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THE AFFINITY OF CERTAIN STREPTOCOCCI FOR THE CENTRAL NERVOUS SYSTEM : THE ROLE OF STREPTOCOCCUS AGALACTIAE AND STREPTOCOCCUS MILLERI IN MENINGITIS AND BRAIN ABSCESS WITH IMPLICATIONS FOR THERAPY

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ABSTRACT

Str. agalactiae is an important cause of meningitis in the newborn and preliminary observations suggested that Str. milleri showed predilection for the central nervous system as a cause of brain abscess.

The effect of Str. agalactiae, Str. milleri and Str.mutans on the central nervous system of white Swiss mice were compared, using different routes of inoculation. Str. milleri was as virulent as Str. mutans following intracranial inoculation but less so by the intravenous route. Str. agalactiae was highly virulent irrespective of the route. The detailed histopathology is described, and its relevance to the pathogenesis of meningitis and brain abscess in man is discussed.

Intracranial and intraspinal abscess in two London neurosurgical units over the past twenty-five years were surveyed, and the results are reported. The Registrar General's returns for inflammatory disease of the central nervous system for 1963-1973 are discussed.

The microbiology and the course of intracranial sepsis were investigated prospectively in a multicentre study of fifty patients. Contrary to previous reports, none of the primary pus samples were sterile. Brain abscess is, essentially, a streptococcal disease (80% of cases) and Str. milleri is the most common causative organism.

Anaerobic bacteria are infrequently isolated (20%), a finding confirmed by gas liquid chromatography. Patients with brain abscess usually have circulating antibodies to the infecting organism. Isolates of Str.milleri belong to Lancefield group F and possess the Ottens and Winkler type O III antigen. Their presence in pus can be detected within one hour by immunoelectrophoresis of the untreated specimen.

A procedure was devised for the assay of antimicrobial drugs in pus. Most antibiotics penetrated adequately although the concentrations obtained were unpredictable. Four of twenty-two pus samples had a profoundly inactivating effect on penicillin, and on cephaloridine to a lesser extent. This phenomenon was investigated further and the results are reported.

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SECTION 1 INTRODUCTION

THE TAXONOMICAL POSITION AND SITES OF ISOLATION OF STREPTOCOCCUS AGALACTIAE AND STREPTOCOCCUS MILLERI

The term streptococcus was first applied by Billroth and Erhlich (1877) to a chain forming coccus which they had seen in infected wounds. Later Rosenbach (1884) used the binomial Streptococcus pyogenes to describe chain forming cocci isolated from suppurative lesions of man. Streptococcal infections of cattle and goats and also of horses were described at about the same time by Nocard and Mollereau (1887) and by Schutz (1887) respectively.

Following this early work, attempts were made to differentiate streptococci from different sites and hosts (Hiss 1902; Gordon 1905; Andrewes and Horder 1906), using a variety of biochemical and cultural characteristics. Streptococcal classification was further studied by Holman (1916) who re-examined the reports of earlier workers and introduced the haemolytic effect on blood agar as an additional test. In spite of this, no correlation emerged between the strains causing a particular disease and their cultural and biochemical characteristics. The haemolytic effects produced by organisms grown on blood agar, first reported by Schottmüller (1903), remained the most useful characteristic for recognising strains from human infection. A thorough examination of the earlier work and of the conditions necessary to demonstrate these haemolytic changes is contained in the monograph by Brown (1919). There was little significant change during the next ten years (Hare 1935a). While streptococci could be divided into groups according to such

characters as haemolysis, mannite fermentation or hydrolysis of sodium hippurate, there were many strains which could not be classified satisfactorily and it was not until the work of Lancefield (1933) that any significant progress was made.

The work of Lancefield (1933,1934) on the serological reactions of the beta-haemolytic streptococci changed the approach of bacteriologists to the study of these organisms. She demonstrated that haemolytic streptococci could be grouped according to their polysaccharide antigens and she showed that the organisms responsible for the majority of severe streptococcal infections in man, for example, scarlet fever and puerpural sepsis, belonged to one antigenic group. This she named Group A. Her work was of immense practical value and was of consequence in elucidating the epidemiology of serious streptococcal disease.

During the whole of this time, the pneumococci had been recognised as distinct from other streptococci. Their solubility in bile solution had been reported first by Neufeld (1900) and had been introduced as a routine differential test by Levy (1907). Later, their ability to ferment inulin was confirmed by Avery et al.(1917). Morgenroth and Levy (1911) observed that trypanosomes were also bile soluble and reasoned that quinine and related trypanosomal drugs might have antipneumococcal activity. They found that optochin (ethylhydrocuprein) had such activity and used it to cure experimental infections in mice. The use of optochin sensitivity as an alternative to bile solubility in the routine identification of pneumococci was proposed by Moore (1915).

In 1937, Sherman proposed that the streptococci be classified thus:-

1. Pyogenic streptococci
2. Enterococci
3. Viridans streptococci
4. Lactic streptococci.

Although Wilson and Miles (1975 p.713) still consider Sherman's divisions to be of value, they point out that they are not so well defined as he suggested. For example, pyogenic streptococci may be found in other groups, and not all the enterococci possess the Lancefield group D antigen. Wilson and Miles also consider that the viridans streptococci should be retitled "other streptococci" in order to accommodate the non-haemolytic strains which can not be included under the other headings and that the pneumococci should be included as a separate well defined group.

Str.agalactiae as described by Lehmann and Neumann (1896, 1907) included many organisms which have since been removed from the species. In 1954, the Judicial Commission conserved the epithet agalactiae for the causative organism of bovine mastitis and the intensive study by Munch-Petersen (1954) on streptococci from such cases provided an elaborate biochemical profile for them. The CAMP reaction (Christie et al.1944), in which sheep or ox red blood cells, in the presence of staphylococcal β-toxin, are lysed by a metabolic product of Str. agalactiae has also proved useful in the recognition of these organisms from higher animals. Carriage of Str.agalactiae in man was first reported by Hare and Colebrook (1934), who found it in the vagina of women before and after childbirth. They did not consider that it was responsible for serious disease in their patients. Lancefield (1933) had identified the group antigen of Str.agalactiae and labelled it Group B.

The strains isolated by Hare and Colebrook were also shown to possess the Lancefield group B antigen (Lancefield and Hare 1935). In addition to the group antigen, Str.agalactiae was shown to possess polysaccharide type antigens (Stapleforth 1932; Lancefield 1934,1938); the four types were designated Ia, Ib, II and III. A fifth type was added later (Wilkinson and Moody 1969).

Until Fry (1938) and Brown (1939) reported on its role in human infections, Str.agalactiae was considered only as a pathogen of animals, usually associated with mastitis. Hill and Butler (1940) reporting on a small number of cases of puerperal fever, concluded that the group B streptococci played a pathogenic role in this condition. There were sporadic reports (see Eickhoff 1972) on the pathogenic role of Str.agalactiae during the next twenty years, but little significance was attached to it as a human pathogen until the studies of Nyhan and Fousek (1958) and Hood et al.(1961), who showed independently that the organisms were pathogens of the newborn and of parturient women. In 1962, Keitel et al. reported cases of neonatal meningitis due to Str.agalactiae and in 1964 Eickhoff et al. reported that it was the most common cause of severe neonatal sepsis at Boston City Hospital, accounting for 25% of such infections. Similar findings have been reported from other centres. Butter and de Moor (1967) reported an increased incidence in Holland and Finn and Holden (1970) found a similar situation in Canada. Str.agalactiae is becoming recognised increasingly as a human pathogen in Australia (Stratford 1976).

Little work has been done on the ecology of Str.agalactiae in man. While the organism is found in the genital tract of 3-6% of normal women (Wilson and Miles 1975 p.379; de Louvois

et al.1975) and its incidence may be associated with sexual activity (Finch et al.1976) it appears probable that the female genital tract is not its natural reservoir (Baker and Barrett 1974).

Studies of the role of <u>Str.agalactiae</u> in neonatal infection showed that strains belonging to type Ia were encountered most frequently (Eickhoff et al.1964; Butter and de Moor 1967), although infections due to all four serotypes occurred. Baker and Barrett (1974) suggested that strains of all serotypes are involved in proportion to their distribution within the population.

Neonatal infections by <u>Str.agalactiae</u> have been shown by Baker and her colleagues (Baker and Barrett 1973, 1974; Baker et al.1973, 1975; Franciosi et al.1973) to be of two types. The first occurs within five days of birth and results, primarily, in septicaemia. The distribution of serotypes in the infants is similar to that found in their mothers and in asymptomatic carriers, with a high incidence of type Ia. The second type of infection occurs in infants who are more than ten days of age; it is associated with meningitis and is almost exclusively due to strains of type III. Meningitis occurring during the first five days of life is also due predominently to strains belonging to type III. These findings have been confirmed by Bascom and Concepcion (1975).

In the majority of cases the type strain causing the meningitis is not present in the genital tract of the mother. Winterbauer et al.(1966) describe a situation in which cross infection was suspected but not proven and Eickhoff (1972) reported a similar experience. MacKnight et al.(1969), studying the carriage of group B streptococci within a neo-

natal unit, found a carrier rate of 31% among the nursing staff from whom nose, throat, vaginal and rectal specimens had been collected. They also noted that the carrier rate dropped following an improvement in nursing technique.

Since the reports of Nyhan and Fousek (1958) and Keital et al.(1962) Str.agalactiae has emerged as an important cause of meningitis, especially during the neonatal period. Mannik et al.(1962), Lazarus et al.(1965) and Butter and de Moor (1967) all reported cases of meningitis due to this organism. The organism has also been implicated in cases of peritonitis, urinary tract infection, endocarditis and abscess formation (Eickhoff 1972).A recent report by Ablow et al.(1976) draws attention to the situation in which group B streptococcal infection during early neonatal life may be confused with hyaline membrane disease (respiratory distress syndrome), both clinically and radiographically.

The classification and identification of the viridans group of streptococci was as much a problem to Sherman in 1937 as it had been to Andrewes and Horder in 1906. In spite of the many observations on the biochemistry and serology of these organisms, (Wannamaker and Matson 1972) the taxonomic situation remained confused due to difficulties over nomenclature and problems regarding the significance and reproducibility of the serological and biochemical results obtained. As a result of later work by Sherman and his colleagues (Sherman et al.1943) the oral viridans streptococci were divided into two groups: Str.salivarius which produced extra-cellular levan from sucrose and Str.mitis which, although accorded specific status, constituted a heterogeneous group. Carlsson (1967) and Guggenheim (1968) suggested further subdivision of the streptococci of

the mouth, but it was not until the work of Colman (1968, 1969, 1970) that an attempt was made to study the viridans and 'other streptococci' as a single group.

The streptococci variously known as viridans streptococci, Str.viridans or Str. mitis have, in the past, been considered to be a heterogeneous group of commensal organisms, including all the aerobic and microaerophilic streptococci which could not be classified into species (Breed et al.1957; Colman and Williams 1972; Austrian et al.1972). The work of Colman and Williams (1965, 1967) and Colman (1968, 1969 and 1970) changed the position dramatically. They carried out quantitative transformation reactions, cell wall analyses and numerical taxonomic procedures on organisms from this group and, as a result of the clustering patterns produced, proposed that it should be divided into six species of the genus Streptococcus. The species' names which they proposed were: Str.milleri, Str.mitior, Str.mutans, Str.pneumoniae, Str.salivarius and Str.sanguis. These names have been accepted for use by Wilson and Miles (1975 p 750) and have been proposed for the Approved List of Bacterial Names by the Judicial Commission of the International Committee on Systematic Bacteriology (1976). The majority of isolates from clinical material can be speciated using a limited range of routine biochemical tests (Colman 1976). The classification of these organisms, together with readily available means of identifying them, has helped to elucidate the role of the viridans and non-haemolytic streptococci as human pathogens. The pathogenic role of Str.pneumoniae, an organism with readily demonstrable biochemical and serological characteristics (White 1938), has long been recognised, as has the association between the dextran producing streptococci, for

example, *Str.sanguis* and subacute bacterial (infective) endocarditis (Hehre and Neill 1946, Niven and White 1946). It is only quite recently, however, that the association between dental caries and *Str.mutans* has become clear (Gibbons 1972). Parker and Ball (1976) drew attention to an association between *Str.milleri* and purulent disease, and Bateman et al.(1975) reported its isolation from liver abscess.

The binomial *Str.milleri* was first proposed by Guthof in 1956 in recognition of the dental bacteriologist Dr.W.D.Miller. Guthof used the name to describe a group of non-haemolytic streptococci which he had isolated from infections of the mouth. The strains grew on 40% bile agar and, at 45°C, hydrolysed arginine and aesculin, but did not ferment mannite, sorbitol or glycerol. Guthof described his strains as lacking a Lancefield antigen. Subsequent work (Colman 1970; Colman and Williams 1972) has shown that the non-haemolytic strains of *Str.milleri* isolated by Guthof, the haemolytic strains reported by Ottens and Winkler (1962) andprobably the 'minute' strains reported by Long and Bliss (1934) together with the similar strains studied by Colman (1970) can, with justification, be recognised as a species of serologically heterogeneous organisms. Streptococcus MG (Mirick et al.1944) has also been identified as *Str. milleri.* The organisms may be alpha, beta or non-haemolytic on horse blood agar. Irrespective of their haemolytic activity they may belong to Lancefield's groups A,C,F or G, or be non-groupable. They can also be typed, using Ottens and Winkler antigens, into five serotypes. Strains of *Str.milleri* and the 'minute' streptococci have not been found to possess a protein or group antigen.

Swift (1952) and Kraus et al.(1953) showed that strepto-

coccus MG formed part of the normal mouth flora although Guthof (1960) studying the mouth flora of 50 healthy school-children, was unable to confirm this. Later studies demonstrated conclusively that Str.milleri was present on dental plaque (Bowden and Hardie 1973; Hardie and Bowden 1974), in carious dentine (Edwardsson 1974), and in infected root canals (Mejàre 1975). Mejàre and Edwardsson (1975 a and b) isolated Str.milleri from the oral cavities of all the 18 patients studied. They found that it constituted 14-56% of the total streptococcal population in gingival crevices and from 4-25% of the population in subgingival plaque. In contrast, it constituted less than 1% of streptococci in saliva and in material from the cheek and tongue surfaces. Thus, like other oral streptococci, it has a very specific and limited area of colonisation within the mouth. Ottens (1961) and Ottens and Winkler (1962) isolated many streptococci of Lancefield groups A, C and G from dental root canals. They prepared typing sera from these strains and described five distinct serological groups, (I - V), some of which overlapped with the earlier types described by Bliss (1937). Ottens and Winkler type antigens have been found among a number of Lancefield groupable streptococci as well as among non-groupable isolates (Colman 1970). It was from this latter group that Ottens and Winkler selected their type strains using the prefix 'O' to denote that the organisms did not possess a Lancefield group antigen. There is also a clear connection between the Lancefield group F antigen and Str.milleri. This is not, however, an exclusive character since the group F antigen is also found among some strains of Str.salivarius (de Moor and Michel 1968).

