done		1000	-	not done	
C27	75	M	3	chest infection	+	+	+	+	not done		60	-	not done	
C28	74	M	9	bronchopneumonia	+	+	+	+	not done		50	-	not done	fatal
C29	79	M	14	chest infection	+	+	+	+	not done		125	-	-	16
C30	70	M	15	chest infection	+	+	+	+	not done		100	-	not done	case No.C84
C31	87	M	17	bronchopneumonia	+	+	+	+	not done		200	-	not done	case No.C2
C32	40	F	17	chronic renal failure/ chest infection	+	+	+	+	not done		200	-	not done	
C33	nk	F	7	chest infection	+	-	+	+	not done		12	4	not done	

C34	nk	M	3	chest infection	+	+	+	+	not done	-	-	not done		
C35	71	F	6	exacerbation of COAD	-	-	+	+	not done	-	-	not done		
C36	78	M	7	chest infection	+	-	(-)	+	not done	-	-	not done		
C37	60	F	8	lobar pneumonia	+	-	+	+	not done	-	-	not done		
C38	58	F	13	chest infection	+	+	+	+	not done	-	-	not done		
C39	64	F	14	cerebrovascular accident	-	-	+	+	not done	-	-	not done		
C40	57	F	3	lobar pneumonia	-	-	-	+	not done	-	4	-	16	
C41	63	M	3	bronchopneumonia	+	+	+	+	not done	-	32	not done		
C42	89	F	3	chest infection	+	+	+	+	not done	-	16	not done		
C43	78	F	4	post-op.	+	+	+	+	not done	-	8	not done		
C44	63	F	4	chest infection	+	+	+	+	not done	-	4	not done		
C45	82	M	6	chest infection	+	+	+	+	not done	-	4	not done		
C46	74	M	6	post-op.	+	+	+	+	not done	-	16	not done		
C47	67	M	6	chest infection	+	+	+	+	not done	-	8	not done		
C48	69	M	6	post-op.	+	+	+	+	not done	-	8	not done		
C49	53	F	6	chest infection	+	+	+	+	not done	-	8	-	8	case NoC83
C50	80	M	6	chest infection	+	+	+	+	not done	-	8	not done		
C51	67	F	6	chest infection	+	+	+	+	not done	-	16	not done		

C52	65	M	6	alcoholic gastritis	+	-	+	+	not done	-	32		not done	
C53	54	M	6	post-op.	+	+	+	+	not done	-	16		not done	case No.C20
C54	nk	F	8	chest infection	+	-	(-)	+	not done	-	4		not done	
C55	72	M	9	chest infection	+	+	+	+	not node	-	16		not done	
C56	48	M	9	chest infection	+	-	(-)	+	not done	-	8		not done	
C57	78	M	9	chest infection	+	+	+	+	not done	-	32		not done	
C58	64	M	9	chest infection	+	+	+	+	not done	-	32		not done	
C59	51	M	9	segmental pneumonia	+	+	+	+	not done	-	8		not done	
C60	67	M	9	chest infection	+	+	+	+	not done	-	4		not done	
C61	67	M	10	chest infection	-	-	+	+	not done	-	32		not done	
C62	56	M	10	chest infection	+	+	+	+	not done	-	32		not done	
C63	84	M	10	chest infection	+	+	+	+	not done	-	8		not done	
C64	69	M	11	chest infection	+	+	+	+	not done	-	8		not done	
C65	66	M	11	post-op.	+	+	+	+	not done	-	8		not done	
C66	75	M	11	chest infection	+	+	+	+	not done	-	16		not done	
C67	66	F	11	chest infection	-	-	+	+	not done	-	4		not done	
C68	82	M	11	chest infection	+	+	+	+	not done	-	4		not done	case No.C69
C69	82	M	11	chest infection	+	+	+	+	not done	-	8		not done	case No.C68
C70	73	M	11	chest infection	+	+	+	+	not done	-	16		not done	
C71	77	F	11	chest infection	+	+	+	+	not done	-	16		not done	case No.C18

C72	35	M	11	post-op.	+	+	+	+	not done	-	16	not done		
C73	77	M	12	pneumonia	+	+	+	+	not done	-	32	not done		
C74	63	M	13	chest infection	+	+	+	+	not done	-	32	not done	case No.C91	
C75	75	F	13	bronchopneumonia	+	-	(-)	+	not done	-	2	not done		
C76	nk	F	14	post-op.	+	+	+	+	not done	-	4	not done		
C77	84	M	14	chest infection	+	+	+	+	not done	-	32	not done		
C78	88	F	15	chest infection	+	+	+	+	not done	-	4	-	32	
C79	70	M	15	pneumonia	+	+	+	+	not done	-	4	not done		
C80	61	M	16	chest infection	+	+	+	+	not done	-	8	not done		
C81	67	M	17	chest infection	+	+	+	+	not done	-	4	not done		
C82	51	F	17	chest infection	+	+	+	+	not done	-	2	not done		
C83	53	F	17	chest infection	+	+	+	+	not done	-	4	not done	case No.C49	
C84	70	M	17	post-op.	+	+	+	+	not done	-	8	not done	case No.C30	
C85	60	F	17	chest infection	-	+	+	+	not done	-	16	not done		
C86	70	F	17	chest infection	-	-	+	+	not done	-	4	not done		
C87	65	F	17	chest infection	+	+	+	+	not done	-	16	not done		
C88	51	M	17	chest infection	+	+	+	+	not done	-	4	not done		
C89	66	M	17	chest infcetion	-	-	(-)	+	not done	-	4	not done		
C90	61	F	18	chest infection	+	-	+	+	not done	-	32	not done		
C91	63	M	18	chest infection	+	+	+	+	not done	-	16	not done	case No.74	

C92	73	M	18	chest infection	+	+	+	+	not done	-	4	not done		
C93	70	M	19	chest infection	+	+	+	+	not done	-	16	not done		
C94	nk	M	19	chest infection	+	+	+	+	not done	-	8	not done		
C95	75	M	19	chest infection	+	+	+	+	not done	-	16	not done		
C96	68	M	19	chest infection	-	-	+	+	not done	-	4	not done	P.M. lung Ag type 19	
C97	65	M	19	chest infection	+	+	+	+	not done	-	4	-	16	
C98	63	F	19	chest infection	+	+	+	+	not done	-	16	not done	case No.C16	
C99	nk	F	19	chest infection	-	-	+	+	not done	-	8	not done	Fatal	
C100	82	F	19	chest infection	+	+	+	+	not done	-	4	not done		
C101	42	M	19	chest infection	+	+	+	+	not done	-	4	not done		
C102	76	M	19	chest infection	+	+	+	+	not done	-	2	not done		
C103	74	M	19	chest infection	+	+	+	+	not done	-	8	not done		
C104	56	F	6	chest infection	+	+	+	+	not done	not done	-	32		
C105	68	M	9	chest infection	+	+	+	+	not done	not done	-	32		
C106	72	M	9	bronchopneumonia	+	+	+	+	not done	not done	-	8		
C107	51	F	13	chest infection	+	+	+	+	not done	not done	-	4		
C108	43	M	17	chest infection	+	+	+	+	not done	not done	-	16		
C109	73	M	19	chest infection	+	+	+	+	not done	not done	-	16		

Table 41

Pneumococcal antigen and antibody in acute serum of patients with acute exacerbation of chronic obstructive airways disease

Acute phase blood	Antibody present	Number of patients
No antigen detected	80 (90%)	89
Antigen present	3 (23%)	13

"p"<0.001(X^2=33, calculated from absolute values)

significant ("p" <0.001).

The results of antibody titres on 6 paired sera showed a significant rise in 5 patients. The titre of type-specific antibody in acute and convalescent sera of one patient was unchanged.

3.4.3. Laboratory tests on patients with acute chest infection secondary to other pathology.

Table 42 shows the results on 166 cases of pneumococcal chest infection secondary to other pathology and includes results of two episodes , due to the same or different pneumococcal types in 3 patients. Of 163 patients, 83 were female and the mean age was 58 years (range 16-94). This was a heterogenous group which consisted of patients with a productive cough and sputum on admission (43), or who developed a lower respiratory infection secondary to cardio-respiratory disease (27), post-operatively (79) or during hospitalisation for treatment of diseases not associated with the respiratory tract (16). A total of 9 patients had clinical evidence of pneumonia and 7 of these were after surgical operation. The remainder (157 cases) had clinical signs consistent with a chest infection but no evidence of pneumonia.

Pneumococcal antigen was detected in 162 of 166 (98%) sputa, but S.pneumoniae was isolated from only 140 (84%). Antibiotics had been prescribed before the collection of the sputum sample in 15 of the 26 culture-negative, antigen-positive sputa. Blood cultures in 36 of the 166 cases were negative. However, antigen was detected in acute phase blood of 7 cases in whom blood culture was negative and in a further 22 cases who did not have blood culture carried out.

Table 42:

Laboratory tests on patients with acute chest infection secondary to other pathology

case No.	age	sex	type	clinical diagnosis	SPUTUM EXAMINATION microscopy pus	microscopy DPC	Pneumococcal Cult.	Pneumococcal Ag.	ACUTE PHASE★ BLOOD Pneumococcal Cult.	Pneumococcal Ag. ng/ml.	Pneumococcal Ab.	CONVALESCENT☆ PHASE BLOOD Pneumococcal Ag. ng/ml.	Pneumococcal Ab.	OTHER SPECIMENS
A1	78	M	3	heart failure & pleural effusion	+	+	+	+	-	4	-	not done		
A2	48	M	3	diabetic & chest infection	+	-	(-)	+	-	4	-	not done		
A3	74	M	17	post-op.	+	+	+	+	-	100	-	not done		
A4	52	F	19	chest infection	+	+	+	+	-	100	-	not done		
A5	79	M	6	myocardial infarction	-	-	-	+	-	1000	-	not done		
A6	58	M	10	chest infection	+	+	+	+	-	12	-	not done		
A7	66	M	19	post-op.	+	+	+	+	-	100	4	not done		
A8	67	F	3	chest infection	+	-	(-)	+	-	-	-	not done		
A9	70	M	6	post-op.	-	-	-	+	-	-	-	not done		
A10	46	F	6	post-op.	+	+	+	+	-	-	-	not done		
A11	59	F	8	chest infection	+	+	(-)	+	-	-	-	not done		
A12	nk	F	11	chest infection	+	-	+	+	-	-	-	not done		
A13	74	M	18	chest infection	+	+	(-)	+	-	-	-	-	4	

A14	65	F	3	post-op.	-	+	+	+	-	-	32	not done		
A15	80	F	3	chest infection	-	-	(-)	+	-	-	8	not done		
A16	nk	F	3	chest infection	+	-	-	+	-	-	8	not done		
A17	nk	F	3	rheumatoid athritis	+	-	(-)	+	-	-	8	not done		
A18	65	M	4	previous tuberculosis	+	+	+	+	-	-	2	not done		
A19	70	F	6	post-op.	+	+	+	+	-	-	8	-	8	case No.A25
A20	67	M	6	heart failure	-	-	+	-	-	-	4	not done		
A21	55	M	6	haemoptysis	+	+	+	+	-	-	8	not done		
A22	43	M	6	lymphoma (pneumonia)	-	-	+	-	-	-	8	not done		
A23	73	F	6	chest infection	+	+	+	+	-	-	2	not done		
A24	16	M	11	aspiration	+	+	+	+	-	-	4	not done		
A25	70	F	12	post-op.	+	+	(-)	+	-	-	8	not done		case No.A19
A26	69	M	13	chest infection	+	-	+	+	-	-	16	not done		
A27	29	F	14	post-op.	+	-	+	+	-	-	32	not done		
A28	62	M	15	chest infection	+	+	+	+	-	-	256	not done		also P.fluid
A29	43	F	17	post-op.	+	+	+	+	-	-	4	not done		
A30	35	F	17	post-op.(pneumonia)	+	-	(-)	+	-	-	4	not done		
A31	67	M	17	infective endocarditis	-	+	+	+	-	-	8	not done		
A32	41	M	17	pancreatitis	+	+	+	+	-	-	8	not done		

A33	50	M	1	post-op.	-	+	+	+	-	-	not done	not done	
A34	42	M	1	chest infection	+	-	+	+	-	-	not done	not done	
A35	44	F	6	post-op.	+	+	+	+	-	not done		-	8
A36	42	F	17	post-op.	+	+	+	+	-	not done		-	32
A37	37	M	1	post-op.	+	+	+	+	not done	25	not done	-	32
A38	73	M	3	post-op	+	+	+	+	not done	500	-	not done	
A39	67	F	3	bronchial carcinoma	+	+	+	+	not done	8	-	not done	
A40	77	M	3	anaemia	+	+	+	+	not done	60	-	not done	
A41	NK	M	3	chest infection	+	+	+	+	not done	8	-	case No.A73(3/12 later)	
A42	61	F	4	post-op	+	+	+	+	not done	500	-	not done	
A43	76	F	8	post-op.	-	+	+	+	not done	4000	-	not done	
A44	73	F	10	myocardial infarction	+	+	+	+	not done	500	-	not done	
A45	79	F	10	chest infection	+	+	+	+	not done	1000	-	not done	
A46	75	F	11	heart failure	-	+	+	+	not done	200	-	not done	
A47	NK	M	13	myocardial infarction	+	+	+	+	not done	25	-	not done	
A48	82	F	13	chest infection	+	-	-	+	not done	50	-	not done	
A49	35	F	15	post.op.(pneumonia)	-	-	+	+	not done	50	-	not done	
A50	41	F	16	post-op.	+	+	+	+	not done	50	-	not done	
A51	76	M	19	bronchial carcinoma	+	+	+	+	not done	500	8	not done	

A52	67	M	4	post myocardial infarction	-	-	+	+	not done	12	2	not done	
A53	58	F	4	chest infection	+	+	+	+	not done	12	2	not done	
A54	71	M	4	cerebrovascular accident	+	+	+	+	not done	25	2	not done	
A55	67	M	7	post-op.	-	-	+	+	not done	25	4	not done	
A56	59	F	7	post-op.	+	+	+	+	not done	25	4	not done	
A57	61	M	9	post-op. respiratory failure	-	-	+	+	not done	25	8	not done	
A58	24	M	11	post-op.	+	+	+	+	not done	60	4	not done	
A59	51	F	3	post-op.	+	+	+	+	not done	-	-	not done	
A60	25	M	4	post-op.	+	+	+	+	not done	-	-	not done	
A61	35	M	6	post-op.	+	+	+	+	not done	-	-	not done	
A62	47	F	12	multiple sclerosis	-	-	-	+	not done	-	-	not done	
A63	20	F	16	post-op.	+	+	+	+	not done	-	-	not done	
A64	46	M	16	post-op.	+	+	+	+	not done	-	-	not done	
A65	72	F	19	chest infection	+	+	+	+	not done	-	-	not done	
A66	18	F	12	post-op.	+	+	+	+	not done	-	-	not done	
A67	65	M	7	bronchial carcinoma	-	-	+	+	not done	-	-	-	16

A68	27	F	19	post-op.	-	-	+	-	not done	-	.	not done		
A69	33	F	3	post-op.	+	+	+	+	not done	-	4	not done		
A70	57	M	3	post-op.	-	-	+	+	not done	-	8	not done		
A71	38	F	3	post-op.	+	+	+	+	not done	-	8	not done		
A72	87	M	3	post-op.	+	+	(-)	+	not done	-	2	not done		
A73	nk	M	3	pneumonia	+	-	-	+	not done	-	8	not done	case No.A41	
A74	75	F	4	post-op.	+	+	+	+	not done	-	8	not done		
A75	89	F	4	myocardial infarction	+	+	+	+	not done	-	2	not done		
A76	51	M	4	chest infection	+	+	+	+	not done	-	8	not done		
A77	74	F	4	myocardial infarction	+	+	+	+	not done	-	32	not done		
A78	86	M	6	post-op.	+	+	+	+	not done	-	4	not done		
A79	56	M	6	post-op.	+	+	(-)	+	not done	-	16	not done		
A80	nk	F	6	post-op.(pneumonia)	+	+	+	+	not done	-	4	not done		
A81	35	F	6	post-op.	+	+	+	+	not done	-	32	not done		
A82	58	F	6	post-op.	+	+	+	+	not done	-	8	not done		
A83	64	F	6	post-op.	+	+	+	+	not done	-	8	not done		
A84	66	F	6	post-op.	+	+	+	+	not done	-	32	not done		
A85	80	M	6	post-op.(pneumonia)	+	+	+	+	not done	-	4	not done		
A86	46	F	6	chest infection	+	+	+	+	not done	-	8	not done		

A87	64	M	6	respiratory failure	+	+	+	+	not done	-	8	not done
A88	71	F	6	bronchial carcinoma	+	+	+	+	not done	-	32	not done
A89	22	F	7	post-op.	+	+	+	+	not done	-	4	not done
A90	31	F	7	post-op.	+	+	+	+	not done	-	16	not done
A91	68	F	7	chest infection	+	-	-	+	not done	-	4	not done
A92	75	F	7	myocardial infarction	+	+	+	+	not done	-	2	not done
A93	69	M	7	bronchial carcinoma	+	+	+	+	not done	-	16	not done
A94	76	M	8	post-op(pneumonia)	-	-	(-)	+	not done	-	16	not done
A95	33	F	8	post-op.	-	+	+	+	not done	-	4	not done
A96	36	F	8	post-op.	+	-	(-)	+	not done	-	16	not done
A97	84	F	8	chest infection	+	+	+	+	not done	-	32	not done
A98	72	M	8	chest infection	+	+	(-)	+	not done	-	16	not done
A99	53	M	8	chest infection	-	-	-	+	not done	-	16	not done
A100	68	M	9	post-op.	+	+	+	+	not done	-	16	not done
A101	37	M	9	post-op.	+	+	+	+	not done	-	32	not done
A102	26	F	9	post-op.	+	+	+	+	not done	-	8	not done
A103	34	F	9	post-op.	+	+	+	+	not done	-	16	not done
A104	22	F	9	post-op.	+	+	+	+	not done	-	16	not done
A105	59	F	9	overdose & cardiac arrest	+	+	+	+	not done	-	16	not done

A106	72	M	9	heart failure	+	+	+	+	not done	-	64	not done
A107	29	M	10	post-op.	+	+	+	+	not done	-	8	not done
A108	17	M	10	post-op.	+	-	-	+	not done	-	16	not done
A109	62	M	10	post-op.	+	+	+	+	not done	-	16	not done
A110	59	M	10	post-op.	+	-	(-)	+	not done	-	32	not done
A111	80	M	10	myocardial infarction	+	+	+	+	not done	-	16	not done
A112	76	M	10	myocardial infarction	-	-	+	+	not done	-	2	not done
A113	85	M	10	chest infection	+	+	+	+	not done	-	8	not done
A114	nk	F	10	chest infection	+	+	+	+	not done	-	16	not done
A115	26	F	11	post-op.	+	+	+	+	not done	-	8	not done
A116	44	F	11	overdose	+	+	+	+	not done	-	16	not done
A117	52	F	12	post-op.	+	-	+	+	not done	-	8	not done
A118	nk	M	12	anaemia	+	+	+	+	not done	-	4	not done
A119	66	F	12	haemoptysis	-	-	+	+	not done	-	8	not done
A120	87	M	12	chest infection	+	+	+	+	not done	-	4	not done
A121	77	M	13	chest infection	+	+	+	+	not done	-	4	not done
A122	23	F	14	post-op.	+	+	+	+	not done	-	8	not done
A123	25	F	14	post-op.	+	+	+	+	not done	-	8	not done
A124	70	M	14	post-op. bronchopneumonia	+	+	(-)	+	not done	-	8	not done

A125	69	M	14	chest infection	+	+	+	+	not done	-	16	not done
A126	94	F	14	cardio-respiratory arrest	+	+	+	+	not done	-	32	not done
A127	50	M	14	heart failure	+	+	+	+	not done	-	16	not done
A128	46	M	14	alcoholism	+	-	+	+	not done	-	16	not done
A129	35	M	15	post-op.	+	+	+	+	not done	-	8	not done
A130	77	M	15	post-op.	+	+	+	+	not done	-	8	not done
A131	52	M	15	post-op.	+	+	+	+	not done	-	8	not done
A132	59	F	15	chest infection	+	+	+	+	not done	-	16	not done
A133	36	F	17	post-op.	+	+	+	+	not done	-	8	not done
A134	54	M	17	post-op.	+	+	+	+	not done	-	4	not done
A135	59	M	17	post-op.(pneumonia)	+	+	+	+	not done	-	16	not done
A136	77	M	17	cerebrovascular accident	+	+	+	-	not done	-	8	not done
A137	78	F	17	chest infection	+	+	+	+	not done	-	16	not done
A138	69	F	17	heart failure	+	+	+	+	not done	-	16	not done
A139	40	F	18	post-op.	+	+	+	+	not done	-	4	not done
A140	65	F	18	post-op.	+	+	+	+	not done	-	8	not done
A141	77	F	18	chest infection	+	+	+	+	not done	-	2	not done
A142	71	F	18	chest infection	+	+	+	+	not done	-	16	not done
A143	68	M	19	post-op.	+	+	+	+	not done	-	16	not done

A144	60	M	19	post-op.	+	+	+	+	not done	-	2	not done
A145	60	M	19	cardiac arrest	+	+	+	+	not done	-	64	not done
A146	33	M	19	post-op.	+	+	+	+	not done	-	16	not done
A147	17	F	19	post-op.	+	+	+	+	not done	-	4	not done
A148	53	M	19	post-op.	+	+	+	+	not done	-	2	not done
A149	nk	M	19	post-op.	+	+	+	+	not done	-	8	not done
A150	96	F	19	post-op.	+	+	+	+	not done	-	8	not done
A151	80	F	19	cerebrovascular accident	+	+	+	+	not done	-	64	not done
A152	91	F	19	chest infection	+	+	+	+	not done	-	4	not done
A153	58	F	19	chest infection	+	+	+	+	not done	-	8	not done
A154	nk	F	19	PUO	+	+	-	+	not done	-	2	not done

A155	47	F	3	chest infection	+	+	+	+	not done	not done	-	16	
A156	72	F	4	heart failure	+	+	+	+	not done	not done	-	2	
A157	79	F	7	chest infection	+	-	-	+	not done	not done	-	8	
A158	78	F	7	heart failure	+	+	+	+	not done	not done	-	8	
A159	72	M	7	leukaemia	+	+	+	+	not done	not done	-	16	
A160	49	M	8	alcoholism	+	+	+	+	not done	not done	-	16	
A161	74	M	12	TB of spine	+	+	+	+	not done	not done	-	8	case No.A165
A162	70	M	14	chest infection	+	+	+	+	not done	not done	-	32	
A163	64	M	17	post-op.	+	+	+	+	not done	not done	-	32	
A164	57	F	17	chest infection	+	+	+	+	not done	not done	-	16	
A165	74	M	17	TB of spine	+	+	+	+	not done	not done	-	64	case No.A161
A166	84	M	18	chest infection	+	+	+	+	not done	not done	-	32	

Antibody was detected in 10 of 28 (36%) acute phase sera which contained pneumococcal antigen. However, 105 of 121 (87%) of acute phase sera without pneumococcal antigen (Table 43) did contain antibody and this difference was statistically significant ("p" <0.001).

The antibody titres on 3 paired sera showed a significant rise in two patients. The titre of type-specific antibody in acute and convalescent sera of one patient was unchanged.

Table 43

Pneumococcal antigen and antibody in acute serum of patients with pneumococcal chest infection secondary to other pathology

Acute phase blood	Antibody present	Number of patients
No antigen detected	105(86%)	121
Antigen present	10(43)	28

"p" <0.001 ($X^2=33$, calculated from absolute values)

3.4.4. Laboratory tests on patients with non-respiratory pneumococcal infection

Table 44 shows the results of laboratory tests on patients with non-respiratory pneumococcal infection. Of 28 patients, 15 were female and there were 7 deaths. The mean age was 51 (range 6 months - 84 years). S.pneumoniae or pneumococcal antigen was detected in acute phase blood of 27 patients. The same type of pneumococcus or pneumococcal antigen was detected in material obtained from the focus of infection in 13 of the 27 patients. The material examined was cerebrospinal fluid (11) an ear swab (1) and a knee aspirate (1). Material for examination was not available from the other 14 patients.

Of 23 patients with pneumococcal bacteraemia, antigen was detected in acute phase serum of 10 (43%). A further 3 patients also had pneumococcal antigen in their acute phase blood , but no bacteraemia.

Antibody was detected in 9 of 23 (39%) patients with bacteraemia or antigenaemia and a significant rise in titre was observed in three paired sera.

Table 44:

Laboratory tests on patients with non-respiratory pneumococcal infection

case no.	age	sex	type	clinical diagnosis	SPECIMEN EXAMINATION Pneumococcal Cult.	Ag.	ACUTE PHASE★ BLOOD Pneumococcal Cult.	Ag. ng/ml.	Ab.	CONVALESCENT☆ PHASE BLOOD Pneumococcal Ag. ng/ml.	Ab.	Outcome
S1	68	F	6	meningitis	+ CSF	+	+	1000	-	not done		R
S2	82	F	7	cholangitis	not available		+	1000	-	not done		R
S3	77	F	7	myeloma	not available		+	100	-	not done		R
S4	19	M	12	meningitis	+ CSF	+	+	1000	-	not done		R
S5	6/12	F	19	meningitis	+ CSF	+	+	100	-	not done		NK
S6	71	F	6	meningitis	+ CSF	+	+	1000	4	not done		F
S7	nk	M	14	pancreatitis	not available		+	100	2	not done		F
S8	84	F	17	no focus of infection	not available		+	100	4	not done		F

S9	2	F	18	meningitis	+ CSF +	+	2000	not done	not done		F
S10	18/12	F	18	meningitis	+ CSF +	+	100	not done	not done		R
S11	6	M	6	nephritis	not available	+	-	-	not done		R
S12	54	M	8	septicaemia	knee aspirate + +	+	-	-	not done		R
S13	58	M	9	no focus of infection	not available	+	-	-	not done		F
S14	22	F	10	meningitis	+ CSF +	+	-	-	-	32	R
S15	nk	M	12	septicaemia	not available	+	-	-	not done		NK
S16	71	M	13	no focus of infection	not available	+	-	-	not done		R
S17	4	F	19	meningitis	+ CSF +	+	-	-	-	32	R
S18	43	M	6	pancreatitis	not available	+	-	8	not done		R
S19	74	F	14	no focus infection	not available	+	-	8	not done		R
S20	68	M	19	septicaemia	not available	+	-	4	not done		NK
S21	61	M	34	pneumonia & meningitis	- CSF +	+	-	32	not done		NK

S22	52	F	3	meningitis	CSF +	+	+	not done		50	-	R
S23	71	F	19	otitis media	ear swab type 19		+	-	not done	not done		NK
S24	72	M	19	no focus of infection	not available		+	-	not done	not done		NK
S25	63	F	4	meningitis	CSF -	+	-	2000	-	not done		NK
S26	80	F	3	no focus of infection	not available		-	250	2	not done		NK
S27	59	M	14	no focus of infection	not available		-	10.000	4	-	32	R
S28	60	M	14	infective endocarditis	P.M. aorta +	+	-	-	16	not done		F

3.4.5. Summary of results of laboratory tests on specimens from patients with pneumococcal infection

Results of culture for S.pneumoniae and tests for pneumococcal antigen carried out on blood and other clinical specimens from patients in each of the four categories are summarised in Table 45. The results show that 49 (48%) cases in Group "P" and 24 (86%) of patients in Group "S" had pneumococcal bacteraemia. In contrast, patients in Groups "C" or " A" did not have bacteraemia although blood culture was carried out in only one fifth of these cases. Pneumococcal antigen was detected in the acute phase blood in 45% and 48% of patients in Groups "P" and "S" respectively. Whereas in patients in Groups "A" and "C" pneumococcal antigen was detected in only 19% and 13% respectively.

Pneumococcal antigen was detected in 98% and S.pneumoniae was isolated from approximately 85% of sputa of cases in Groups "C" and "A". However, the difference in positivity between culture and antigen detection in respiratory specimens from patients in Group "P" was 42%. In Group "S" material from the infection focus was examined in only half of the patients. In these pneumococcal antigen was detected in 100%, whereas S.pneumoniae was isolated from 86%.

Table 45:

Summary of the results of laboratory tests on specimens from patients with pneumococcal infection.

	Group P	Group C	Group A	Group S
number of cases	123	109	166	28
Acute phase blood				
Bacteraemia	48%	0%	0%	86%
Antigenaemia	45%	14%	19%	48%
Material from the focus of infection				
pneumococci isolated	42%	85%	85%	85%
pneumococcal antigen present	98%	98%	98%	100%

Group P = pneumococcal pneumonia

Group C = acute exacerbation of chronic obstructive airways disease.

Group A = acute pneumococcal chest infection secondary to other pathology.

Group S = non-respiratory pneumococcal infection

Results of sputum examination, when cases were categorised according to clinical diagnosis, are shown in Table 46. They show the similarity between Groups "A" and "C", whereas in cases of pneumonia only the results of antigen tests were comparable with the other 2 groups. Pneumococcal antigen in sputum identified the greatest number of pneumococcal chest infections.

Results of blood culture and tests for pneumococcal antigen and antibody in acute phase blood of patients with pneumococcal infections are summarised in Table 47. They show that in all groups type-specific pneumococcal antibody was present in acute phase blood of three-quarters of the patients when bacteraemia or antigenaemia were absent. However, the percentage of patients with antibody in acute phase serum was reduced when pneumococcal infection was accompanied by bacteraemia or antigenaemia.

Table 48 shows the results of tests for pneumococcal antigen and antibody in acute blood of patients on whom blood culture was not done. They show, also, that the percentage of patients with type-specific antibody in acute phase blood was markedly reduced amongst patients with pneumococcal antigen in their serum.