TISSUE SPECIFICITY OF STREPTOCOCCI

The concept that streptococci might have a predeliction for certain tissues is not a new one. In 1915 Rosenow put forward the theory that streptococci possess the power of elective localisation and substantiated his statements by animal experiments. He maintained that streptococci isolated from cases of appendicitis, endocarditis, myositis and cholecystitis, when injected into animals, produced lesions similar in both location and kind to those from which they were originally obtained and that this selective character was lost on repeated subculture. The publication of Rosenow's paper provoked a great deal of discussion and widely divergent views on the subject of streptococcal localisation. Moody (1916), in a study of streptococci from chronic alveolar abscesses, could find no evidence of a localising effect in rabbits although he did note differences in pathogenicity between fresh clinical isolates and strains which had been repeatedly subcultured. On the other hand Irons et al.(1916) claimed support for Rosenow's theory by producing iridocyclitis in rabbits from a strain of viridans streptococci isolated from a clinical case. They found that the early isolates from the clinical case produced the condition in rabbits but that those obtained four weeks later did not, neither did the previous isolates which had been subcultured. Similar relationships were reported by Oftedal (1916) with streptococci isolated from the sputum of asthmatic patients. In a more comprehensive investigation, Detweiler and Maitland (1918) were far less convinced about the localising properties of streptococci. They concluded from their studies, which were confined to "Str.viridans", that while a few strains showed a remarkable consistency between infection site in man

and animals this was confined to a minority of isolates and that, in general, lesions in animals bore no relation to the origin of the organism. They found that, irrespective of the site of origin in the patient, the strains of "Str. viridans" studied produced most lesions in the heart and joints of the experimental animals. Henrici (1916), in a study of streptococci from patients with rheumatic fever, found that rheumatic lesions in rabbits were produced equally by haemolytic and non-haemolytic strains and concluded that no particular group of streptococci was responsible for rheumatic fever in man.

STREPTOCOCCAL INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

The affinity of certain streptococci for the central nervous system is notorious. For more than 50 years Str.pneumoniae has been incriminated as one of the major causes of meningitis (Wieder 1924; White 1938), and a number of workers have isolated it from the pus of brain abscesses (McFarlan 1943; Garfield 1969; Shaw and Russell 1975). More recently, bacteriologists have become aware that Str.agalactiae is prominent amongst the bacteria causing meningitis in neonatal life and early infancy (Eickhoff et al.1964; Quirante et al. 1974; Bascom and Concepcion 1975). In a retrospective study of 200 cases of brain abscess Garfield (1969) observed that organisms of this group had been isolated from 56% of cases. Similar findings were contained in the report by McFarlan (1943).

In 1973, when this work began, there was no evidence that a particular species or group of streptococci was responsible for brain abscess formation. Many of the investigations described in this thesis were prompted by

observations on three patients with cerebral abscess. Streptococci were isolated from all three and in two of them the infecting organisms were identified as Str.milleri. The coincidence of two brain abscess yielding streptococci of unusual type suggested that Str.milleri might have a predilection for the central nervous system as a cause of abscess rather than meningitis, and the records of Dr.M.T.Parker at the Cross Infection Reference Laboratory, Colindale, confirmed that it is often associated with brain abscess.

PATHOGENESIS OF BRAIN ABSCESS

The pathogenesis of brain abscess is complex. The lesions may arise as the result of direct spread from contiguous anatomical structures, following injury or local infection; alternatively, they may occur as the result of metastatic spread from a distant focus.

Forty percent of all brain abscesses are the result of direct spread of sepsis from the middle ear or mastoid (Pennybacker 1950; Tutton 1953). Such abscesses usually occur in the temporal lobe although they may also be found in the cerebellum or frontal region. Abscesses also develop as the result of direct spread of infection from the nasal air sinuses, the commonest foci of infection being the frontal sinus, the ethmoidal sinuses or the sphenoidal sinus. Abscesses arising from these foci are usually found in the frontal lobes. Less commonly, abscesses develop as the result of penetrating injury of the cranium, following compound fracture of the skull, or post-operatively. Brain abscesses are a complication of cavernous sinus thrombosis, itself arising from septic thrombophlebitis of the anterior

facial vein, or of malignant tumours involving the cranial bones. In these instances the location of the abscess is less predictable.

Metastatic abscesses most commonly follow suppurative infection in the lungs, especially bronchiectasis, or acute or chronic pelvic infection. They may arise as complications of acute bacterial endocarditis, but only rarely in cases of subacute bacterial endocarditis, dental abscesses, bacter-aemia or suppurative lesions elsewhere in the body, (Gregory et al.1967). Metastatic abscesses are more likely to be multiple, and are more lethal than single abscesses.

Studies on the routes of entry of bacteria into brain tissue (Dow and Muruzzi 1958) suggest that infections of the middle ear spread to the tegmen tympani, setting up a local petrositis which is followed by a local pachymeningitis. The infection crosses the subarachnoid space without causing a spreading leptomeningitis and tracks through the cortex of the brain to the white matter. It is thought that bacteria are likely to traverse the pia-arachnoid and the cortex in the penetrating vessels of the pia probably in the form of microemboli originating as septic thrombi on the damaged endothelium of vessels of the middle ear. These emboli are arrested at the junction of the grey and white matter, where the pia-arachnoid vessels divide into several branches, and the multiplying bacteria invade the white matter which, compared to the grey matter, has a scanty blood supply. Sometimes the abscess is more superficial and is contiguous with the tegmen. Abscesses may communicate by fistulas with the outer surface of the skull (Tutton 1953), or with the middle ear cavity by a bony deficit in the middle fossa, an appearance noted at necropsy. Abscesses of

otogenic origin may arise in the cerebellum as the result of direct extension from a suppurative labyrinthitis or by retrograde thrombosis from the lateral, the inferior petrosal or the superior petrosal sinuses (Dow and Muruzzi 1958). Other less frequent pathways are the preformed canals in the bone and by direct extension from the air cells. The findings of Shaw and Russell (1975) confirm the view that an infective thrombophlebitis involving the inferior and superior petrosal sinuses is the most important pathway for the spread of infection from the ear to the cerebellum. The studies of Pennybacker (1948), Krayenbuhl (1966) and Shaw and Russell (1975) into cerebellar abscesses of otogenic origin show that dural attachment of the abscess is commonly superior, anterior and medial to the external auditory meatus at a point where the venous connections between the inferior and superior petrosal sinuses are found.

Abscesses resulting from frontal sinusitis are usually attached to the posterior wall of the frontal sinus low down and medially. Alternatively, they may be attached to the orbital roof as a result of chronic infection of the orbital extension of the frontal sinus. It is in this region that the communicating veins from the parietes to the brain are found (Tutton 1953). The spread of infection from the focus to the brain substance is again thought to be by the peri- or intravascular route.

A classical theory in pathology is that the route of spread of infection from the lungs or pelvic region to the brain is via the paravertebral veins. This view is supported by the work of Batson (1940) and Collis (1944) who showed, experimentally and on cadavers, that retrograde venous spread from the abdomen and thorax could take place via the valveless

system of vertebral veins. This is now accepted as the most important route of infection for metastatic spread (Tutton 1953). Metastatic abscesses occur widely throughout the brain substance, although they tend to form in areas supplied by the middle cerebral artery. They are frequently multiple or multiloculated, and are characteristically less well encapsulated, tending to spread. The septic process may also give rise to septic infarcts.

Once the bacteria have lodged in the brain the capillaries dilate and neutrophils and monocytes enter the tissues. The adventitial cells of the blood vessels separate off as fibroblasts and begin to lay down granulation tissue and collagen fibres (Berry and Alpers 1972), thus beginning the delimitation process of the potential abscess. This is one of the few lesions in which collagen plays a prominent role in the reactive responses of neural tissues. Waggener (1974) points out that the findings of such prominent deposits of collagen around brain abscesses is remarkable in view of the limited collagenic capabilities of normal cerebral tissues. Lymphocytes, plasma cells and cerebral histiocytes (Hortega cells) cluster about the focus of infection. The surrounding astrocytes become active and show signs of toxic damage -clasmatodendrosis, but their activity seems to be limited to the surrounding area of granulation. The vessels surrounding the capsule become dilated and show perivascular cuffing and, sometimes, thrombosis, which acts as a stimulus for capillaries to proliferate in the granulation tissue layer.

The development of abscesses near the corticomedullary junction results in the capsule being thinner on the medial side due to the relative hypovascularity of the white matter (Waggener 1974). This may account for their tendency to

rupture into the ventricle rather than into the subarachnoid space (Falconer et al.1943). The area of oedema surrounding an abscess is often of greater volume than the abscess itself and contributes to the space occupying effects of the lesion. Following the work of Schoefl (1963) who established that proliferating capillaries leak protein-containing fluid, Waggener (1974) suggested that the young capillary population of the granulation tissue might be a major source of oedematous fluid which surrounds brain abscesses.

The failure of an infective focus to lay down granulation tissue, and ultimately a capsule, results in a spreading encephalitis, more often seen in metastatic abscesses than in those of otogenic origin. Unless surgically treated the abscess will rupture either into a ventricle or onto the surface of the brain causing a terminal leptomeningitis. Whatever the origin of the infection, a brain abscess is thought to develop only in an area of necrosis which is a prime requirement for its initiation (Waggener 1974).

BACTERIOLOGY OF BRAIN ABSCESS

Although there are many reports relating to the surgery and treatment of brain abscess, little attention has been paid to the bacteriology. The report of McFarlan (1943) appears to be the only published work devoted solely to this aspect of intracranial sepsis. Many reports give details of the bacteriological findings (Table 1-1) but, with the exception of those of McFarlan (1943) and Heinemann and Braude (1963), details of laboratory procedures are not stated. During the pre-antibiotic and early antibiotic era, *Staph.aureus* was the most common single isolate, but, *Str.pneumoniae* was also often found, usually in abscesses complicating pneumococcal meningitis

Table 1-1

BRAIN ABSCESS

BACTERIOLOGICAL FINDINGS*

	McFarlan (1943)	Beller et al. (1973)	Tutton (1953)	Shaw & Russell (1975)	Samson & Clark (1973)	Gregory et al. (1967)	Kao (1973)	Heinemann & Braude (1963)
Period of Study	NS	1941-70	1948-52	1950-73	1961-71	NS	NS	1957-63
No. of cases	48	74	60	47	42	13	26	18
No. of:								
Pure cultures	32	45	) 50	29	21	10	7	5
Mixed cultures	14	12	)	11	9	2	2	11
Sterile cultures	2(4%)	17(23%)	10(17%)	7(15%)+	12(29%)	1(8%)	15(62%)	2(11%)
Bacteria isolated								
Staph.aureus	15	32	15	6	11	2	3	0
Str.pneumonia	6	1	0	5	0	2	0	0
β.haemolytic strept.	4**	4	4	0	7	0	0	0
α Haemolytic strept.	2	0	3	1	1	0	0	2
Non-haemolytic strept.	2	8	9	0	0	5	2	0
Anaerobic strept.	10	0	3	4	5	0	0	11
Bacteroides ssp.	8	2	5	0	5	1	0	16
Coliforms	0	4	4	2	)	2	4	2
Proteus	6	10	11	9	) 10++	0	0	2
Miscellaneous	4	8	3	6	)	3	0	4

* All the studies were retrospective, except McFarlan (1943)

** Lancefield Group A.

\+ Excluding sterile cultures with positive films

++ Includes Proteus Pseudomonas and H.influenzae

NS Period of study not stated.

(Wieder 1924). Other streptococci were also isolated.

Anaerobic culture was not always carried out by early workers and if undertaken no special arrangements were made for rapid transport of specimens to the examining laboratory in anaerobic media. Heinemann and Braude (1963) emphasised the importance of anaerobic culture of specimens from patients with brain abscess and stressed that prompt inoculation of cultures was essential. They isolated anaerobes more frequently than any previous workers, culturing them from 14 of 18 patients, and they commented that they believed them to be the aetiological agents in most cases of brain abscess. Aerobes were isolated from only 7 patients in their study. A high incidence of anaerobic organisms has also been reported in this country (Ingham et al.1975a and b).

Pus from brain abscess is frequently sterile (Table 1-1). This may be due to the sterilising effects of prior antibiotic treatment, or to inadequate methods of culture, as Heinemann and Braude suggest.

PATHOGENESIS OF SUBDURAL EMPYEMA

Since the description of subdural empyema by Ceci and Onetti in 1886, the syndrome has been studied by a number of workers. It is recognised as a clinical entity distinct from other forms of intracranial suppuration, notably brain abscess, meningitis and suppurative thrombophlebitis. Various names have been applied to it in the past but the term most widely accepted is subdural empyema, since this denotes a collection of pus in a preformed space (Bhandari and Sarkari 1970).

Early reports on subdural infection usually referred to otitic disease as the cause but from the late 1930's sinus infection has become increasingly responsible (Hitchcock and

Andreadis 1964). Smith (1956), Platou etal.(1959) and Benson et al. (1960) report that previous meningitis was also a common cause in the cases which they studied.

According to Courville (1944) infection of the paranasal sinuses spreads by a progressive thrombophlebitis of the mucosal veins, extending to the perforating cerebral veins and the venous sinuses. Once infection has entered the subdural space, pus forms rapidly and it can spread widely. The extension of pus from the various primary sites follows a definite course (Bhandari and Sarkari 1970).

Stern and Boldrey (1952) observed that the incidence of subdural empyema has increased over the last thirty years relative to that of brain abscess. Reports by Hitchcock and Andreadis (1964) and by other workers have confirmed this observation.

The bacteriological findings from the major reports on subdural empyema are shown in Table 1-2. There is a high reported incidence of sterility, as in brain abscess, from 14% (Benson et al.1960) to 53% (Anagnostopoulos and Gortvai 1973). The report by Schiller et al.(1948) was confined to 27 cases for which the culture results were positive. In a review of 327 cases of subdural empyema recorded in English, Yoshikawa et al.(1975) reported that 27% of 234 cases examined bacteriologically were culturally sterile. Surprisingly, only 5% of 78 cases reported before 1960 were sterile. The figure rose to 29% ($^{12}/42$) during the period 1960-69 and to 42% ($^{48}/114$) in the reports published between 1970-73. Anaerobic bacteria were isolated from 12% of the 234 cases. The analysis suggests that anaerobic bacteria may play an increasingly important role in subdural empyema. The isolation by Frederick and Braude (1974) of anaerobic

Table 1-2

SUBDURAL EMPYEMA - BACTERIOLOGICAL FINDINGS IN RETROSPECTIVE STUDIES

	Schiller et al. (1948)	Anagnostopoulos and Gortvai (1973)	Benson et al.[+] (1960)	Hitchcock & Andreadis (1964)	Coonrod & Dans (1972)	Bhandari & Sarkari (1970)
Period of Study	NS	1947-63	1949-59	1949-62	1952-69	1954-68
Number of cases	33	32	67	29	7	37
Number cultured	27	30	67	27	7	37
No. of positive cultures	27	14	58	23	4	24
No. of negative cultures	0	16(53%)	9(14%)	4(15%)	3(43%)	13(35%)
Bacteria isolated:						
Staph.aureus	2	2	0	6	2	6
Staph.epidermidis	0	2	0	0	0	0
Str.pneumoniae	1	0	20		0	0
β-haemolytic strept.)		2	0	)	0	0
α-haemolytic strept.)	14	0	0	) 6	0	1
Non-haemolytic strept.)		4	0	)	0	12
Anaerobic strept.)	10	1	0	6	2	2
Bacteroides ssp.)		1	0	0	0	0
H.influenzae	0	0	30	0	0	0
Coliforms	0	0	0	2	0	1
Proteus ssp.	0	2	0	4	0	3
N.meningitidis	0	0	6	0	0	0
Miscellaneous	0	0	2	8	1	2

+ Effusions complicating pyogenic meningitis
NS Period of study not stated

bacteria from $^{23}/83$ specimens aseptically removed from chronically infected paranasal sinuses suggests that they are a probable source for anaerobic as well as aerobic bacteria isolated from subdural empyemas.

Hitchcock and Andreadis (1964) found that streptococci were the organisms isolated most commonly in their series, as in other studies. Yoshikawa et al.(1975) isolated streptococci from $^{4}/5$ of their cases. Except where subdural empyema occurs as a complication of pyogenic meningitis, Str.pneumoniae, H.influenzae and N.meningitidis are rarely found.

Prior to the introduction of antibiotics the mortality among patients with subdural empyema was high. Kubick and Adams (1943) reported that $^{12}/14$ patients who had not received antibiotics had died. Deaths fell dramatically with the introduction of these drugs, although Hitchcock and Andreadis (1964) reported a 34% mortality, and in the study by Bhandari and Sarkar (1970) 35% of patients died. A Lancet annotation (1972) suggested that the overall mortality was probably around 40% since a number of cases are only diagnosed at postmortem. Three of the five patients reported by Yoshikawa et al.(1975) died.

Extradural suppuration usually arises from pre-existing otorhinologic infection, or following accidental or surgical trauma. Exceptions to this general case are the extradural suppurative lesions of the spinal canal. In this area the dura is less firmly and widely attached and abscesses arise as a complication of bacteraemia, osteomyelitis or surgical trauma.

EXPERIMENTAL INTRACRANIAL SEPSIS

There are few reports of attempts to produce abscesses in the

brains of experimental animals. While naturally occurring abscesses in the central nervous system of domestic animals have been reported (Field et al.1954; Finley 1975) attempts to produce similar lesions in laboratory animals have proved remarkably unsuccessful.

The first attempt to produce experimental brain abscesses was by Malinovsky (1891). He found that injection of a virulent culture would only result in abscess formation if the site had been injured previously. This he did by puncturing the cortex with a hypodermic needle ten days before injecting the virulent culture. Essick (1919) produced acute abscesses in cats by injuring the brain and then inoculating it with organisms and obtained the most consistent results when the organism had been isolated from a previous experimental brain abscess. Most of the cats died within 48 hours of an overwhelming toxaemia or meningitis and provided little information on the development of intracerebral lesions. In a continuation of this work Groff (1934) gave details of a number of techniques which he had used to produce brain abscesses in cats. He found that intracerebral injections with Str.haemolyticus, Staph.aureus or the 'colon' bacillus did not induce abscess formation even when the organism was accompanied by marine sponge, clotted blood, macerated brain tissue, or agar, or when the brain had been previously injured by a stab wound. Meningitis, on the other hand, was a common sequel as were generalised overwhelming infections. At autopsy the animals showed marked evidence of toxicity within the brain tissue but no focal lesions or other signs of localised infection. Intracerebral injection of Diplococcus pneumoniae, type III, suspended in molten blood agar proved more successful and resulted in abscess formation in two cats.

One animal died two days after injection and a well circumscribed abscess, 6mm in diameter, was demonstrated at postmortem. The second cat survived for seven days and during this time it remained apathetic and irritable. The animal showed signs of a contralateral, slowly developing, hemiplegia and a constant rotary nystagmus. It died in a tonic convulsion with the entire body turned away from the side of the lesion. At post mortem an abscess was found adjacent to the motor area. Groff (1934) also described a two stage technique which, although not wholly reliable, resulted in brain abscess formation in five cats which survived for periods of 3 - 8 days. The first stage of the technique consisted of making a small cranial defect which resulted in adhesions between the dura and the cortex. This was followed by a second operation at which blood agar suspensions of D.pneumoniae, type III, or Str.pyogenes were injected into the brain through the exposed dura. These experiments were successfully repeated and constitute the first reported cases of experimental brain abscess in which the animal survived for longer than seven days.

Markley (1941) first injected sodium ricinoleate in agar into the brains of twelve anaesthetised dogs and then five days later injected D.pneumoniae, type III, into the same site. D.pneumoniae was cultured from six of them, one contained an anaerobic Gram-negative rod and one was reported to be sterile. The most common neurological finding was a right hemiparesis ($^{4}/8$). Three dogs showed no neurological signs whatever. Three of the abscesses were drained and in these cases the animals made a complete recovery without

antibiotic therapy. All the other dogs died of the infection or were sacrificed during the experiment. The extensive investigations by Falconer, Dorothy Russell and McFarlan (Russell et al.1941; Falconer et al.1943; McFarlan 1943) provide the most comprehensive studies to date on experimental brain abscesses. These workers produced abscesses in the brains of $^{63}/130$ adult rabbits inoculated with a variety of bacteria. All the micro-organisms used had come from clinical material and the majority of them ($^{11}/16$) were from brain abscesses. A two stage experimental system was used with an inoculum of 10^6 - 10^7 bacteria in 0.1 ml 2% agar. The animals were followed for up to six weeks and the histopathology mimicked very closely that occurring in brain abscess in man, including the formation of collagen rich capsules. The intracerebral injection of Str.pneumoniae failed to produce an abscess, although three strains were used in nine separate experiments; in each case the result was a rapidly fatal meningoencephalitis or meningitis.

In addition to abscess formation there were a number of diffuse spreading infections. These were found in 31% of infections due to Str.pyogenes but in only 17% of those caused by Staph.aureus. The tendency to localisation was even more evident with the anaerobic streptococci where only one out of 12 animals showed a diffuse reaction. They also found that positive blood cultures accompanied a diffuse meningitis or meningoencephalitis, but were rare among rabbits with a localised infection. They observed that the inoculated micro-organism could be isolated from a localised lesion for a considerable period of time, thus demonstrating that the pathological findings represented stages in the evolution of an abscess and not merely the reaction to a transitory

infection. Finally, they found that capsule formation occurred just as readily in anaerobic infections as in those due to aerobic bacteria. The process of encapsulation always started in the cortex and could be detected at the end of the first week. Capsule formation took place last in the white matter and the lesion was often not completely encapsulated for 3 - 4 weeks. The slow development of a complete capsule was attributed to the paucity of both microglial cells and blood vessels in this part of the brain.

The route by which bacteria pass from the nasal cavities into the brain has also been investigated experimentally. Le Gros Clark (1929) showed that if a solution of potassium ferrocyanide and ferric ammonium citrate was dropped into the nose of a rabbit it was quickly absorbed through the olfactory submucosa, as shown by the Prussian Blue reaction. The solution then passed along neural and perivascular sheaths, through the cribriform plate to the olfactory bulb and the pia arachnoid. From there the solution passed into the brain substance. Moxon et al.(1974) studied the incidence of Haemophilus influenzae meningitis in infant mice following intranasal inoculation. They observed that it was rare for meningitis to develop without an accompanying bacteraemia. Fluorescent antibody studies suggested that bacteria penetrated the nasal mucosa and entered the systemic circulation but that the spread to the meninges was via the dorsal longitudinal and the lateral dural venous sinuses.

The range of bacterial species used to induce cerebral abscess in experimental animals has been wide. Early workers used any human pathogen that was easy to grow and it was not until the 1930's that organisms isolated from clinical lesions of the central nervous system were used experimentally.

As far as is known the only report in which anaerobic and microaerophilic streptococci from clinical lesions of the central nervous system have been used experimentally was by Falconer et al.(1943). Three isolates were used for twelve experiments and abscesses were produced in $^{8}/12$ rabbits. Although Collis (1944) suggested that the streptococci responsible for intracranial abscess may have some specific affinity for brain tissue and McFarlan (1943) and Garfield (1969) observed that streptococci were the group of organisms isolated most commonly from cerebral pus, there has not, apparently, been any further attempt to study the role of streptococci in intracranial sepsis experimentally.

THE MANAGEMENT AND TREATMENT OF BRAIN ABSCESS IN MAN

Although there are a number of individual reports on the successful treatment of brain abscess dating back to the 17th century (Grant 1941), it is generally considered that modern neurosurgical practice stems from the publication, in 1893, of the monograph by Sir William MacEwen entitled "Pyogenic and Infective Diseases of the Brain and Spinal Cord". The reports by Braun (1890) of eleven patients with brain abscess who were successfully treated and by MacEwen (1893) of 18 of 19 patients who recovered after treatment, established the need for surgical drainage in treatment. MacEwen remarked in his monograph that

> "I now regard an uncomplicated cerebral abscess, early recognised, accurately localised, and promptly operated on, as one of the most satisfactory of intracranial lesions, the patient at once being relieved from a perilous condition and usually restored to sound health."

Sadly, the favourable prognoses expected by him have not been realised.

The majority of reports describe a large number of patients who died before operation (Morgan et al.1973), emphasising that the problems of early recognition and accurate localisation are as important today as they were eighty years ago. Localisation of brain abscess has improved progressively with the development of ventriculography, angiography and electroencephalography. However, it is only the more recent development of the gamma isotope scan and computerised axial tomography (EMI scan) which have really provided the means for safe, accurate location of abscesses in the brain.

The success rate reported by MacEwen (1893) was so much higher than that of any report during the next 35 years that Grant (1941) attempted to find reasons for this, other than the one of higher surgical competence. He suggested that MacEwen's success may have been due in part to the fact that operation was postponed, whether by choice or force of circumstances, until the abscess had had time to encapsulate and that the original focus of infection was always eradicated before drainage was instituted. Mortality rates reported over the next 35 years were consistently in excess of 60% (Adson 1920; Koerner 1925; Beck and Pollach 1927; Neumann 1930). Grant observed that, although the number of cases in these reports was large, no individual surgeon had seen as many patients with brain abscess as MacEwen, nor in such a short period of time. Thus, they had been unable to develop satisfactory techniques based on experience.

A dramatic reduction in the mortality associated with brain abscess was expected following the introduction of antimicrobial drugs. The early study of Tutton (1953) who reported a 13% operative mortality rate supported this expectation. In 1961 Pennybacker reported a 6% mortality rate in 35 cases of brain abscess following mastoid infection for the years 1950-60 and contrasted this with the 36% mortality in 50 similar patients for the years 1938-50. However, the anticipated benefits of antibiotics were not fully realised and an editorial in the British Medical Journal (1969) described these earlier reports as the high point of achievement in the field. This is not to say that the effects of antibiotics were insignificant. Jooma et al. (1951), in a review of 295 cases, showed that the mortality rate in 145 patients who did not receive penicillin was 53%, compared to 27% in 150 who did receive the drug. Similarly, Pennybacker (1948), in a study of cerebellar abscesses, found that the 97% ($^7/9$) mortality before penicillin was reduced to 11% ($^1/9$) for patients who received penicillin. In contrast to these encouraging findings the study by Liske and Weikers (1964) showed that in Wisconsin the introduction of penicillin had not affected the mortality rate which remained at 60%. The report by Garfield (1969), on 200 unselected cases of brain abscess treated over two periods of seven years, is also depressing. He showed that the overall mortality rate was 40% and that it was the same for the years 1951-7 as for the period 1962-7. He attributed these findings to the continuing problem of accurate localisation of brain abscess and to the inadequate or inappropriate usage of antibiotics. The findings of Morgan et al.(1973) are even more disturbing, for in their report on operative mortality

(using Cushing's criteria) they found that $^{11}/58$ (19%) of patients died during the period 1946-60 but that this figure had risen to 57% ($^{12}/21$ cases) during the period 1961-71. Shaw and Russell (1975) reported similar findings in their series of cerebellar abscesses, where the mortality rate rose from 25% for the period 1950-59 to 45% for the period 1960-69 and finally to 55% for the years 1970-73.

Attempts have been made to determine the factors associated with mortality in brain abscess (Karandanis and Shulman 1975). In common with other workers (Griffith 1968; Samson and Clark 1973; Shaw and Russell 1975) they found that mortality was higher in patients admitted in coma and in their study only one of the nine patients thus admitted survived. In contrast three of MacEwen's surviving patients had been so stuporous when operated upon that no anaesthesia was required (Grant 1941). Similarly, for patients with multiple abscess or those where the abscess is deep in the brain tissue or has ruptured the prognosis is also poor (Gregory et al.1967; Shaw and Russell 1975). The location of the lesion is also significant in this context, patients with cerebral lesions having a better prognosis than those with cerebellar abscesses (Shapiro 1973).

The space occupying effect of a brain abscess has been widely quoted as one of the major factors contributing to mortality (Kiser and Kendig 1963; Wright and Ballantine 1967; Samson and Clark 1973). It is clear from these reports that where the space occupying effect extends to compression of the brain stem, the outlook becomes very grave. Shaw and Russell (1975) suggested that a further significant factor in deciding the outcome of an abscess was the ability of the individual patient to cope with the infection, although they

did not elaborate further on this subject. It is difficult to compare the mortality from brain abscess in one unit with that of another. What is clear is that in spite of the widespread use of antibiotics, the overall mortality rate published by neurosurgeons is still in excess of 40% (Table 1-3).

Tutton (1953) was quite clear that the incidence of brain abscess was declining as a result of the higher standards of living of the lower classes, which he believed provided the majority of cases, and from the widespread use of antibiotics which prevented infections from becoming chronic. In their review of otogenic abscesses, Wright and Grimaldi (1973) confirmed this view. They reported that the incidence of otogenic abscess in Oxford for the years 1938-49 was 50 with a 36% mortality, but, for the period 1950-60, the numbers had dropped to 35 with a mortality rate of 5.7%, and during the period 1961-71 there were only 18 cases with no deaths. In contrast to these findings Shaw and Russell (1975) reported no decline in cerebellar abscesses of otogenic origin in Glasgow. They reported that there were 16 cases for the period 1950-59, 20 cases for the period 1960-69 and 11 cases between 1970-73. As mentioned earlier, they also reported an increasing mortality over this period, from 25% (1950-59) to 55% (1970-73). Beller et al. (1973), working in Israel, also suggested that the incidence of brain abscess is on the increase. Their group of 89 patients is somewhat unusual since 43% of cases occurred in patients under 15 years of age.

Samson and Clark (1973), who studied 42 patients in the United States, found an increased incidence with 11 of their group presenting in the period 1961-65 and the remaining 31

Table 1-3

ABSCESSES OF THE CENTRAL NERVOUS SYSTEM

PREVIOUS STUDIES

		Number of cases	Sterile cultures	Overall mortality	Treated mortality
Pennybacker	1938-49	110	-	-	36%
Beller et al.	1941-71	89	23%	45%	40%
Morgan et al.	1946-71	88	39%	36%	29%
Tutton	1948-52	68	17%	30%	13%
Garfield*	1951-67	200	9%	40%	-
Samson et al.	1961-71	42	29%	45%	18%
Kao	1964-72	26	62%	-	23%

*This series of supratentorial abscesses includes subdural abscesses.

presenting between 1966 and 1971. The authors state that the reason for this apparent increase remains obscure. In this group also there are a large number of children (25% under ten years of age).