Table 46:

Sputum examination in patients with pneumococcal infection of the lower respiratory tract.

	Group P	Group C	Group A
Number of patients	122	101	163
Number of sputa	84	109	166
purulent sputum	49(59%)	95(87%)	143(86%)
Diplococci seen	32(39%)	80(73%)	131(79%)
Culture positive	45(54%)	93(85%)	140(84%)
Antigen positive	80(95%)	107(98%)	162(98%)

Group P = pneumococcal pneumonia

Group C = pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease

Group A = acute pneumococcal chest infection secondary to other pathology

Table 47:

Summary showing incidence of antibody in relation to bacteraemia and antigenaemia in acute blood of patients with pneumococcal infections.

	Group P		Group C		Group A		Group S	
	No. of cases	antibody present	No. of cases	antibody present	No. of cases	antibody present	No. of cases	antibody present
Bacteraemia + Antigenaemia	26	15%	not applicable		not applicable		8	38%
Bacteraemia/ No antigenaemia	18	55%	not applicable		not applicable		11	36%
Antigenaemia/ No bacteraemia	21	48%	5	40%	7	29%	3	66%
No bacteraemia/ No antigenaemia	28	79%	19	84%	25	76%	1	100%

Table 48:

Summary of results of tests for antigen and antibody in acute blood of patients on whom blood culture was not carried out.

	Group P		Group C		Group A	
	No. of cases	antibody present	No. of cases	antibody present	No. of cases	antibody present
Antigen in blood	5	20%	8	13%	21	38%
No antigen in blood	11	73%	70	91%	96	90%

3.4.6. Urinary antigen in relation to bacteraemia and antigenaemia in patients with pneumococcal infection.

Results of tests for pneumococcal antigen in urine of 91 patients are compared with the results of culture and presence of antigen in blood taken at or about the same time (Table 49). A number of the patients included in Tables 33,40,42 and 44 were studied and are identified by their case numbers. Hence, specimens of urine were examined from patients with pneumonia (49), non-respiratory pneumococcal infection (13), acute chest infection secondary to other pathology (17) and pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease(12).

The scatter diagram (Fig. XXVII) shows the correlation between pneumococcal antigen in urine and blood. Clearly, in about half of these patients antigenuria did not correlate with antigenaemia. Furthermore, the relative amounts of antigen detected in paired specimens of blood and urine differed widely in most patients.

Figure XXVII:

Correlation between pneumococcal antigen in the urine and blood of patients with pneumococcal infection.

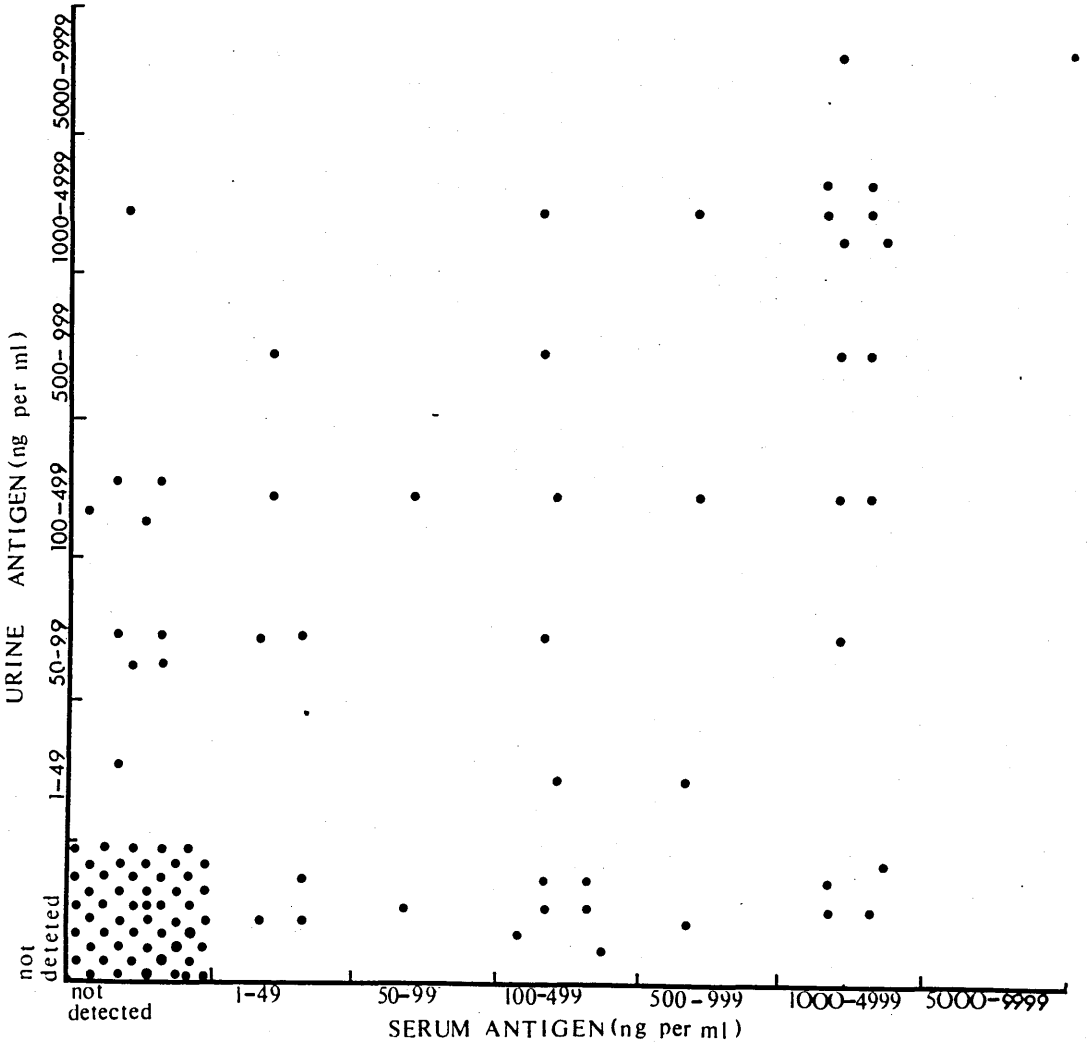


Table 49:

Tests for pneumococcal antigen in urine of patients with pneumococcal infections.

case No.	type	clinical diagnosis	ACUTE PHASE★ BLOOD pneumococcal cult.	Ag. ng/ml	Ab.	URINE pneumococcal Ag. ng/ml	CONVALESCENT PHASE☆ BLOOD pneumococcal Ag. ng/ml	Ab.	URINE pneumococcal Ag. ng/ml
P2	3	pneumonia	+	4000	-	1000	125	8	not done
P3	8	pneumonia & septicaemia	+	125	-	25	-	32	-
P4	1	pneumonia	+	125	-	125	500	-	125
P5	9	lobar pneumonia	+	1000	-	200	1000	-	500
P13	6	lung abscess & pneumonia	+	1000	-	50	not done		not done
P14	7	bronchopneumonia	+	50	-	250	not done		not done
P15	8	lobar pneumonia	+	250	-	-	not done		not done
P18	9	bronchopneumonia	+	250	-	-	not done		not done
P20	16	bronchopneumonia	+	+	-	+	not done		not done
P22	23	pneumonia	+	50	-	-	not done		not done
P23	29	lobar pneumonia	+	+	8	-	not done		not done
P26	8	pneumonia	+	500	4	1000	not done		not done
P27	1	lobar pneumonia	+	-	-	-	-	16	not done
P29	5	pneumonia	+	-	-	-	-	32	-

P30	8	pneumonia	+	-	-	100	-	-	-
P31	1	pneumonia	+	-	-	10	not done		not done
P32	8	pneumonia	+	-	-	-	not done		not done
P35	1	pneumonia	+	-	2	100	-	64	-
P37	14	lobar pneumonia	+	-	2	-	-	64	not done
P39	8	atypical pneumonia	+	-	4	-	not done		not done
P42	14	bronchopneumonia	+	-	8	-	not done		not done
P43	14	pneumonia	+	-	2	-	not done		not done
P45	34	lobar pneumonia	+	-	not done	-	not done		not done
P51	8	lobar pneumonia	-	1000	-	not done	1000	-	1000
P53	1	lobar pneumonia	-	1000	-	2000	not done		not done
P57	4	pneumonia	-	4000	-	250	not done		not done
P58	7	pneumonia	-	200	-	1000	-	32	not done
P61	1	lobar pneumonia	-	4000	16	4000	2000	16	4000
P62	3	pneumonia	-	100	4	not done	100	16	500
P63	3	pneumonia	-	30	2	60	15	4	60
P65	3	pneumonia	-	50	4	not done	-	8	1000
P66	3	lobar pneumonia	-	4	2	500	not done		not done

P68	3	pneumonia & septicaemia	-	8	2	125	not done		not done
P71	3	pneumonia	not done	500	-	-	500	8	25
P73	8	lobar pneumonia	not done	100	-	-	-	-	-
P77	3	lobar pneumonia	-	-	-	-	100	-	50
P81	9	lobar pneumonia	-	-	-	-	not done		-
P82	22	atypical pneumonia	-	-	-	-	not done		not done
P90	3	lobar pneumonia	-	-	256	-	not done		not done
P91	3	lobar pneumonia	-	-	16	-	not done		-
P93	6	lobar pneumonia	-	-	32	-	not done		not done
P96	8	lobar pneumonia	-	-	16	-	not done		not done
P97	8	pneumonia	-	-	2	-	not done		not done
P102	33	lobar pneumonia	-	-	16	-	not done		not done
P103	35	bronchopneumonia	-	-	4	-	not done		not done
P104	41	bronchopneumonia	-	-	2	-	not done		not done
P105	19	lobar pneumonia	-	-	not done	50	not done		not done
P106	19	lobar pneumonia	-	-	not done	-	not done		not done
P121	29	lobar pneumonia	not done	-	not done	50	not done		-

S1	6	meningitis	+	1000	-	500	not done	not done
S2	7	cholangitis	+	1000	-	-	not done	not done
S3	7	myeloma	+	100	-	-	not done	not done
S4	12	meningitis	+	1000	-	-	not done	not done
S8	17	no focus	+	100	4	-	not done	not done
S10	18	meningitis	+	100	not done	-	not done	not done
S11	6	nephritis	+	-	-	50	not done	not done
S15	12	septicaemia	+	-	-	-	not done	not done
S17	19	meningitis	+	-	-	-	- 32	not done
S18	6	pancreatitis	+	-	8	100	not done	not done
S19	14	no focus	+	-	8	-	not done	not done
S23	19	otitis media	+	-	not done	-	not done	not done
S25	4	meningitis	not done	2000	-	1000	not done	not done
A1	3	heart failure	-	4	-	-	not done	not done
A5	6	myocardial infarction	-	1000	-	-	not done	not done
A10	6	post-op.	-	-	-	-	not done	not done
A14	3	post-op.	-	-	32	-	not done	not done

A17	3	rheumatoid arthritis	-	-	8	-	not done	not done
A21	6	haemoptysis	-	-	8	-	not done	not done
A23	6	chest infection	-	-	2	-	not done	not done
A24	11	aspiration	-	-	4	-	not done	not done
A27	14	post-op.	-	-	32	-	not done	not done
A32	17	pancreatitis	-	-	8	-	not done	not done
A34	1	chest infection	-	-	not done	-	not done	not done
A70	3	post-op.	not done	-	8	-	not done	not done
A75	4	myocardial infarction	not done	-	8	-	not done	not done
A101	9	post-op.	not done	-	32	-	not done	not done
A121	13	chest infection	not done	-	4	-	not done	not done
A139	18	post-op.	not done	-	4	-	not done	not done
A150	19	post-op.	not done	-	8	-	not done	not done
C3	9	bronchopneumonia	-	12	-	-	not done	not done
C5	6	bronchopneumonia	-	25	4	-	not done	not done
C7	3	chest infection	-	-	2	-	not done	not done
C8	3	lobar pneumonia	-	-	4	-	not done	not done

C9	3	chest infection	-	-	4	-	not done		not done
C11	6	chest infection	-	-	2	-	not done		not done
C19	4	chest infcection	-	-	8	-	not done		not done
C24	14	respiratory arrest	-	-	16	-	not done		not done
C26	3	pneumonia	not done	1000	-	1000	not done		not done
C40	3	lobar pneumonia	not done	-	4	100	-	16	not done
C42	6	alcholic gastritis	not done	-	32	-	not done		not done
C92	19	chest infection	not done	-	4	-	not done		not done

The relationship between antigen in the acute phase blood and urine of patients in each of the four categories are summarised in Table 50. The results show that antigenuria occurred in 20 (43%) patients in Group "P", 4 (13%) patients in Group "S" and 3 (23%) patients in Group "C". However pneumococcal antigen was not found in the acute phase urine of 17 patients in the less severely ill patients of Group "A". Antigenuria therefore was more likely in more severely ill patients.

Table 51 shows that in patients with pneumonia the incidence of antigenuria was significantly higher in those with antigenaemia than those who did not have antigenaemia (<0.001).

Specimens of urine and blood from 14 pneumonia patients were examined for pneumococcal antigen during convalescence - these included 3 patients from whom acute phase urine was not tested. Of 10 patients with pneumococcal antigenaemia, with or without antigenuria during the acute phase, pneumococcal antigen was detected in convalescent urine of 8 and the blood of 7. However, one patient who had neither antigenaemia nor antigenuria during the acute phase had both antigenaemia and antigenuria during convalescence.

Table 50:

Relationship between the results of acute phase blood and presence of pneumococcal antigen in urine.

Results of acute phase blood	Group P	Group S	Group C	Group A
	Number of urines positive (%)			
Antigenaemia (with or without bacteraemia)	15 of 21(71%)	2 of 7(16%)	1 of 3(33%)	not applicable
Bacteraemia only	3 of 11(27%)	2 of 6 (33%)	not applicable	not applicable
No bacteraemia/ No antigenaemia	2 of 14 (14%)	not applicable	2 of 10(11%)	0 of 15 (0%)
Total	20 of 46(43%)	4 of 13(31%)	3 of 13(23%)	0 of 17(0%)

Table 51

Pneumococcal antigen in acute phase urine of patients with pneumococcal pneumonia with and without antigenaemia.

Acute phase blood	Antigen present	Number of patients
No antigenaemia	5(20%)	25
antigenaemia	15(71%)	21
Total		46

"p" <0.001 ($X^2 = 12.2$, calculated from absolute values)

3.4.7. Type-specific antibody in 45 paired sera of patients with pneumococcal infection.

Table 52 shows the results of antibody titrations on 45 paired sera of patients with pneumococcal infection included in Tables 33,40,42 and 44. The majority of patients studied had pneumonia (33) and, together with a few patients from the other three groups, are identified by their case number.

The majority of patients (67%) had a 4-fold or greater rise in antibody titre. In six, the increase in titre was found despite the presence of homologous pneumococcal antigen in the convalescent serum.

The results are summarised in relation to antigenaemia and bacteraemia in Table 53 and show a 4-fold or greater rise in 11 of 19 (58%) patients with antigenaemia (both with or without bacteraemia) compared to 8 of 9 (89%) with bacteraemia alone.

Five of 8 patients with antigenaemia during the acute phase but who did not respond with a significant rise in antibody titre, had homologous antigen in both acute and convalescent sera. Of the remaining three, antibody was present in both acute and convalescent serum of one patient and the other two had no demonstrable antibody in either acute or convalescent sera. The latter were infected with S.pneumoniae type 8. Similarly, the one patient with bacteraemia alone, who did not have a detectable antibody response, also was infected with

Table 52*:

Titres of type-specific antibody in paired sera of patients with pneumococcal infection.

Case No.	type	ACUTE BLOOD Cult.	Ag.	Ab.	CONVALESCENT BLOOD Ag.	Ab.
P1	1	+	+	<2	+	16
P2	3	+	+	<2	+	8
P3	8	+	+	<2	-	32
P4	1	+	+	<2	+	<2
P5	9	+	+	<2	+	<2
P27	1	+	-	<2	-	16
P28	4	+	-	<2	-	16
P29	5	+	-	<2	-	32
S14	10	+	-	<2	-	32
S17	19	+	-	<2	-	32
P35	1	+	-	2	-	64
P37	14	+	-	16	-	64
P30	8	+	-	<2	-	-
P50	8	-	-	<2	+	32
P71	3	not done	+	<2	+	8
C2	3	-	+	<2	-	16
C29	14	not done	+	<2	-	16
P58	7	-	+	<2	-	32
S27	14	not done	+	4	-	32
P62	3	-	+	4	+	16
P64	19	-	+	4	+	16
P61	1	-	+	16	+	16

P63	3	-	+	2	+	4
P65	3	-	+	4	-	8
P73	8	not done	+	-	-	-
P51	8	-	+	-	+	-
P52	8	-	+	-	-	-
A13	18	-	-	-	-	4
P110	5	not done	-	-	-	512
A67	7	not done	-	-	-	16
P85	3	-	-	4	-	16
P86	7	-	-	16	-	128
P87	9	-	-	4	-	16
P88	9	-	-	2	-	32
P114	3	not done	-	4	-	16
C40	3	not done	-	4	-	16
C78	15	not done	-	4	-	32
C97	19	not done	-	4	-	16
P84	3	-	-	4	-	8
P83	1	-	-	8	-	8
A19	6	-	-	8	-	8
C49	6	not done	-	8	-	8
P77	3	-	-	-	+	-
P111	23	not done	-	-	-	-

* antibody titre is the reciprocal of the highest dilution of serum giving clear peripheral fluorescence with homologous pneumococci

Heavy type are the serum pairs which do not show a > 4 fold rise in titre.

Table 53:

Summary of results of antibody titres in acute and convalescent sera of patients relative to the results of acute phase blood.

	Number of convalescent sera showing a >4 fold rise (%)
Antigenaemia (with or without bacteraemia)	11 of 19 (58%)
Bacteraemia alone	8 of 9 (89%)
No bacteraemia/ or antigenaemia	11 of 17 (65%)

S.pneumoniae type 8.

A 4-fold or greater rise in antibody titre was not observed in six patients who had neither antigenaemia nor bacteraemia during the acute phase. However 4 of them had approximately the same titre of antibody in both samples. Of the remaining two, one had antigen present in convalescent serum and the other was a type 23 infection.

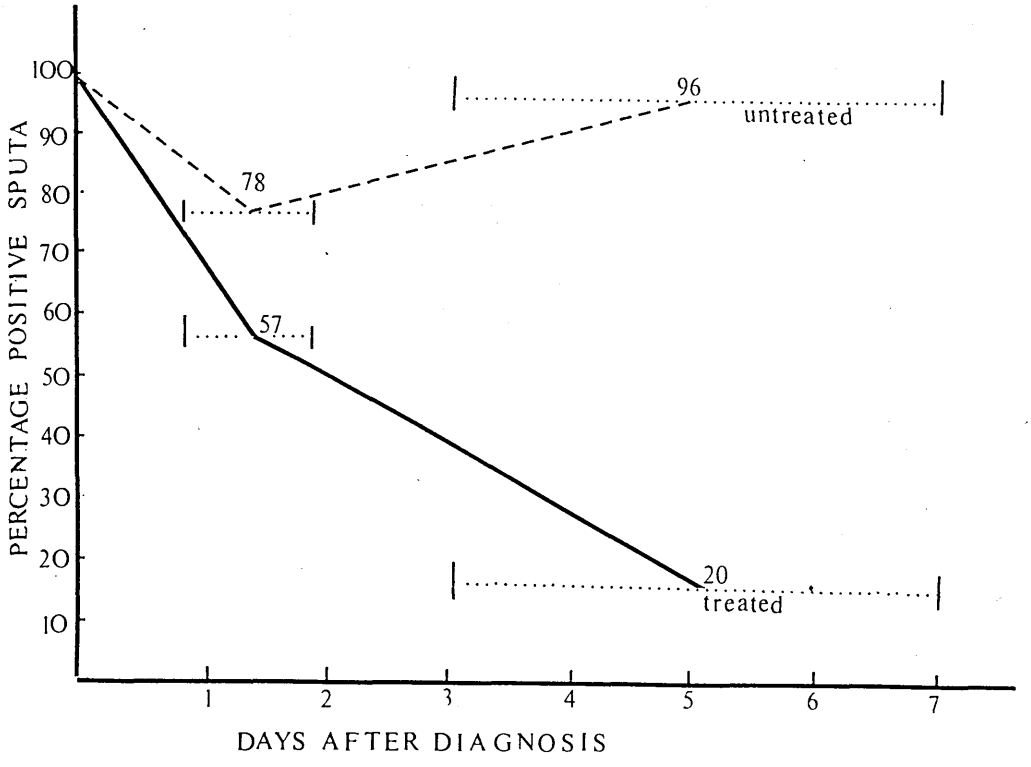
3.4.8. Results of culture and antigen tests for pneumococci in sputum relative to time and treatment.

From 398 patients two or more specimens of sputum were examined for pneumococci by culture and antigen tests. Specimens were not necessarily serial samples from the same patient and therefore analysis was limited to the recognition of general trends which relate to changes in status of culture and antigen tests with respect to both time and treatment. Moreover, each episode recorded was not necessarily restricted to an individual patient ; several occurred in the same patient. The results of culture and tests for pneumococcal antigen on specimens of sputum which were examined after (and including) the initial diagnostic specimen were compared in the different clinical groups (P,A and C) and divided further into treated and untreated patients. Subsequent sputum specimens examined on days 1 to 7 after the initial specimen were considered part of the initial episode, whereas those examined after 7 days were considered to represent a new episode.

Differences between treated and untreated patients with respect to the detection of pneumococci or pneumococcal antigen in subsequent specimens of sputum (Fig. XXVIII) indicates that following antibiotic therapy, pneumococci were eliminated from the sputum of

Figure XXVIII:

Trends in sputum positivity with respect to the detection of pneumococci or pneumococcal antigen in treated and untreated patients.



Note : These data are based on results from a total of 55 untreated and 209 treated patients; not every patient was sampled at both 1-2 days and 3-7 days.

43% of the patients in 1 or 2 days and from 80% in 3 to 7 days. Conversely, in patients who were not treated, pneumococci were present in the sputum of 96% after 3 to 7 days. A total of 55 patients with pneumococci in their initial sputum were not treated before a second specimen was examined. Of these, 32 were tested after 1 or 2 days and 7 of them (22%) were negative by culture and in antigen tests. The remainder were still culture positive, although 7 of 25 (28%) were antigen negative. These results suggest that pneumococci and pneumococcal antigen can be eliminated from patients' sputum without antibiotic treatment. However, at 3 to 7 days, 22 of 23 untreated patients (96%) were culture positive with unchanged antigen status relative to the results of the initial specimen.

Only 17% of treated patients, in whom the initial sputum was culture-positive, remained culture-positive after 1 or 2 days treatment. The other positive specimens detected were culture-negative but contained the same pneumococcal antigen type found in the first specimen. **However, the titre of pneumococcal antigen in these specimens was reduced by four fold or more in 62% of cases.**

These results show that, in patients who were treated with antibiotics pneumococci were rapidly killed. In all cases cultures were negative after 2 days treatment, although pneumococcal antigen persisted in the sputum of some patients for longer periods.

Results of treatment in relation to pathology

Of 209 episodes (in 203 patients), the majority of whom were treated with a penicillin or cotrimoxazole, 20% had pneumonia, 42% acute chest infection secondary to other pathology and 38% pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease. The cumulative percentage of negative specimens at day 7 was 80%, but the results of certain groups of patients differed significantly from this (Table 54). For example, only 51% of patients with pneumonia were negative by day 7, compared to 87% of the others ($p < 0.001$). Overall, only 50% of all patients in whom the initial sputum was culture-negative but antigen-positive, were negative by day 7 and differed significantly when compared to 89% of patients in whom the initial sputum was culture positive ($p < 0.001$).

Table 54:

Cumulative percentage of sputum samples negative, in tests for the detection of pneumococci, after 7 days treatment in patients with pneumococcal chest infection.

Clinical diagnosis	No. of cases	RESULT* OF FIRST SPUTUM SPECIMEN: Culture and antigen positive	Culture positive	Culture negative
		cumulative percentages of cases negative after 7 days treatment		
Group "P"	41	51%	81%	32%
Group "A"	89	87%	90%	68%
Group "C"	79	87%	89%	42%
		cumulative percentage of all patients whose sputa were negative after a total of 7 days treatment		
total	209	80%	89%	50%

Patients in Groups "P","A" and "C" were grouped according to clinical diagnosis as defined previously.

* On the basis of culture and test for pneumococcal antigen

Recurrence and relapse

Forty five of 203 patients had a recurrence of pneumococcal infection. In 30 episodes the pneumococcal type was different and in 19 the pneumococcal type was the same. In 17 of the 19 patients infected with the same type sputum culture and antigen tests carried out while the patient was being treated showed that pneumococci had been eliminated from the patient's respiratory tract before the second episode. Table 55 gives the details of recurrence in sputa from patients previously treated for pneumococcal chest infection. The results show that previous pneumococcal infection and treatment does not preclude further episodes due to the same pneumococcus. However, after 2 weeks, the type of pneumococcus involved is more likely to differ from the initial organism.

In all, 87 episodes (in 76 patients) were due to the same pneumococcal type, 167 episodes (in 132 patients) to different types. Pneumococci or pneumococcal antigen were detected in sputum of 36 patients between 7 and 14 days after initial diagnosis and the start of antibiotic treatment. In 9 of these patients, the pneumococci were different from the types detected in the initial sputum. However, the pneumococcal type detected in the other 27 was the same as that detected initially. These "relapsing" patients fitted into one of three categories.

1. Four patients with pneumonia from whom the initial

Table 55:

Recurrence of pneumococci in the sputum of patients[$] previously treated for pneumococcal infection.

Recurrence	Sputum positive[*] when tested at 4 to 14 days	2 weeks to 11 months	1 to 3 years
Total number of cases	36	150	68
percentage recurrence with the same type	75	33	15
percentage recurrence different type	25	66	85

*= based on results of culture and tests for pneumococcal antigen.

$ = most of these patients had chronic obstructive airways disease.

sputum was culture-negative but antigen-positive. These included one patient whose sputum was culture-negative but antigen-positive for a total of 25 days after initial diagnosis. This patient was treated with a number of antibiotics, active against pneumococci, both singly and in combination during the period of convalescence.

2. Antibiotic treatment was ineffective in 13 patients. Consequently their sputum remained culture-positive and with one exception antigen-positive also. Cephalosporins (cephradine and cephalexin) were used to treat 7 patients and septrin, tetracycline and moxalactam was used to treat one each of three others. The remainder had been given ampicillin, but during treatment a beta-lactamase producing bacterium was isolated from the sputum on culture i.e. H.influenzae, B.cattarhalis and S.aureus in three different patients. Beta -lactamase activity was also demonstrated directly in a sample of the homogenised sputum from all three .

3. Ten patients treated for pneumococcal infection became culture and antigen-negative while receiving an antimicrobial agent. However within a week of stopping the antibiotic their sputum contained the same pneumococcal type as was detected in the initial sputum. Homologous pneumococcal antibody was detected in the convalescent serum, taken between episodes, in eight of these patients. Thus, although the number of patients tested was small, the results indicate that the

presence of antibody in serum does not prevent recurrence of respiratory tract infection.

3.4.9.Presence of pneumococci in the respiratory tract as an indicator of future development of a pneumococcal chest infection.

Two studies were carried out :

1. Nasopharyngeal swabs, from 94 patients admitted to hospital for elective surgery, were tested for pneumococci before surgery.

2.Specimens of sputum from 30 patients with chronic bronchitis were tested at different times during a two year period.

Although the numbers of patients who developed a chest infection after initial testing were too small to be significant, the results suggested that there was no correlation between the carriage of pneumococci in the respiratory tract and the future development of a pneumococcal chest infection. For example, none of the surgical patients with S.pneumoniae in their nasopharynx developed chest infection post-operatively. Furthermore none of the patients who had a chest infection after operation carried pneumococci in their nasopharynx before surgery. Similarly, in chronic bronchitics, S.pneumoniae was not isolated, at the time of clinical exacerbation, from patients who had pneumococci in their sputum before exacerbation, whereas in patients with pneumococcal infection associated with acute exacerbation of chronic bronchitis, only a few had the same type of pneumococcus in their sputum before the exacerbation.

3.5.PNEUMOCOCCAL TYPE DISTRIBUTION

3.5.1. Comparison of the distribution of pneumococcal types in the sputum of patients with pneumonia, acute chest infection secondary to other pathology and pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease.

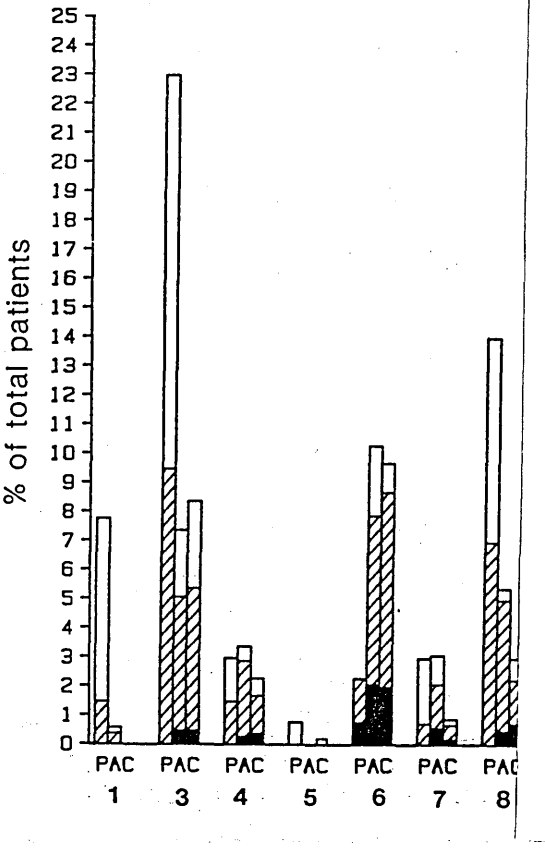
A total of 1682 episodes of pneumococcal chest infection were investigated by culture and tests for pneumococcal antigen on sputum.

The distribution of types in relation to the three clinico-pathological groups (Fig. XXIX) shows that the types detected were similar. However, there were notable differences. Type 1 accounted for 7.8% of cases in Group "P", less than 1% in Group "A" and was not found amongst the cases in Group "C". There were fewer episodes of infection due to types 24 and the higher types in each of the three groups; for example types 24,25,27,28,31,32,36,37,38,39,47 and 48 were not found amongst patients with pneumonia and only rarely in the other two groups.

The frequency of the 15 most common types detected in all cases, is shown for each clinical group in Table 56. Types 3 and 19 predominated in all groups but there was some variation in the distribution of other types. Types 6 and 17 were isolated 4 times more often from patients in Groups "A" and "C" than in patients in Group "P", whereas types 3 and 8 were isolated 2 to 3 times more

Figure XXIX:

Comparison of the distribution of pneumococcal types in the sputum of patients with pneumonia, acute chest infection secondary to other pathology and pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease.



C+A- : S.pneumoniae isolated on culture but pneumococcal antigen was not detected in the sputum.

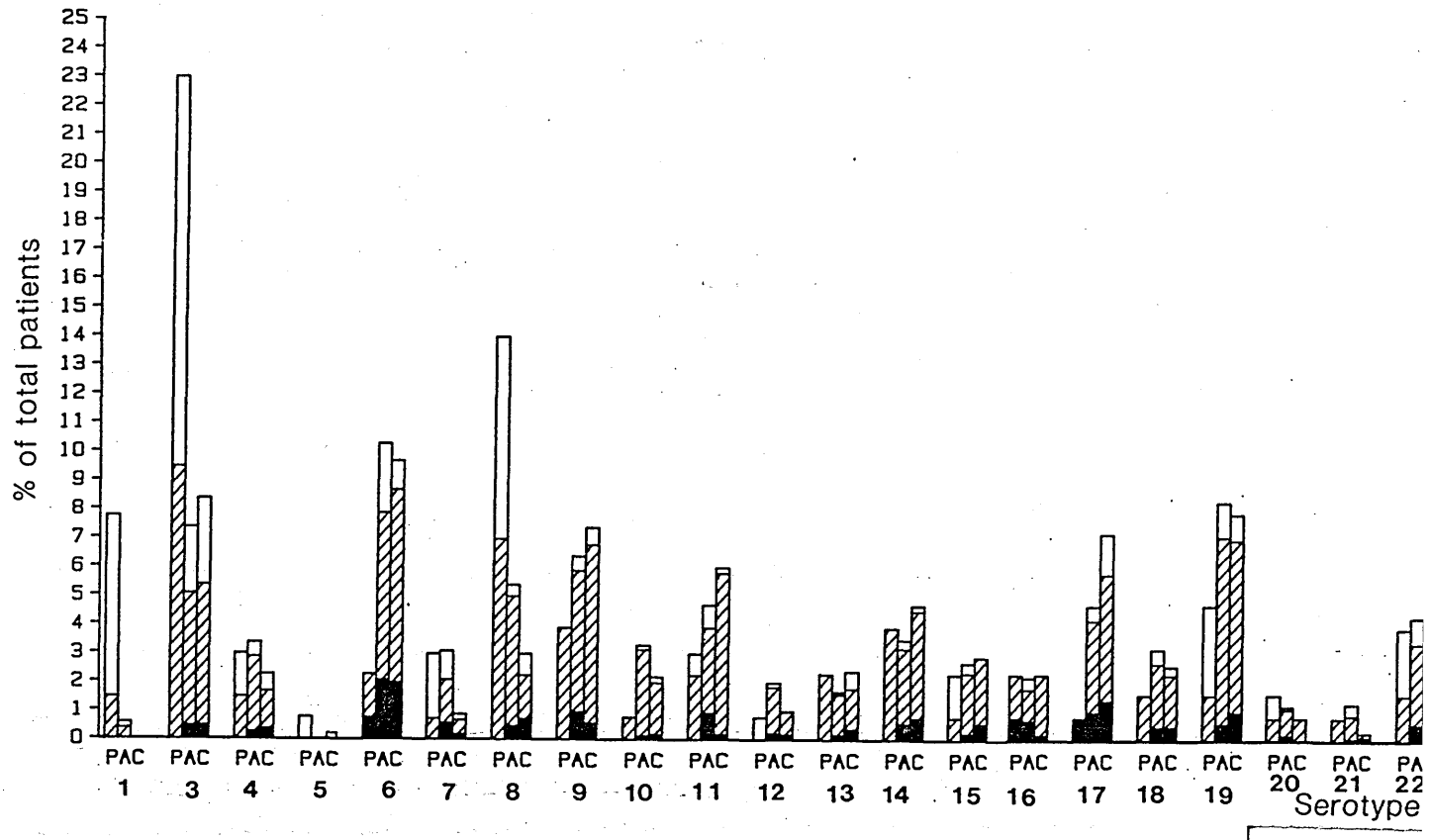
C+A+ : S.pneumoniae isolated on culture and pneumococcal antigen detected in the sputum.

C-A+ : S.pneumoniae not isolated on culture but pneumococcal antigen was detected in the sputum.

P : pneumococcal pneumonia.
A : acute chest infection secondary to other pathology.
C : acute exacerbation of chronic obstructive airways disease.

Figure XXIX:

Comparison of the distribution of pneumococcal types in the sputum of patients with pneumonia, acute chest infection secondary to other pathology and pneumococcal infection associated with acute exacerbation of chronic obstructive airways disease.



C+A- : S.pneumoniae isolated on culture but pneumococcal antigen was not detected in the sputum.

C+A+ : S.pneumoniae isolated on culture and pneumococcal antigen detected in the sputum.

C-A+ : S.pneumoniae not isolated on culture but pneumococcal antigen was detected in the sputum.

P : pneumococcal pneumonia.
A : acute chest infection secondary to other pathology.
C : acute exacerbation of chronic obstructive airways disease.

Table 56:

Frequency of the 15 most common types detected in the sputum of patients with pneumococcal chest infection.*

type	CLINICAL DIAGNOSIS					
	Group "P"$		Group "A"		Group "C"	
	No. of cases	%	No. of cases	%	No. of cases	%
3	29	22.7	69	7.4	52	8.4
4	4	3.1	32	3.4	14	2.3
6	3	2.3	96	10.3	60	9.7
8	18	14.0	51	5.4	19	3.1
9	5	3.9	60	6.4	46	7.4
10	1	<1.0	31	3.3	14	2.3
11	4	3.1	44	4.7	37	6.0
14	5	3.9	33	2.8	29	4.7
15	3	2.3	25	2.7	18	2.9
17	1	<1.0	44	4.7	44	7.1
18	2	1.6	30	3.2	16	2.6
19	6	4.7	78	8.3	49	7.9
22	5	3.9	40	4.3	20	3.2
23	4	3.1	49	5.2	40	6.5
42	1	<1.0	28	3.0	18	2.8
Others	37	29.0	226	24.0	142	23.0
Total	128	100	936	100	618	100

$ Type 1 was the third most common type detected in Group "P" (7.8%) but was seldom isolated from the other two groups therefore it was not considered to be amongst the most common 15 types.

* Patients in Groups P, A and C were grouped according to clinical diagnosis as defined previously.

often from patients in Group "P". In addition it was found that, when the distribution in Groups "A" and "C" were compared, type 17 was prevalent in Group "C" whereas type 8 was more common in Group "A". Table 57 summarises the statistical analysis of types associated with each of the three groups. Types 3 and 8 were isolated significantly more often from patients with pneumonia than from patients in Groups "A" and "C" ($p < 0.001$), whereas type 6 was significantly associated with chest infections without pneumonia ($p<0.001$). Similarly type 17 was also associated with chest infections without pneumonia ($p < 0.05$) but was more often found in patients with chronic obstructive airways disease ($P < 0.05$). Conversely type 8 was significantly associated with acute chest infections secondary to other pathology ($p<0.05$). No significant difference was found in the incidence of other types in the three clinico-pathological groups.

Table 57:

The association of particular types of *Streptococcus pneumoniae* with clinical presentation.

a. Types associated with pneumonia.

	No pneumonia[$]		No significant difference		Pneumonia
p value[*]	<0.001	<0.05		<0.05	<0.001
	6	17	9,11,14,19,23		3,8

[$] No pneumonia = acute chest infection plus acute exacerbation of chronic obstructive airways disease.

b. Types associated acute exacerbation of chronic obstructive airways disease and acute chest infection secondary to other pathology.

	Acute chest infection		No significant difference		Chronic obstructive airways disease
p value[*]	<0.05				<0.05
	8		3,6,11,14,19,23		17

[*] Determined using X^2 in two by two contingency tables and incorporating Yates Correction for Continuity (Siegal,1956).

3.5.2. Comparison of the distribution of pneumococcal types in systemic disease and the upper respiratory tract in adults and children.

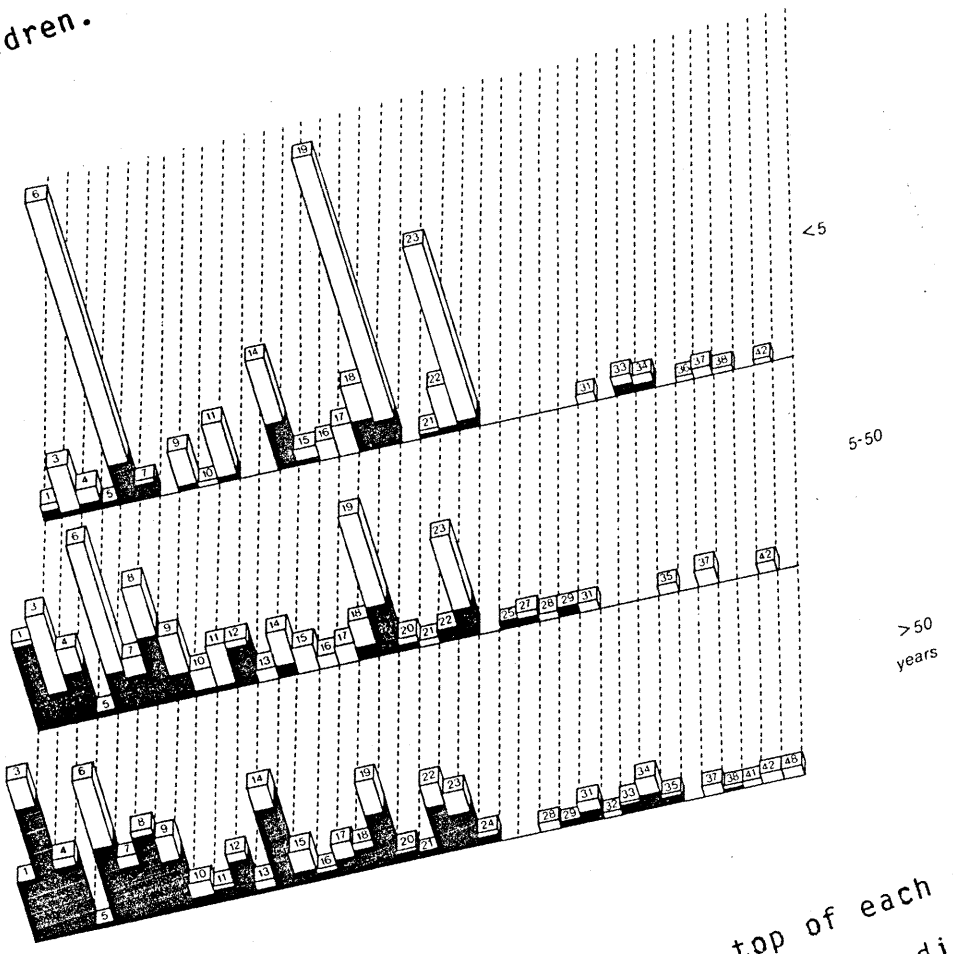
The sources of 874 S.pneumoniae strains together with the age distribution of patients are shown in Table 58. Three hundred and sixty were from cases of unequivocal pneumococcal disease and 514 were isolated from the upper respiratory tract. Blood culture was the most common source of strains from systemic disease (235) and 67% of blood culture isolates were from patients over 50 years of age. Of the upper respiratory isolates, 73% were obtained from the nasopharynx of children under 5 years of old whereas only 6% of the strains examined were from patients over 50 years of age. In contrast, 68% of sputum isolates (see Table 58 for definition of these strains) were from patients over 50.

The distribution of types in relation to age (Fig.XXX) shows that the strains belonged to 38 types (20 monotypes and 18 serogroups). Isolates that belonged to types 24 and the higher types formed a small proportion of isolates in all age groups. Types 8 and 12 were not found in pre-school children but were found in both the upper respiratory tract and implicated in systemic pneumococcal infection in the other age groups.

The frequency of the 15 most common types in systemic disease and the upper respiratory tract is shown in Table 59. Types 6 and 19 were predominant amongst strains isolated from the upper respiratory tract and

Figure XXX:

Comparison of the distribution of pneumococcal types in systemic disease and the upper respiratory tract in adults and children.



Pneumococcal types are shown at the top of each column.
Shaded area : types associated with systemic disease.
Unshaded area : types associated with upper respiratory tract carriage.

Table 58:

Sources and age distribution of *Streptococcus pneumoniae* strains.

	Numbers of isolates and age group			
Source	< 5 years	5-50 years	>50 years	Total
Blood	34	43	158	235
Cerebrospinal fluid	17	20	29	66
Pleural fluid and post-mortem lung	6	6	27	39
Others *	1	11	8	20
Total systemic disease	58	80	222	360
Nasopharynx	289	80	25	394
Commensal in sputum$	0	38	82	120
Total upper respiratory tract isolates	289	118	107	514

* Isolates from normally sterile sites, eg. joint fluid and peritoneal fluid.

$ Sputa with no pus in gram film; no infection noted in case sheet; no pneumococcal antigen detected in sputum; and mixed growth of *S.pneumoniae* and upper respiratory tract organisms isolated.

Table 59:

Frequency of the 15 most common types isolated from systemic disease and the upper respiratory tract.

	Systemic disease		Upper respiratory tract	
Type	Number of isolates	%	Number of isolates	%
1	30	8.3	3	<1.0
3	38	10.5	32	6.2
4	25	6.9	11	2.1
6	35	9.7	105	20.0
7	22	6.1	6	1.2
8	29	8.0	10	1.9
9	17	4.7	21	4.1
11	5	1.4	21	4.1
12	16	4.4	3	<1.0
14	34	9.4	27	5.3
17	4	1.1	15	2.9
18	15	4.2	15	2.9
19	26	7.2	89	17.3
22	14	3.9	17	3.3
23	14	3.9	63	12.3
Others	36	10.0	76	14.8
Total	360	100	514	100

were also amongst the most common types associated with systemic disease in all age groups. There was considerable variation in the distribution of types in each category. Types 1,4,7,8, and 12 were isolated between 3 and 8 times more often from systemic disease than from the upper respiratory tract, whereas types 6,11,17,19 and 23 were isolated from 2 to 3 times more often from the upper respiratory tract than from systemic disease.

Table 60 summarises the statistical analysis of types associated with systemic disease and upper respiratory carriage respectively. Because too few nasopharyngeal strains were available from older people, the serotype distribution was assessed in only two age groups - first children under 5 years of age and, second, older children and adults. In pre-school children serotypes 14 and 18 were isolated significantly more often from systemic disease than from the upper respiratory tract $p < 0.001$). In older patients, however, the serotypes significantly associated with systemic disease were types 1,4,7,8 and 12 ($p < 0.05$). Type 23 in pre-school children and type 6 in older patients was significantly associated with upper respiratory tract carriage ($p < 0.05$). No significant difference was found in the frequency of isolation other types from systemic disease compared to upper respiratory carriage.

Table 60:

The association of particular serotypes of *Streptococcus pneumoniae* with systemic disease and upper respiratory carriage

	Upper respiratory carriage		Systemic disease	
p value*	<0.5	no significant difference	<0.05	<0.001
< 5 years	23	3,6,9,19,22		14,18
>5 years	6	3,9,11,14,17 18,19,22,23	4,7,8,12	1

* determined using X^2 in two by two contingency tables incorporating Yates Correction for Continuity (Siegal,1956).

CHAPTER 4

DISCUSSION

Acute respiratory tract infections in the United Kingdom are responsible anually for 3.3 million recorded spells of sickness (about 36% of the total), over half a million admissions to hospital and an estimated one million episodes of pneumonia (Kennedy, 1985). Of infectious diseases, 90% of deaths in the developed world are due to acute respiratory infections and in contrast to other infectious diseases, the mortality rate has not improved in 20 years (Table 61). Cockburn (1979) concluded therefore that the greatest decrease in death rate had been in diseases for which preventative measures had been available and had been adequately applied :the decrease was less marked in diseases where reliance has been on treatment.

Nowadays most patients with acute respiratory disease in hospital show signs of lower respiratory tract infection. Lower respiratory tract infection includes a wide range of clinico-pathological conditions which overlap.

Recent studies showed, in hospitals, that 20-47% of chest infections without pneumonia and 68-89% of cases of lobar pneumonia were due to pneumococcal infection (El-Refaie and Dulake, 1975; Dulake, 1979). These figures were based on the results of antigen tests, rather than on culture results of sputum. Kennedy (1985) reviewed the results of three recent studies of community-acquired pneumonia in the United Kingdom. He showed that, in Glasgow and Bristol, approximately 10%

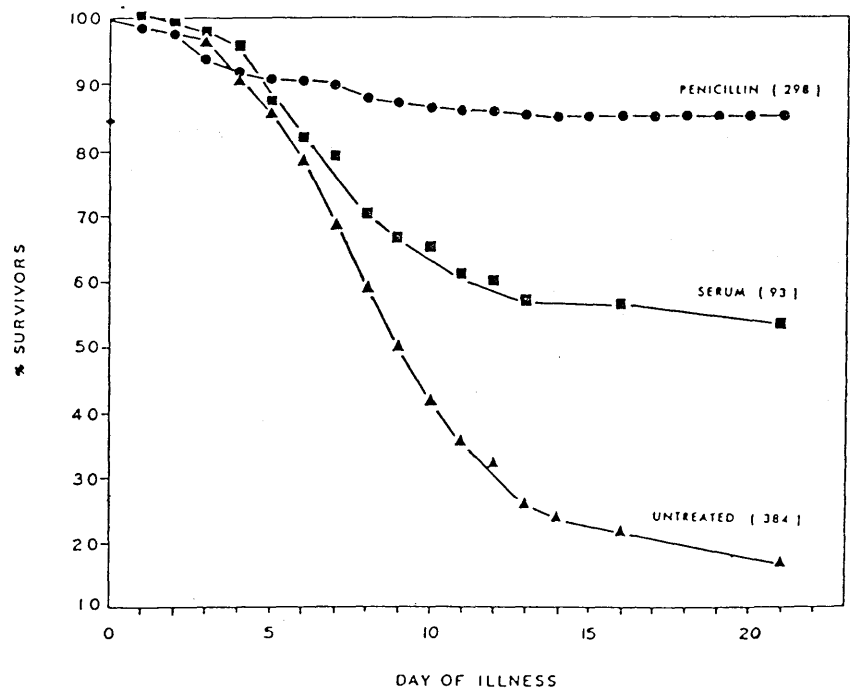
of patients had pneumococcal pneumonia but in 50% and 40% of patients respectively no causal organism could be demonstrated. However, in a study carried out in Nottingham (Macfarlane et al., 1982) tests for pneumococcal antigen were carried out on patients' sputum and showed that 76% had pneumococcal pneumonia : only 3% of cases were undiagnosed bacteriologically. This suggests that pneumococci are still the major cause of pneumonia. Clearly the high diagnostic rate (97%) in the Nottingham series was due to the use of antigen tests for pneumococci in sputum.

The case fatality rate in pneumococcal pneumonia ranges from 10 to 20%, but is higher in patients with bacteraemia (Austrian and Gold, 1964; Calder, McHardy and Schonell, 1970). Furthermore many patients die in spite of antibiotic therapy. About 60% of fatal cases of pneumococcal bacteraemia die in the first five days after onset (Austrian and Gold, 1964). The way in which the pneumococcus damages the host it invades is not clearly understood but appears not to be reversed by antibiotic therapy (Austrian, 1981). Moreover, Austrian and Gold (1964) found that the mortality rate, in bacteraemia over the first five days, had not changed since before the advent of serotherapy in 1934 (Fig. XXXI).

Antibiotics such as penicillin have had a dramatic effect on the course and outcome of pneumococcal pneumonia, but antibiotic usage has altered conventional bacteriological findings (Spencer and Philp, 1973) and

Figure XXXI:

Effect of therapy on % survival in pneumococcal bacteraemia.



Numbers in parentheses indicate size of each group of patients. Data for untreated and serum-treated patients (capsular Types I and II only) from Tilghman and Finland (1).

Reproduced from Austrian & Gold (1964)

Table 61:

Causes of death (per 100,000 per annum) in 14 developed countries (WHO statistics *).

Causes of death	1955-9	1974-5
All causes	947.72	911.35
infectious diseases	26.28	7.42
respiratory diseases	63.73	71.07

* reproduced and adapted from Cockburn(1979).

thereby created diagnostic problems that were not in existence before their use (Shulman, Phillips and Petersdorf, 1965).

Clearly Streptococcus pneumoniae remains a major cause of respiratory infection in hospital patients and since clinical presentation and pathology is diverse requires precise laboratory diagnosis.

Serotyping

Characterisation of pneumococcal antisera

In the studies reported here, co-agglutination was shown to be more sensitive than the capsular reaction test, but showed a high concordance with that test (Smart, 1986 ; Smart and Henrichsen, 1986). Most of the cross-reactions observed here have been previously noted in the capsular reaction test (Morch, 1943). However this study found, by co-agglutination, antigen sharing between pneumococcal types which had not previously been reported. Differences between co-agglutination and the capsular reaction test may reflect increased sensitivity of the co-agglutination test or differences in the antigenic structure of the strains used for antiserum production.

Absorption with the C-mutant strain reduced the range of cross-reactions observed with antisera to types 17A, 22F and 22A. Thus the reaction observed between 22F antiserum with type 36 and 22A antiserum with type 17F was clearly due to antibody to pneumococcal C-polysaccharide. Absorption of other sera with the C-

mutant strain had little or no effect on antibody titres for other types. Conversely, absorption with the appropriate homologous type eliminated all cross-reactions : the cross-reacting types therefore have antigenic determinants in common with the types used to raise the antiserum.

Antisera for diagnostic use showed no cross-reactions. For example, antisera to types 1,3,4 and 8 were monospecific whereas type 6B, 7A, 9N, 10F,12F, 17F, and 19F antisera reacted only with a related type. However antisera for diagnosis of types 11,14,15,18,20,22 and 23 were absorbed before use to remove cross-reacting antibodies. Since absorption also reduced the titre of type-specific antibody, the antiserum and absorbing type were selected on the basis of these cross-reactions. For example, type 22A antiserum was used after absorption with type 31: conversely antiserum to type 22F required absorption with types 20 and 31. Similarly, type 18C antiserum was absorbed with type 20 since this one absorption removed cross-reacting antibody for other types. Most of the other antisera were used to produce factor sera for typing within groups.

Although not every antiserum has been tested in this way by co-agglutination, this study shows that there is considerable overlap in the antigenic structure of pneumococci of different types.

Serotyping by co-agglutination

The results of serotyping clinical isolates of S.pneumoniae by co-agglutination and the capsular reaction test were in complete agreement. Furthermore serotypes within groups were determined using commercially-available group antisera. This method, which used the factors remaining in group antiserum after absorption with a type within the group, would suit laboratories in which factor sera were not available. Serotyping by co-agglutination has other advantages over both the capsular reaction test and counterimmumoelectrophoresis (CIE). These include ease of the test (no special equipment is required), economical use of antisera, and since viable cultures are not required, pneumococcal suspensions can be stored for days or even weeks before testing. Russell et al. (1978) suggested that capillary precipitation had the same advantages, but they did not carry out typing within groups and used 16 hour broth cultures of pneumococci. Co-agglutination on the other hand is applicable to suspensions of pneumococci taken directly from the initial culture plate or broth. The major weakness of CIE for typing pneumococci is the inability to detect capsular polysaccharide antigens of types 7F,7A,14,33F,33A and 37 which are neutrally-charged in conventional buffers (Henrichsen et al., 1980). Sottile and Rytel (1975) could not type one of 71 strains by CIE. Since this strain was isolated from blood and was

virulent in a mouse it may have been one of these types.

The increased sensitivity of co-agglutination compared to the capsular reaction test may explain the differences observed in the antigenic structure of S.pneumoniae type 7F (Table 11). Alternatively, the strain of S.pneumoniae type 7F used both in this study and by the State Serum Institute, Copenhagen, for antibody production may have differed from that originally described by Morch (1943).

Cross-reactions

Pneumococcal antisera also react with bacteria which share an antigen in common with pneumococci, and consequently serological tests cannot be considered totally specific. In most cases however, cross-reacting bacteria are morphologically different from pneumococci although strains of alpha-haemolytic streptococci e.g. S.mitis and S.sanguis are more difficult to recognise. The latter however are readily distinguished from pneumococci by the capsular reaction test, which, when positive, demonstrates the location of the reactive carbohydrate (Austrian et al., 1972). Although such visual distinction is not possible in co-agglutination tests, co-agglutination with cross-reactive streptococci is less intense than with pneumococci, and cross-reactions can be recognised by testing bacteria-free supernatants, prepared from the original suspension, since the reactive carbohydrate remains cell associated.

Sottile and Rytel (1975) identified strains of alpha-

haemolytic streptococci which reacted with type-specific pneumococcal antisera in CIE tests. Austrian et al. (1972), isolated 250 strains of streptococci, from sputum and nasopharyngeal secretions, which reacted with pneumococcal antisera in the capsular reaction test. In all, 28 pneumococcal types of antigen were detected in streptococci and 82% of these were accounted for by types 19,9,45,13,36,43 and 21 - in descending order of frequency. In the series presented here, streptococci which reacted with group 19 pneumococcal antiserum comprised 28%. Typing with factor sera was unsuccessful, but the results were broadly in accord with Austrian (1975b) who found that streptococci which reacted with pneumococcus group 19 antiserum were more closely related to types 19B and 19C than to types 19F or 19A - the predominant types in pneumococcal disease (Smart, Dougall and Girdwood, 1987b). Similarly, Morch (1944) recognised streptococci which reacted with pneumococcus group 7 antiserum. In the series presented here typing with factor sera showed that they more closely resembled pneumococcus type 7A than type 7F the predominant type, in this group, in human infection (Smart et al., 1987b). Macleod (1947) drew attention to the possible relationship between streptococci in the nasopharynx which cross-reacted with pneumococcus type 7 and the presence of "natural antibody" to this organism in serum. Austrian et al.(1972) noted that streptococci cross-reactive with some of the more pathogenic types

of pneumococci, for example 1,2,3,4,5,6,8 and 14, were seldom if ever found. Some streptococcal strains studied by Austrian et al. (1972) reacted with antiserum to pneumococcal C-polysaccharide as well as type-specific pneumococcal antisera : this was found with several strains of S.mitis in this study, although type-specific antigens could not be demonstrated. C-polysaccharide may therefore be the precursor to more complex polysaccharides in both pneumococci and the alpha-haemolytic streptococci to which pneumococci are genetically related (Yurchak and Austrian, 1966).

Enterobacteria have also been reported to cross-react with pneumococcal antisera. A cross-reaction between E.coli and pneumococcus type 1 was reported by Barnes and Wight (1935) and between E.coli K30,K42,K85 with pneumococcus antisera which corresponded to types IV,I and II respectively (Heidelberger et al., 1968). Most of the cross-reactive E.coli in the study reported here reacted with these pneumococcal antisera but some strains of E.coli and Klebsiella aerogenes reacted with other pneumococcal antisera also . Although a cross-reaction between Klebsiella pneumoniae and pneumococcus type 2 is well known (Avery, Heidelberger and Gobel, 1925) it was found here that a third of the cross-reactions observed with E.coli strains was with pneumococcus type 2 antiserum and is of interest. In the 7 years since typing was re-introduced in the west of Scotland, a type 2 pueumococcus has not been isolated

from a patient, although antibody to type 2 pneumococci was present in the serum of a number of patients with pneumonia (unpublished observation). Perhaps colonisation of the gastro-intestinal tract by E.coli which share an antigen with pneumococcus type 2 in a proportion of the population has been sufficient to prevent the development of pneumococcal pneumonia due to this type.

Although most of the cross-reactive bacteria studied here were not clinically significant, the results show the need for care with the interpretation of pneumococcal serological tests carried out on clinical material. Furthermore the isolation and study of capsular polysaccharides from bacteria which cross-react with pneumococcal antisera may reveal useful alternatives to pneumococci for the production of antigenic material for immunisation.