While any change in the overall incidence of brain abscess is difficult to assess, it is apparent that there have been changes with regard to abscesses of metastatic origin. Some causes of metastatic brain abscess, for example puerperal sepsis, have virtually disappeared from modern medicine, although forty years ago this was a relatively common condition (Colebrook (1935) and McFarlan (1943)) studied a case of cerebral abscess following puerperal sepsis. Tutton (1953) suggested that one of the major factors affecting the incidence of brain abscess was the decrease in the number of cases of chronic bacterial infection, resulting from the introduction of antibiotics.

ANTIMICROBIAL TREATMENT OF BRAIN ABSCESS

The complexity of the physiological, clinical, pharmacological and bacteriological factors affecting the successful treatment of brain abscess, together with the complete lack of prospective information, have made it difficult for satisfactory therapeutic recommendations to be made. Garfield (1969) found very few penicillin resistant bacteria in his study and suggested therefore that penicillin was the drug of choice. Del Bene and Farrer (1972), recognising that brain abscesses often contain penicillin resistant organisms, suggested that the combination of penicillin and tetracycline, as previously recommended by Heinemann and Braude (1963) should be changed to high dosage penicillin (20 mega units per day) and chloramphenicol (3-4g/day).

The absence of adequate clinical evidence to support either of these regimes must raise doubts about their efficacy, in the light of experience of patients with meningitis. Garrod et al.(1973) quoted Lepper and Dowling (1951) who found that if high dosage penicillin was used to treat patients with meningitis the mortality rate was 30% ($^{13}/43$). When however, tetracycline was administered together with the same dosage of penicillin, the mortality rate rose to 79% ($^{11}/14$) even though when used alone tetracycline had been successful. Similarly, Mathies et al.(1967) found that the mortality rate of 4.3% among patients with meningitis treated with ampicillin rose to 10.5% when patients were treated with ampicillin plus chloramphenicol and streptomycin. These observations suggest that genuine antagonism between drugs is being expressed in vivo.

It has been suggested (Heinemann et al.1971; Bartlett 1974) that, before bacteriological results are available, the most appropriate therapeutic regime consists of high doses of chloramphenicol. More recently, however, Ingham et al.(1975a and b) have drawn attention to the use of metronidazole in the treatment of brain abscesses. While this drug is only active against anaerobic organisms, the evidence to date (Salem et al.1975) is that it can be administered in combination with any of the common chemotherapeutic agents and shows no evidence of antagonistic interaction.

THE BLOOD-BRAIN BARRIER

The peculiar impermeability of the vasculature of the central nervous system, known generally as the blood-brain barrier, was first demonstrated by Goldman in 1913. He noted that intravenous trypan blue failed to penetrate the brain while

other organs were deeply stained, and proposed that the central nervous system possessed a special mechanism for the selective exchange of substances between the circulating blood and the brain. In their studies on the blood-brain barrier, Eckman et al.(1958) review much of the previous work. They reiterate the earlier suggestions that impermeability must be a property of either the vessel walls, or their pial coverings or of the glial perivascular membrane. Electron microscopical studies have shown that the cytoplasmic foot processes of the astrocytes are closely applied to the capillary endothelium and it has been suggested that this is an important factor in the barrier (Farquhar and Hartman 1957, Waggener 1974).

The work of Broman and his colleagues (Broman and Lindberg-Broman 1945, Broman 1949) showed that the functional integrity of the blood-brain barrier was maintained under conditions of anoxia, vasodilation or alteration in osmotic pressure or pH. They reported that certain toxic agents, for example bile salts and some radio-opaque contrast media, produce reversible alterations of the barrier which allow intravenous dyes to enter brain tissue. Eckman et al.(1958) showed that intravenous endotoxin had the same effect.

Studies have been carried out on the penetration of antimicrobial drugs from the vascular system into brain tissue through either inflamed or non-inflamed meninges (Wellman et al.1954, Kramer et al.1969). Davson and Smith (1957) showed that the blood-brain barrier and the blood-cerebrospinal fluid barrier were not the same and concluded that drug penetration into CSF differs from that into avascular brain tissue. Their opinion was confirmed by subsequent workers (Ruedy 1965, Kramer et al.1969, Picardi et al.1975).

A recent study (Adinolfi et al.1976) suggests that the barriers exhibit different permeability characteristics during the perinatal period.

The directional flow of CSF affects the local concentration of antimicrobial drug. The primary flow is from the ventricles to the cisterna magna, thence to the basal cisterns, around the brain stem in the ambient cisterns, into the corpus callosal cisterns and finally into the cerebral subarachnoid spaces (Lerner 1975). Ingraham et al. (1948) showed that small amounts of drug injected into the lumbar theca were unlikely to reach the ventricular fluid and McCracken (1972) confirmed this. Lerner (1975) pointed out that the degree of penetration of a drug into the ventricular system following intrathecal injection was dependent on the volume administered. Thus, if the volume injected were equivalent to 10% of the total CSF volume the drug would not penetrate beyond the basal cisterns, whereas if the same amount of drug were contained in a volume equivalent to 25% of the total CSF volume, then significant levels would be detected throughout the CSF, subarachnoid and ventricular systems.

PENETRATION OF ANTIBIOTICS INTO THE CENTRAL NERVOUS SYSTEM OF MAN

Evaluation of the potential therapeutic benefits of antimicrobial drugs is based on the minimum inhibitory concentration of the drug for the infecting organism and the concentration of active agent present in the serum or cerebrospinal fluid of the host after a standard dose. Serum antibiotic levels may provide useful information on the absorption, metabolism and excretion of the drug. They do not, however, in the majority of cases, give any

indication that the drug has penetrated to the site of infection in effective concentrations. This can only be assumed in the light of a favourable clinical response. In contrast to the extensive reports on the absorption of anti-microbial drugs into the vascular system and the cerebro-spinal fluid, there is very little published information on the penetration of these agents into other sites, especially their penetration into the parenchyma of the central nervous system (Ruedy 1965).

The efficiency with which an antibiotic passes from the serum to the CSF depends, in part, on its physical properties, including its ability to pass the blood/CSF barrier and, in part, on host factors, in particular whether or not the meninges are inflamed. Penicillin and ampicillin pass into the CSF far more effectively if there is meningeal inflammation (Lithander and Lithander 1962). Taber et al.(1967) reported that as much as 35% of the serum level of ampicillin could be found in the CSF if the meninges were inflamed, compared to 9% in patients with viral meningitis and only 4% in healthy controls. Reports on the penetration and effectiveness of cephalothin in the treatment of infection of the central nervous system are contradictory. Flux et al.(1963) treated 100 children and found the drug to be effective in treatment of pneumococcal and staphylococcal meningitis. However, Winterbauer et al.(1967) and Westenfelder and Paterson (1969) reported unpredictable CSF levels and a number of treatment failures. Lerner (1969) found that cephalothin only penetrated the CSF if the protein content was in excess of 50 mg/dl. The report by Mangi et al.(1973) of five patients who developed meningitis due to cephalothin-sensitive organisms whilst receiving the drug at

a dosage of 6g per day supports the assertion that it does not pass satisfactorily into the CSF. These reports have les to the widespread opinion that cephalothin is unsatisfactory for the treatment of meningitis due to Gram-negative bacteria (Flux et al.1973, Westenfelder and Paterson 1969, Lerner 1969).

Lerner (1969) studied the penetration of lincomycin into the CSF of 11 patients. He found that the drug was absent from the CSF of eight cases and only present in low levels in two. The remaining patient had a spinal fluid level of 7mg/l. Penetration was found to be independent of CSF protein content. A study by Picardi et al.(1975) indicated that the related drug clindamycin penetrates the CSF more satisfactorily. Working with rhesus monkeys, these workers showed that, in the absence of meningeal inflammation and with antibiotic serum levels in excess of 5 mg/l, the CSF concentration was 10-25% of the serum level. They also studied the penetration of clindamycin into monkey brain tissue and found it to be erratic, poor and unrelated to CSF levels. The only published report on the use of clindamycin for the treatment of brain abscess (Khuri-Bulos et al.1973) showed that it entered the CSF at a concentration adequate to resolve a bacteroides meningitis but that it did not penetrate a posterior fossa abscess which harboured this and other sensitive organisms. Taber et al.(1967) reported that chloramphenicol and the four analogues of tetracyline all pass the blood/CSF barrier, whether the meninges are inflamed or not, and attain serum/CSF levels of between 30% and 50%.

Davson and Smith (1957) considered that the blood/brain

barrier and the blood/cerebrospinal fluid barrier were not the same and that it could be expected that drug penetration into the extravascular cerebral tissue would differ from that into the CSF. Wellman et al.(1954) were the first to determine levels of antimicrobial drugs in fresh human brain tissue. They administered penicillin, tetracycline, erythromycin and streptomycin to a group of 27 psychiatric patients before prefrontal lobotomy. At the time of operation blood, CSF and brain tissue were collected for assay. They found that neo-penil (penicillin G-2 diethylaminoethyl ester hydriodide) and the tetracyclines penetrated brain tissue in significant amounts while streptomycin and erythromycin did not. They also reported that penicillin G did not penetrate brain tissue. However, the samples tested were collected four hours after an intramuscular dose of 600,000 units at a time when the serum levels were in the range 0.06 - 2.0 mg/l, and the CSF samples were free of detectable penicillin. The findings of Wellman et al.(1954) support the view of Davson and Smith (1957) that estimation of the level of antimicrobial drug in the CSF does not necessarily provide any indication of the concentration present in brain tissue. This subject was further studied by Kramer et al.(1969). In a most careful piece of work they determined the concentrations of penicillin, ampicillin, chloramphenicol, cephaloridine and cephalothin in the serum and brain tissue of patients undergoing neurosurgery. Each patient received one drug in a single intravenous or intramuscular injection of 2g. Brain samples of between 50-300 mg were frozen, ground to a powder, and suspended in buffer solution to a standard volume. The haemoglobin content of the homogenate was determined. Following antibiotic assay of the serum and brain

tissue samples a correction was applied to the brain tissue assay result to allow for the level of blood-antibiotic contamination and thus the antibiotic content of avascular brain tissue was calculated. Their results are shown in Table 1-4. The variation in assay results for each vertical column in the Table is due to differences in the time since injection that the specimens were collected, but each pair of serum and brain sample specimens was taken at the same time. They found that chloramphenicol is concentrated nine-fold in brain tissue but the other antibiotics are present at much lower levels than in serum. There were a number of instances in which serum samples contained significant amounts of penicillin, ampicillin or cephaloridine, while no detectable drug could be found in the corresponding brain samples. The average level of cephalothin in the specimens of brain tissue was 10% of the serum concentration, while for cephaloridine the level was less than 5%. Penicillin and ampicillin penetrated poorly into brain tissue, attaining brain tissue/serum ratios of 5% and less than 2% respectively.

Garfield (1969) believed that in many instances the antibiotics prescribed for treatment of brain abscess were either inappropriate for the infecting organism(s) or were administered in inadequate dosage. In contrast, Black et al. (1973a) thought that the failure of antibiotics to control intracerebral infection was due to poor antibacterial activity within the abscess cavity, related in part to the protective action of the purulent milieu within the abscess. This was supported by the finding of viable sensitive bacteria in samples of pus which contained therapeutic levels of anti-microbial drug. They also questioned whether the place of

Table 1-4

BLOOD AND CORRECTED BRAIN CONCENTRATIONS OF ANTIBIOTICS

(Results of Kramer et al.1969)

Chloramphenicol		Cephalothin		Ampicillin		Penicillin		Cephaloridine	
Blood ug/cc	Brain ug/gm	Blood ug/cc	Brain ug/gm	Blood ug/cc	Brain µg/gm	Blood ug/cc	Brain ug/gm	Blood ug/cc	Brain ug/gm
-	47.0	46.8	0.4	38.0	0	15.0	0	15.8	1.97
	54.0	13.4	1.7	27.0	0	6.0	0.10	19.4	0
6.2	19.2	-	3.0	45.0	0	14.6	1.38	22.6	0.57
3.5	18.0	1.8	0.8	10.0	2.0	2.2	0	22.8	1.58
4.6	25.1	27.0	*20.2	24.0	0	8.9	0.07	20.4	0
3.4	24.0	1.4	1.7	19.0	0.3	4.9	0.66	16.8	1.6
3.2	10.0	2.1	2.7	15.0	0.3	6.1	0.02	8.5	0.6
-	30.0	0.7	1.9	6.0	0.5	2.3	0.07		
3.4	*412.0	-	0.6			-	0.56		
-	63.0								
4.0	36.0	11.7	1.6	21.0	0.4	7.5	0.32	18.05	0.9

* Not included in calculation

antibiotics in the management of brain abscess is other than to prevent further spread of the lesion, or to control the residual local infection following aspiration. They suggested that antibiotic therapy may be of prophylactic value at the pre-abscess stage which, in their view, presents as a cerebritis. This hypothesis had first been developed by Heinemann et al,(1971) who, in an extension of earlier work (Heinemann and Braude, 1963), successfully treated, with antibiotics alone, six patients who had neurological signs and in some cases electroencephalographic traces suggestive of localised intracranial infection. Chow et al.(1975) also reported the successful treatment, by antibiotics, of a patient with bacteraemia due to Listeria monocytogenes who had pulmonary and cerebral (multiple) abscesses assumed to be caused by listeria. Black et al. (1973b) refuted any suggestion that an abscess could be treated by antibiotics alone.

In their study of six patients with brain abscess, Black and his co-workers found that chloramphenicol, methicillin and penicillin all penetrated into the abscess cavity in therapeutic concentrations. They reported, however, that antibiotics alone had no effect on the clinical state of the patients who continued to deteriorate until the abscess was aspirated, after which recovery began promptly. In the conviction that circulating antibiotic penetrated an abscess cavity in adequate amounts, these workers questioned the value and justification of the local instillation of chemotherapeutic agents.

SECTION 2 A STUDY OF THE EFFECT OF SOME STREPTOCOCCI ON WHITE SWISS MICE (WSM)

BACTERIAL STRAINS

Isolates of *Streptococcus agalactiae* (NCTC 9993), *Streptococcus milleri* (NCTC 10708) and *Streptococcus mutans* (NCTC 10449) were supplied by Dr. S.P. Lapage, Curator of the National Collection of Type Cultures, Colindale. *Streptococcus milleri* (R 74/338) was a clinical isolate used in some later experiments. The organisms were cultured in nutrient broth (Oxoid - No.2) with added glucose. Strains were preserved in liquid nitrogen after being grown for 18 hours in this medium, to which had been added 16% glycerol and 5% fresh horse blood.

EXPERIMENTAL ANIMALS

The animals used for these experiments were male white Swiss mice (WSM) aged 4-6 weeks and weighing approximately 15g. For intracranial and intraoticular injections they were anaesthetised with ether in a closed container for 45 seconds. Otherwise they were not anaesthetised. The site of injection was swabbed with a weak iodine solution (BP). After charging the syringe, but before injection, the outer surface of the needle was wiped with a cotton swab soaked in 70% isopropyl alcohol. During withdrawal of the needle, following intracerebral injection, negative pressure was applied, to close the needle track as far as possible. The injections were performed using 25G or 30G hypodermic needles (Beckton Dickinson) and 0.25ml glass syringes.

Autopsies were carried out on most of the animals that died and appropriate sites were cultured to confirm that death was due to infection by the organisms injected.

ROUTES AND SITES OF INOCULATION

Intracranial

Determination of route of injection and depth of penetration

The location of suitable routes for penetration of the frontal and temporal poles of the brain was determined by injecting powdered sudan black using 25G hypodermic needles. The anaesthetised mice were killed and the brain and skull were examined for traces of the dye. The optimal depth of penetration from the external surface of the skull was shown to be 3.5 mm. Needle guards were constructed from rigid plastic needle covers, cut down so that the point of the needle protruded by 3.5 mm. They were stored in 70% alcohol.

Tolerance to intracranial injection

The tolerance of white mice to intracranial injection was demonstrated by injecting 0.1 ml phosphate buffered saline (PBS) into the temporal and frontal lobes of two groups of ten mice who had been anaesthetised previously. They all recovered from the anaesthetic rapidly and, apart from head droop for the first hour, suffered no after effects from the injection.

Routes of Inoculation Used

1. Temporal lobe

The mouse was anaesthetised and placed on its right side.

The temporal lobe was entered by inserting a guarded needle to a depth of 3.5 mm in the coronal plane at an angle of 65-70° to the midsagittal plane, at the point where the medial $^{2}/3$rds joins the lateral third of the line joining the outer canthus to the external auditory meatus. The angulation served to avoid arteries of the external carotid circulation.

2. Frontal lobe

The anaesthetised mice were placed in a crouching position. The guarded needle was inserted vertically through the scalp and skull to a depth of 3.5 mm at a point 3 mm above and 3 mm to the left of the line joining the left and right outer canthi.

3. Ear drum

The anatomy of the ear of the white Swiss mouse was studied using a dissecting microscope. With the aid of an illuminated magnifying glass and using a fine bore sterile pipette drawn from glass tubing 4 mm in diameter, bacterial cultures were passed into the external auditory meatus and were inoculated onto the ear drum.

4. Mastoid cavity and nasal sinuses

Mice, 4-6 weeks of age, do not have mastoid air cells or paranasal sinuses that are detectable by X-ray (Figures 2-1 and 2-2). These routes of inoculation could, therefore, not be used.

5. Intravenous and intraperitoneal injection

These injections were carried out on unanaesthetised animals into the tail vein and into the lower half of the abdomen to the left of the midline respectively.

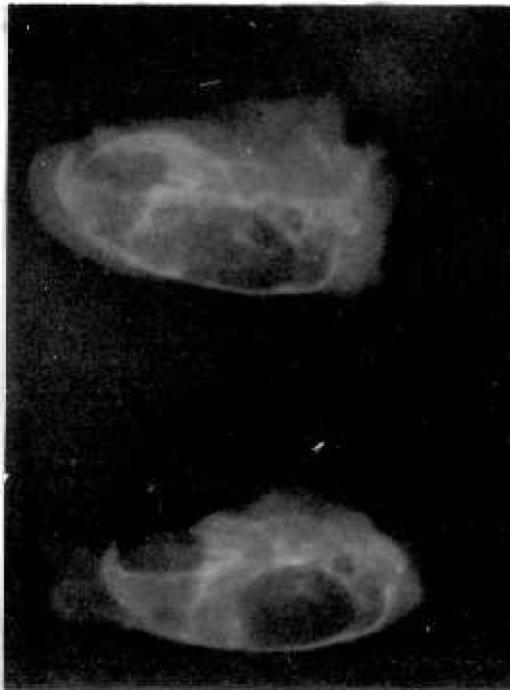

Fig.2-1 Skull x-rays of 4-6 week old white Swiss mice. No mastoid air cells or paranasal sinuses are discernible. (Exposure 200 mA 40 kV for 0.01 seconds.)

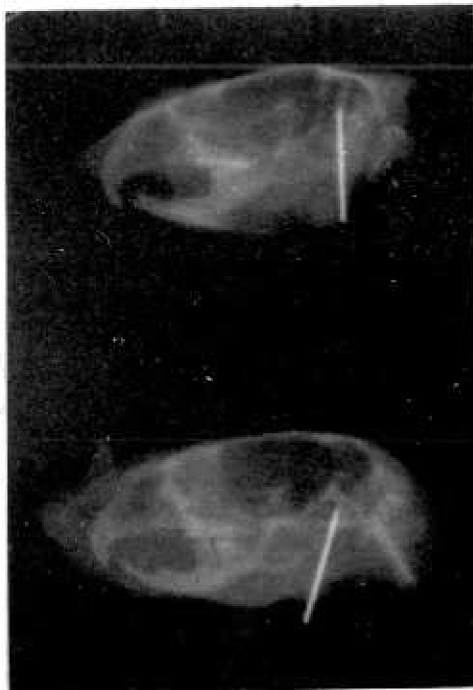

Fig.2-2 Skull x-ray of 4-6 week old white Swiss mice indicating the auditory canal in relation to the cranium. (Exposure 200 mA 40 kV for 0.01 seconds.)

6. Intrahepatic injection

The right hypochondrium of unanaesthetised mice was injected at a point immediately below the bottom rib.

HISTOLOGICAL METHODS

Mice were killed in an ether jar and dissected immediately. The brain was removed, bisected longitudinally, and placed in 10% formol saline (4% formaldehyde in saline) for a minimum of 24 hours. Four procedures were employed in order to find a technique which would give reproducible results of high quality.

1. Paraffin processing by use of an automatic processer

A Histokinette automatic tissue processer (Elliott Automation) programmed to give the following process was used:-

Beaker No.	Reagent	Time in	Time out	Duration
1	Formol-saline	-	8.00pm	
2	70% Alcohol	8.00pm	10.00pm	2 hours
3	90% Alcohol	10.00pm	1.00am	3 hours
4	Alcohol I	1.00am	2.00am	1 hour
5	Alcohol II	2.00am	3.00am	1 hour
6	Alcohol III	3.00am	4.00am	1 hour
7	Chloroform I	4.00am	5.00am	1 hour
8	Chloroform II	5.00am	6.00am	1 hour
9	Wax I	6.00am	7.30am	1½ hours
10	Wax II	7.30am	9.30am	2 hours

Tissues were embedded in paraffin wax without vacuum treatment.

The results were disappointing. Tissues showed considerable shrinkage and distortion and the hardening effect on mouse brain tissue was such that complete fragmentation

occurred on cutting.

2. Paraffin processing by hand

Various time schedules were tried and the following was found to be the most satisfactory.

1. Fixation in 10% formol saline
2. 50% alcohol for 1 hour - 2 changes
3. 70% alcohol for 1 hour - 2 changes
4. 80% alcohol for 1 hour - 2 changes
5. 95% alcohol for 1 hour - 2 changes
6. Absolute alcohol for 1 hour - 2 changes
7. Chloroform for 1 hour - 2 changes
8. Chloroform overnight
9. Paraffin wax at 56°C for ½ hour
10. Paraffin wax at 56°C for 3 hours - 3 changes.

The tissues were embedded without vacuum treatment. Sections were cut at a thickness of 8µm on a base sledge microtome. They were floated out on a cool waterbath, mounted on albumenised slides and dried at 37°C overnight before being stained.

3. Celloidin processing

The procedure used was that described by Culling (1974) using low viscosity nitrocellulose (LVN) at concentrations of 5, 10 and 20%. Sections were cut in the conventional way at a thickness of 10 and 20 µm in alternate groups of three, to provide sections of thickness appropriate for various neurological staining methods.

4. Frozen sections

Blocks of fixed and unfixed tissue were mounted on 1 cm diameter cork mats and frozen onto the chuck holder of a cryostat freezing microtome with compressed carbon dioxide.

Sections were cut at 15 µm and mounted on albumenised slides. Sections of fixed tissues were dried at 37°C while those from unfixed tissue were kept at -20°C.

With the exception of the automatic processing procedure, the methods all produced good quality sections. However, the slowness of the celloidin method and the problems of cutting serial sections with a cryostat together with the difficulties of long term storage of frozen sections discouraged the use of these methods in the present investigation. Since the results were not appreciably better than those obtained by hand processing and wax embedding this method was adopted for all future studies. Serial sections of the left hemisphere were cut in the sagittal plane to a depth of 4 mm. All sections were cut at 8 µm and were mounted two to a slide. Every fifth slide was stained by haematoxylin and eosin and the two adjacent slides by cresyl violet for Nissl substance and by Gram's method.

STAINING METHODS

All sections were treated with iodine and sodium thiosulphate to remove formalin pigment.

The haematoxylin and eosin method used was that described by Culling (1974).

Stain for Nissl substance

5% Cresyl violet	5 ml
1% Acetic acid	5 ml
Distilled water	90 ml

Method

1. Take sections to water.
2. Stain in a closed coplin jar for 10 minutes.
3. Wash and decolourise in 70% alcohol until the background is pale blue.
4. Drain and pass quickly through absolute alcohol and two changes of xylol.
5. Mount.

Results

Nucleoli	- blue
Nissl substance	- deep blue to mauve
Other tissue elements	- colourless

A section of human brain tissue was included as a control.

Stains for bacteria in sections

Method of Gram

1. Take sections to water.
2. Stain for 30 seconds in 1% crystal violet, wash.
3. Treat with Gram's iodine for 2 minutes, wash.
4. Decolourise rapidly with acetone, wash.
5. Counterstain with 1% neutral red for 10 seconds, wash.
6. Blot and dehydrate rapidly in absolute alcohol.
7. Clear and mount.

Results

Nucleoli	- red
Connective tissue	- pink/red
Gram-positive bacteria	- blue/black
Gram-negative bacteria	- pink/red

Method of Sowter and McGee (1976)

Methyl green (C.I. 142585)	0.15 g
Pyronin (C.I. 45005)	0.50 g
96% Ethyl alcohol	2.50 ml
Glycerin	20.00 ml
0.5% Phenol (Analar) in distilled water	100.00 ml

Dissolve the dyes in the alcohol and mix with the other ingredients. Add 100 ml chloroform, shake well and allow to stand overnight. Carefully remove the top aquous layer.

Method

1. Take sections to water.
2. Stain with 1% crystal violet for 30 seconds, wash.
3. Treat with Gram's iodine for 2 minutes, wash.
4. Decolourise with acetone for 10 seconds, wash.
5. Counterstain with aqueous methyl green/pyronin solution for 30 seconds.
6. Rinse in distilled water and stain with 0.5% light green in distilled water for 10-15 seconds.
7. Rinse in distilled water, blot dry and dehydrate rapidly in absolute alcohol.
8. Clear in xylol and mount.

Results

Nuclei	- blue
Cytoplasm	- green/blue
Collagen	- green
Gram-positive bacteria	- magenta
Gram-negative bacteria	- scarlet red

Fluorescent Antibody Method

The sandwich technique (Nairn 1969, p 111) was used with a fluorescein isothiocyanate/antirabbit immunoglobulin conjugate (Dako). The maximum dilutions of antistreptococcal sera and conjugate which gave specific high intensity fluorescence were determined using films prepared from broth cultures of Str.agalactiae, Str.mutans and Str.milleri.

Method

1. Take sections to water.
2. Treat sections with appropriate antistreptococcal serum in a moist chamber at 37°C for twenty minutes.
3. Wash for twenty minutes in repeated changes of phosphate buffer pH 8.0.
4. Drain until almost dry and treat sections with dilute fluorochrome solution for twenty minutes at 37°C in a moist chamber.
5. Wash for twenty minutes in repeated changes of phosphate buffer pH 8.0.
6. Drain until almost dry and mount in glycerol. Seal coverslip edges with varnish.
7. Examine under a fluorescence microscope.

Results.

Homologous bacteria and bacterial antigen - Bright apple green

Heterologous bacteria - not demonstrated

Tissue structures - very pale grey/green

RESULTS

MORTALITY OF WHITE SWISS MICE INJECTED WITH THREE SPECIES OF STREPTOCOCCI

1. Effect of the Route of Administration and Inoculum Size

Cultures of the three species were grown for 18 hours in nutrient broth (Oxoid - No.2) with 1% added glucose. They were centrifuged, washed three times in PBS, and standardised to contain 10^8 and 4×10^6 colony forming units (CFU) per ml. Surface viable counts, in triplicate, were carried out on horse blood agar to confirm the viable number and purity of the culture.

a) Comparison of mortality following injection by the intravenous and intracerebral routes

One hundred and twenty mice were divided into 12 groups of ten and inoculation made by the intravenous, intraperitoneal or intracranial route, either into the temporal or the frontal lobe, using 0.1 ml of a suspension containing 10^8 CFU per ml. A further nine groups of ten mice received injections into the left temporal or frontal lobe of the brain, or intravenous injections with 0.1 ml containing 4×10^6 CFU per ml of the test organisms. In addition, two groups of control mice, with ten in each group, received 0.1 ml PBS either into the left frontal or the left temporal lobe. The 20 control animals remained well for the three week duration of the experiment and there were no anaesthetic deaths.

The fate of the mice injected by the intravenous, intraperitoneal and intracerebral routes with 10^7 CFU of the three strains is shown in Tables 2-1, 2-2 and 2-3, and the fate of mice injected with 4×10^5 CFU via the intravenous and

Table 2-1

MORTALITY OF WSM INJECTED INTRAVENOUSLY WITH STREPTOCOCCI (1 x 10^7/0.1 ml PBS)

	Number Dead						Survivors
	24 hrs.	48 hrs.	72 hrs.	4 days	5 days	6 days	
Str.agalactiae	Nil	6/10	10/10	-	-	-	Nil
Str.milleri	Nil	Nil	1/10	1/10	2/10	2/10	8/10
Str.mutans	Nil	3/10	4/10	6/10	6/10	8/10	2/10

Table 2-2

MORTALITY OF WSM INJECTED INTRAPERITONEALLY WITH STREPTOCOCCI (1 x 10^7/0.1 ml PBS)

	Number Dead						Survivors
	24 hrs.	48 hrs.	72 hrs.	4 days	5 days	6 days	
Str.agalactiae	4/10	6/10	10/10	-	-	-	Nil
Str.milleri	Nil	Nil	3/10	6/10	6/10	7/10	3/10
Str.mutans	Nil	2/10	8/10	8/10	10/10	-	Nil

Table 2-3

MORTALITY OF WSM INJECTED INTRACEREBRALLY WITH STREPTOCOCCI (1 x 10^7/0.1 ml PBS)

Into Frontal Pole

	Number Dead			Survivors
	24 hrs.	48 hrs.	72 hrs.	
Str.agalactiae	5/10	10/10	-	Nil
Str.milleri	2/10	6/10	10/10	Nil
Str.mutans	1/10	8/10	10/10	Nil

Into Temporal Lobe

	Number Dead			Survivors
	24 hrs.	48 hrs.	72 hrs.	
Str.agalactiae	6/10	10/10	-	Nil
Str.milleri	2/10	6/10	10/10	Nil
Str.mutans	3/10	9/10	10/10	Nil

intracerebral routes is shown in Tables 2-4 and 2-5.