Serological identification of S.pneumoniae

Serological identification is a rapid and precise method for identifying isolates of S.pneumoniae and permits definite identification 24 hours earlier than is possible by current procedures for presumptive identification (such as optochin susceptibility). Indeed recent reports of optochin-resistant pneumococci (Burdash and West, 1982; Sottile and Rytel, 1975) and of an optochin-sensitive strain of S.sanguis (Burdash and West, 1982) cast doubt on the reliability of the optochin test. The simplest form of serological

identification of pneumococci is carried out using a commercially-available pool of concentrated rabbit antiserum which reacts with all 83 serotypes of S.pneumoniae (Omni serum). Because Omni serum contains antibody against pneumococcal C-polysaccharide - the cell wall antigen of S.pneumoniae - in addition to type-specific pneumococcal antibodies, it is recommended for use in the capsular reaction test. In contrast, type-specific antibodies are present in Omni serum with a titre almost half that in the pools from which it is made (Henrichsen et al., 1980). Consequently, bacteria which contain the C-antigen (e.g. non-capsulate pneumococci, S.mitis or group C beta-haemolytic streptococci) will react in tests with Omni serum and may be indistinguishable from capsulate pneumococci unless the capsular reaction test is carried out.

These potential problems associated with the use of Omni serum, together with cross-reactions between other bacteria and pneumococcal antisera, may have contributed to discrepancies in identification which have been reported when Omni serum has been used. For example, Sottile and Rytel (1975) found that 31% of alpha-haemolytic streptococci reacted in CIE tests with Omni serum although 13% of these did not react with type-specific antisera : These strains may therefore have contained C-antigen. Burdash and West (1982) observed two strains of alpha-haemolytic streptococci which reacted in the co-agglutination test with Omni serum,

whereas Smith and Washington (1984) found a strain of S.salivarius which reacted with latex particles sensitised with Omni serum. Wicher et al.(1982) used direct immunofluorescence (IF) and Omni serum and found that 23 % of beta-haemolytic and 7% of alpha-haemolytic streptococci fluoresced. However, they noted that the intensity of fluorescence was weaker than with S.pneumoniae as was found here. Recently, Holmberg et al. (1985) have confirmed these observations and found that Omni serum in CIE, IF and latex agglutination tests carried out on alpha-haemolytic streptococci were positive in 51%, 46% and 21% respectively. These results show that Omni serum, when used in tests other than the capsular reaction test, cannot be relied upon to distinguish pneumococci from other respiratory alpha-haemolytic streptococci. In addition, Henrichsen et al. (1980) have shown that Omni serum forms a precipitate in CIE with thioglycollate- containing media. This finding may invalidate attempts to identify pneumococci in blood culture broth.

Contrary to all these findings, the co-agglutination test used here - which did not use Omni serum - proved reliable for the presumptive identification of pneumococci. Non-capsulate pneumococci gave negative or equivocal reactions but reacted strongly in the co-agglutination test with antiserum to pneumococcal C-polysaccharide (Smart, Dougall and Girdwood, 1987a). Cross-reactive alpha-haemolytic streptococci were easily

distinguished from pneumococci by testing bacteria-free supernatants. However, there is little doubt that the cultural conditions employed in this study simplified the differentiation of pneumococci and alpha-haemolytic streptococci.

Detection of pneumococcal capsular polysaccharide

Most of the clinical studies in this thesis depended on the results of pneumococcal antigen tests carried out on clinical material. It was therefore essential to assess critically different methods for detecting pneumococcal antigen with particular reference to sensitivity and specificity when carried out on a variety of clinical material. On the basis of the relative amounts of pneumococcal antigen present in different specimens, the most appropriate methods of antigen detection were then chosen for use in clinical studies.

Sensitivity of antigen tests using purified preparations

Five different antigen detection methods, which employed polyvalent, pools and type-specific pneumococcal antisera, were tested in parallel with purified pneumococcal polysaccharides.

CIE tests

Types 7F,14 and 33F, were not detectable by CIE - an observation reported by other workers (Kenny *et al.*,1972; Kalin, Lindberg and Olausson, 1982). However, Coonrod and Rytel (1973) detected type 14 capsular

polysaccharide using CIE - but only with type-specific antiserum. The difficulty with types 7F and 14 result from the neutral charge of the polysaccharide and makes detection dependent on the buffer used (Anhalt and Yu, 1975; Szu and Oravec, 1982).

In this study, Omni serum was as sensitive in CIE as antiserum pools or type-specific antisera and supports the work of Coonrod and Rytel (1973) and Kalin et al. (1982). However these workers observed a greater intensity of precipitation with type antisera than with Omni serum. On the other hand, Kenny et al. (1972) found that type antisera were ten times more sensitive than Omni serum in CIE tests. Previous reports of the sensitivity of CIE, in tests with purified pneumococcal capsular polysaccharides, showed that the sensitivity of CIE was between 25 and 1,000 ng/ml but with considerable variation in the amount of pneumococcal antigens detected by different workers (Kenny et al., 1972; Coonrod and Rytel, 1973; Coonrod and Drennan, 1976; Coonrod and Rylco-Bauer, 1976; Leinonen, 1980; Burdash and West, 1982; Kalin et al., 1982). This may have been due to differences in CIE technique or to variation in the potency of purified pneumococcal capsular polysaccharide from different sources. The minimum amounts of capsular polysaccharides detected in this study by CIE with type-specific antisera, agreed broadly with other studies (Kenny et al. 1972; Coonrod and Rytel, 1973; Coonrod and Drennan, 1976; Leinonen,

1980). However in this study, CIE with Omni serum was less sensitive than that reported by others (Burdash and West, 1982; Coonrod and Rylco-Bauer, 1976; Kalin et al., 1982). The data reported by Kalin et al. (1982) were particularly impressive. In CIE tests with Omni serum, they detected concentrations of pneumococcal capsular polysaccharides which were rarely detected in CIE tests with type-specfic antisera or Omni serum reported by the workers cited above. This may result from contamination with pneumococcal C-polysaccharide which accounts for 15% of the total polysaccharide content of pneumococcal vaccine (Sorensen and Henrichsen, 1984). It is therefore likely that the precipitation reactions which Kalin et al. (1982) observed with Omni serum and individual pneumococcal polysaccharides (which are incorporated in the polyvalent vaccine) may, in some cases, have been due to a nonspecific reaction of anti- C-polysaccharide antibody in Omni serum with the C-polysaccharide component in the capsular polysaccharide preparation.

Co-agglutination tests- Kronvall, Trollfors

Co-agglutination by the method of Trollfors et al. (1983) was 2 to 10 times more sensitive than by the Kronvall technique using the same kind of antiserum. However the concentrations of antigen detected by Omni serum in the Trollfors co-agglutination test were similar to results obtained by CIE (except for types 7F,14 and 33F as discussed above). Tests with pool and type-specific antisera were more sensitive by Trollfors

method.

Mayer et al. (1983) found the Phadebact co-agglutination test which used Omni serum more sensitive than tests with pool and type-specific co-agglutination reagents prepared in their laboratory. But it should be noted that the Phadebact reagent was prepared by the Trollfors method whereas their pool and typing reagents were produced by the Kronvall method and this invalidates direct comparisons.

The study reported here was the first to assess the sensitivity of the Trollfors co-agglutination test with pools and type-specific antisera although the sensitivity of this technique using Omni serum has been reported by others (Burdash and West, 1982; Mayer et al., 1983; Kalin et al., 1982). Some polysaccharides varied considerably in the minimum amounts detectable by different workers. In the present study, the Trollfors co-agglutination test with Omni serum was less sensitive than reported by other workers (Burdash and West, 1982; Mayer et al., 1983, Kalin et al., 1982). Batch-to-batch variation of Omni serum, differences in the potency or purity of pneumococcal capsular polysaccharides from different sources or lack of uniformity amongst operators in distinguishing between a positive and negative reaction may have contributed to the relatively low sensitivity of Omni serum found here and also may explain the wide variation in the minimal amounts of certain polysaccharides detected in the

other studies.

Latex agglutination tests

Latex agglutination was slightly more sensitive than the Trollfors co-agglutination test; positive reactions developed rapidly and were easier to read. In tests with Omni serum, latex agglutination was generally somewhat more sensitive than CIE, (although with a few types CIE was more sensitive). These results agree, in general, with those reported by others (Coonrod and Rylco-Bauer, 1976, Leinonen, 1980), although the results in tests with certain types varied. In contrast to Whittle et al. (1974), who found that latex particles, sensitised with Omni serum had a granular appearance which made interpretation difficult, the method used here to sensitise latex particles gave a smooth suspension and clear end-points of antigen titrations. Latex agglutination tests with pool or type-specific antisera were more sensitive than tests with Omni serum and this agrees with the findings of Leinonen (1980).

ELISA tests

In the studies presented here, ELISA with antiserum pools and type-specific antisera was the most sensitive method for the detection of purified capsular polysaccharide antigen. Most antigens were detected at concentrations between 1 and 10 ng/ml - a sensitivity that compares favourably with radioimmunoassay (RIA). Leinonen (1980) for example, found that the detection limit for types 3,6,19 and 23 in RIA with type-specific

antisera was between 5 and 10 ng/ml.

Nolan and Ulmer (1980) developed a direct ELISA which detected purified type 3 capsular polysaccharide in concentrations as low as 0.15 ng/ml. In contrast, Drow and Manning (1980) with an indirect sandwich ELISA found that the minimal concentration detectable of type 3 capsular polysaccharide was from 1 to 3 ng/ml. In the direct ELISA method, the same type-specific antibody used to capture the antigen was also used for detection after conjugation with enzyme. However, in the indirect assay, the antisera for antigen detection and capture were prepared in different animals - the presence of antigen-bound detecting antibody being demonstrated by addition of the appropriate enzyme-conjugated anti-species antibody. A number of pneumococcal antisera, prepared in animals other than rabbits, will be required before the clinical value of the indirect ELISA can be assessed. In contrast, the method of De Jong (1983) for the preparation of antibody-enzyme conjugates was adapted for rabbit pneumococcal antisera in the studies reported here. Thus the commercially-available rabbit pneumococcal antisera were used to capture and detect pneumococcal antigen without the need to prepare conjugates as described by Nolan and Ulmer (1980). Furthermore, pooled pneumococcal antisera were as effective as type-specific antisera in the ELISA tests.

Harding et al.(1979) conjugated Omni serum with enzyme

and detected 1 to 3 ng/ml of pneumococcal polysaccharide in samples prepared from pneumococcal vaccine although different batches of Omni serum varied in their ability to coat ELISA plates. The method used in the study reported here differed from that used by Harding et al. (1979) but tests which used Omni serum as the "capture" antibody were not successful. Alternatively, when type-specific or antiserum pools were used as the "capture" antibody and Omni serum as the "detecting" antibody, the sensitivity of the assay was low (> 50 ng/ml). The reason for this is not clear, but when the ratio of enzyme to antibody was increased there was a slight improvement in sensitivity at the expense of specificity. Since Omni serum is a concentrated preparation of the IgG fraction of different rabbit pneumococcal antisera and contains 83 different antibody specificities, it is possible that the antibody-enzyme complexes interfered with the fixation of each other to antigen bound on the ELISA plate. Leinonen (1980) has proposed the same mechanism to explain the difference observed in the sensitivity of latex particles sensitised with Omni serum compared to latex particles sensitised with pool or type-specific antisera. There was no difference in the sensitivity of CIE for the detection of pneumococcal antigen in tests with Omni serum compared to pool or type-specific antisera. Thus, in CIE, differences in antibody mobility (Holliday, 1981) reduced interference with the

formation of precipitin bands. The polyvalent antiserum which reacted with the serotypes in the 23 valent pneumococcal vaccine was no better than Omni serum or antiserum pools in CIE tests. However, the minimum amounts of some pneumococcal antigens detected by it in the Kronvall and Trollfors co-agglutination and latex agglutination tests, were lower than that detected by Omni serum. Since the polyvalent serum did not react in the ELISA with pneumococcal antigens at a concentration of less than 50 ng/ml the factors affecting its performance may have been the same as those which inhibited the reaction of Omni serum in ELISA tests.

Antisera for pneumococcal antigen detection

In four of five methods studied here, in which the same pneumococcal antisera were tested in parallel with different pneumococcal capsular polysaccharides, antiserum pools and type-specific antisera were more sensitive than the polyvalent serum or Omni serum. Although not apparent with CIE, the latter method was insensitive and types 7F,14 and 33F were not detected.

The Kronvall co-agglutination test, which was used primarily for serotyping was the least sensitive method for the detection of pneumococcal antigen when Omni serum was used, whereas in tests with pools and type-specific antisera it was as sensitive as CIE. Since the infecting type of pneumococcus was known in cases studied (because it had been cultured or serotyped in clinical material), the problem with serological

detection using polyvalent antisera was irrelevant. It was concluded therefore that for clinical studies in which sensitivity of antigen detection was of primary importance (e.g. serum and urine) specimens were tested by the Trollfors' co-agglutination and latex agglutination tests with pools and type-specific antisera, and, if negative, further tested by ELISA. These conclusions were supported by preliminary clinical studies.

Specificity of antigen tests on clinical material

Nonspecific reactions can be a problem in tests for pneumococcal antigen carried out on clinical material and they fall into two broad categories:

1. Bacterial antigens which cross-react with pneumococcal antisera
2. Extraneous proteins which react with the material (such as staphylococcal protein A, latex particles or plastic ELISA plates) which carry the pneumococcal antiserum. Nonspecific agglutination of staphylococcal protein A by clinical material is particularly common (Thirumoorthi and Dajani, 1979; Edwards and Coonrod, 1980; Mayer et al., 1983). Similarly nonspecific agglutination of latex particles by rheumatoid factors and other nonspecific factors have been noted (Coonrod and Rylco-Bauer, 1976; Cerosaletti et al., 1985). Doskeland and Berdal (1980) found that urine, serum and CSF sometimes contained substances which gave rise to nonspecific reactions in ELISA techniques. Boiling

(3 minutes at 100 °C) has been shown to be the most effective way of eliminating these nonspecific factors (Mayer et al., 1983; Smith et al., 1984) and heating also caused the liberation of antigen complexed with antibody which increases the sensitivity of antigen tests (Doskeland and Berdal, 1980). In contrast, nonspecific reactions are rare in CIE tests and are easily recognised (Tilton et al., 1984).

Tests for antigen in clinical material

1.Serum

Preliminary studies carried out on the serum of patients with pneumococcal pneumonia showed that the amounts of pneumococcal antigen present varied over a wide range (4ng to 4000 ng/ml). The number of sera which gave positive reactions in different antigen detection methods, using the same pneumococcal antiserum depended on the amount of antigen in individual patients sera. Only a third of sera were positive by CIE and the Kronvall co-agglutination test, whereas two-thirds were positive by latex agglutination or the Trollfors co-agglutination test. The remainder were positive only in ELISA tests. This is consistent with the findings using purified polysaccharides since serum often contains little antigen and its detection is directly related to the sensitivity of the method used.

2.Cerebrospinal fluid

S.pneumoniae was isolated from specimens of

cerebrospinal fluid which did not contain detectable pneumococcal antigen and this parallels reports by other workers who used CIE (Fossieck et al., 1973; Whittle et al., 1974), co-agglutination (Olcen, 1978; Thirumoorthi and Dajani, 1979), or latex agglutination (Whittle et al., 1974). The sensitivity of antigen tests carried out on cerebrospinal fluid depends upon the numbers of bacteria present before antibiotic treatment (Feldman, 1976; Olcen, 1978). Olcen's results showed that in the case of S.pneumoniae the bacterial count did not correlate with the results of the Kronvall co-agglutination test or CIE although these tests were usually negative when the CSF bacterial count of H.influenzae or N.meningitidis was between 10^4 to 10^5 cfu/ml. Perhaps this indicates that other factors (e.g. pneumococcus type or the presence of type-specific antibody) limit the sensitivity of antigen tests in pneumococcal meningitis.

The preliminary studies on CSF reported here were carried out before other more sensitive tests (e.g. latex agglutination, ELISA) for pneumococcal antigen were developed. Sippel et al. (1984) showed that a direct ELISA with Omni serum was less sensitive than the Kronvall co-agglutination test or CIE for the detection of antigen in CSF. In view of the poor performance of Omni serum in ELISA tests as previously described, subsequent specimens of CSF in this study were tested, first by Kronvall co-agglutination, and if

negative, they were re-tested in the same way as serum using type-specific antisera if S.pneumoniae had been isolated on culture.

3.Sputum

Detection of pneumococcal antigen in sputum using CIE and Omni serum is widely, but not universally, accepted as a valuable tool for the diagnosis of pneumococcal chest infection. Although Verhoef and Jones (1974), Tugwell and Greenwood (1975), El-Refaie and Dulake (1975) and Spencer and Savage (1976) argued in its favour, Edwards and Coonrod (1980) and Kalin et al. (1982) found that co-agglutination was more sensitive than CIE for detecting pneumococcal antigen in sputum, particularly in patients who had received antibiotic treatment. The preliminary studies described here support the latter view and further in comparisons with antiserum pools, pneumococcal antigens were not detected in a number of sputa when Omni serum was used in CIE, Kronvall co-agglutination and latex agglutination tests. Moreover, CIE with type-specific antisera was less sensitive than the Kronvall co-agglutination test with the same serum but CIE was more sensitive than the co-agglutination test with Omni serum. Thus CIE in general, was less sensitive than other tests and this was only in part accounted for by the occurrence of antigen types (7F,14,37 and 33F) which did not react by this method.

In the studies reported here, a number of sputa,

which did not contain type-specific pneumococcal antigen reacted in tests with Omni serum. More than half of the reactions detected in CIE with Omni serum on sputa sent to another laboratory and which were subsequently tested here were due to pneumococcal C-polysaccharide antigen. However, the other sputa which reacted in CIE with Omni serum were not confirmed by co-agglutination with type-specific antisera although previous studies had shown the latter to be more sensitive and this accords with the observations by Kalin et al. (1982).

Nonspecfic reactions were a major problem when sputa were examined with a commmercial latex suspension (Wellcogen). However, these were mostly non-purulent sputa with oropharyngeal contamination and although they did not react with the control latex suspension supplied by the manufacturer, further tests with a variety of pneumococcal latex suspensions confirmed that the reactions were nonspecific. In contrast to CIE and latex agglutination, non-specific reactions were rarely found in the less sensitive Kronvall co-agglutination test and only occasionally with antiserum pools. Nonspecific reactions with pooled antisera in CIE tests have been previously reported (Perlino and Shulman, 1976).

Clearly heat stable factors in sputum, perhaps related to commensal flora (a proportion of which possess antigens which react with pneumococcal antisera) or to

sputum mucopolysaccharide, were a major problem in tests for pneumococcal antigen in sputum. Although nonspecific reactions were common to all methods in this study, they were particularly troublesome in latex agglutination tests and this may reflect the increased sensitivity of this test or the presence of substances in sputa which interact with latex particles sensitised with pneumococcal antisera.

Because of the differences in sensitivity and specificity of tests for pneumococcal antigen in sputum which were found here, preliminary studies were extended to evaluate each antigen detection method for the laboratory diagnosis of pneumococcal chest infection. Of the four methods used, the Kronvall co-agglutination test with type-specific antisera correlated best with the results of culture and Gram's stain in infected patients. In addition, most of the sputa which were positive in co-agglutination tests with type-specific antisera were also positive by other methods and this contrasts with the findings of Whitby, Kristinsson and Brown (1985) who found poor concordance between the results of different antigen tests (i.e. latex agglutination, co-agglutination and CIE) which used Omni serum. In this study, detection of type-specific pneumococcal antigen by the Kronvall co-agglutination test was the most efficient method for the laboratory diagnosis of pneumococcal chest infection. Furthermore, although detection of type-specific

antigen was more sensitive than culture, the presence of antigen correlated with the isolation of more than 10^7 cfu/ml S.pneumoniae, which is regarded by other workers (Dixon and Miller, 1965; Wilson and Martin, 1972; Bartlett and Finegold, 1978) as the lower limit of quantitative sputum culture which distinguishes between infected and non-infected patients.

Preliminary studies on pathologically confirmed infection (post-mortem lung tissue and pleural fluid) showed that the Kronvall co-agglutination test was a better indicator than culture and this agrees with the findings of other workers (El-Refaie et al.,1976; Lampe, Chottipitayasunondh and Sunakorn, 1976).

Requirements for optimal antigen detection in clinical material

Although the Kronvall co-agglutination test was less sensitive than other methods for the detection of purified pneumococcal capsular polysaccharide and pneumococcal antigen in serum, it was reliable, easy to carry out, detected all types of pneumococci and was suitable for detailed serotyping with factor sera. Therefore in contrast to serum and urine, the presence of pneumococcal antigen in material, from which S.pneumoniae could be cultured directly (sputum, pleural fluid, post-mortem lung tissue and cerebrospinal fluid), depended on the results of the Kronvall co-agglutination test. In most cases these specimens were tested for antigen before the serotype of the infecting

pneumococcus was known and as a result they were tested with nine reagents (pools A to I), which when taken together covered all 83 pneumococcal antigen types.

The results of sensitivity and specificity were used to decide the way in which different tests were deployed to investigate clinical material and were as follows;

1. Serum, urine and cerebrospinal fluid

Antigen concentrations varied over a wide range and deproteinization by heat eliminated false positive and negative reactions. Therefore antigen tests with high sensitivity were used.

2. Sputum and other respiratory specimens

Antigen concentrations were high, but deproteinization with heat failed to eliminate all nonspecific factors which react with pneumococcal antisera. Therefore antigen tests with high specificity and lower sensitivity were used and are summarised in Table 62 .

Microscopic and Bacteriological analysis of sputum

This study confirmed the relationship between the presence of pneumococcal antigen, diplococci in Gram's stained smears and large number of S.pneumoniae in cultures of sputum from patients with pneumococcal chest infection. However the value of these tests in clinical diagnosis depends largely on the quality of the sputum obtained and they require precise evaluation.

Macroscopic appearance of sputum did not correlate with the cellular composition determined microscopically.

Table 62:

Classification of antigen tests and resultant application to pneumococcal antigen detection in clinical material.

Test	Sensitivity	Specificity	Application *
CIE	+	++	limited screening test.
CO-AGGLUTINATION			
1. Kronvall	+	++++	respiratory material
2. Trollfors	++	+++	serum, urine & CSF
LATEX	+++	++	serum, urine & CSF.
ELISA	++++	+	confirmatory test

* Application based on performance characteristics of the test in terms of economics, speed, reliabilty and the level of subjectivity in reading the test results.

For example, the number of purulent sputa were overestimated on the basis of macroscopical appearance and this confirms the findings of Miller (1963), whereas many sputa which were moderately or heavily contaminated with oropharyngeal material, were not recognised by their gross appearance and this agrees with the findings of other workers (Chodosh, 1970; Bartlett, 1974; Murray and Washington, 1975). Examination of Gram's stained smears was a better indicator than macroscopical inspection in the assessment of sputum quality. However, contrary to the findings of Murray and Washington (1975), pus cells, broncho-pulmonary cells and squamous epithelial cells were often incorrectly identified in Gram's stained smears. As a result, a number of sputa which did not contain pus cells were described as purulent or mucopurulent whereas others were incorrectly described as predominatly saliva.

In this study, presumptive identification of pneumococci by Gram's stain was confirmed by culture when sputa contained pus cells (defined by crystal violet microscopy) and the criteria defined by Rein et al. (1978) were used. This parallels the experience of other workers (Heineman et al., 1977; Drew, 1977). However this was not the case when respiratory material did not contain pus cells and substantiates claims that without microscopy, culture results are of unknown relevance (Heineman et al.,1977). Further support for the importance of microscopic evaluation of sputum was

provided by the observation that, after dilution, S.pneumoniae was isolated more often from purulent than from non-purulent respiratory specimens and indicates that growth of S.pneumoniae from undiluted cultures of non-purulent material probably resulted from contamination by pneumococci from the nasopharynx (Dixon and Miller, 1965).

Determination of pneumococcal antibodies by indirect immunofluorescence assay

Current methods for pneumococcal antibody determination include radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) and indirect haemagglutination (IHA). However, specially prepared pneumococcal capsular polysaccharides are required, so that for practical purposes , the range of pneumococcal antibody types which can be studied by these methods is limited to types contained in the pneumococcal vaccines. Furthermore, Schiffman et al. (1980) found that RIA was unsuitable for detection of antibody to types 5 and 11. These methods (i.e. RIA, ELISA and IHA), generally require preparation of antigen for use in the assays immediately before use and are therefore more suitable for testing a large number of sera against a small number of types. In contrast, indirect immuno-fluorescence assay (IFA) has seldom been used to detect pneumococcal antibodies although it is technically simpler to carry out and uses reagents which are

available in most diagnostic bacteriology laboratories. Moreover, there is no limit to the range of pneumococcal antibody types which can be studied and IFA is suitable for testing individual sera against a wide range of pneumococcal antigens simultaneously.

Because the interpretation of indirect immunofluorescent reactions depends on visual inspection of changes at the surface of whole pneumococcal cells, (the actual site of antibody deposition) interference due to factors which cause non-specific reactions in tests with purified pneumococcal polysaccharides are not encountered.

In this study antibody to pneumococcal C-polysaccharide in human sera was removed by absorption which made the IFA specific for antibody to pneumococcal capsular polysaccharide. Heidelberger and Anderson (1944) considered that preliminary absorption to remove C-polysaccharide antibody was essential for reliable antibody determination. However absorption may not be essential for antibody studies which use RIA, since the pneumococcal cell wall polysaccharide does not incorporate significant amounts of C^{14} from glucose into its structure (Schiffman et al., 1980). In contrast Pedersen et al. (1982) concluded that absorption of C-polysaccharide antibody was necessary for ELISA tests for antibody detection. Because pneumococcal antibody determinations by IFA, ELISA and IHA (Sloyer et al., 1974 ; Berntsson et al., 1978 ; Amman and Pelger, 1972) were carried out without preliminary absorption of

pneumococcal anti-C-polysaccharide antibody, direct comparisons with the results presented here would not be valid.

In this study the results of antibody tests by IFA for types other than 1 and 12F correlated with the results of RIA. Although the IFA was less sensitive than RIA it was estimated that the minimum amounts of pneumococcal antibody nitrogen detected by IFA, for the majority of types studied, was below 300 ng Ab N/ml- a level currently considered to be the lower limit of type-specific antibody in serum affording protection against bacteraemic pneumococcal disease (Robbins et al., 1983). However for types 1 and 12F, IFA was less sensitive even when different strains of these types were used to prepare the whole cell antigens. In comparisons with RIA, the sensitivity of the IFA varied considerably with the pneumococcal type and this may result from different amounts of capsular polysaccharide on individual types or relate to the class of human antibody.

Clinical studies

The patients included in the clinical studies were selected on the basis that the clinical diagnosis was unequivocal. The study was designed to relate aspects of the disease process with the microbiological determinants such as the distribution of antigen, results of pneumococcal culture and antibody

development. Epidemiological parameters were not directly studied and the cases described may not reflect the patterns of pneumococcal disease in hospitals or the community. For example, a considerable number of patients with unequivocal pneumococcal disease were excluded because laboratory investigations were incomplete. Patients infected with the higher and in general rarer pneumococcal types were also excluded.

The role of sputum examination in the laboratory diagnosis of pneumococcal chest infection

Before the introduction of antigen tests in the mid 1970s, bacteriological diagnosis of pneumococcal chest infection was unsatisfactory. It is now generally accepted that the presence or absence of pneumococci in sputum cultures does not correlate well with lung infection. There are two main reasons for this. First, pneumococci in expectorated sputa may reflect upper respiratory tract colonisation, local bronchitis or definite pulmonary infection. Second, failure to isolate pneumococci from sputum in cases of bacteraemic pneumococcal pneumonia (Barrett-Connor, 1971) and from patients treated with antibotics (Spencer and Philp, 1973) does not indicate that pneumococci are not involved in lower respiratory tract infection. It has therefore been suggested that wider use of Gram's stained smears (Rein et al., 1978) and quantitative culture (Wilson and Martin, 1972) would improve the

specificity of sputum culture and that antigen tests would increase the sensitivity of sputum examination for the laboratory diagnosis of pneumococcal chest infection (El-Refaie and Dulake, 1975).

Bacteriological examination

Preliminary studies reported here showed that the Gram's stained smear was as sensitive, but was more specific than culture of sputum for the laboratory diagnosis of pneumococcal chest infection. Moreover, it was found that the Gram's stain allowed distinction between infected and non-infected patients in whom sputum cultures were positve. However, in the clinical studies that followed, Gram's stains of sputum were, in general, unhelpful in patients with pneumococcal pneumonia. However, it should be noted that different criteria were used to select patients in the two studies and this may contribute to the inconsistency of the results. Thus patients in the preliminary studies were chosen on the basis that, in them, more than one laboratory test carried out on the sputum was positive regardless of the clinical picture. The study itself therefore included both clinically infected and non-infected patients of whom patients with pneumonia accounted for only a small proportion. In the subsequent clinical studies reported here all the patients were clinically infected and were grouped on the basis of the clinical diagnosis. A pneumococcal aetiology was established by the results of a range of laboratory

tests on a variety of clinical material. Several studies have demonstrated good correlation between the results of Gram's stained smears and sputum culture (Drew, 1977) particularly when interpretation of the smear was supported by the capsular reaction test (Merrill et al., 1973 ; Perlino, 1984) or when strict criteria, which related to predominance, were used to define a positive Gram's stain smear (Rein et al.,1978). These criteria were also discriminatory in the studies reported here, but only when pneumococci were isolated from the sputum. In contrast, Gram's stained smears were of no value when carried out on non-purulent sputa from patients with pneumococcal pneumonia, a result that broadly agrees with the findings of Boerner and Zwadyk (1982). These authors investigated patients with community-acquired pneumonia and found that Gram's stains of sputum suggested pneumococcal infection in more than half. Furthermore, they found that these patients responded quickly to penicillin in contrast to patients whose Gram's stained smear showed no predominant pathogen. Although these workers were unable to determine the cause of the pneumonia in most of the other patients, they suggested that a proportion had pneumococcal pneumonia but had spontaneously cleared the organism from their sputum by the time they presented at hospital. A number of the patients investigated in this study with bacteraemic pneumococcal pneumonia did not have pneumococci in either a Gram's

stain or culture of sputum a fact that confirms the findings of Barrett-Connor (1971). Although poor sputum quality could explain the lack of correlation between sputum and blood culture, other investigations carried out on the sputa (i.e. microscopical assessment and antigen tests) suggested that this was not the case. The most likely reason for this discrepancy was that blood culture was carried out at the time the patient was admitted, whereas sputum for culture was not obtained until later, perhaps after physiotherapy and antibiotic treatment.