Str. milleri (NCTC 10708) and Str.mutans (NCTC 10449) at a dose of 10^6 CFU, were injected into the temporal lobes of two further groups of ten mice whose fate is shown in Table 2-6.

At a dose of 10^7 CFU, Str.agalactiae killed all animals in 48 hours when injected by the intracerebral route, and within 72 hours when injected by the intravenous or the intraperitoneal routes. Str.milleri was less lethal than Str.agalactiae when injected via the intravenous route, for the majority of animals survived a single dose of 10^7 CFU. Using the intraperitoneal route, at 72 hours only 3/10 mice injected with Str.milleri had succumbed compared to 10/10 injected with Str.agalactiae. However, animals in the group that had received Str.milleri continued to die and by the sixth day 7/10 had perished. When Str.milleri was injected by the intracranial route, all the animals succumbed, although not so rapidly as those infected with Str.agalactiae. Str.mutans produced the same effect as Str.milleri when injected intracerebrally. Following intravenous and, to a lesser extent, intraperitoneal injection, however, Str.mutans proved more lethal.

Reduction of the infective dose of Str.agalactiae from 10^7 to 4×10^5 CFU produced no appreciable change in the mortality rate 72 hours post intravenous injection, although survival time was lengthened, none of the animals dying before 72 hours with the lower dose. Similarly, following intracerebral injection with the lower dose the mortality rate at 48 hours showed no change, although, again in contrast to the group receiving the higher dose, the survival time was lengthened.

Table 2-4

MORTALITY OF WSM INJECTED INTRAVENOUSLY WITH STREPTOCOCCI (4 x 10^5/0.1 ml PBS)

	Number Dead			Survivors
	24 hrs.	48 hrs.	72 hrs.	at 2 wks:
Str.agalactiae	Nil	Nil	9/10	1/10
Str.milleri	Nil	Nil	Nil	10/10
Str.mutans	Nil	Nil	2/10	8/10

Table 2-5

MORTALITY OF WSM INJECTED INTRACEREBRALLY WITH STREPTOCOCCI (4 x 10^5/0.1 ml PBS)

Into Frontal Pole

	Number Dead			Survivors
	24 hrs.	48 hrs.	72 hrs	
Str.agalactiae	Nil	10/10	-	Nil
Str.milleri	Nil	Nil	Nil	10
Str.mutans	Nil	Nil	Nil	10

Into Temporal Pole

	Number Dead			Survivors
	24 hrs.	48 hrs.	72 hrs.	
Str.agalactiae	8/10	10/10	-	Nil
Str.milleri	Nil	Nil	Nil	10
Str.mutans	Nil	Nil	Nil	10

Table 2-6

MORTALITY OF WSM INJECTED INTRACEREBRALLY WITH STREPTOCOCCI (1×10^6/0.1 ml PBS)

	Temporal Pole			
	Number Dead			Survivors
	24 hrs.	48 hours	72 hrs.	
Str.milleri	2/10	6/10	10/10	Nil
Str.mutans	2/10	8/10	10/10	Nil

Table 2-7

COMPARATIVE MORTALITY OF WSM 6 DAYS AFTER INJECTION OF SPECIES OF STREPTOCOCCI (%)

	I.V.		I.P.	I.C.		
	1×10^7	4×10^5	1×10^7	1×10^7	1×10^6	1×10^5
Str. agalactiae	100	90	100	100	NT*	100
Str. milleri	20	Nil	70	100	100	Nil
Str. mutans	80	20	100	100	100	Nil

* Not tested

I.V. = Intravenous injection

I.P. = Intraperitoneal injection

I.C. = Intracerebral injection

Table 2-5 shows that intracerebral injection with a dose of 4 x 10^5 CFU of Str.milleri or Str.mutans caused no deaths in contrast to the 100% mortality among animals receiving these streptococci at a higher dose. When the dose was increased from 4 x 10^5 to 10^6 CFU a mortality rate similar to that produced by a dose of 10^7 CFU resulted. (Table 2-6). No difference in mortality was found to follow injection into either the frontal pole or the temporal pole (Table 2-3 and 2-5). Subsequently, intracranial injections were therefore made only into the temporal pole.

The experiments indicate the comparatively greater virulence of Str.agalactiae, irrespective of the route of injection, and suggest that for young mice the lethal dose is less than 40,000 CFU. They indicate that Str.mutans is more virulent when injected into the blood stream than is Str.milleri, the lethal dose, following intravenous injection, being approximately 10^7 CFU. Str.milleri and Str.mutans when injected intracerebrally are lethal at doses of 10^6 CFU. The comparative mortality rates from these experiments are shown in Table 2-7.

b) Effect of injecting streptococci by the otogenous route

The drums and auditory canals of 30 anaesthetised mice were scarified using sterile fine bore pasteur pipettes. The following day, the mice were divided into three groups of ten, anaesthetised and inoculated on to the ear drum with 10^5 CFU Str.agalactiae, Str.milleri and Str.mutans respectively. Three days later the mice were all well and the ear drums were reinoculated with the same organisms at the same dosage.

After a further four days, during which the mice remained well, a third injection, this time of 5×10^5 CFU was given. Within three days of the last injection eight out of the ten mice receiving Str.agalactiae had died, but those who received Str.milleri or Str.mutans were alive and well and remained so. Postmortem examination showed that death was due to acute meningitis.

c) Effect of injecting streptococci by the intrahepatic route

The three test strains of streptococci were each injected intrahepatically into ten mice in doses of 10^5 CFU Str.milleri and Str.mutans and 3×10^4 CFU Str.agalactiae, in 0.05 ml PBS. The results are shown in Table 2-8. Whereas nine of ten receiving Str.agalactiae died after two days from generalised infection, none of those receiving Str.milleri or Str.mutans suffered any ill effect.

2. Effect of Preceding Cerebral Trauma on Mortality Rate of Mice Injected Intravenously with Streptococci

Ninety, four week old mice were divided into nine groups of ten.

Str.agalactiae, Str.milleri and Str.mutans were each injected into three groups of 30 mice as follows:-

1. 4×10^5 CFU injected intravenously (10 mice).
2. 4×10^5 CFU injected intravenously one hour after the mouse had received 0.1 ml PBS into the temporal lobe (10 mice).
3. 4×10^5 CFU into the temporal lobe (10 mice).

Table 2-9 shows that where intravenous inoculation was preceded by intracerebral trauma there was increased mortality from each of the streptococcal species studied.

Table 2-8

MORTALITY OF WSM INJECTED INTRAHEPATICALLY WITH STREPTOCOCCI (0.05 ml)

	Inoculum	Number Dead 24 hrs.	Number Dead 48 hrs.	Survivors at 2 weeks
Str. agalactiae	3×10^4	4/10	9/10	1/10
Str. milleri	10^5	0/10	0/10	10/10
Str. mutans	10^5	0/10	0/10	10/10

Table 2-9

PERCENTAGE MORTALITY OF WSM AT SIX DAYS SHOWING EFFECT OF PRECEDING CEREBRAL TRAUMA ON MORTALITY AFTER INTRAVENOUS INJECTION

	4×10^5 I.V.	4×10^5 I.V. + PBS I.C.	4×10^5 I.C.
Str. agalactiae	80%	90%	100%
Str. milleri	Nil	20%	20%
Str. mutans	Nil	20%	Nil

I.V. = Intravenous injection
I.C. = Intracerebral injection

3. Effect of Physical Stress on Sublethal Doses of Streptococci

Two groups of mice were kept at $4^{o}C$ for four hours before and for 18 hours after intracerebral injection of 10^5 CFU Str.milleri or Str.mutans. Equal numbers of control animals were kept at room temperature before and after injection. There was no difference between the refrigerated group and the control group, (Table 2-10).

4. Effect of Animal Passage on Sublethal Doses of Streptococci

Str.milleri (NCTC 10708), Str.milleri (R 74/338 - clinical isolate) and Str.mutans (NCTC 10449) were passaged four times through mice, using the intracerebral route, before being injected intracerebrally at a dose of 10^5 CFU each into ten mice. Control mice received the same dose of unpassaged organisms. Further experiment would be required to assess the significance of the difference observed (Table 2-11).

5. Allergy

Two groups of ten mice which had previously received 10^5 CFU Str.milleri (NCTC 10708) or Str.mutans (NCTC 10449) intracerebrally were reinoculated with 10^5 CFU at the same site with the same organism four weeks later to show whether repeated infection or hypersensitivity played a part in the establishment of intracranial abscess by sub-lethal doses. None died.

6. Immunity following Intracerebral Inoculation

Twenty-three mice surviving intratemporal inoculation

Table 2-10

EFFECT OF COLD ON THE MORTALITY OF WSM INOCULATED INTRACEREBRALLY

		Percentage Mortality	
	Inoculum	Mice at 4°C	Mice at 25°C
Str.milleri (NCTC 10708)	10^5	0	0
Str.mutans	10^5	0	0

Table 2-11

EFFECT OF INTRACEREBRAL PASSAGE ON THE PATHOGENICITY OF STREPTOCOCCI

		Percentage Mortality	
	Inoculum	Passaged Strain	Unpassaged Strain
Str.milleri (NCTC 10708)	10^5	10	0
Str.milleri (R74/338)	10^5	10	0
Str.mutans	10^5	0	0

were injected at the same site seven days later (Table 2-12). Irrespective of the size of the first inoculation, the five previously injected with Str.agalactiae were given 10^5 CFU, and died within 48 hours. None of 18 mice which had survived lower doses of Str.milleri or Str.mutans died as a result of a second injection of 10^6 CFU, although this is a lethal dose in previously unchallenged mice (Table 2-6).

STUDY OF THE FATAL DISEASE FOLLOWING INFECTION WITH STREPTOCOCCI

Bacteriological Investigations

The majority of animals were examined bacteriologically and the appropriate organism was recovered from the tissues at postmortem examination. Many of the animals dying 4-6 days after infection could not be adequately examined due to the deterioration which occurred between death and postmortem.

Thirty-nine of 90 mice injected by the intravenous route died. Purulent meningitis was a common feature in the 27 mice infected with Str.agalactiae but was found in only three of the twelve animals infected with Str.milleri or Str.mutans. All the animals examined had white nodules less than 1 mm in diameter scattered on the cut and uncut surfaces of the liver, spleen and/or kidneys and many of them exhibited hyperaemia of the endocardium. Viable bacteria were isolated from the heart blood of all 39. Twenty-seven of 30 mice injected by the intraperitoneal route died. All those examined showed signs of peritonitis and, in the majority, culture of the heart blood was positive. Many of these animals also had lesions in the liver, kidney

Table 2-12

NUMBER OF MICE RECEIVING TWO TEMPORAL LOBE INJECTIONS AT AN INTERVAL OF SEVEN DAYS

	1st injection (CFU)	Number of Survivors	2nd injection (CFU)	Number of Survivors
Str. agalactiae	3×10^4	0/10	-	0
	3×10^3	2/10	10^5	0/2
	3×10^2	3/10	10^5	0/3
Str. milleri	10^6	1/10	10^6	1/1
	10^5	3/10	10^6	3/3
	10^4	3/10	10^6	3/3
Str. mutans	10^6	3/10	10^6	3/3
	10^5	4/10	10^6	4/4
	10^4	4/10	10^6	4/4

CFU = Colony forming units

and spleen from which viable bacteria could be isolated and $^5/10$ mice injected with Str.agalactiae had a purulent meningitis. None of the 17 infected with Str.milleri or Str.mutans had purulent meningitis.

The nine mice dying following intrahepatic injection of Str.agalactiae all had a generalised infection and viable bacteria were isolated from the blood, peritoneum and meninges as well as the liver.

All animals showed signs of severe meningitis following intracranial injection. In half of the twenty mice injected with Str.agalactiae culture of the heart blood was also positive. In contrast mice injected with Str.milleri and Str.mutans rarely had a positive blood culture, 2/20 and 1/20 respectively. All nine animals that died following injection of Str.agalactiae by the otogenous route had meningitis and in four there was also a bacteraemia. Viable streptococci could not be isolated from the ears of the survivors.

Histological Studies

Animals that were to be studied in detail histologically were the subjects of two separate experiments.

Str.agalactiae, Str.milleri and Str.mutans were each injected into the temporal lobes of ten mice at a concentration of 5×10^5 CFU in 0.01 ml using 30G needles. Control mice received 0.01 ml PBS into the temporal lobe. The mice were killed by exposure to ether for three to four minutes at predetermined intervals (Table 2-13) and their brains were dissected out immediately. They were bisected

Table 2-13

NUMBER OF MICE KILLED AT PREDETERMINED INTERVALS AFTER TEMPORAL LOBE INJECTION (5 x 10^5 CFU) OF THREE STREPTOCOCCAL STRAINS

	Time in hours after injection									
	1/4	1	2	18	20	22	24	26	42	50
Control group (PBS) only		1		1			1	1	1	1
Str. agalactiae	1	1	1	1^{++}	1^{+}	1^{++}		1^{+}		
Str. milleri	1	1	1	1			1	1	1	1
Str. mutans	1	1	1	1			1	1	1	1

+ Moribund

++ Dead

Table 2-14

INOCULUM SIZES AND NUMBER OF MICE KILLED AT PREDETERMINED INTERVALS FOLLOWING TEMPORAL LOBE INJECTION

	Inoculum in CFU	Time in hours 2	6	18	24	44	48	50	72	Time in Days 5	6	7
Str. agalactiae	3×10^4	1	1	1	1	3^{++}	1^{+}	1^{++}	1	-	-	-
	3×10^3	-	1	1	1	-	1	-	1	1	1	1
	3×10^2	-	-	1	2	-	1	1	1	1	1	-
Str. milleri	10^6	1	1	1	1	-	1	-	1	1	1	1
	10^5	-	1	1	1	-	1	-	1	-	1	1
	10^4	-	-	1	2	-	1	-	1	1	1	-
Str. mutans	10^6	1	1	1	2	-	-	-	1	1	-	-
	10^5	-	1	1	2	-	-	-	1	1	-	-
	10^4	-	1	1	2	-	-	-	1	1	-	-

$^{+}$ Moribund

$^{++}$ Dead

longitudinally down the midline, and the two hemispheres were fixed in 10% formol saline.

In another experiment, the strains of streptococci were injected into the temporal lobes of groups of ten mice, at three concentrations. Again, the mice were killed at predetermined intervals (Table 2-14) during the next seven days, and, following dissection, the brains were fixed in formol saline. Any mouse appearing unwell was sacrificed in preference to his healthier contemporaries.

Serial sections of the left hemisphere were cut in the sagittal plane from lateral to medial, so that the track of the inoculating needle could be studied. They were cut at a thickness of 8 µm, mounted in pairs and dried at 37°C for 24-48 hours. Every fifth slide was stained by haematoxylin and eosin and the sections adjacent were stained by Gram's method, using neutral red as counterstain. All stained sections were examined using low power (x 6.3) and high power (x 25, x 100) objectives.

<u>Histological Findings</u>

The histology of the brain of 4-6 week old white Swiss mice was studied using two uninoculated subjects. Microscopy showed that there were two distinct types of nuclei in the temporal lobe (Figure 2-3).

<u>Type A</u> These nuclei are approximately 14 µm in diameter and are densely packed in certain areas of the brain, being only 15-30 µm apart. They are usually round, although triangular forms do occur, and they have prominent nucleoli. They contain 2-6 Nissl granules per cell. The nuclear membrane is not marked; the nuclear cytoplasm is coarsely granular and in some cells there is vacuolation. These nuclei,

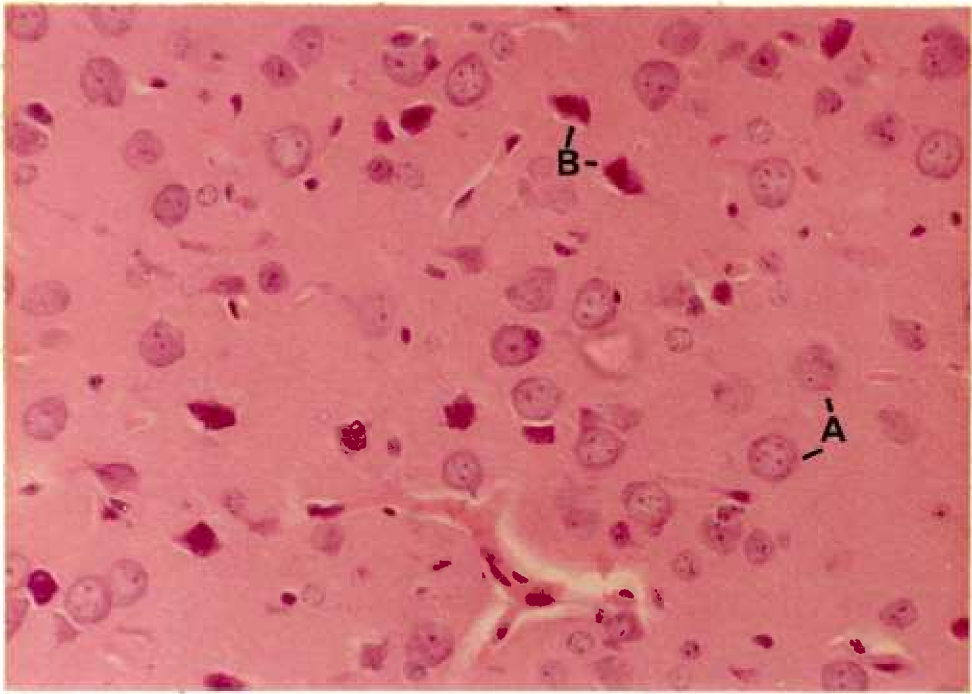

Fig.2-3 Mouse brain, showing types of cell nuclei.
(Haematoxylin and eosin x 460)

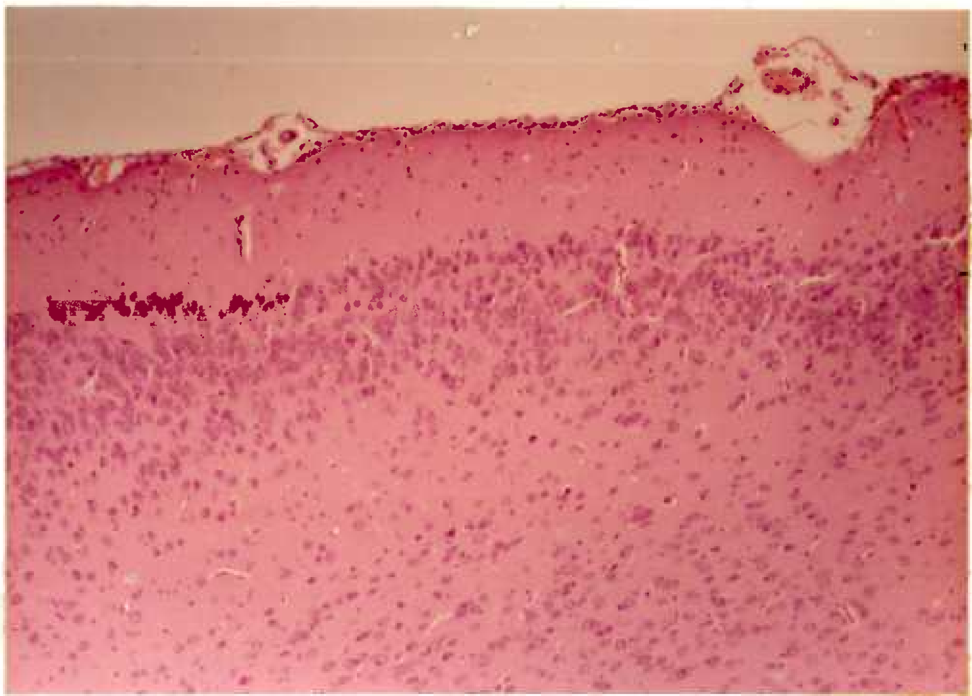

Fig.2-4 Mouse brain, showing general topography. The limiting membrane is one cell thick.
(Haematoxylin and eosin x 120)

together with those of the capillary endothelium, constitute the bulk of nuclear elements in the temporal lobe.

<u>Type B</u> These nuclei are approximately 7 μm in diameter and are densely staining. They are less common than Type A.

The nature of these cells is unclear. They appear throughout the grey matter although there is considerable variation in relative numbers. Occasionally foci are found in which the majority of cells have this appearance. No report could be found that these cells formed part of the normal histology of the mouse brain and the similarity between these cells and those cells showing ischaemic changes reported in the rat brain by Brown and Brierley (1972) suggest that they may be the result of local trauma during removal of the brain. Brown and Brierley also report the presence of vacuoles similar to those seen in the Type A cells in the brains of rats following nitrogen intoxication. The fact that these cells are not found uniformally throughout the brain substance and that they occur predominantly in the outer layers would support this premise and suggest that the appearance is not due either to anaesthesia or to a fixation artefact. That they are of traumatic origin is supported by their presence around the haemorrhagic needle track 18 hours after intracerebral injection of phosphate buffered saline.

The low power appearance of the outer layers of the temporal lobe of uninoculated mouse brain is shown in Figure 2-4. The vascular limiting membrane is one cell thick and is closely attached to the outer surface. Its nuclei often appear dense and flattened. The underlying brain tissue, to a depth of 150-200 μm, contains very few nuclei, and with higher magnification these can be recognised as

belonging to Type B. Deep to this is a layer 150-200 μm in thickness where the nuclei, predominantly those of Type A, are very closely packed. Deeper to this and extending to the ventricles, the distribution of nuclei becomes more uniform and both types are found.

A haemorrhagic needle track is seen after injection of a small volume of sterile buffered saline into the brain. Two hours after injection the area surrounding the track appears undamaged. There is no polymorphonuclear infiltration and the only discernible change is that some of the nuclei immediately adjacent to the track have become pycnotic and stain densely. Twenty-four hours following injection the site, which is still haemorrhagic, is surrounded by a small area of oedema which contains fewer nuclei than the surrounding brain tissue. These are mainly of Type B.

Streptococci deposited down the needle track can be seen after intracerebral injection of Str.agalactiae (Figure 2-5). Within one hour, the organisms are invading the surrounding tissue (Figure 2-6) and can be seen in the capillaries (Figures 2-7 and 2-8). They are also present throughout the meninges (see below). At two hours they are seen in the ventricles (Figure 2-9). Defined abscesses (Figure 2-10), surrounded by areas of polymorphonuclear infiltration, (Figure 2-11) have formed 18 hours following injection. Microabscesses can be seen developing adjacent to the main lesion (Figure 2-12) and, in remote parts of the brain, areas of intense polymorphonuclear activity can also be found (Figure 2-13). Some of these contain few, if any discernible bacteria.

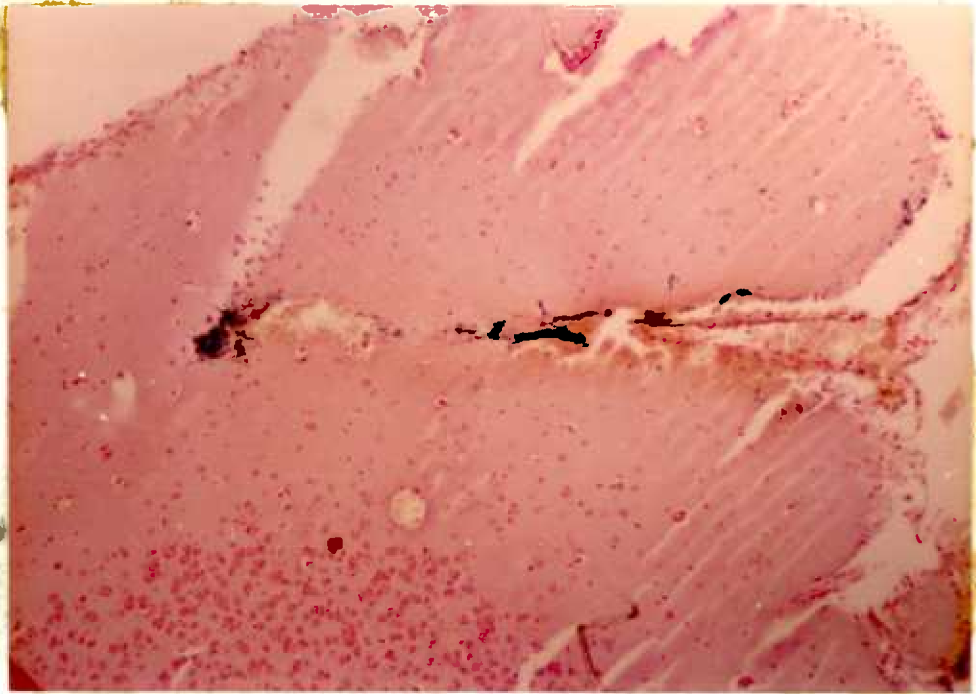

Fig.2-5 Temporal lobe showing Gram-positive cocci throughout the length of the haemorrhagic needle track. Fifteen minutes after injection of Str.agalactiae. (Tissue Gram x 150)

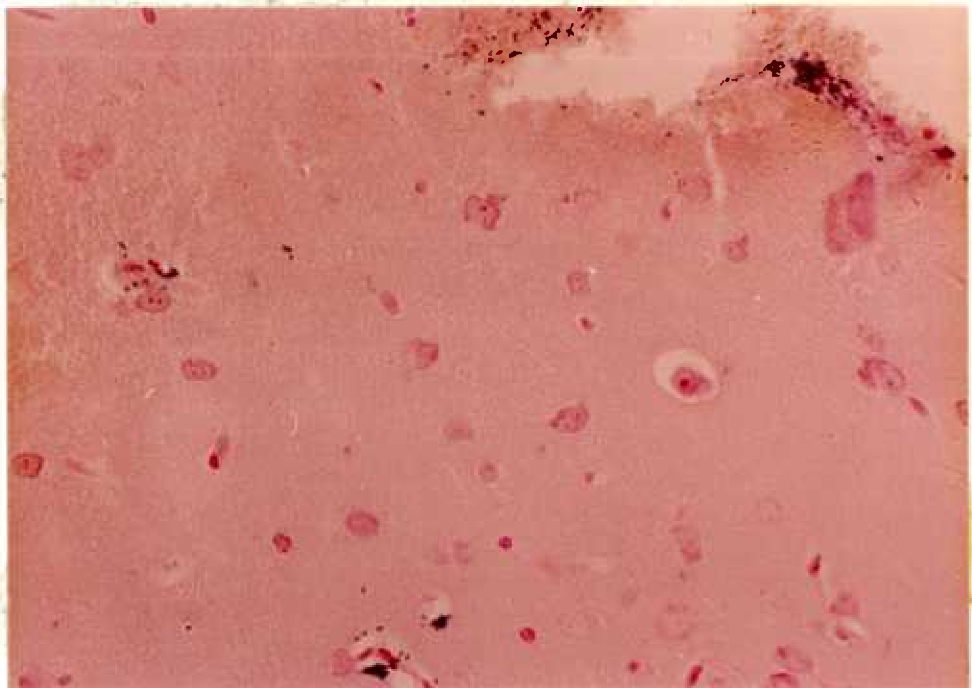

Fig.2-6 Gram-positive cocci invading tissue remote from infected needle track (upper righthand corner). One hour after injection of Str. agalactiae. (Tissue Gram x 460)

Fig.2-7 From the same brain as Fig.2-6. Gram-positive cocci in the wall of a dilated (surface) capillary. One hour after injection of Str.agalactiae. (Tissue Gram x 460)

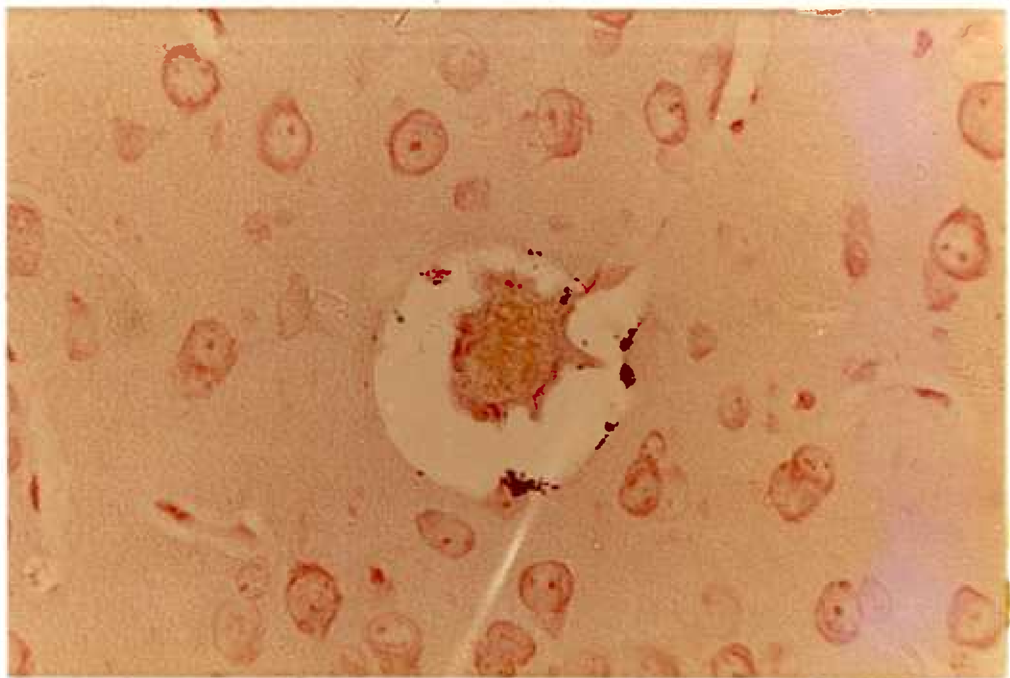

Fig.2-8 Gram-positive cocci in the wall of a capillary. In spite of the distortion it is apparent that the bacteria are in the perivascular areas. One hour after injection of Str.agalactiae. (Tissue Gram x 600)