Other respiratory specimens from patients with pneumonia

It was found here that many patients with severe pneumococcal pneumonia were unable to produce a specimen of sputum early in the course of the disease and this has been noted by other workers (Barrett-Connor, 1971; Guzzetta et al., 1983; Kalin and Lindberg, 1983). Consequently, laboratory diagnosis in a number of these patients was made by culture of pleural fluid, bronchial aspirates or, retrospectively, using post-mortem lung tissue. In addition, pneumococci isolated from a throat swab obtained on admission from two patients with pneumonia was taken as proof of a pneumococcal aetiology on the basis of Kalin's observations (1982). He isolated pneumococci from the nasopharynx of one third of patients with bacteraemic pneumococcal pneumonia who could not produce a pre-treatment specimen of sputum. Moreover, since it has been suggested that

colonisation of the upper respiratory tract with S.pneumoniae is decreased in patients with pneumonia due to other pathogens (Schmid et al., 1979), it might be argued that the presence of pneumococci in the upper respiratory tract of an adult patient with pneumonia is adequate evidence of a pneumococcal aetiology.

Quantitative culture

Attempts to distinguish pneumococcal infection from colonisation by quantitative culture of sputum have not proved reliable (Barrett-Connor, 1971; Downes and Ellner, 1979) and this was confirmed in preliminary clinical studies reported here. Furthermore, no correlation was found between the numbers of pneumococci present in sputum cultures and the clinical diagnosis of pneumococcal chest infection and supports the findings of Downes and Ellner (1979) and Kalin, Lindberg and Tunevall (1983). Consequently, estimates of the relative numbers of pneumococci isolated from the sputum of patients included in the clinical studies were not carried out and sputum culture was used to confirm the detection of pneumococci or pneumococcal antigen in the sputum or other clinical material.

Detection of sputum antigen in the diagnosis of pneumococcal chest infection

Pneumococcal antigens were detected in most of the culture-positive sputa confirming the findings of Spencer and Savage(1976) that sputum antigen does not distinguish between pneumonia and other pneumococcal

chest infections. These results differ from those reported by other workers (Leach and Coonrod, 1977; Perlino and Shulman, 1976; Verhoef and Jones, 1974), who failed to detect pneumococcal antigen in a number of culture-positive sputa from patients with chronic bronchitis. However, the patients with chronic bronchitis who were studied here had clinical signs of a chest infection. The results of the present study do not necessarily disagree, therefore, with the suggestion of these workers that the demonstration of sputum antigen can distinguish between colonisation of the upper airways with pneumococci and a chest infection. Indeed preliminary studies showed that sputum antigen was useful for this purpose. Pneumococcal antigens were detected in culture-negative sputa and this identified additional patients with pneumococcal chest infection in each of the three clinico-pathological groups. Antigen tests were particularly important for the diagnosis of pneumococcal pneumonia, because in contrast to patients in the other two groups, a positive sputum antigen was the only indication of pneumococcal infection - an observation that agrees with that of Kalin and Lindberg (1983). Furthermore, pneumococcal antigens were often found in non-purulent sputa.

Detection of pneumococcal antigens in pleural fluid, bronchial aspirates and, retrospectively, in post-mortem lung tissue of patients with pneumonia was further proof that S.pneumoniae was the cause of

pneumonia and this confirms the findings of other workers (El-Refaie et al., 1976; Coonrod and Wilson, 1976). Detection of antigen in other respiratory specimens supplemented the results of culture or antigen tests carried out on sputum or blood.

In the current study, pneumococcal antigen was detected in the sputum of most of the patients in each clinico-pathological group. Somewhat surprisingly pneumococcal antigen was not detected in the sputum of three patients with pneumococcal pneumonia - a result similar to that reported by Schmid et al., (1979). However, the amount of antigen in sputum may be influenced by the patient's state of hydration, the amount of saliva mixed with sputum and the bacterial counts in the respiratory tract (Miller et al., 1978). Clearly these factors are more likely to contribute to the poor quality of sputa obtained from patients with severe pneumonia than in other milder chest infections where sputum is produced freely. Consequently, Kalin and Lindberg (1983) found that a second specimen for antigen testing was valuable when the first specimen had been obtained early in the course of the disease and suggested that the concentration of antigen in the initial specimen was insufficient to be detectable by antigen tests.

Antigen persistence

Persistence of pneumococcal antigen in the sputum of pneumonia patients after treatment with antibiotics has

been widely reported (Tugwell and Greenwood, 1975; El-Refaie and Dulake, 1975; Perlino and Shulman, 1976; Spencer and Savage, 1976; Leach and Coonrod, 1977; Downes and Ellner, 1979; Edwards and Coonrod, 1980). Kalin and Lindberg (1983) observed that the mere initiation of antibiotic therapy (i.e.< 24 hours) was enough to render the results of microscopy and culture for pneumococci unreliable and stressed the importance of performing antigen tests on sputa obtained from patients treated with antibiotics. El-Refaie and Dulake (1975) found that in patients with lobar pneumonia, antigen remained in the sputum for longer periods than in patients with bronchitis and suggested that this was related to the pathology of lung tissue in lobar pneumonia. Demonstration of antigen persistence in sputum may therefore have diagnostic potential which permits laboratory differentiation between pneumonia and other less severe forms of pneumococcal chest infection. However, it has been shown that pneumococci persisted in the respiratory tract of patients with chronic bronchitis, despite antibiotic treatment (Calder and Schonell, 1971) and nosocomial pneumococcal infections have been reported in hospital patients after antibiotic treatment (Mylotte and Beam, 1981).

To clarify the relationships between a positive culture and antigen test with respect to time and antibiotic treatment pneumococcal culture and antigen tests were carried out on specimens of sputum after the

initial laboratory diagnosis. It must be stressed however, that it was not routine practice to examine serial samples from patients with pneumococci in their sputum nor were these requested by laboratory staff. Collection of repeat sputa for examination depended on the patient's physician, and therefore varied from patient to patient with regard to time and treatment. Nevertheless, the results for patients whose sputum was sampled irregularly were grouped with the results of appropriate samples obtained from patients who were tested systematically in order to establish trends. A number of patients included in this part of the study did not have symptoms of infection and did not require antibiotic treatment. It was found that pneumococci or pneumococcal antigen usually persisted in the sputum of untreated patients for two days or more. However, S.pneumoniae, and in some cases pneumococcal antigen also, was eliminated spontaneously from the sputum of a few patients a result that parallels the findings in the patients with bacteraemic pneumococcal pneumonia discussed above. Factors which prevented the isolation or detection of pneumococci in subsequent sputa were not identified but inadequate specimens or antibiotic therapy - unknown to the clinician - may have been responsible. Despite these limitations the results were unequivocal in one patient with a type 3 pneumococcal infection : both pneumococci and pneumococcal antigen were eliminated from her sputum in two days without

antibiotic treatment and coincided with a significant rise in homologous serum antibody titre. Furthermore, the presence of a wide range of type-specific pneumococcal antibodies in the serum of patients with no immediate history of pneumococcal infection (Ward, 1930; Roberston and Cornwell, 1930 ; Gwaltney et al., 1975) supports the view that individuals in the community are continuously exposed and respond to different pneumococcal types : some of these exposures could have resulted in a chest infection which might not have been treated with antibiotics. These results suggest therefore that some pneumococcal chest infections can be controlled by the host's respiratory defences.

After two days of appropriate antibiotic treatment, pneumococci were generally not isolated from patients' sputum but pneumococcal antigen often persisted and this was related to underlying lung pathology. This observation agrees with that of other workers (El-Refaie and Dulake, 1975). In a number of patients failure to isolate pneumococci from sputum was paralleled by a reduction in sputum antigen concentration and suggests a close relationship between these two parameters. However, in other patients sputum antigen remained essentially unchanged despite effective anti-pneumococcal therapy and was often paralleled by the presence of higher concentrations of antigen in pleural fluid or post-mortem lung tissue. These results support the argument that in patients with pneumonia,

infected lung tissue or fluid is the source of sputum antigen rather than the product of viable pneumococci in the patient's sputum (El-Refaie and Dulake, 1975). Thus pneumococcal antigen in sputum may indicate either an infected respiratory secretion or a deep-seated pulmonary infection and these can be distinguished by testing further specimens of sputum after antibiotic therapy has cleared the sputum of viable pneumococci.

Serial sampling of patients' sputum highlighted several other features which are important in the management of patients with pneumococcal chest infection. Firstly, pneumococci and pneumococcal antigen were detected in the sputum of a few patients during antibiotic treatment and indicated that treatment was ineffective. Secondly, a number of patients with COAD had a recurrence of infection shortly after antibiotic treatment as has been shown by others (Calder and Schonell, 1971). Serotyping of isolates showed that some patients had relapsed with the same pneumococcal type whereas others had become re-infected with a different type. Serotyping was particularly valuable when patients had a recurrence of infection after only 7 days when antigen persistence might otherwise have suggested a more serious pathology (i.e. pneumonia or antibiotic failure). In any event most of these patients were culture and antigen-positive.

Although it was found in the studies reported here that no sputum was produced early in the course of

pneumococcal pneumonia, antigen tests carried out on good quality sputum were extremely valuable for laboratory diagnosis of pneumococcal chest infections. Furthermore the results of culture and antigen tests carried out on additional specimens obtained during and after antibiotic treatment proved useful in patient management. There was usually sufficient antigen in sputum for serotyping and this provided information of use in other ways. For example knowledge of the type guided the choice of more sensitive antigen detection techniques for analysis of serum or urine and was essential for antibody studies. The importance of these parameters in diagnosis and prognosis will now be discussed.

Bacteraemia and Antigenaemia

Bacteraemia

In the studies reported here, two groups of patients developed pneumococcal bacteraemia namely, those with pneumonia (Group "P") and non-respiratory pneumococcal disease (Group "S"). Blood cultures from other patients with pneumococcal chest infection (Groups "A" and "C") were sterile although a few patients in these groups had clinical signs of pneumonia. Unfortunately blood cultures were seldom taken from these patients and therefore the possibility cannot be excluded that at least some were also bacteraemic. In contrast pneumococcal bacteraemia was demonstrated in almost half

of the patients with pneumonia and, in a few, the laboratory diagnosis was made from blood culture alone. The incidence of bacteraemic pneumococcal pneumonia reported here was higher than that found by other workers (Macfarlane et al., 1982) and was probably the result of patient selection. It has been generally accepted that bacteraemia is a complication of between a quarter and a third of patients with pneumococcal pneumonia (Austrian and Gold, 1964) and this proportion has not changed since before the introduction of antibiotics (Cole, 1902; Heffron, 1939 ; Tilghman and Finland, 1937). In these reports, bacteraemia was associated with high mortality in pneumococcal pneumonia but in the study reported here, the number of patients with pneumococcal pneumonia who died was no different in bacteraemic and non-bacteraemic patients. Before the introduction of antigen tests, blood culture was preferred to sputum culture to establish the diagnosis of pneumococcal infection in patients with pneumonia (Austrian and Gold, 1964). However antigen tests were used here and the results agreed with other recent studies (Macfarlane et al.,1982; Morgan et al., 1984). These workers showed that the diagnostic value of blood culture was limited in patients with pneumococcal pneumonia - findings which parallel the experience of workers in the pre-antibiotic era when one worker suggested that " The test is of greater prognostic than diagnostic value" (Cole, 1902).

Nevertheless it is my view that blood cultures should be obtained from all patients with clinical signs of pneumonia **before antibiotic treatment** to allow a more detailed study of the factors which contribute to the development and pathology of bacteraemic pneumococcal pneumonia. Until the results of such studies are known our understanding of the pathology of pneumococcal disease comes mainly from the study of pneumococcal antigen since this is not affected by factors which contribute to the failure of culture methods (e.g. antibiotic therapy).

Antigenaemia

In the studies reported here antigenaemia was more common than bacteraemia in patients with pneumococcal chest infection and this agrees with the observations of other workers (Tugwell and Greenwood, 1975; Dulake, 1979). Moreover, in contrast to bacteraemia, pneumococcal antigen was detected in the serum of patients who did not have clinical pneumonia a result that agrees with the observations made by Bartram et al. (1974). Also, it was found here that bacteraemia in patients with pneumonia did not necessarily correlate with the presence of antigen in the serum as has been reported by other workers (Dorff et al., 1971; Kenny et al.,1972; Coonrod and Rytel, 1973; Kalin and Lindberg, 1983). Conversely , pneumococcal antigen was detected in the serum of patients with non-bacteraemic pneumococcal pneumonia. This confirms the findings of Tugwell and

Greenwood (1975) who pointed out that failure to isolate the organism from blood was not necessarily the result of antibiotic therapy. The results reported here suggest that pneumococci and pneumococcal antigen enter the ciculation independently. Serum antigen studies on the patients reported here with bacteraemic pneumococcal meningitis and who had pneumococci and pneumococcal antigen in CSF, support this view. It has been shown by other workers (Tilghman and Finland, 1937; Finland, Spring and Lowell, 1940) that patients with bacteraemic pneumococcal pneumonia have only a few hundred organisms per ml of blood. In contrast, in vitro studies carried out here showed that between 10^5 and 10^9 cfu/ml of viable pneumococci were required to produce 1 ug/ml of free capsular polysaccharide in the growth medium - a figure similar to that reported by Coonrod and Drennan (1976). As a result they calculated that 500 pneumococci/ml would produce no more than 5 ng/ml of antigen per day which has been shown here to be the minimum amount detectable by ELISA. Most of the patients with pneumococcal antigenaemia studied here had antigen levels higher than this and in patients with accompanying bacteraemia, antigenaemia persisted after antibiotic treatment had eliminated pneumococci from the circulation. These findings indicate that antigen in the circulation was derived from an extravascular site of infection such as consolidated lung or CSF and supports the conclusion of other workers (Coonrod and

Drennan, 1976). Clearly both culture and antigen tests on acute phase blood of patients with pneumococcal infection were of limited diagnostic value. However, unlike sputum examination, they were useful as a laboratory index of severe disease in patients with a pneumococcal chest infection as discussed below.

Outcome in relation to bacteraemia and antigenaemia

The present study confirmed early reports (Blake, 1918; Bukantz et al., 1942) that, in patients with pneumococcal pneumonia, antigenaemia was associated with a significantly higher death rate than either bacteraemia alone or neither bacteraemia nor antigenaemia. Moreover, there was no correlation between the actual amount of antigen in the serum and death. For example two patients with low levels of antigen (4 and 8 ng/ml) died whereas the patient with the highest level found in this study (10 ug/ml) survived - a result that paralleled the findings of Spencer and Savage (1976). Bukantz et al. (1942) observed that the incidence of antigenaemia varied with pneumococcal type. Typing of pneumococci and pneumococcal antigen in blood was performed here and showed that type 3 was the most commonly detected serum antigen and, in contrast to other common types, was not found as a cause of bacteraemia without antigenaemia. Thus the high mortality rate associated with type 3 pneumococcal pneumonia (Austrian and Gold, 1964) is probably related to its capacity to cause

antigenaemia.

Antigenuria

In the present study, pneumococcal antigen was detected in the urine of almost half of the patients with pneumonia (Group "P"), a quarter of patients with non-respiratory pnemococcal disease but in only a few patients in Group "C" who had clinical signs of pneumonia. Antigen was therefore more likely to appear in the urine of severely ill patients and in this respect resembled antigenaemia and bacteraemia as an indicator of prognosis. However antigenuria did not necessarily correlate with antigenaemia and vice versa - an observation which agreed with the findings of Kalin and Lindberg (1983) but differed from that reported by Coonrod and Rytel (1973). The latter detected antigen in the urine of all patients with antigenaemia.

Many years ago Blake (1918) reported the value of testing urine for antigen in patients with pneumococcal pneumonia and his results are summarised below, together with the findings of this study :

1. All patients who failed to excrete antigen in their urine recovered and this agreed with the findings of Dochez and Avery (1917). They were the first workers to describe antigenuria in patients with pneumococcal pneumonia and found that only 2 of 88 patients who did not excrete antigen in their urine died. However, in the study reported here there was no correlation between the outcome and the presence or absence of antigenuria.

2. Patients who failed to excrete antigen in their urine during the acute phase of the disease often began to excrete small quantities during convalescence: This was observed in two patients who were studied here. Blake(1918) suggested that antigenuria in convalescence was associated with the liberation of antigen from the resolving pneumonic lesion.

3. The presence of precipitating antibody in the blood was inversely related to the presence of antigen in urine. In the patients studied here however, there was no difference in the frequency of antibody detection relative to the presence or absence of antigenuria. Blake (1918) reasoned that either antigen and antibody were not completely bound in urine or that the kidney possessed the ability to separate the two and allow antigen to be excreted while retaining antibody within the kidney tissues. Coonrod (1974) found that antigen in urine was partially degraded - an observation which partly supports Blake's hypothesis. However he also referred to animal studies (Howard et al., 1970) which showed that complexing of antibody with persisting polysaccharide may eventually cause degradation and disappearance of the polysaccharide. Therefore antigenuria may indicate an active host immune response in pneumococcal disease. If complexing is a major feature of resolution of the disease then regular testing of urine for antigen would be important in patient management.

Tests to detect antigenuria in acute phase urine were carried out here but proved to be of limited diagnostic value, although antigen was detected in the urine of a few patients with pneumonia who did not have either bacteraemia or antigenaemia. Moreover detection of antigenuria was of limited prognostic value - in contrast to Blake's findings (1918). However it should be noted that neither the laboratory tests nor patient treatment used in the present study were comparable to those of Blake. For example, Blake (1918) used the precipitation reaction to detect antigen and antibody whereas the equivalent tests carried out here were considerably more sensitive. Furthermore it is not certain that Blake (1918) detected type-specific antibody since his work was carried out before the discovery of other factors which also precipitate pneumococcal antigen(e.g. C-reactive protein, anti-C polysaccharide antibody). Finally , Blake's study was carried out before the introduction of specific antimicrobial agents or serum therapy and he therefore observed the full effect of the host's immune response. In the present study all patients were, of course, treated with antibiotics.

It is recognised that antibiotic therapy has markedly reduced the number of deaths in patients with pneumonia (Austrian and Gold, 1964). Part of the reason of this success may be that antibiotic therapy prevents the development of bacteraemia, antigenaemia or antigenuria

in most patients.

Pathological aspects of systemic pneumococcal infection

In the study reported here no attempt was made to study other pathological features in patients with systemic pneumococcal disease. However, certain aspects of the disease in some patients warrant further consideration.

Other workers have observed a high incidence of jaundice, diarrhoea and persistent pyrexia in patients with systemic antigen (Tugwell and Greenwood, 1975). In addition, Coonrod and Rytel (1973) noted that patients with pneumococcal antigen in their serum or urine had a higher incidence of pleural effusion than those without demonstrable antigen. Pleural fluid from a few patients with pneumococcal pneumonia were examined in this study although other patients may have had an effusion which was not tapped or examined bacteriologically. It was found here that 6 of 8 (75%) of patients with a pleural effusion had antigenaemia or antigenuria. Of the two remaining who did not have systemic antigen, one showed a high level of type-specific antibody in her serum whereas the other patient had neither serum antigen nor antibody and the urine was not tested.

No satisfactory explanation for the development of pleural effusions in patients with pneumococcal pneumonia has been offered (Coonrod and Rytel, 1973), but it has been suggested that immune complexes are involved when effusions develop in patients with a wide

range of lung diseases (Andrews et al.,1981).

Other notable features in a few severely ill patients studied here were leucopenia on admission, followed by the development of a leucocytosis, thrombocytopenia and disseminated intravascular coagulation (DIC). Each of a number of patients, infected with different pneumococcal types showed these haematological abnormalities.

Leucopenia in bacteraemic pneumococcal pneumonia has been shown to be associated with increased mortality (Austrian and Gold, 1964). Studies of pneumococcal infection in rabbits showed that neutropenia was induced by pneumococcal cell wall components rather than capsular polysaccharide (Goldblum, Reed and Barton, 1981). They suggested that activation of complement released products which facilitated neutrophil margination and caused a reduction of cirulating neutrophils.

Thrombocytopenia has been described in patients with pneumococcal pneumonia (Reimann, 1924), although the mechanism which causes it is still not clearly understood. However, it has been shown that pneumococci induce platelet aggregation and cause release of serotonin in vitro (Zimmerman and Spiegelberg, 1975). These workers also found that immunoglobulins present in most human sera reacted with pneumococci to produce antigen-antibody complexes which then interacted with platelets to cause aggregation.

Clotting abnormalities in patients with lobar pneumonia were first reported by Dochez (1912 b). Recently,

pneumococcal septicaemia accompanied with DIC has been reported and patients with asplenia were found to be particularly likely to develop this (Bisno and Freeman, 1970). Fulminant pneumococcal disease with DIC has been observed by other workers (Rytel et al., 1974; Coonrod and Leach, 1976). Rytel et al. (1974) reported that the levels of pneumococcal antigen in the serum of patients with DIC were higher than that found in other patients with antigenaemia but without DIC. They suggested therefore, that pneumococcal capsular polysaccharide antigens initiate DIC either directly or as part of an antigen- antibody complex by activation of complement. In contrast, Coonrod and Leach (1976) did not find a correlation between DIC and the level of circulating antigen but demonstrated in patients with DIC the presence of a massive bacteraemia (more than 10^6 organisms /ml of blood) or C-substance in serum. Since pneumococci were rarely seen in blood films and C-substance was not present in the antigen-positive serum of other patients with bacteraemic pneumococcal pneumonia, (Coonrod and Rytel, 1973) Coonrod and Leach (1976) concluded that in fulminant pneumococcal bacteraemia, complement could be activated and DIC would result. Results of laboratory tests and clinical investigation carried out here tend to favour this view. Two patients with DIC studied here had previously undergone splenectomy and the results of laboratory tests, though different, paralleled those reported by

Coonrod and Leach, 1976).

The results of the studies reported here showed that patients with pneumococcal bacteraemia or antigenaemia may develop pathological changes some of which can contribute to death. However the data presented precludes any firm conclusions regarding the mechanisms which cause these changes.

Detection of type-specific pneumococcal antibody in the serum of patients with pneumococcal infection

Acute phase serum

In the present study homologous type-specific pneumococcal antibody was detected in the acute phase serum of more than three quarters of the patients with pneumococcal infection (Groups "P", "A", "C" and "S"). Moreover, antibody was detectable in the acute phase blood of half of the patients who had either bacteraemia or antigenaemia. Riley and Douglas (1981) have shown that bacteraemia can be demonstrated in spite of detectable homologous antibody : Co-circulating antibody and antigen have also been detected in the serum of patients with pneumococcal pneumonia (Blake, 1918; Bullowa et al., 1941). In contrast, most of the patients in this study who had both bacteraemia and antigenaemia, had no demonstrable antibody.

It should be pointed out that patients were not investigated for evidence of dysfunction of WBCs, complement or T-cells - although the latter appear to

be unnecessary for the control of pneumococcal infection (Johnston, 1981).

Antibody has often been detected in acute phase blood of patients with pneumococcal pneumonia (Ward, 1930; Robertson et al., 1930; Bullowa, de Gara and Bukantz,1942; Coonrod and Drennan, 1976 ; Riley and Douglas, 1981) and otitis media (Sloyer et al., 1974). Factors which may contribute to this apparent anomaly (i.e.that patients with demonstrable antibody nevertheless develop disease with homologous pneumococci) are:-

First, patients might have acquired their pneumococcus a few days before the onset of symptoms and admission to hospital. Therefore the presence of type-specific antibody in acute phase sera may simply reflect an early immune response.

Second, a wide range of type-specific pneumococcal antibodies are found in the serum of patients with no immediate history of pneumococcal infection (Ward, 1930; Robertson and Cornwell, 1930; Robertson et al., 1930). Demonstration of antibody in the acute phase of infection may merely reflect the background antibody levels in the general population.

Third, serum antibody has been detected in adult patients before colonisation with a homologous strain of S.pneumoniae (Gwaltney et al., 1975) : The presence of antibody does not therefore prevent colonisation - and presumably also may not always protect against disease.

It therefore seems likely that type-specific pneumococcal antibody in the acute phase serum of adult patients with pneumococcal infection is the result of previous exposure to particular pneumococcal types - although there is also the possibility that this antibody may be stimulated by cross-reacting antigens from other bacteria. Early immune responses may also be the result of primary sensitisation at different stages during the life of an individual and the response in early childhood may be very different from that in an adult - particularly if the immune system has already encountered pneumococcal antigens albeit of a different type. These observations do not negate the importance of serum antibody protection but do emphasize the need to consider other host defence mechanisms (such as respiratory IgA, cell-mediated immunity) at the level of the upper and lower respiratory tract mucosa. These may well be reduced or ineffective in patients who develop pneumococcal infection although there is as yet no evidence for this.

Convalescent serum

In the present study, titrations of pneumococcal antibody in paired sera from patients with pneumococcal infection showed three patterns of antibody response. First, two-thirds of the patients showed a significant (i.e. four-fold) rise in titre in the convalescent serum. Moreover, in those patients who had antigenaemia, rise in antibody titre was usually

associated with reduction in the concentration of serum antigen. Coonrod and Drennan (1976) observed a correlation between the development of antibody and the disappearance of circulating antigen and suggested that antibody production might be associated with the elimination of antigen from serum. Second, patients who had detectable antibody but no antigen in the acute phase often did not show a significant rise in titre during the convalescent phase - an observation also reported by Coonrod and Drennan (1976) and also by Riley and Douglas (1981). It is therefore likely that these patients had had an early immune response to the infection and that the rise was "missed". Third, some patients failed to show a significant rise in antibody titre during convalescence and this was associated with antigen in the serum - a result that confirms the findings of Riley and Douglas (1981). Furthermore, a few patients with acute phase antigenaemia became antigen-negative during convalescence without developing demonstrable antibody and this has also been observed by Coonrod and Drennan (1976).

Failure to detect antibody in convalescent serum has been shown to be due to the formation of antigen-antibody complexes - these have been demonstrated by RIA (Schiffman et al., 1980; Riley and Douglas, 1981) and this may have been the case in some patients in this study. Although RIA was not used here, the ELISA method for antigen detection was as sensitive as RIA and was

carried out on serum after heat-treatment to liberate antigen from complexes with antibody. On the other hand, detection of pneumococcal antibody by immumo-fluorescence, which was used here is less sensitive than RIA and therefore low levels of antibody may have been present but undetected in convalescent sera examined by this method.

Despite these results, it is possible that the ability to mount an antibody response is reduced in some patients with pneumococcal infection as was reported by Onyemelukwe et al. (1985). Such a reduction could be due to characteristics - possibly a deficiency - of the immune response of the individual or immunological paralysis induced by the polysaccharide antigen. Animal experiments have shown that mice develop antibody in response to low but not high doses of pneumococcal capsular polysaccharide antigen. However there is evidence that immunologically "paralysed" mice may produce antibody that is undetectable because of continuous neutralistion by antigen (Howard, Christie and Courtenay, 1970). Furthermore, these workers have suggested that production of antibody may be delayed due to selective suppression or elimination of clones of B lymphocytes. In human pneumococcal infection, a transient state of unresponsiveness develops rather than immune paralysis and this may be important when considering vaccination after pneumococcal infection (Onyemelukwe et al., 1985).

Antigen-antibody balance in the acute phase blood of patients with pneumococcal infection

Patients with no demonstrable antibody

In the present study, those patients who did not have demonstrable antibody in their acute phase serum generally had bacteraemia and antigenaemia ; this situation is well recognised (Blake, 1918; Bullowa et al., 1942). However, neither bacteraemia nor antigenaemia was present in a small number of patients in each of the four clinico-pathological groups studied who did not have detectable antibody. It is noteworthy that absence of antibody during the acute phase was more often a feature of patients with pneumonia than patients with a milder chest infection without clinical evidence of pneumonia and perhaps reflects the severity of the disease. These results therefore suggest that patients who lack demonstrable antibody may not have been previously exposed to the infecting type of pneumococcus and as a consequence were "susceptible" to infection. Alternatively, acute phase serum may have been taken too early in the course of the infection or when antibody levels were low because of neutralisation by antigen from infected lung tissue. In these antibody-negative patients with severe disease, the underlying lung pathology and the production of large amounts of pneumococcal capsular antigen by viable pneumococci could explain why, in contrast to other patients, few

patients with bacteraemia, antigenaemia (or both) had detectable antibody.

Patients with demonstrable antibody

Circulating antibody prevented neither pneumococcal infection nor bacteraemia nor antigenaemia and this also has been observed by other workers (Bullowa et al., 1942; Riley and Douglas, 1981). Indeed Blake (1918) who first introduced the concept of antigen-antibody balance in patients with pneumococcal pneumonia, suggested that both may have been present in the serum of some patients when he was unable to detect serum antibody. Because antibody to pneumococcal capsular polysaccharide antigen plays an important role in the resolution of pneumococcal pneumonia, has been used to treat pneumococcal disease, and vaccination with capsular polysaccharide is currently suggested as prophylaxis in patients " at risk" of pneumococcal disease the significance of this apparent failure of antibody to protect individuals from infection shown by me and by other workers needs to be considered further.

Since pneumonia can develop in the presence of circulating antibody (as seems evident from the observations here), what is the actual mechanism of infection and the role of capsular polysaccharide antibody ? The most reasonable explanation would seem to be that local changes take place in the lung during the evolution of the pneumonic lesion which provide

conditions for growth of pneumococci while protecting the organism from the action of antibody in the blood. It has been suggested that the role played by circulating antibody in the process of localisation is to eliminate pneumococci at the periphery of the lesion thus retarding the spread of the process and preventing blood invasion (Robertson et al., 1930). However, as noted earlier, the presence of antibody in the circulation does not prevent bacteraemia.

It has also been suggested that pneumococcal antibody has two functions, namely, neutralisation of free capsular polysaccharide antigen and sensitisation of pneumococci for phagocytosis. Furthermore, it is believed that, if antigen is in excess, pneumococci would not be sensitised. In other words, if antibody is to be effective in preventing infection there must be enough remaining in excess after antigen neutralisation to sensitise the viable pneumococci. However, the results reported here showed that, in general, there was no difference between the titre of antibody in the acute phase blood of patients with bacteraemia compared to that in those without bacteraemia. Moreover, when antibody titres in patients' sera and the reference serum were compared, it was found that more than two-thirds of the patients (whether or not they had bacteraemia) had an antibody level greater than the level currently considered to be the minimum which affords protection against bacteraemic pneumococcal

disease (Robbins et al., 1983).

Although it is not yet possible to provide a complete explanation, the following arguments may reconcile some of the apparent disparate findings.

First, it must be said that one cannot argue from an artificial and incomplete combination of reacting substances in vitro to the actual process in the lesion. Thus antibody is only one component of the phagocytic system. Perhaps a comparison of the potential of both infected and non-infected patients' serum and leucocytes to opsonise and phagocytose the infecting pneumococcus would be informative. Second, differences in antibody class or the extent to which antibody is complexed with antigen in the circulation may account for failure to prevent blood invasion in patients with bacteraemic disease. Third, the antigen-antibody balance in serum probably bears little relationship to that in the consolidated lung - the site where effective opsonisation and phagocytosis must take place to halt the progress of infection.

Pneumococcal antibody in relation to prognosis

The present study confirms the observations of other workers (Blake, 1918; Bukantz et al., 1942; Coonrod and Drennan, 1976) that antigenaemia in pneumococcal pneumonia is associated with delayed appearance of antibody. Nevertheless, this did not prevent a slow but satisfactory clinical recovery - an observation also made by Tugwell and Greenwood (1975) and Bullowa et al.

(1941). Bullowa and his co-workers suggested that, since capsular antigen continued to be detected in the blood after recovery, it was not toxic per se. However, despite this apparent lack of toxicity, the results of studies carried out here and elsewhere have shown that acute phase antigenaemia is associated with the development of complications and death in pneumococcal pneumonia. Furthermore is was observed here that death often followed several days after antibiotic therapy had eliminated viable pneumococci from the blood and tissues - a finding that agrees with that of Austrian and Gold (1964). Observations such as these suggested that a toxin derived from S.pneumoniae may be involved in the pathogenic process of pneumococcal pneumonia but this has not been convincingly demonstrated (Johnston, 1981).

In the present study, there were no complications or deaths in patients with bacteraemia alone or with neither bacteraemia nor antigenaemia providing that antibody was not present in the circulation. Conversely, all patients who had serum antibody and who had bacteraemia together with antigenaemia, died. These observations support the conclusions of others (Lord and Persons, 1931; Bullowa et al., 1941) that antibody production during the course of pneumococcal infection is no assurance against further progress of the infection and a fatal outcome.

Pneumococcal type distribution

The distribution of pneumococcal types was investigated in two unrelated studies. One of these compared the distribution of types from systemic disease with those from the upper respiratory tract of adults and children and included a few strains of S.pneumoniae from sputum. The other comprised the type distribution of pneumococci and pneumococcal antigen from the sputum of patients with clinical evidence of a chest infection. In both studies a wide range of pneumococcal types was detected which were similar to those reported by other workers in the UK (Hince and Howard, 1978) and the USA (Broome et al., 1980). Certain types were not encountered, notably, types 2,40,43,44,45 and 46. Although types 40,43,and 46 were found by Hince and Howard (1978), they did not find types 5 and 47. These differences reflect either temporal or geographical variations in the distribution of types within the United Kingdom. Types 45 and 46 often cause pneumococcal disease in Papua New Guinea and South Africa but they have rarely been found in other parts of the world (Austrian, 1981). The virtual disappearance of type 2 from North America and Northern Europe since World War II has often been noted (Colman and Hallas, 1983; Broome et al.,1980) though type 2 remains a common cause of pneumococcal infection in Senegal and Spain (Robbins et al., 1983) and France (Colman and Hallas, 1983).

Comparison of the distribution of pneumococcal types in systemic disease and the upper respiratory tract of adults and children

The distribution of pneumococcal types isolated from cases of systemic disease in this study was similar to that reported from England (Colman and Hallas,1983) and the USA (Broome et al.,1980).

However, there were some differences. Type 12 was an important cause of systemic disease in adults here but was an uncommon cause of systemic disease in England - and this supports the suggestion that the distribution of pneumococcal types varies in different areas within the United Kingdom. The low incidence of systemic disease due to types 8 and 12 in children that was found here was also reported by others workers (Colman and Hallas, 1983; Austrian, 1981) and was paralleled by a low rate of nasopharyngeal carriage in children studied here. The types most often carried in the upper respiratory tract of patients in this study were similar in all age groups and were the same as those reported in the USA (Kaiser and Schaffner, 1974; Gray et al., 1979). Statistical analysis of the distribution of types indicates three patterns of isolation. Types 1,4,7,8 and 12 in children and adults and types 14 & 18 in children under 5 years of age are clearly associated with systemic disease and, as suggested by Austrian (1981), this may be related to the chemical composition of their capsular polysaccharide. Conversely, other types notably

type 23 in pre-school children and type 6 in older children and adults were associated with nasopharyngeal carriage rather than systemic disease. The remaining types showed no significant difference between frequency of isolation from the nasopharynx and from systemic disease.

These included type 3 - well recognised as a virulent type - and often associated with high mortality (Austrian and Gold, 1964).

Type 3 was the most common type isolated from patients with severe pneumonia. However, it ranked only third in order of frequency amongst isolates from blood and cerebrospinal fluid. It was isolated twice as often from patients over 50 years of age with systemic disease as from those under 50 years. In contrast type 1, uncommon as a commensal, was isolated three times as often from patients with systemic disease who were less than 50 years old than from those over 50 years of age. This study demonstrates that, for a few serotypes of S.pneumoniae (i.e. types 1,4,7,8,12,14 and 18) frequency of isolation from cases of systemic disease was not directly related to their frequency of occurrence in the nasopharynx of carriers and that this was age-dependent. However, for the majority of types the incidence of systemic disease was proportional to their frequency of isolation from the nasopharynx. Thus serotyping of nasopharyngeal isolates of pneumococci from patients with pneumonia who are unable to produce a

sputum can not only establish the diagnosis (Kalin, 1982) but also indicates the potential of that type to cause bacteraemia with the further complications associated with systemic disease.

Distribution of pneumococcal types in the sputum of patients with lower respiratory tract infection

It was found, in this study, that the distribution of pneumococcal types in the sputum of patients in the three broad clinico-pathological groups (Groups "P","A" and "C") was similar. However, types 1 to 8 predominated in patients with pneumonia (Group "P") whereas types higher than 8 were prevalent in patients in Groups "A" and "C". The distribution in Groups "A" and "C" paralleled that found in adult carriers whereas the distribution of types associated with systemic disease in adults was generally somewhere between that found in carriers and in patients with pneumonia - an observation which is consistent with the findings of Finland and Barnes (1977). However, since bacteraemia is seen in only a quarter of patients with pneumococcal pneumonia and bacteraemia can be detected in adult patients who do not have pulmonary infection, it is not surprising that the types associated with systemic disease were different from those causing pneumococcal pneumonia.

Although types 1 to 8 predominated in patients with pneumonia, comparison with past studies in Glasgow (Christie, 1932; Grist et al., 1952) indicate a recent

tendency for other higher types to emerge as a cause of pneumonia. Furthermore there has been a dramatic change in the prevalence of the lower numbered types. For example, types 1 and 2 accounted for almost 80% of pneumococcal pneumonia in Glasgow in the 1930s (Christie,1932; 1934) whereas type 3 was then regarded as a commensal. In the 1950s, Grist and his colleagues (1952) found that types 1 to 8 accounted for 74% of pneumococcal pneumonia, types 1 and 2 being isolated from 44% of all patients. In this study types 1 to 8 accounted for only half of the pneumococci which caused pneumonia - type 2 was not isolated and types 3 and 8 were predominant. The distribution of pneumococcal types from patients with pneumonia in this study is similar to that found in other recent studies in the UK (Calder and Schonell, 1971; Macfarlane et al., 1982; Morgan et al., 1984). However the latter authors noted that pneumococcal pneumonia is now a disease of the elderly whereas in the 1930s it more often affected young or middle aged adults (Christie, 1932). The effect that this change has had on type distribution requires further investigation but may have been influenced by changes in social conditions.

Statistical analysis of the distribution of types associated with pneumococcal chest infection indicated three patterns of infection. Types 3 and 8 were clearly associated with pneumonia and, together with type 1, were the types most often detected by antigen tests

carried out on culture-negative sputa. Conversely, other types, notably types 6 and 17, were associated with chest infections without pneumonia and the latter type was particularly associated with acute exacerbations of COAD. The remaining types showed no statistically significant difference between frequency of isolation within the three clinico-pathological groups. Although type 1 could not be included in the statistical analysis for lack of representation in Groups "A" and "C, it was a major cause of pneumonia here - an observation which agrees with that reported in Nottingham (Macfarlane et al., 1982). In addition, half of the patients with pneumonia due to types 1 and 8 had bacteraemia in contrast to patients infected with type 3 in whom bacteraemia was detected in only a fifth. These observations are consistent with the findings in systemic disease which were previously discussed.

The studies reported here are in accord with others (cited by Shapera and Matsen, 1972) and demonstrate a similarity between the distribution of pneumococcal types in the nasopharynx of carriers, the sputum of patients with a chest infection and in the blood and body fluids of patients with systemic disease. However, it was also shown here that a few types have a greater capacity to produce severe disease and this varied with the age of the patient and clinical presentation. Since disseminated pneumococcal infections invariably originate from organisms carried in the nasopharynx

(Austrian, 1974) it seems that vaccination may be the only practical method of reducing the incidence of severe disease in the population. This aspect has been discussed in detail by Smart et al., 1987b).

Final considerations

The results of studies reported here showed that tests for pneumococcal antigen carried out on clinical material were the best single method for the diagnosis of pneumococcal infection : they were particularly useful when patients had been treated with antimicrobial agents. In addition antigen tests on serum and urine were a useful indication of systemic disease. Serotyping also helped here in that certain types were shown to be inherently capable of causing systemic disease. These tests identified patients who were likely to develop complications or to die as a result of the infection.

In contrast, antibody studies were less useful in diagnosis although they generally supported the conclusions of antigen studies as to the likely course of patients' response to infection. The tests used here however were unable to identify the mechanisms responsible for complications and death. For example, capsular polysaccharide antigen and antibody were often present in serum simultaneously - which therefore suggests the presence of antigen-antibody complexes. However this observation did not necessarily

correlate with death or complications. The pathological processes associated with immune complexes are usually mediated by complement activation. Since pneumococcal antibodies are of the IgG2 subclass (Siber et al., 1980), and so do not fix complement as effectively as antibodies of subclass IgG1 or IgG3 (Spiegelberg, 1974), immune complex disease seems unlikely. Nevertheless in some patients complement-fixing antibodies have been demonstrated (Coonrod and Rylco-Bauer, 1977) - and account for some of the clinical problems. Pneumococcal C-antigen reacts with both C-reactive protein (a beta-globulin) and with antibodies to C-polysaccharide and so activates complement by the classical pathway. Pneumococcal teichoic acid, on the other hand is a potent activator of the alternate pathway and little if any antibody is required for this. Further studies in this area may clarify a link between pneumococcal infection and the development of immune complex disease in some patients with pneumococcal pneumonia.

Detection of pneumococcal C-antigen and antibody

In contrast to pneumococcal capsular polysaccharide antigen, C-substance antigenaemia is rarely found in patients with bacteraemic pneumococcal pneumonia (Coonrod and Rytel, 1973) although it has been reported that some patients with very high levels of circulating capsular polysaccharide may have C-substance antigenaemia also (Coonrod and Leach, 1976). Holberg et al. (1985) used ELISA to detect pneumococcal C-substance in the

sputum of patients with pneumococcal pneumonia and found that only 85% of culture-positive sputa contained C-substance. This suggests that the concentration of C-substance in clinical material is considerably less than that of capsular polysaccharide antigen.

On the other hand , it has been known for some time that the titre of antibody to pneumococcal C-polysaccharide in serum is often equal to (or even greater) than that of the type-specific antibody resulting from infection. The serum of normal individuals however almost always contains detectable antibody to pneumococcal C-polysaccharide (Heidelberger and Anderson, 1944). Recently it has been shown that C-antibody seldom increases after vaccination (Pederson et al., 1982) or pneumococcal infection (Holmberg et al., 1985). In contrast, Gray et al. (1983) found that in infants and children, antibody to pneumococcal C-polysaccharide often increased after pneumococcal infection or carriage - an observation which may have implications when considering vaccination in this age group - since these patients generally respond poorly to vaccination with capsular polysaccharides.

Detection of pneumococcal C-polysaccharide antigen and antibody therefore seems (generally) unhelpful for diagnosing pneumococcal infection. This is best achieved by tests directed towards the capsular polysaccharide antigen. Conversely, tests for C-antigen or antibody - or their complexes - may be important

for the prediction of the outcome of infection.

FUTURE INVESTIGATIONS

The results presented here suggest several avenues for the further investigation of pneumococci and pneumococcal infection.

Development of improved diagnostic tests

The materials and reagents were either commercially-available or readily prepared in the laboratory. However other diagnostic reagents may be developed and become generally available which could improve the specificity and sensitivity of tests for the detection of pneumococcal capsular polysaccharide antigen and antibody carried out by non-specialist bacteriology laboratories. These might include, first, a polyvalent antiserum, with high titres of type-specific capsular polysaccharide antibody which react specifically with invasive pneumococcal types. This would be particularly useful for the detection of small amounts of antigen often found in CSF, serum and urine. Moreover, were this antiserum to be prepared in an animal other than a rabbit it could be used as the second antibody in antigen-capture ELISA tests. However the maximum number of type-specific antibodies which can be combined in a polyvalent serum for use in antigen tests by latex agglutination or ELISA needs to be determined. The results of this study suggest that the number is probably more than 5 but is certainly less than 23.

Second, preparation of radiolabelled capsular polysaccharides for use in RIA would provide a more sensitive method for the detection of pneumococcal antibody: this same technique (i.e. RIA) could also be used to detect antigen even when complexed with antibody. Moreover, the use of radiolabelled pneumococci for serum opsonisation assay might be a better method of assessing a patient's response to infection and so the level of resistance to systemic pneumococcal disease.

Third, as with other serological procedures, suitable reference materials should be prepared which would allow accurate quantitation of antigen or antibody irrespective of the assay method used.

Further laboratory studies on pneumococci

It is important to maintain surveillance of prevalent, infective pneumococcal types for the formulation of appropriate pneumococcal vaccines. If penicillin resistance in pneumococci were to become common, pneumococcal vaccines might be useful in selected groups, to prevent systemic disease due to penicillin-resistant strains.

Serotyping by co-agglutination proved a rapid, economical and accurate method of identifying pneumococcal isolates. The role of serotyping, but using a limited number of typing reagents, in the investigation of pneumococcal infection is being

evaluated in several other laboratories at present. Further studies however are also needed to identify those bacterial antigens which cross-react with pneumococcal antisera in the co-agglutination test.

C-polysaccharide is a species-specific cell wall antigen common to all pneumococci but a few serotypes incorporate the phosphorylcholine moeity of C-substance into their capsular polysaccharide. Further studies are needed to develop a test for the detection of C-substance in clinical material and to determine if this is serotype-dependent. Moreover the biological mechanisms which cause pneumococci to lose their capacity to produce a capsule require further study. The results might help to explain findings such as the observation that non-capsulate pneumococci are often isolated from eye swabs.

Antibody response to pneumococci

Antibody studies are an important component of epidemiological investigation. Further studies on the antibody response in pneumococcal infection are clearly necessary. A wide range of antibodies to different pneumococcal capsular polysaccharides are present in the serum of healthy individuals. A detailed investigation of the antibody class (or subclass) and the titres associated with infection might reveal a parameter which could be used as an epidemiological or diagnostic tool. However, the host response to other cellular

components would require investigation before a reliable diagnostic test based on serology could be introduced.

Nevertheless it is probable that antibody assays to determine the response to pneumococcal capsular polysaccharides will be used to determine the efficiency of pneumococcal vaccines. Their use could be extended to estimate the response to individual capsular polysaccharides contained in the vaccine and whether this is likely to protect the individual from infection - either with a cross-reactive type within the group or with other antigenically-related types of pneumococci.

Pneumococcal infections

Although pneumococcal infections have been investigated for almost a century many problems in this interesting field remain. Some have been described by Austrian (1975a) and by Johnston (1981) and both authors agree that the principle problem posed by the pneumococcus - to both research worker and clinician - is an understanding of the way in which the organism damages the host it invades. Despite an enormous amount of research this is still virtually unknown. Johnston (1981) proposed that a combination of the host defences with antigenaemia and possibly also with immune complexes was probably responsible for tissue damage and eventually death. The problem is clearly multi-factorial and further studies are needed to assess the

role of complement activation in human pneumococcal disease. In conjunction with such studies, the development of an animal model would be beneficial although laboratory animals such as rabbits and mice are unsuitable for this as they do not respond immunologically to pneumococci in the same way as humans.

Proof that immune complexes are involved in the pathology of pneumococcal infection might lead to a change in the current treatment regimes recommended for severely ill patients. For example, plasmapheresis, leucapheresis and blood exchange have been successfully used to treat patients with meningococcal septicaemia who develop signs of immune complex disease (Bjorvatn et al., 1984) and may have application to pneumococcal disease. Finally, it is not inconceivable that studies which identified the precise nature of the antigen and antibody involved in immune complexes might prompt a return to serum therapy or a modern equivalent.

REFERENCES

Abernethy, T.J. & Avery, O.T. (1940) The occurrence during acute infections of a protein not normally present in the blood. I. Distribution of the reactive protein in patients' sera and the effect of calcium on the flocculation reaction with C polysaccharide of pneumococcus. Journal of Experimental Medicine, 73, 173-199.

Allibone, E.C., Allison, P.R. & Zinnemann, K. (1956) Significance of H. influenzae in bronchiectasis of children. British Medical Journal, 1, 1457-1460.

Ammann, A.J., Addiego, J., Wara, D.W., Lubin, B., Smith, W.B. & Mentzer, W.C. (1977) Polyvalent pneumococcal polysaccharide immunization of patients with sickle-cell anemia and patients with splenectomy. New England Journal of Medicine, 297, 897-900.

Ammann, A.J. & Pelger, R.J. (1972) Determination of antibody to pneumococcal polysaccharides with chromic chloride-treated human red blood cells and indirect hemagglutination. Applied Microbiology, 24, 679-683.

Amoss, H.L. (1930) Specific soluble substances of the pneumococcus in the blood in pneumonia. Proceedings of the Society of Experimental Biology and Medicine, 28, 23-25.

Andersson, B., Svanborg-Eden, C. & Hanson, L.A. (1982) Pneumococcal adhesion to human pharyngeal cells. Scandinavian Journal of Infectious Diseases, 33, supplement, 96-97.

Andrews, J.S., Arora, N.S., Shadforth, M.F., Goldberg, S.K. & Davis, J.S. (1981) The role of immune complexes in the pathogenesis of pleural effusions. American Review of Respiratory Disease, 124, 115-120.

Anhalt, J.P. & Yu, P.K.W. (1975) Counterimmunoelectrophoresis of pneumococcal antigens: Improved sensitivity for the detection of types VII & XIV. Journal of Clinical Microbiology, 2, 510-515.

Armstrong, R.R. (1931) A swift and simple method for deciding pneumococcal "type". British Medical Journal,1, 214-215.

Armstrong, R.R. (1932) Immediate pneumococcal typing. British Medical Journal, 1, 187-188.

Armstrong, R.R. & Johnson, R.S. (1932) Treatment of lobar pneumonia by anti-pneumococcal serum. British Medical Journal, 2, 662-665.

Austrian, R. (1953) Morphologic variation in pneumococcus. I. An analysis of the bases for morphologic variation in pneumococcus and description of a hitherto undefined morphologic variant. Journal of Experimental Medicine, 98, 21-34.

Austrian, R. (1964) The role of toxemia and of neural injury in the outcome of pneumococcal meningitis. American Journal of Medical Sciences, 247, 257-262.

Austrian, R.(1974) Streptococcus pneumoniae (Pneumococcus). In Manual of Clinical Microbiology, 2nd edition, ed. Lennette, E.H., Spaulding, E.H. & Truant, J.P. pp 109-115. Washington, D.C: American Society for Microbiology.

Austrian, R. (1975a) Random gleanings from a life with the pneumococcus. Journal of Infectious Diseases, 131, 474-484.

Austrian, R.(1975b) Cross-reactions between pneumococcal and streptococcal polysaccharides. In Symposium of new approaches for inducing natural immunity to pyogenic organisms, ed. Robbins, J.B., Horton, R.E. & Krause, R.M. pp 39-44. U.S. Department of Health and Human Services Publication (N.I.H.) 74-553 Washington, D.C. : U.S. Government printing office.

Austrian, R. (1981) Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention. Reviews of Infectious Diseases, 3, supplement, S1-S17.

Austrian, R. (1985) Life with the pneumococcus. Notes from the bedside, laboratory and library. Philadelphia : University of Pennsylvania Press.

Austrian, R., Buettger, C. & Dole, M. (1972) Problems in the classification and pathogenic role of alpha and nonhemolytic streptococci of the human respiratory tract. In Streptococci and streptococcal diseases, ed. Wannamaker, L.W. & Matson, J.M. Ch. 21, pp 355-370. New York : Academic Press.

Austrian, R., Boettger, C., Dole, M., Fairly, L. & Freid, M. (1985) Streptococcus pneumoniae Type 16A, a hitherto undescribed pneumococcal type. Journal of Clinical Microbiology, 22, 127-128

Austrian, R. & Collins, P. (1966) Importance of carbon dioxide in the isolation of pneumococci. Journal of Bacteriology, 92, 1281-1284.

Austrian, R., Douglas, R.M., Shiffman, G., et al. (1976) Prevention of pneumococcal pneumonia by vaccination. Transactions of the Association of American Physicians, 89, 184-194.

Austrian, R. & Gold, J. (1964) Pneumococcal bacteremia with especial reference to bacteremic pneumococcal pneumonia. Annals of Internal Medicine, 60, 759-776.

Austrian, R., Howie, V.A. & Ploussard, J.H. (1977) The bacteriology of pneumococcal otitis media. The John Hopkins Medical Journal, 141, 104-111.

Austrian, R. & MacLeod, C.M. (1949) A type-specific protein from pneumococcus. Journal of Experimental Medicine, 89, 439-450.

Avery, O.T. (1915) A further study on the biologic classification of pneumococci. Journal of Experimental Medicine, 22, 804-819.

Avery, O.T. (1918) Determination of types of pneumococcus in lobar pneumonia. A rapid cultural method. Journal of the American Medical Association, 70, 17-19.

Avery, O.T., Heidelberger, M. & Gobel, W.F. (1925) The soluble specific substance of Friedlander's bacillus. Paper II. Chemical and immunologic relationships of pneumococcus type II and of a strain of Friedlander's bacillus. Journal of Experimental Medicine, 42, 709-725.

Avery, O.T., MacLeod, C.M. & McCarty, M. (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. Journal of Experimental Medicine, 79, 137-158.

Baker, P.J., Stashak, P.W. & Prescott, B. (1969) Use of erythrocytes sensitized with purified pneumococcal polysaccharides for the assay of antibody and antibody-producing cells. Applied Microbiology, 17, 422-426.

Baldwin H.S. & Rhoades, D.R. (1925) Studies on pneumococcus immunity. VII. The protective substance in pneumococcus pneumonia with special reference to bacteriemia and specific treatment. Hygienic Laboratory Bulletin, 141, 43-67.

Barach, A.L. (1931) Immune transfusion in lobar pneumonia. American Journal of Medical Sciences, 182, 811-821.

Barnes, L.A. & Wight, E.C. (1935) Serological relationship between pneumococcus type I and an encapsulated strain of Escherichia coli. Journal of Experimental Medicine, 62, 281-287.

Barrett-Connor, E. (1971) The nonvalue of sputum culture in the diagnosis of pneumococcal pneumonia. American Review of Respiratory Disease, 103, 845-848.

Bartlett, R.C. (1974) In Medical Microbiology: Quality Cost and Clinical Relevance. pp 24-31. New York: John Wiley & Sons.

Bartlett, J.G. & Finegold, S.M. (1978) Bacteriology of expectorated sputum with quantitative culture and wash technique compared to transtracheal aspirates. American Review of Respiratory Disease, 117, 1019-1027.

Bartram, C.E., Crowder, J.G., Beeler, B. & White, A. (1974) Diagnosis of bacterial diseases by detection of serum antigens by counterimmunoelectrophoresis, sensitivity and specificity of detecting Pseudomonas and pneumococcal antigens. Journal of Laboratory and Clinical Medicine, 83, 591-598.

Beebe, R.T. & Sutliff, W.D. (1930) The treatment of lobar pneumonia with homologous convalescent serum. New England Journal of Medicine, 203, 823-825.

Bergqvist, G. & Trovik, M. (1985) Neonatal infections with Streptococcus pneumoniae. Scandinavian Journal of Infectious Diseases, 17, 33-35.

Berntsson, E., Broholm, K.A. & Kaijser, B. (1978) Serological diagnosis of pneumococcal disease with enzyme-linked immunosorbent assay (ELISA). Scandinavian Journal of Infectious Diseases, 10, 177-181.

Bezancon, F. & Griffon, V. (1897) Pouvoir agglutinatif du serum dans les infections experimentales et humaines a pneumocoques I. Comptes Rendus Societe de Biologie 49, 551-553.

Bezancon, F. & Griffon, V. (1900) Etude de la reaction agglutinante du serum dans les infections experimentales et humaines a pneumocoques. Annals of the Institute Pasteur, 14, 449-

Bisno, A.L. & Freeman, J.C. (1970) The syndrome of asplenia, pneumococcal sepsis, and disseminated intravascular coagulation. Annals of Internal Medicine, 72, 389-393.

Bjorvatn, B., Bjertnaes, L., Fadnes, H.O., et al. (1984) Meningococcal septicaemia treated with combined plasmapheresis and leucapheresis or with blood exchange. British Medical Journal, 288, 439-441.

Blake, F.G. (1917) Methods for the determination of pneumococcus types. Journal of Experimental Medicine, 26, 67-80.

Blake, F.G. (1918) Antigen-antibody balance in lobar pneumonia. Archives of Internal Medicine, 21, 779-790.

Boerner, D.F. & Zwadyk, P. (1982) The value of the sputum Gram's stain in community-acquired pneumonia. Journal of the American Medical Association, 247, 642-645.

Bornstein, D.L., Schiffman, G., Bernheimer, H.P. & Austrian, R. (1968) Capsulation of pneumococcus with soluble C-like (C_S) polysaccharide. I. Biological and genetic properties of C_S pneumococcal strains. Journal of Experimental Medicine, 128, 1385-1400.

Bortolussi, R., Marrie, T.J., Cunningham, J. & Schiffman, G. (1981) Serum antibody and opsonic responses after immunization with pneumococcal vaccine in kidney transplant recipients and controls. Infection and Immunity, 34, 20-25.

Bowers, E.F. & Jeffries, L.R. (1955) Optochin in the identification of Str. pneumoniae. Journal of Clinical Pathology, 8, 58-60.

Brook, I. (1980) Anaerobic and aerobic bacterial flora of acute conjunctivitis in children. Archives of Ophthalmology, 89, 833-835.

Brook, I., Pettit, T.H., Martin, W.J. & Finegold, S.M. (1979) Anaerobic and aerobic bacteriology of acute conjunctivitis. Annals of Opthalmology, 11, 389-393.

Broome, C.V., Facklam, R.R., Allen, J.R. & Fraser, D.W. (1980) Epidemiology of pneumococcal serotypes in the United States, 1978-1979. Journal of Infectious Diseases, 141, 119-123.

Brown, C.C., Coleman, M.B., Alley, R.D., Stranahan, A. & Stuart-Harris, C.H. (1954) Chronic bronchitis and emphysema: significance of the bacterial flora in the sputum. American Journal of Medicine, 17, 478-484.

Browne, K., Miegel, J. & Stottmeier, K.D. (1984) Detection of pneumococci in blood cultures by latex agglutination. Journal of Clinical Microbiology, 19, 649-50.

Brumfitt, W., Willoughby, M.L.N. & Bromley, L.L. (1957) An evaluation of sputum examination in chronic bronchitis. Lancet, 2, 1306-1309.

Bukantz, S.C., de Gara, P.F. & Bullowa, J.G.M. (1942) Capsular polysaccharide in the blood of patients with pneumococcic pneumonia. Detection, incidence, prognostic significance and relation to therapies. Archives of Internal Medicine, 69, 191-212

Bullowa, J.G.M., Bukantz, S.C. & de Gara, P.F. (1941) The balance between capsular polysaccharide and antibody in relation to the prognosis and therapy of pneumococcal pneumonia. Annals of Internal Medicine, 14, 1348-1359.

Bullowa, J.G.M., de Gara, P.F. & Bukantz, S.C. (1942) Type-specific antibodies in the blood of patients with pneumococcic pneumonia. Detection, incidence, prognostic significance and relation to therapies. Archives of Internal Medicine, 69, 1-14.

Burdash, N.M. & West, M.E. (1982) Identification of Streptococcus pneumoniae by the Phadebact coagglutination test. Journal of Clinical Microbiology, 15, 391-394.

Calder, M.A., Lutz, W. & Schonell, M.E. (1968) A five year study of bacteriology and prophylactic chemotherapy in patients with chronic bronchitis. British Journal of Diseases of the Chest, 62, 93-99.

Calder, M.A., McHardy, V.U. & Schonell, M.E. (1970) Importance of pneumococcal typing in pneumonia. Lancet, 1, 5-7.

Calder, M.A. & Schonell, M.E. (1971) Pneumococcal typing and the problem of endogenous or exogenous reinfection in chronic bronchitis. Lancet, 1, 1156-1159.

Cerosaletti, K.M., Roghmann, M.C. & Bentley, D.W. (1985) Comparison of latex agglutination and counterimmuno-electrophoresis for the detection of pneumococcal antigen in elderly pneumonia patients. Journal of Clinical Microbiology, 22, 553-557.

Chickering, H.T. (1914) Agglutination phenomena in lobar pneumonia. Journal of Experimental Medicine, 20, 599-606.

Chodosh, S. (1970) Examination of sputum cells. New England Journal of Medicine, 282, 854-857.

Christie, I.M. (1932) Type-specific organisms in acute pneumonia and in convalescents and contacts. Lancet, 2, 1173-1176.

Christie, I.M. (1934) Epidemiological significance of the serological types of pneumococci. Lancet, 2, 39-42.

Clough, P.W. (1913) The development of antibodies in the serum of patients recovering from acute lobar pneumonia. Johns Hopkins Hospital Bulletin, 24, 295-306.

Clough, P.W. (1919) Phagocytosis and agglutination in the serum in acute lobar pneumonia; the specificity of these reactions and the regularity of their occurrence. Johns Hopkins Hospital Bulletin, 30, 167-176.

Clough, P.W. (1924) The protective power of normal human serum against pneumococcus infection. Johns Hopkins Hospital Bulletin, 35, 330-335.

Cockburn, W.C., (1979) The importance of infections of the respiratory tract. Journal of Infection, 1, supplement 2, 3-8.

Cole, R.I. (1902-3) Blood cultures in pneumonia. Johns Hopkins Hospital Bulletin, 13-14, 136-139.

Cole, R.I. (1913) Treatment of pneumonia by means of specific serums. Journal of the American Medical Association, 61, 663-666.

Cole, R.I. (1914) Pneumococcus hemotoxin. Journal of Experimental Medicine, 20, 346-362.

Cole, R.I. (1929) Serum treatment in type 1 lobar pneumonia. Journal of the American Medical Association, 93, 741-747.

Colman, G. & Hallas, G. (1983) Systemic disease caused by pneumococci. Journal of Infection, 7, 248-225.

Congeni, B.L. & Nankervis, G.A. (1978) Diagnosis of pneumonia by counterimmunoelectrophoresis of respiratory secretions. American Journal of Diseases of Children, 132, 684-687.

Coonrod, J.D. (1974) Physical and immunologic properties of pneumococcal capsular polysaccharide produced during human infection. Journal of Immunology, 117, 2183-2201.

Coonrod, J.D. & Drennan, D.P. (1976) Pneumococcal pneumonia: Capsular polysaccharide antigenemia and antibody responses. Annals of Internal Medicine, 84, 254-260.

Coonrod, J.D. & Leach, R.P. (1976) Antigenemia in fulminant pneumococcemia. Annals of Internal Medicine, 84, 561-563.

Coonrod, J.D. & Rylko-Bauer. (1976) Latex agglutination in the diagnosis of pneumococcal infection. Journal of Clinical Microbiology, 4, 168-174.

Coonrod, J. D. & Rylko-Bauer, B. (1977) Complement-fixing antibody response in pneumococcal pneumonia. Infection and Immunity, 18, 617-623.

Coonrod, J.D. & Rytel, M.W.(1972) Determination of aetiology of bacterial meningitis by counterimmuno-electrophoresis. Lancet, 1, 1154-1157.

Coonrod, J.D. & Rytel, M.W. (1973) Detection of type-specific pneumococcal antigens by counterimmunoelectrophoresis. II Etiologic diagnosis of pneumococcal pneumonia. Journal of Laboratory and Clinical Medicine, 81, 778-786.

Coonrod, J.D. & Wilson, H.D. (1976) Etiologic diagnosis of intrapleural empyema by counterimmunoelectrophoresis. American Review of Respiratory Disease, 113, 637-641.

Cooper, G., Rosenstein, C., Walter, A. & Peizer, L. (1932) The further separation of types among the pneumococci hitherto included in Group IV and the development of therapeutic antisera for these types. Journal of Experimental Medicine, 55, 531-554.

Cronberg, S. & Nilsson, I.M. (1970) Pneumococcal sepsis with generalised Shwartzman reaction. Acta Medicine Scandinavica, 188, 293-299.

Davey, P.G., Cruikshank, J.K., McManus, I.C., Mahood, B., Snow, M.H. & Geddes, A.M. (1982) Bacterial Meningitis - ten years experience. Journal of Hygiene Cambridge, 88, 383-401.

De Jong, P.J. (1983) Simple method for preparation of antibody-enzyme conjugates for enzyme-linked immunosorbent assays. Journal of Clinical Microbiology, 17, 928-930.

Dirks-Go, S.I.S. & Zanen, H.C. (1978) Latex agglutination, counterimmunoelectrophoresis, and protein A co-agglutination in diagnosis of bacterial meningitis. Journal of Clinical Pathology, 31, 1167-1171.

Dixon, J.M.S. & Miller, D.C. (1965) Value of dilute inocula in cultural examination of sputum. Lancet, 2, 1046-1048.

Dochez, A.R. (1912a) The presence of protective substances in human serum during lobar pneumonia. Journal of Experimental Medicine, 16, 665-681.

Dochez, A.R. (1912b) Coagulation time of the blood in lobar pneumonia. Journal of Experimental Medicine, 16, 693-700.

Dochez, A.R. & Avery, O.T. (1917) Soluble substance of pneumococcus origin in the blood and urine during lobar pneumonia. Proceedings of the Society of Experimental Biology and Medicine, 14, 126-127.

Dochez, A.R. & Avery, O.T. (1917) The elaboration of specific soluble substance by pneumococcus during growth. Journal of Experimental Medicine, 26, 477-493.

Dochez, A.R. & Gillespie, L.J. (1913) A biologic classification of pneumococci by means of immunity reactions. Journal of the American Medical Association, 61, 727-730.

Dorff, G.J., Coonrod, J.D. & Rytel, M.W. (1971) Detection by immunoelectrophoresis of antigen in sera of patients with pneumococcal bacteraemia. Lancet, 1, 578-579.

Doskeland, S.O. & Berdal, B.P. (1980) Bacterial antigen detection in body fluids : Methods for rapid antigen concentration and reduction of nonspecific reactions. Journal of Clinical Microbiology, 11, 380-384.

Downes, B.A. & Ellner, P.D. (1979) Comparison of sputum counterimmunoelectrophoresis and culture in diagnosis of pneumococcal pneumonia. Journal of Clinical Microbiology, 10, 662-665.

Drew, W.L. (1977) Value of sputum culture in diagnosis of pneumococcal pneumonia. Journal of Clinical Microbiology, 6, 62-65.

Drow, D.L. & Manning, D.D. (1980) Indirect sandwich enzyme-linked immunosorbent assay for rapid detection of Streptococcus pneumoniae Type 3 antigen. Journal of Clinical Microbiology, 11, 641-645.

Dubos, R.J. (1938) The effect of formaldehyde on pneumococci. Journal of Experimental Medicine, 67, 389-398.

Dulake, C. (1979) Counter-current immunoelectrophoresis for the diagnosis of pneumococcal chest infection. Journal of Infection, 1, supplement 2, 45-51.

Eddy, B.E. (1944) Nomenclature of pneumococcal types. U.S. Public Health Report, 59, 449-451.

Edwards, E.A. & Coonrod, J.D. (1980) Coagglutination and counterimmunoelectrophoresis for detection of pneumococcal antigens in the sputum of pneumonia patients. Journal of Clinical Microbiology, 11, 488-491.

Eggers, H.E. (1912) On the antipneumococcal powers of the blood in pneumonia. Journal of Infectious Diseases, 10, 48-56.

Elmes, P.C. & White, J.C. (1953) Deoxyribonuclease in the treatment of purulent bronchitis. Thorax, 8, 295-300.

El-Refaie, M. & Dulake, C. (1975) Counter-current immunoelectrophoresis for the diagnosis of pneumococcal chest infection. Journal of Clinical Pathology, 28, 801-806.

El-Refaie, M., Tait, R., Dulake, C. & Dische, F.E. (1976) Pneumococcal antigen in pneumonia. A post-mortem study with the histological and bacteriological findings. Postgraduate Medical Journal, 52, 497-500.

Ernst, J. D., Hartiala, K.T., Goldstein, I.M. & Sande, M.A.(1984) Complement (C5)-derived chemotactic activity accounts for accumulation of polymorphonuclear leukocytes in cerebrospinal fluid of rabbits with pneumococcal meningitis. Infection and Immunity, 46, 81-86.

Evans, W. & Hansman, D. (1963) Tetracycline-resistant pneumococcus. Lancet, 1, 451.

Feldman, W.E. (1976) Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. Journal of Pediatrics, 88, 549-552.

Felton, L.D. & Bailey, G.H. (1926) Biologic significance of the soluble specific substances of pneumococci. Journal of Infectious Diseases, 38, 131-144.

Ferrante, A., Rowan-Kelly, B. & Paton, J.C. (1984) Inhibition of in vitro human lymphocyte response by the pneumococcal toxin Pneumolysin. Infection and Immunity, 46, 585-589.

Fine, D.P. (1975) Pneumococcal type-associated variability in alternate complement pathway activation. Infection and Immunity, 12, 772-778.

Finland, M. (1930) The serum treatment of lobar pneumonia. New England Journal of Medicine, 202, 1244-1247.

Finland, M. (1979) Pneumonia and pneumococcal infections, with special reference to pneumococcal pneumonia. The 1979 J. Burns Amberson Lecture. American Review of Respiratory Disease, 120, 481-502.

Finland, M. & Barnes, M.W. (1977) Changes in occurrence of capsular serotypes of Streptococcus pneumoniae at Boston City Hospital during selected years between 1935 and 1974. Journal of Clinical Microbiology, 5, 154-166.

Finland, M. & Brown, J.W. (1939) Specific treatment of pneumococcus type 1 pneumonia. American Journal of Medical Sciences, 197, 151-168.

Finland, M. & Brown, J.W.(1939) Specific treatment of pneumococcus type 11 pneumonia . American Journal of Medical Sciences, 197, 369-381.

Finland, M. & Brown, J.W. (1939) Specific treatment of pneumococcus type V and VII pneumonias. American Journal of Medical Sciences, 197, 381-393.

Finland, M., Spring, W.C. & Lowell, F. C. (1940) Immunological studies on patients with pneumococcic pneumonia treated with sulfapyridine. Journal of Clinical Investigation, 19, 179-199.

Finland, M. & Sutliff, W.D. (1932) Specific antibody response of human subjects to intracutaneous injection of pneumococcus products. Journal of Experimental Medicine, 55, 853-865.

Fossieck, B., Craig, R. & Paterson, P.Y. (1973) Counterimmunoelectrophoresis for rapid diagnosis of meningitis due to Diplococcus pneumoniae. Journal of Infectious Diseases, 127, 106-109.

Francis, T. (1932) The identity of the mechanisms of type-specific agglutinin and precipitin reactions with pneumococcus. Journal of Experimental Medicine, 55, 55-62.

Frankel, A. (1885) Bakteriologische Mittheilungen. Zeitschrift fur klinische Medicin, 10, 401-461.

Giebink, G.S., Foker, J.E., Kim, Y. & Schiffman, G. (1980) Serum antibody and opsonic responses to vaccination with pneumococcal caspular polysaccharide in normal and splenectomized children. Journal of Infectious Diseases, 141, 404-412.

Goldblum, S.E., Reed, W.P. & Barton, L.L. (1981) Reduction of circulating granulocytes induced by type 1 pneumococcal cell walls in New Zealand white rabbits. Infection and Immunity, 33, 1-6.

Goodwin, R.A., Wilcox, C. & Finland, M. (1945) Persistence of pneumococci in sulfonamide treated cases of pneumonia. American Journal of Medical Sciences, 209, 628-639.

Gransden, W.R., Eykyn, S.J. & Phillips, I. (1985) Pneumococcal bacteraemia : 325 episodes diagnosed at St. Thomas's Hospital. British Medical Journal, 290, 505-508.

Grant, M.H. (1922) Pneumonias in Glasgow and their pneumococcal types.Glasgow Medical Journal,198, 193-195.

Gray, B.M., Converse, G.M. & Dillon, H.C. (1979) Serotypes of Streptococcus pneumoniae causing disease. Journal of Infectious Diseases, 140, 979-983.

Gray, B.M., Dillon, H.C. & Briles, D.E. (1983) Epidemiological studies of Streptococcus pneumoniae in infants: Development of antibody to phosphocholine. Journal of Clinical Microbiology, 18, 1102-1107.

Green, G.M. & Kass, E.H. (1964) The role of the alveolar macrophage in the clearance of bacteria from the lung. Journal of Experimental Medicine, 119, 167-176.

Griffith, F.(1928) The significance of pneumococcal types. Journal of Hygiene, 27, 113-159.

Grist, N.R., Landsman, J.B., Anderson, T., Rowan, G. & Smith, A.(1952) Studies in the aetiology of pneumonia in Glasgow 1950-1951. Lancet, 1, 640-646.

Gruer, L. D., Collingham, K.E. & Edwards, C.W. (1983) Pneumococcal peritonitis associated with an IUCD. Lancet, 2, 677.

Guckian, J.C. (1975) Effect of pneumococci on blood clotting, platelets and polymorphonuclear leukocytes. Infection and Immunity, 12, 910-918.

Guzzetta, P., Toews, G.B., Robertson, K.J. & Pierce, A.K.(1983) Rapid diagnosis of community-acquired bacterial pneumonia. American Review of Respiratory Disease, 128, 461-464.

Gwaltney, J.M., Sande, M.A., Austrian, R. & Hendley, J.O. (1975) Spread of Streptococcus pneumoniae in families. II Relation of transfer of S. pneumoniae to incidence of colds and serum antibody. Journal of Infectious Diseases, 132, 62-69.

Hansman, D. & Bullen, M,M, (1967) A resistant pneumococcus. Lancet, 2, 264-265.

Harding, S.A., Scheld, W.M., McGowan, M.D. & Sande, M.A. (1979) Enzyme-linked immunosorbent assay for detection of Streptococcus pneumoniae antigen. Journal of Clinical Microbiology, 10, 339-342.

Heffron, R. (1939) Pneumonia with special reference to pneumococcus lobar pneumonia. New York :The Commonwealth Fund.

Heidelberger, M. & Anderson, D.G. (1944) The immune response of human beings to brief infections with pneumococcus. Journal of Clinical Investigation, 23, 607-612.

Heidelberger, M., Jann, B., Jann, F., Orskov, I., Orskov, F. & Westphal, O. (1968) Relations between structures of three K polysaccharides of Escherichia coli and cross - reactivity in antipneumococcal sera. Journal of Bacteriology, 95, 2415-2417.

Heidelberger, M. & Kabat, E.A. (1936) Chemical studies on bacterial agglutination. II. The identity of precipitin and agglutinin. Journal of experimental Medicine, 63, 737-744.

Heidelberger, M. & Kabat, E.A. (1938) Chemical studies on bacterial agglutination. IV. Quantitative data on pneumococcus R (Dawson S) - anti-R (S) systems. Journal of Experimental Medicine, 67, 545-550.

Heidelberger, M. & Kendall, F.E. (1929) A quantitative study of the precipitin reaction between type III pneumococcus polysaccharide and purified homologous antibody. Journal of Experimental Medicine, 50, 809-823.

Heidelberger, M. & Kendall, F.E. (1935) The precipitin reaction between type III pneumococcus polysaccharide and homologous antibody. II Conditions for quantitative precipitation of antibody in horse sera. Journal of Experimental Medicine, 61, 559-562.

Heidelberger, M., Sia, R.H.P. & Kendall, F.E. (1930) Specific precipitation and mouse protection in type I antipneumococcus sera. Journal of Experimental Medicine, 52, 477-483.

Heineman, H.S., Chawla, J.K. & Lofton, W.M. (1977) Misinformation from sputum cultures without microscopic examination. Journal of Clinicial Microbiology, 6, 518-527.

Heltberg, O., Korner, B. & Schouenborg, P. (1984) Six cases of acute appendicitis with secondary peritonitis caused by Streptococcus pneumoniae. European Journal of Clinical Microbiology, 3, 141-143.

Hendley, J.O., Sande, M.A., Stewart. P.M. & Gwaltney, J.M. (1975) Spread of Streptococcus pneumoniae in families. I. Carriage rates and distribution of types. Journal of Infectious Diseases, 132, 55-61.

Henrichsen, J., Berntsson, E. & Kaijser, B.(1980) Comparison of counterimmunoelectrophoresis and the capsular reaction test for typing of pneumococci. Journal of Clinical Microbiology, 11, 589-592.

Hince, C.J. & Howard, A.J. (1978) Serotypes of Streptococcus pneumoniae in the United Kingdom. Current Chemotherapy, Proceedings of the 10th International Congress of Chemotherapy Zurich , 1, 543-545.

Holliday, M.G. (1981) Pneumococcal typing by polyvalent counterimmunoelectorphoresis (PIE). Journal of Immunological Methods, 46, 243-249.

Holmberg, H., Danielsson, D., Hardie, J., Krook, A. & Whiley, R. (1985) Cross-reactions between alpha - streptococci and Omniserum, a polyvalent pneumococcal serum, demonstrated by direct immunofluorescence, immunoelectroosmophoresis, and latex agglutination. Journal of Clinical Microbiology, 21, 745-748.

Holmberg, H., Holme, T., Krook, A., Olsson, T., Sjoberg, L. & Sjogren, A-M. (1985) Detection of C polysaccharide in Streptococcus pneumoniae in the sputa of pneumonia patients by an enzyme-linked immunosorbent assay. Journal of Clinical Microbiology, 22, 111-115.

Holmberg, H., Krook, A. & Sjogren, A-M. (1985) Determination of antibodies to pneumococcal C polysaccharide in patients with community-acquired pneumonia. Journal of Clinical Microbiology, 22, 808-814.

Holt, L.B. (1962) The culture of Streptococcus pneumoniae. Journal of General Microbiology, 27, 327-330.

Howard, J.G., Christie, G.H. & Courtenay, B.M.(1970) Treadmill neutralization of antibody and central inhibition. Separate components of pneumococcal polysaccharide paralysis. Transplantation, 10, 251-253.

Howard. J.G., Christie, G.H. Jacob, M.J. & Elson, J. (1970) Studies on immunological paralysis. III. Recirculation and antibody-neutralizing activity of C^{14}-labelled type III pneumococcal polysaccharide in paralysed mice. Journal of Clinical and Experimental Immunology, 7, 585-596.

Howard, M.W., Strauss, R.G. & Johnston, R.B. (1977) Infections in patients with neutropenia. American Journal of Diseases of Children, 131, 788-790.

Howden, R. (1976) Use of anaerobic culture for the improved isolation of Streptococcus pneumoniae. Journal of Clinical Pathology, 29, 50-53.

Howden, R. (1979) A rapid bile solubility test for pneumococci. Journal of Clinical Pathology,32, 1293-1294.

Hurlimann, J., Thorbecke, G.J. & Hochwald, G.M. (1966) The liver as the site of C- reactive protein formation. Journal of Experimental Medicine, 123, 365-378.

Ingram, D.L., Pearson, A.W. & Occhiuti, A.R. (1983) Detection of bacterial antigens in body fluids with the Wellcogen Haemophilus influenzae b, Streptococcus pneumoniae and Neisseria meningitis (ACYW135) latex agglutination tests. Journal of Clinical Microbiology, 18, 1119-1121.

Irwin, R.S., Demers, R.R., Pratter, M.R., Erickson, A.D., Farrugia, R. & Teplitz, C.(1980) Evaluation of methylene blue and squamous epithelial cells as oropharyngeal markers: A means of identifying oropharyngeal contamination during transtracheal aspiration. Journal of Infectious Diseases,141, 165-171.

Jacobs, M.R., Koornhof, H.J., Robins-Browne, R.M.,et al. (1978) Emergence of multiply resistant pneumococci. New England Journal of Medicine, 299, 735-740.

Jacox, R.F. (1947) A new method for the production of non-specific capsular swelling of the pneumococcus. Proceedings of the Society of Experimental Biology and Medicine, 66, 635-638.

Jennings, H. J., Lugowski, C. & Young, N.M. (1980) Structure of the complex polysaccharide C-substance from Streptococcus pneumoniae Type 1. Biochemistry, 19, 4712-4719.

Johansen, K.S. & Pedersen, F.K. (1982) Antibody response and opsonization after pneumococcal vaccination in splenectomized children and healthy persons. Acta Pathologica et Microbiologica et Immunologica Scandinavica Section C, 90, 265-270.

Johnson, M.K., Boese - Marrazzo, D. & Pierce, W.A. (1981) Effects of pneumolysin on human polymorphonuclear leukocytes and platelets. Infection and Immunity, 34, 171-176.

Johnston, R.B., Klemperer, M.R., Alper, C.A. & Rosen, F.S. (1969) The enhancement of bacterial phagocytosis by serum. Journal of Experimental Medicine, 129, 1275-1290.

Johnston, R.B. (1981) The host response to invasion by Streptococcus pneumoniae: Protection and the pathogenesis of tissue damage. Reviews of Infectious Diseases 3, 282-288.

Kaiser, A.B. & Schaffner, W. (1974) Prospectus: The prevention of bacteremic pneumococcal pneumonia. A conservative appraisal of vaccine intervetion. Journal of the American Medical Association, 230, 404-408.

Kaldor, J. Asznowicz, R. & Buist, D.G.P. (1977) Latex agglutination in diagnosis of bacterial infections, with special reference to patients with meningitis and septicemia. American Journal of Clinical Pathology, 68, 284-289.

Kalin, M. (1982) Bacteremic pneumococcal pneumonia : value of culture of nasopharyngeal specimens and washed sputum specimens. European Journal of Clinical Microbiology,1, 394-395

Kalin, M., Kanclerski, K., Granstrom, M. & Mollby, R.(1987) Diagnosis of pneumococcal pneumonia by enzyme-linked immunosorbent assay of antibodies to pneumococcal hemolysin (Pneumolysin). Journal of Clinical Microbiology, 25, 226-229.

Kalin, M. & Lindberg, A.A (1983) Diagnosis of pneumococcal pneumonia : A comparison between microscopic examination of expectorate, antigen detection and cultural procedures. Scandinavian Journal of Infectious Diseases, 15, 247-255.

Kalin, M. & Lindberg, A.A. (1985) Antibody response against the type specific capsular polysaccharide in pneumococcal pneumonia measured by enzyme-linked immunosorbent assay. Scandinavian Journal of Infectious Diseases, 17, 25-32.

Kalin, M., Lindberg, A.A. & Olausson, E-H. (1982) Diagnosis of pneumococcal pneumonia by coagglutination and counterimmunoelectrophoresis of sputum samples. European Journal of Clinical Microbiology, 1, 91-96.

Kalin, M., Lindberg, A.A. & Tunevall, G. (1983) Etiological diagnosis of bacterial pneumonia by Gram stain and quantitative culture of expectorates. Scandinavian Journal of Infectious Diseases, 15, 153-160.

Kaplan, M. H. & Volanakis, J.E. (1974) Interaction of C-reactive protein complexes with the complement system I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. Journal of Immunology, 112, 2135 -2146.

Kass, E.H. (1981) Assessment of the pneumococcal polysaccharide vaccine. Reviews of Infectious Diseases, 3 supplement, 1-197.

Kauffmann, F., Lund, E. & Eddy, B.E. (1960) Proposal for a change in the nomenclature of Diplococcus pneumoniae and a comparison of the Danish and American type designations. International Bulletin of Bacteriological Nomenclature and Taxonomy, 10, 31-40.

Kauffmann, F., Morch, E. & Schmith, K. (1940) On the serology of the pneumococcus - group. Journal of Immunology, 39, 397-426.

Kelly, R.T., Greiff, D. & Farmer, S. (1966) Neuraminidase activity in Diplococcus pneumoniae. Journal of Bacteriology, 91, 601-603.

Kennedy, D, (1985) Respiratory infections. Serious Infections Update, 6

Kenny, G. E., Wentworth, B.B., Beasley, R.P. & Foy, H.M. (1972) Correlation of circulating capsular polysaccharide with bacteremia in pneumococcal pneumonia. Infection and Immunity, 6, 431-437.

Kirkman, J.B., Fisher, J. & Pagano, J.S. (1970) A microtitre plate technique for the agglutination typing of Diplococcus pneumoniae. Journal of Infectious Diseases, 121, 217-221.

Klebs, E. (1875) Beitrage zur kenntiss der pathogenen schistomyceten. VII Die monadinen. Archiv Fur Experimentelle Pathologie und Pharmakologie (Lepzig), 4, 409-488.

Klein, J.O. (1981) The epidemiology of pneumococcal disease in infants and children. Reviews of Infectious Diseases, 3, 246-253.

Klemperer, G. & Klemperer, F. (1891) Versuche uber immunisirung und heiling bei der pneumokokkeninfection. Berliner Klinische Wochenschrift, 28, 833-835, 869-875.

Kneeland, Y. & Mulliken, B. (1940) Antibody formation in cases of lobar pneumonia treated with sulfapyridine. Journal of Clinical Investigation, 19, 307-312.

Kneeland, Y. & Mulliken, B. (1940) Antibody formation in cases of lobar pneumonia treated with sulfathiazole. Journal of Clinical Investigation, 19, 735-738.

Koskela, M. & Leinonen, M. (1981) Comparison of ELISA and RIA for measurement of pneumococcal antibodies before and after vaccination with 14-valent pneumococcal capsular polysaccharide vaccine. Journal of Clinical Pathology, 34, 93-98.

Kronvall, G. (1973) A rapid slide-agglutination method for typing pneumococci by means of specific antibody absorbed to protein A-containing staphylococci. Journal of Medical Microbiology, 6, 187-190.

Krumwiede, C. & Noble, W.C. (1918) A rapid method for the production of precipitin antigen from bacteria : An attempt to apply it to the determination of the type of pneumococcus in sputum. Journal of Immunology, 3, 1-10.

Krumwiede, C. & Valentine, E. (1918) Determination of the type of pneumococcus in the sputum of lobar pneumonia : A rapid, simple method. Journal of the American Medical Association, 70, 513-514.

Lampe, R.M., Chottipitayasunondh, T. & Sunakorn, P. (1976) Detection of bacterial antigen in pleural fluid by counterimmunoelectrophoresis. Journal of Pediatrics, 88, 557-560.

Laurenzi, G.A., Potter R.T. & Kass, E.H. (1961) Bacteriologic flora of the lower respiratory tract. New England Journal of Medicine, 265, 1273-1278.

Leach, R.P. & Coonrod, J.D. (1977) Detection of pneumococcal antigens in the sputum in pneumococcal pneumonia. American Review of Respiratory Disease, 116, 847-852.

Lees, A. W. & McNaught, W. (1959a) Non-tuberculous bacterial flora of sputum and of the upper and lower respiratory tract in pulmonary tuberculosis. Lancet 2, 1115-1117.

Lees, A.W. & McNaught, W. (1959b) Bacteriology of lower-respiratory-tract secretions, sputum, and upper-respiratory-tract secretions in "normals" and chronic bronchitics. Lancet, 2, 1112-1115.

Leinonen, M.K. (1980) Detection of pneumococcal capsular polysaccharide antigens by latex agglutination, counterimmunoelectrophoresis, and radioimmunoassay in middle ear exudates in acute otitis media. Journal of Clinical Microbiology, 11, 135-140.

Lister, F.S. (1913) Specific serological reactions with pneumococci from different sources. Publications of the South African Institute for Medical Research, 1, 103-116.

Logan, W.R. & Smeall, J.T. (1932) A direct method of typing pneumococci. British Medical Journal, I, 188-189.

Lord, F.T. & Nesche, G.E. (1929) Antibody and agglutinin in pneumococcus pneumonia. Journal of Experimental Medicine, 50, 449-453.

Lord, F.T. & Persons, E.L. (1931) Certain aspects of mouse protection tests for antibody in pneumococcus pneumonia. Journal of Experimental Medicine, 53, 151-158.

Lowell, F.C., Strauss, E. & Finland, M. (1940) Observations on the susceptibility of pneumococci to sulfapyridine, sulfathiazole and sulfamethylthiazole. Annals of Internal Medicine, 14, 1001-1023.

Lund, E. (1950a) Four new pneumococcal types. Acta Pathologica et Microbiologica Scandinavica, 27, 720-725.

Lund, E. (1950b) Antigenic relationship between pneumococci and non-hemolytic streptococci. Acta Pathologica et Microbiologica Scandinavica, 27, 110-118.

Lund, E. (1959) Diagnosis of pneumococci by optochin and bile tests. Acta Pathologica et Microbiologica Scandinavica, 47, 308-315.

Lund, E. (1962) Type 48, A new pneumococcus type. Acta Pathologica et Microbiologica Scandinavica, 56, 87-88.

Lund, E. (1970) Types of pneumococci found in blood, spinal fluid and pleural exudate during a period of 15 years. Acta Pathologica et Microbiologica Scandinavica Section B, 78, 333-336.

Lund, E. & Henrichsen, J. (1978) Laboratory diagnosis, serology and epidemiology of Streptococcus pneumoniae. In Methods in Microbiology. ed. Bergen, T. & Norris, J.R., 12, Ch. 11. London : Academic Press.

Lund, E. & Munksgaard, A. (1967) A new pneumococcus type. Type 12A. Acta Pathologica et Microbiologica Scandinavica, 70, 305-310.

Lund, E., Munksgaard, A. & Stewart, S.M. (1972) A new pneumococcus type. Type 47A. Acta Pathologica et Microbiologica Scandinavica Section B, 80, 497-500.

Lund, E. & Rasmussen, P. (1966) Omni-Serum. A diagnostic pneumococcus serum, reacting with the 82 known types of pneumococcus. Acta Pathologica et Microbiologica Scandinavica, 68, 458-460.

McCarthy, L.R. & Senne, J.E.(1980) Evaluation of acridine orange stain for detection of microorganisms in blood cultures. Journal of Clinical Microbiology, 11, 281-285.

McCartney, J.E. & Fraser, J.(1921-22) Pneumococcal peritonitis. British Journal of Surgery, 9, 479-488.

Macfarlane, J.T., Finch, R.G., Ward, M.J. & Macrae, A.D. (1982) Hospital study of adult community-acquired pneumonia. Lancet, 2, 255-258.

MacLeod, C.M. (1947) Natural antibodies to pneumococcus in man. Transactions of the Association of American Physicians, 60, 22- 26

MacLeod, C.M. & Krauss, M.R. (1950) Relation of virulence of pneumococcal strains for mice to the quantity of capsular polysaccharide formed in vitro. Journal of Experimental Medicine, 92, 1-9.

MacLeod, C.M., Hodges, R.G., Heidelberger, M. & Bernhard, W.G. (1945) Prevention of pneumococcal pneumonia by immunisation with specific capsular polysaccharides. Journal of Experimental Medicine, 82, 445-465.

Mair, W. (1929) A system of bacteriology. Medical Research Council, London, 2, 168-

Mandell, G.L. & Hook, E.W. (1969) Leukocyte bactericidal activity in chronic granulomatous disease: Correlation of bacterial hydrogen peroxide production and susceptibility to intracellular killing. Journal of Bacteriology, 100, 531-532.

Mantovani, B.(1975) Different roles of IgG and complement receptors in phagocytosis by polymorpho-nuclear leukocytes. Journal of Immunology, 115, 15-17.

Massaro, D., Fedorko, J. & Katz, S. (1964) Bacterial species isolated from the sputum of patients with chronic bronchitis. American Review of Respiratory Disease, 89, 116-118.

May, J. R. (1952) The bacteriology of chronic bronchitis. Lancet, 2, 1206-1207.

May, J. R. (1953) The bacteriology of chronic bronchitis. Lancet, 2, 534-537.

Mayer, M.E. Jan Geiseler, P. & Harris, B. (1983) Coagglutination for detection and serotyping of bacterial antigens: Usefulness in acute pneumonias. Diagnosis of Microbiological Infectious Diseases, 1, 277-285.

Meads, M., Harris, H.W., Finland, M. & Wilcox, C. (1945)) Treatment of pneumococcal pneumonia with penicillin. New England Journal of Medicine, 232, 747-755.

Merrill, C.W., Gwaltney, J.M., Hendley, J.O. & Sande, M.A. (1973) Rapid identification of pneumococci. Gram stain vs the Quellung reaction. New England Journal of Medicine, 288, 510-512.

Miller, D.L. (1963) A study of techniques for the examination of sputum in a field survey of chronic bronchitis. American Review of Respiratory Disease,88, 473-483.

Miller, J., Sande, M.A., Gwaltney, J.M. & Hendley, J.O. (1978) Diagnosis of pneumococcal pneumonia by antigen detection in sputum. Journal of Clinical Microbiology, 7, 459-462.

Mitchell, O.W.H. & Muns, W.E., (1917) A rapid method for the determination of pneumococcus types. Journal of Medical Research, 37, 339-343.

Monroe, P.W., Muchmore, H.G., Felton, F.G. & Pirtle, J.K.(1969) Quantitation of microorganisms in sputum. Applied Microbiology, 18, 214-220.

Moore, H.F.(1915) The action of ethylhydrocuprein (optochin) on type strains of pneumococci in vitro and in vivo, and on some other micro-organisms in vitro. Journal of Experimental Medicine, 22, 269-285.

Moore, H.F. & Chesney, A. M. (1917) A study of ethylhydrocuprein (optochin) in the treatment of acute lobar pneumonia. Archives of Internal Medicine, 19, 611-682.

Moore, H.F. & Chesney, A.M. (1918) A further study of ethylhydrocuprein (optochin) in the treatment of acute lobar pneumonia. Archives of Internal Medicine, 21, 659-681.

Morch, E. (1942) Further studies on the serology of the pneumococcus group. Journal of Immunology, 43, 177-202.

Morch, E. (1943) Serological studies on the Pneumococci. Copenhagen: E. Munksgaard.

Morch, E. (1944) Serological relationship between a streptococcus strain and some pneumococcus types. Acta Pathologica Microbiological Scandinavica, 21, 96-101.

Morch, E. (1946) Pneumococcal hemolysin. Acta Pathologica et Microbiologica Scandinavica, 23, 555-575.

Morgan, A.D., Rhind, G.B., Connaughton, J.J. & Calder, M.A.(1984) Pneumococcal serotyping and antigen detection in pneumococcal pneumonia of adults. Journal of Infection, 9, 134-138.

Morgenroth, J. & Kaufmann, M. (1912) Arzneifestigkeit bei bakterien (Pneumokokken).Zeitschrift fur Immunitats-forschung, und Experimentelle Therapie, 15, 610-624.

Morgenroth, J. & Levy, R. (1911) Chemotherapie der Pneumokokkeninfektion. Berliner Klinische Worchensehrshrift, 48, 1560-1561.

Morrello, J.A. & York, M.K. (1982) Rapid identification procedures for blood culture isolates. In Rapid Methods and Automation in Microbiology: Proceedings of the Third International Symposium on Rapid Methods and Automation in Microbiology. ed. Tilton, R.C. pp 348-352. Washington, D.C : American Society for Microbiology.

Mufson, M.A., Kruss, D.M., Wasil, R.E. & Metzger, W.I. (1974) Capsular types and outcome of bacteremic pneumococcal disease in the antibiotic era. Archives of Internal Medicine, 134, 505-510.

Mulder, J. (1956) Bacteriology of bronchitis. Proceedings of the Royal Society of Medicine, 49, 773-776.

Mulks, M.H., Kornfeld, S.J. & Plaut, A.G. (1980) Specific proteolysis of human IgA by Streptococcus pneumoniae and Haemophilus influenzae. Journal of Infectious Diseases, 141, 450-456.

Murray, P.R. & Washington, J.A. (1975) Microscopic and bacteriologic analysis of expectorated sputum. Mayo Clinic Proceedings, 50, 339-344.

Mylotte, J.M. & Beam, T.R. (1981) Comparison of community-acquired and nosocomial pneumococcal pneumonia. American Review of Respiratory Disease, 123, 265-268.

Neufeld, F. (1900) Ueber eine specifische bakteriolytische wirkung der galle. Zeitschrift fur Hygeine und Infektionshrenkeiten (Leipzig), 34, 454-464.

Neufeld, F. (1902) Ueber die agglutination der pneumokokken und uber die theorieen der agglutination. Zeitschrift fur Hygeine und Infektionshrenkeiten, 40, 54-72.

Neufeld, F. & Etinger-Tulczynska, R. (1931) Schnell-diagnose der pneumokokkentypen aus dem auswurf. Zeitchrift fur Hygeine und Infektionshrenkeiten, 115, 431-435

Neufeld, F.L. & Handel, L. (1910) Weitere untersuchungen uber pneumokokken-heilsera III. Mitteilung uber vorkommen und bedeutung atypicher varietaten der pneumokokkus. Arbeiten aus dem Kaiserlichen Gesundheit-samte, 34, 293-304.

Nolan, C.M. & Ulmer, W.C. (1980) Enzyme immumoassay of the capsular polysaccharide of Streptococcus pneumoniae type 3 in cerebrospinal fluid in experimental meninigitis. Journal of Medical Microbiology, 13, 551-560.

Nuckols, H.H. & Hertig, A.T. (1938) Pneumococcus infection of the genital tract in women. Especially during pregnancy and the puerperium. American Journal of Obstetrics and Gynaecology, 35, 782-793.

Olcen, P. (1978) Serological methods for rapid diagnosis of Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae in cerebrospinal fluid: A comparison of co-agglutination, immunofluorescence and immunoelectroosmophoresis.Scandinavian Journal of Infectious Diseases, 10, 283-289.

Onyemelukwe, G.C., Leinonen, M., Makela, H. & Greenwood, B.M. (1985) Response to pneumococcal vaccination in normal and post-infected Nigerians. Journal of Infection, 11, 139-144.

Ordman, D. (1938) Pneumococcus types in South Africa. A study of their occurrence and distribution in the population and the effect thereon of prophylatic inoculation. Publications of the South African Institute of Medical Research, 9, 1-27.

Pasteur. (1881) Note sur la maladie nouvelle provoquee par la salive d'un enfant mort de la rage. Bulletin de L'Academie de Medecine (Paris), 10, 2 serie , 94-103.

Paton, J.C., Rowan-Kelly, B. & Ferrante, A. (1984) Activation of human complement by the pneumococcal toxin pneumolysin. Infection and Immunity,43, 1085-1087.

Pecora, D.V. & Yegian, D. (1958) Bacteriology of the lower respiratory tract in health and chronic diseases. New England Journal of Medicine, 258, 71-74.

Pedersen, F.K., Henrichsen, J. & Schiffman, G.(1983) Comparison of enzyme-linked immunosorbent assay and radioimmunoassay for determination of anti-pneumococcal polysaccharide antibodies. Acta Pathologica et Microbiologica et Immunologica Scandinavica Section C, 91, 251-255.

Pedersen, F.K., Henrichsen, J., Sorensen, U.S. & Nielsen, J.L. (1982). Anti-C-carbohydrate antibodies after pneumococcal vaccination. Acta Pathologica et Microbiologica et Immunologica Scandinavica Section C, 90, 353-355.

Penn, R.L., Lewin, E.B., Douglas, R.G., Schiffman, G., Lee, C-J. & Robbins, J.B. (1982) Antibody responses in adult volunteers to pneumococcal polysaccharide types 19F and 19A administered singly and in combination. Infection and Immunity, 36, 1261-1262.

Perlino, C.A. (1984) Laboratory diagnosis of pneumonia due to Streptococcus pneumoniae. Journal of Infectious Diseases, 150, 139-144.

Perlino, C.A. & Shulman, J.A. (1976) Detection of pneumococcal polysaccharide in the sputum of patients with pneumococcal pneumonia by counterimmunoelectrophoresis. Laboratory and Clinical Medicine, 87, 496-502.

Pickering, C.A.C. (1975) Antibiotics for chest diseases. British Journal of Hospital Medicine, 14, 508-514.

Ragsdale, A.R. & Sanford, J.P. (1971) Interfering effect of incubation in carbon dioxide on the identification of pneumococci by optochin discs. Applied Microbiology, 22, 854-855.

Rawlins, G.A. (1953) Liquefaction of sputum for bacteriological examination. Lancet, 2, 538-539.

Rees, T. & Waterworth, P.M. (1980) A problem with the recognition of penicillin-resistant pneumococci. Journal of Clinical Pathology, 33, 1092-1094.

Reimann, H.A. (1924) Blood platelets in pneumococcus infections. Journal of Experimental Medicine,40,553-565.

Reimann, H.A.(1925) Variations in specificity and virulence of pneumococci during growth in vitro. Journal of Experimental Medicine, 41, 587-600.

Rein, M.F., Gwaltney, J.M., O'Brien, W.M., Jennings, R.H. & Mandell, G.L. (1978) Accuracy of Gram's stain in identifying pneumococci in sputum. Journal of the American Medical Association, 239, 2671-2673.

Ries, K., Levison, M.E. & Kaye, D. (1974) Transtracheal aspiration in pulmonary infection. Archives of Internal Medicine, 133, 453-458.

Riley, I.D. & Douglas, R.M. (1981) An epidemiological approach to pneumococcal disease. Reviews of Infectious Diseases, 3, 233-245.

Robbins, J.B., Austrian, R., Lee, C-J., et al. (1983) Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. Journal of Infectious Diseases, 148, 1136-1159.

Robertson, O.H. & Cornwell, M.A. (1930) A study of the resistance of normal human beings to recently isolated strains of pathogenic pneumococci. Journal of Experimental Medicine, 52, 267-277.

Robertson, O.H., Terrell, E.E., Graeser, J.B. & Cornwell, M.A. (1930) The relation of natural humoral antipneumococcal immunity to the inception of lobar pneumonia. Journal of Experimental Medicine, 52, 421-433.

Russell, H., Facklam, R.R., Padula, J.F. & Cooksey, R. (1978) Capillary precipitin typing of Streptococcus pneumoniae. Journal of Clinical Microbiology, 8, 355-359.

Rytel, M.W., Dee, T.H., Ferstenfeld, J.E. & Hensley, G.T. (1974) Possible pathogenetic role of capsular antigens in fulminant pneumococcal disease with disseminated intravascular coaggulation (DIC). American Journal of Medicines, 57, 889-896.

Schiffman, G. (1981) Chemistry and immunochemistry of the pneumococcal polysaccharide vaccine with special reference to cross-reactions and immunologic factors. Reviews of Infectious Diseases, 3 supplement, S18-S31.

Schiffman, G. & Austrian, R. (1971) A radioimmunoassay for the measurement of pneumococcal capsular antigens and of antibodies thereto. (abstract) Federation Proceedings, 30, 658.

Schiffman, G., Douglas, R.M., Bonner, M.J., Robbins, M. & Austrian, R. (1980) A radioimmunoassay for immunologic phenomena in pneumococcal disease and for the antibody response to pneumococcal vaccines. I. Method for the radioimmunoassay of anticapsular antibodies and comparison with other techniques. Journal of Immunological Methods, 33, 133-144

Schiffman, G., Summerville, J.E., Castagna, R., Douglas, R.M., Bonner, M.J. & Austrian, R. (1980) Quantitation of antibody, antigen, and antigen-antibody complexes in sera of patients with pneumococcal pneumonia. American Society for Microbiology, abstract 3105

Schmid, R.E., Anhalt, J.P., Wold, A.D., Keys, T.F. & Washington, J.A. (1979) Sputum counterimmunoelectrophoresis in the diagnosis of pneumococcal pneumonia. American Review of Respiratory Disease, 119, 345-348.

Schulkind, M.L., Ellis, E.F. & Smith, R.T. (1967) Effect of antibody upon clearance of ^{125}I labelled pneumococci by the spleen and liver. Pediatric Research , 1, 178-184.

Seigal, S. (1956) Non-parametric statistics for the behavioral sciences. New York : McGraw-Hill.

Shapera, R.M. & Matsen, J.M. (1972) Host factors and capsular typing of body fluid isolates in fulminant pneumococcal infections. Infection and Immuntity, 5, 132-136.

Shayegani, M., Parsons, L.M., Gibbons, W.E. & Campbell, D. (1982) Characterization of nontypable Streptococcus pneumoniae-like organisms isolated from outbreaks of conjunctivitis. Journal of Clinical Microbiology,16, 8-14.

Shulman, J.A., Phillips, L.A. & Petersdorf, R.G. (1965) Errors and hazards in the diagnosis and treatment of bacterial pneumonias. Annals of Internal Medicine,61,41-58.

Siber, G.R., Ambrosino, D.M., McIver, J., et al. (1984) Preparation of human hyperimmume globulin to Haemophilus influenzae b, Streptococcus pneumoniae and Neisseria meningitidis. Infection and Immunity, 45, 248-254.

Siber, G.R., Schur, P.H., Aisenberg, A.C., Weitzman, S.A. & Schiffman, G.(1980) Correlation between serum IgG_2 concentrations and the antibody response to bacterial polysaccharide antigens. New England Journal of Medicine, 303, 178-182.

Sickles, G.M. (1927) Effect of Type I pneumococcus culture broth on the protective action of Type 1 antiserum. Journal of Infectious Diseases, 40, 369-376.

Simberkoff, M.S., Schiffman, G., Katz, L.A., Spicehandler, J.R., Moldover, N.H. & Rahal, J.J.(1980) Pneumococcal capsular polysaccharide vaccination in adult chronic hemodialysis patients. Journal of Laboratory and Clinical Medicine, 96, 363-370.

Sippel, J.E., Prato, C.M., Girgis, N.I. & Edwards, E.A. (1984) Detection of Neisseria meningitidis Group A, Haemophilus influenzae type b, and Streptococcus pneumoniae antigens in cerebrospinal fluid specimens by antigen capture enzyme-linked immunosorbent assays. Journal of Clinical Microbiology, 20, 259-265.

Sloyer, J.L., Howie, V.M., Ploussard, J.H., Amman, A.J., Austrian, R. & Johnston, R.B. (1974) Immune response to acute otitis media in children. I. Serotypes isolated and serum and middle ear fluid antibody in pneumococcal otitis media. Infection and Immunity, 9, 1028-1032.

Smart, L.E. (1986) Serotyping of Streptococcus pneumoniae strains by coagglutination. Journal of Clinical Pathology, 39, 328-331.

Smart, L.E. & Henrichsen, J. (1986) An alternative approach to typing of Streptococcus pneumoniae strains by coagglutination. Acta Pathologica et Microbiologica et Immunologica Scandinavica Section B, 94, 409-413.

Smart, L.E., Dougall, A.J. & Girdwood, R.W.A. (1987a) Identification of non-capsulate strains of Streptococcus pneumoniae by coagglutination. Journal of Clinical Pathology, 40, 243.

Smart, L.E., Dougall, A.J. & Girdwood, R.W.A. (1987b) New 23-valent pneumococcal vaccine in relation to pneumococcal serotypes in systemic and non-systemic disease. Journal of Infection, 14, 209-215.

Smith, L.P., Hunter, K.W., Hemming, V.G. & Fischer, G.W. (1984) Improved detection of bacterial antigens by latex agglutination after rapid extraction from body fluids. Journal of Clinical Microbiology, 20, 981-984.

Smith, S.K. & Washington, J.A. (1984) Evaluation of the Pneumoslide latex agglutination test for identification of Streptococcus pneumoniae. Journal of Clinical Microbiology, 20, 592-593.

Sorensen, U.B.S., Agger, R., Bennedsen, J. & Henrichsen, J. (1984) Phosphorylcholine determinants in six pneumococcal capsular polysaccharides detected by monoclonal antibody. Infection and Immunity, 43, 876-878.

Sorensen, U.B.S. & Henrichsen, J. (1984) C-polysaccharide in a pneumoccocal vaccine. Acta Pathologica et Microbiologica et Immunologica Scandinavica Section C, 92, 351-356.

Sottile, M.I. & Rytel, M.W. (1975) Application of counterimmunoelectrophoresis in the identification of Streptococcus pneumoniae in clinical isolates. Journal of Clinical Microbiology, 2, 173-177.

Spencer, R.C. & Philp, J.R. (1973) Effect of previous antimicrobial therapy on bacteriological findings in patients with primary pneumonia. Lancet, 2, 349-351.

Spencer, R.C. & Savage, M.A. (1976) Use of counter and rocket immunoelectrophoresis in acute respiratory infections due to Streptococcus pneumoniae. Journal of Clinical Pathology, 29, 187-190.

Spiegelberg, H.L. (1974) Biological activities of immunoglobulins of different classes and subclasses. In Advances in Immunology ed. Dixon, F. and Kunkel, H.G. pp 259-294. New York :Academic Press Inc.

Stephens, C.G., Reed, W.P., Kronvall, G. & Williams R.C. (1974) Reactions between certain strains of pneumococci and Fc of IgG. Journal of Immunology, 112, 1955-1959.

Stephens, C.G., Williams, R.C. & Reed, W.P. (1977) Classical and alternative complement pathway activation by pneumococci. Infection and Immunity, 17, 296-302.

Sternberg, G.M. (1881) A fatal form of septicaemia in the rabbit, produced by subcutaneous injection of human saliva. An experimental reasearch. Bulletin of the National Board of Health (U.S.), 2, 781-783.

Sutliff, W.D. & Finland, M. (1932) Antipneumococcic immunity reactions in individuals of different ages. Journal of Experimental Medicine, 55, 837-851.

Sutliff, W.D. & Finland, M. (1931) Type 1 lobar pneumonia treated with concentrated pneumococcic antibody (Felton). The clinical course. Journal of the American Medical Association, 96, 1465-1469.

Szu, S.C., Lee, C-J, Parke, J.C., et al. (1982) Cross-immunogenicity of pneumococcal Group 9 capsular polysaccharides in adult volunteers. Infection and Immunity, 35, 777-782.

Szu, S.C. & Oravec, L.S. (1982). Detection of 'neutral' type 7F and type 14 pneumococcal capsular polysaccharides by immunoelectrophoresis. Journal of Clinical Microbiology, 15, 1172-1175.

Thirumoorthi, M.C. & Dajani, A.S. (1979) Comparison of staphyloccocal coagglutination, latex agglutination and counterimmunoelectrophnoresis for bacterial antigen detection. Journal of Clinical Microbiology, 9, 28-32.

Thompson, W.E. & Wise, R. (1982) Detection of Streptococcus pneumoniae in blood culture specimens using counterimmunoelectrophoresis. European Journal of Clinical Microbiology, 1, 123-124.

Thompson, W.E. & Wise, R. (1983) Comparison of counter-immunoelectrophoresis and latex particle agglutination in the detection of Streptococcus pneumoniae in blood cultures. Journal of Hospital Infection, 4, 165-171.

Tilghman, R.C. & Finland, M. (1937) Clinical significance of bacteremia in pneumococcic pneumonia. Archives of Internal Medicine, 59, 602-619.

Tillett, W.S. & Francis, T. (1930) Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. Journal of Experimental Medicine, 52, 561-571.

Tillett, W.S., Goebel, W.F. & Avery, O.T. (1930) Chemical and immunological properties of a species-specific carbohydrate of pneumococci. Journal of Experimental Medicine, 52, 895-900

Tilton, R.C., Dias, F. & Ryan, R.W. (1984) Comparitive evaluation of three commercial products and counterimmunoelectrophoresis for the detection of antigens in cerebrospinal fluid. Journal of Clinical Microbiology, 20, 231-234.

Toews, G.B. & Vial, W.C. (1984) The role of C5 in polymorphonuclear leukocyte recruitment in response to Streptococcus pneumoniae. American Review of Respiratory Disease, 19, 82-86.

Tomasz, A.(1967) Choline in the cell wall of a bacterium : Novel type of polymer-linked choline in pneumococcus. Science, 157, 694-697.

Tompkins, D.S. (1983) Comparison of Phadebact coagglutination tests with counterimmunoelectrophoresis for the detection of bacterial antigens in cerebrospinal fluid. Journal of Clinical Pathology, 36, 819-212.

Trollfors, B., Burman, L., Dannetun, E., Llompart, J. & Norrby, R. (1983) Serotyping of Streptococcus pneumoniae strains by coagglutination and counterimmunoelectrophoresis. Journal of Clinical Microbiology, 18, 978-980.

Tugendreich, J. & Russo, C.(1913) Ueber die wirkung von chinaalkaloiden auf pneumokokkenkulturen. Zeitschrift fur Immunitatsforschung und Experimentelle Therapie, 19, 156-171.

Tugwell, P. & Greenwood, B.M. (1975) Pneumococcal antigen in lobar pneumonia. Journal of Clinical Pathology, 28, 118-123.

Tuomanen, E., Liu, H., Hengstler, B., Zak, O. & Tomasz, A. (1985) The induction of meningeal inflammation by components of the pneumococcal cell wall. Journal of Infectious Diseases, 151, 859-868.

Ungermann, E. (1910) Beitrag zur kenntnis der ursachen der pneumokokkenimmunitat, insbesondere zum verhalten "serumfester" pneumokokkenstamme. Zeitschrift fur Immunitatsforschung und Experimentelle Therapie, 5, 269-279.

Verhoef, J. & Jones, D.M., (1974) Pneumococcal antigen in sputum. Lancet, 1, 879.

Vial, W.C., Toews, G.B. & Pierce, A.K. (1984) Early pulmonary granulocyte recruitment in response to Streptococcus pneumoniae. American Review of Respiratory Disease, 129, 87-91.

Volanakis, J.E. & Kaplan, M.H. (1971) Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide (35323). Proceedings of the Society of Experimental Biology and Medicine 236, 612-615.

Wadsworth, A.B. (1903) The agglutination of the pneumococcus with certain normal and immune sera. Journal of Medical Research, 10, 228-242.

Wara, D.W. (1981) Host defense against Streptococcus pneumoniae: The role of the Spleen. Reviews of Infectious Diseases, 3, 299-309.

Ward, H.K. (1930) Observations on the phagocytosis of the pneumococcus by human whole blood. I. The normal phagocytic titre, and the anti-phagocytic effect of the specific soluble substance. Journal of Experimental Medicine, 50, 675-702.

Ward, H.K. & Enders, J.F. (1933) An analysis of the opsonic and tropic action of normal and immune sera based on experiments with the pneumococcus. Journal of Experimental Medicine, 57, 527-547.

Webster, L.T. & Hughes, T.P. (1931) The epidemiology of pneumococcus infection. The incidence and spread of pneumococci in the nasal passages and throats of healthy persons. Journal of Experimental Medicine, 53, 535-552.

Weichselbaum, A. (1886) Ueber die aetiologie der akuten lungen und rippenfellentzundungen. Medizinische Jahrbucher (3rd Series), 1, 483-554

Whitby, M., Kristinsson, K.G. & Brown, M. (1985) Assessment of rapid methods of pneumococcal antigen detection in routine sputum bacteriology. Journal of Clinicl Pathology, 38, 341-344.

White, B. (1938) The Biology of Pneumococcus. New York :The Commonwealth Fund.

Whittle, H.C., Tugwell, P., Egler, L.J. & Greenwood, B.M. (1974) Rapid bacteriological diagnosis of pyogenic meningitis by latex agglutination. Lancet, 2, 619-621.

Wicher, K., Kalinka, C., Mlodozeniec, P. & Rose, N.R. (1982) Fluorescent antibody technic used for identification and typing of Streptococcus pneumoniae. American Journal of Clinical Pathology, 77, 72-77.

Wilson, M.J.B. & Martin, D.E. (1972) Quantitative sputum culture as a means of excluding false positive reports in the routine microbiology laboratory. Journal of Clinical Pathology, 25, 697-700.

Wimberley, N., Faling, L.J. & Bartlett, J.G. (1979) A fiberoptic bronchoscopy technique to obtain uncontaminated lower airway secretions for bacterial culture. American Review of Respiratory Disease, 119, 337-343.

Winkelstein, J.A., Abramovitz, A.S. & Tomasz, A. (1980) Activation of C3 via the alternative complement pathway results in fixation of C3b to the pneumococcal cell wall. Journal of Immunology, 124, 2502-2506.

Winkelstein, J.A., Bocchini, J.A. & Schiffman, G., (1976) The role of the capsular polysaccharide in the activation of the alternative pathway by the pneumococcus. Journal of Immunology,116, 367-370.

Winkelstein, J.A. & Drachman, R.H. (1968) Deficiency of pneumococcal serum opsonizing activity in sickle cell disease. New England Journal of Medicine, 279, 459-466.

Winkelstein, J.A. & Tomasz, A. (1977) Activation of the alternative pathway by pneumococcal cell walls. Journal of Immunology, 118, 451-454.

Winkelstein, J.A. & Tomasz, A. (1978) Activation of the alternative complement pathway by pneumococcal cell wall teichoic acid. Journal of Immunology, 120, 174-178.

Yurchak, A.M. & Austrian, R. (1966) Serologic and genetic relationships between pneumococci and other respiratory streptococci. Transaction of the Association of American Physicians, 79, 368-375.

Zighelboim, S. & Tomasz, A. (1981) Multiple antibiotic resistance in South African strains of Streptococcus pneumoniae: Mechanism of resistance to B-Lactam antibiotics. Reviews of Infectious Diseases, 3, 267-276.

Zimmerman, T.S. & Spiegelberg, H.L. (1975) Pneumococcus-induced serotonin release from human platelets. Identification of the participating plasma/serum factor as immunoglobulin. Journal of Clinical Investigation, 56, 828-834.

PUBLISHED MATERIAL

Published material submitted in support of the candidature of Leslie E. Smart for the Doctor of Philosophy examination.

1. Smart, L.E. (1986) Serotyping of Streptococcus pneumoniae strains by coagglutination. Journal of Clinical Pathology, 39, 328-331.

2. Smart, L.E. and Henrichsen, J. (1986) An alternative approach to typing of Streptococcus pneumoniae strains by coagglutination. Acta Patologica et Microbiologica et Immunologica Scandinavica Section B, 94, 409-413.

3. Smart, L.E., Dougall, A.J. and Girdwood, R.W.A. (1976) Identification of non-capsulate strains of Streptococcus pneumoniae by coagglutination. Journal of Clinical Pathology, 40, 243.

4. Smart, L.E., Platt, D.J. and Timbury, M.C. (1987) A comparison of the distribution of pneumococcal types in systemic disease and the upper respiratory tract in adults and children. Epidemiology and Infection, 98, 203-209.

5. Smart, L.E., Dougall, A.J. and Girdwood, R.W.A. (1987) New 23-valent pneumococcal vaccine in relation to pneumococcal serotypes in systemic and non-systemic disease. Journal of Infection, 14, 209-125.