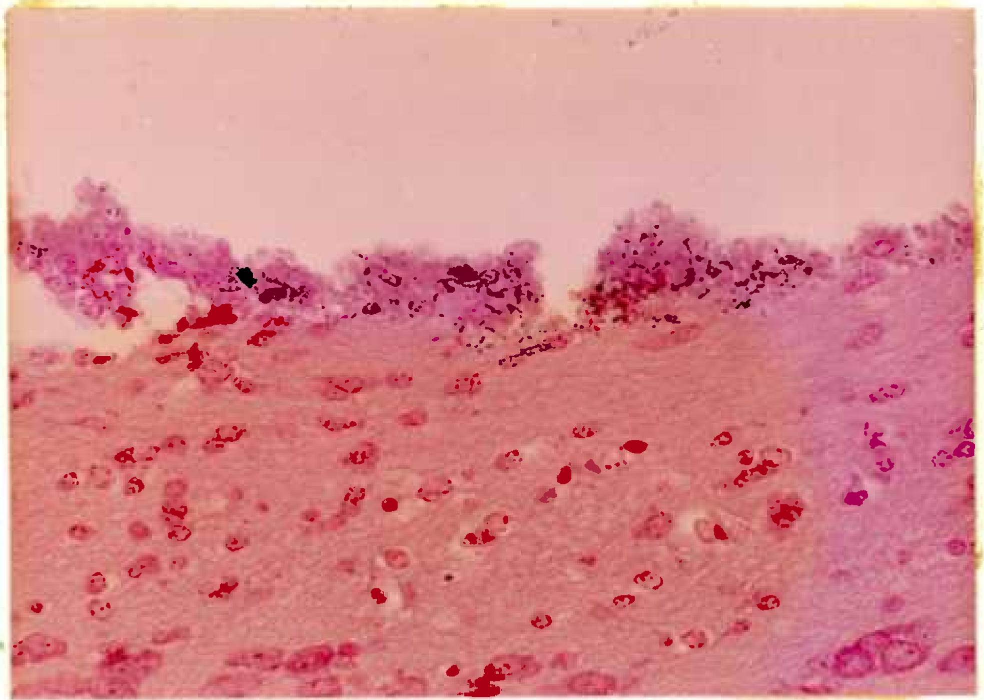

Fig.2-9 Gram-positive cocci invading ventricle. There is haemorrhage but no inflammatory reaction. Two hours after injection of Str.agalactiae. (Tissue Gram x 460)

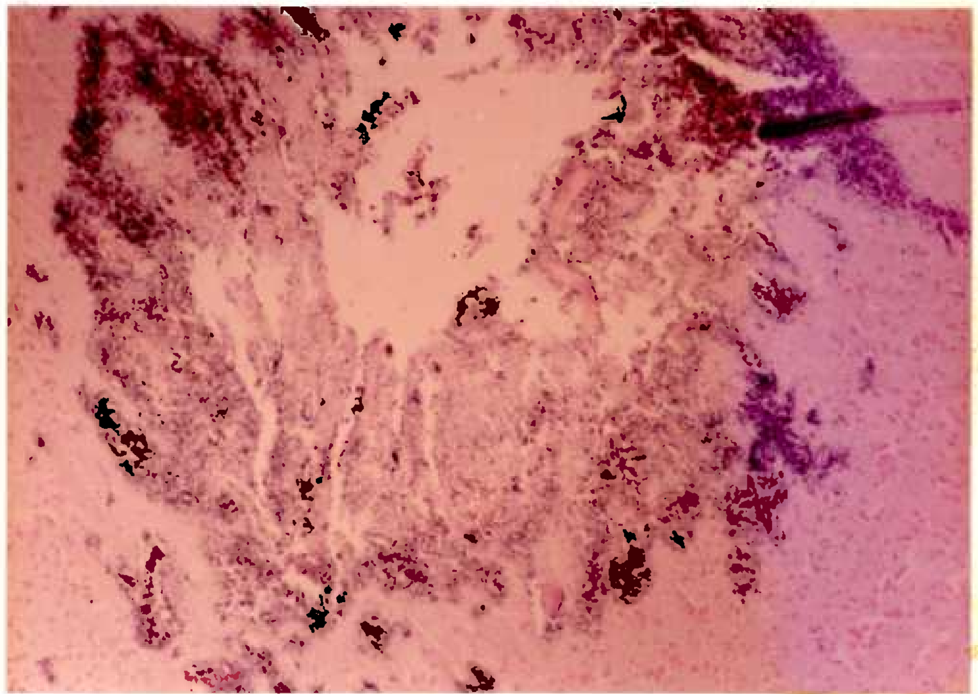

Fig.2-10 Necrotic cavitating lesion of brain related to needle track, showing abundant Gram-positive cocci. Eighteen hours after injection of Str. agalactiae. (Tissue Gram x 120)

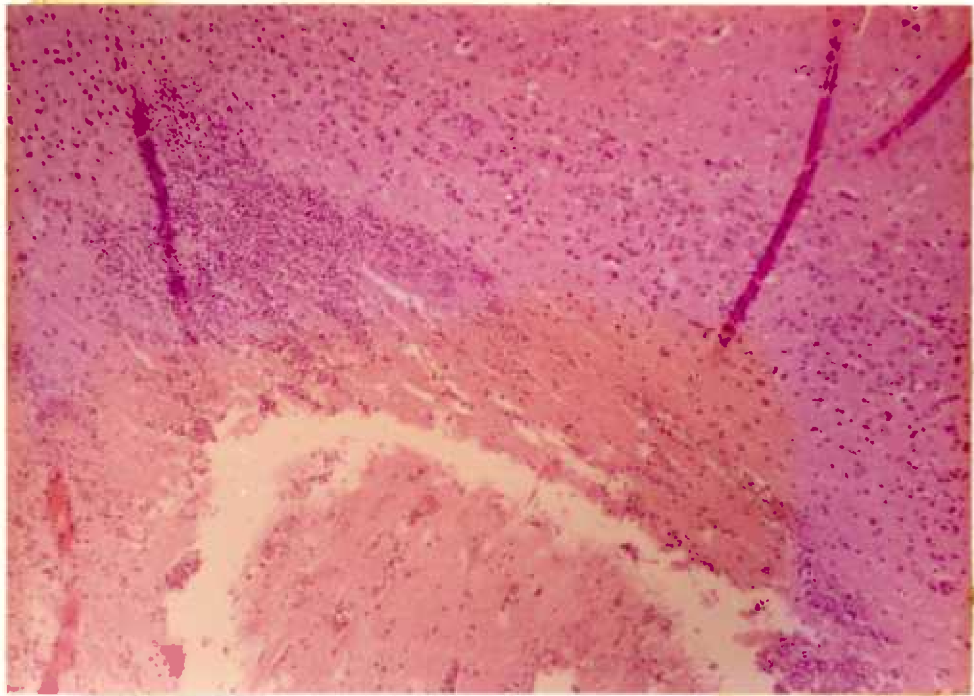

Fig.2-11 Region of ventricle. Inflammatory exudate abutting on necrotic lesion at base of needle track. Eighteen hours after injection of Str. agalactiae. (Haematoxylin and eosin x 120)

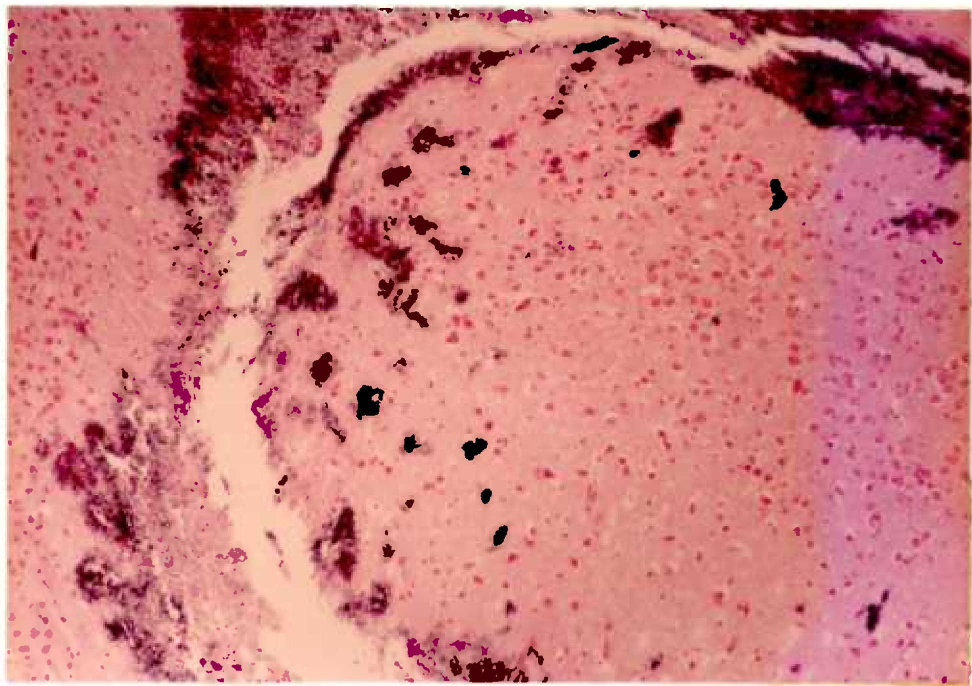

Fig.2-12 Ventricle, packed with Gram-positive cocci. There is necrosis of the ventricular wall, but little inflammatory reaction. Dense colonies of cocci are seen in adjacent areas of brain. Eighteen hours after injection of Str.agalactiae. (Tissue Gram x 120)

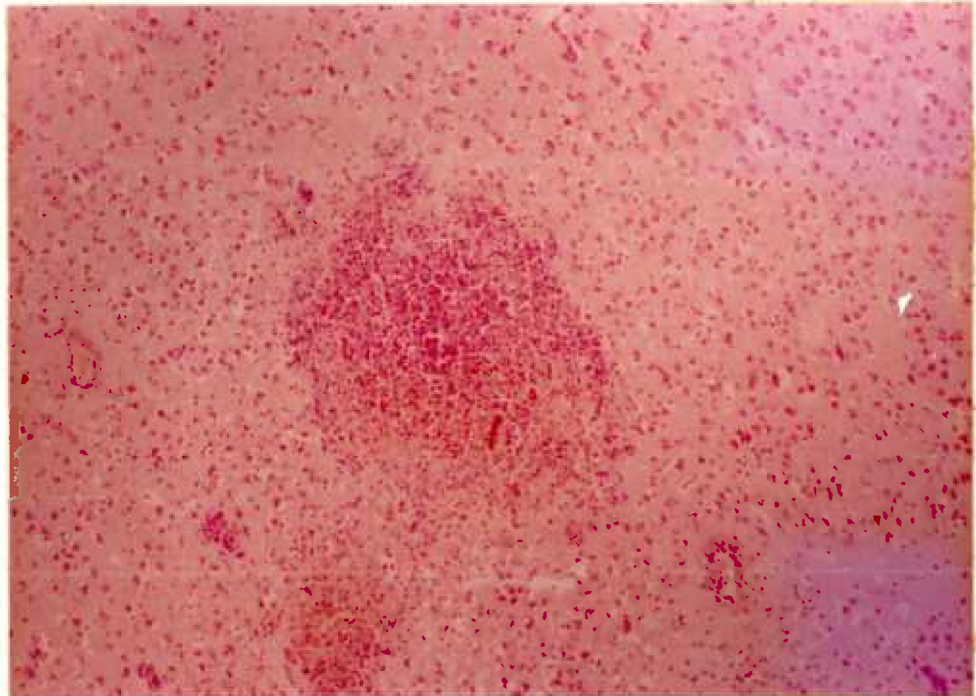

Fig.2-13 Brain, remote from needle track. Intense inflammatory reaction round discrete Gram-positive cocci. Eighteen hours after injection of Str.agalactiae. (Tissue Gram x 100)

The common feature of infection by Str.agalactiae is a severe spreading purulent meningitis, which had been apparent to the naked eye at necropsy.

Bacteria are discernible in the meninges 15 minutes after injection (Figure 2-14). Within one hour they are multiplying freely and there may be signs of a cellular response (Figure 2-15). Figure 2-16 shows a massive polymorphonuclear response six hours after injection with characteristically few Gram-positive cocci visible. Eighteen hours after injection the inflammatory response is greatly reduced and is confined to the submeningeal region (Figure 2-17). Gram-positive cocci can, however, still be seen (Figure 2-18). A polymorphonuclear response of this sort is characteristic of animals who survive for eighteen hours or longer and show macroscopic signs of meningitis at autopsy. Alternatively, the animal may fail to show a cellular response, the bacteria multiply freely and within 18 hours the animal has died from an overwhelming generalised infection (Figure 2-19).

All of the changes described follow inocula of 3×10^4 and 5×10^5 colony forming units; when smaller doses are used no circumscribed lesions develop, and the only histological feature is a mild polymorphonuclear infiltration around the needle track, which does not persist.

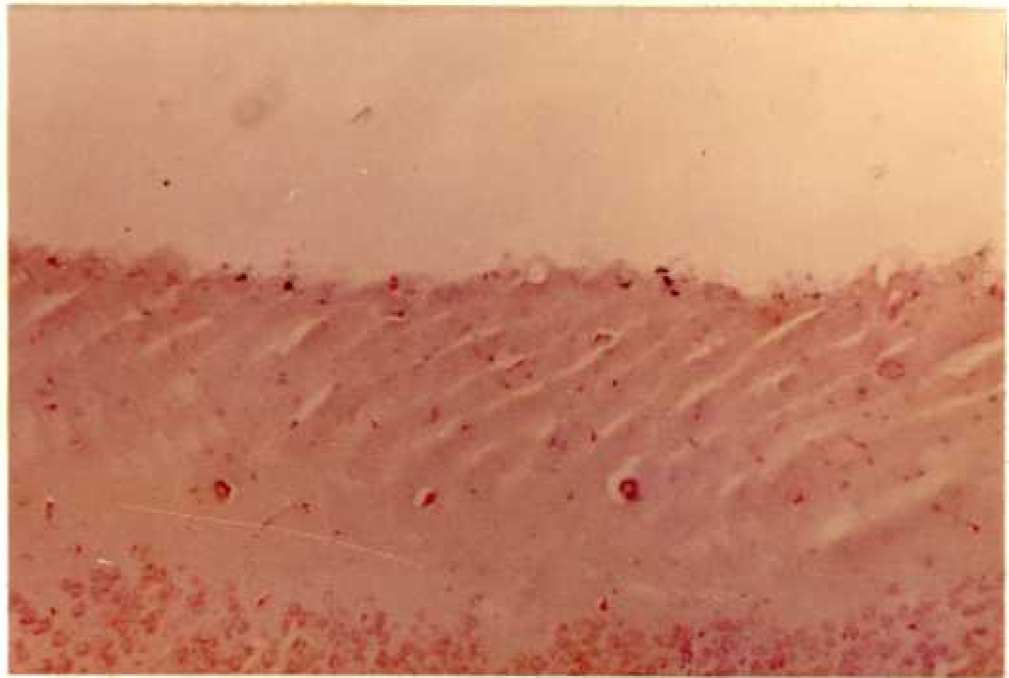

Fig.2-14 Temporal lobe showing Gram-positive cocci in the meninges. Fifteen minutes after injection of Str.agalactiae. (Tissue Gram x 150)

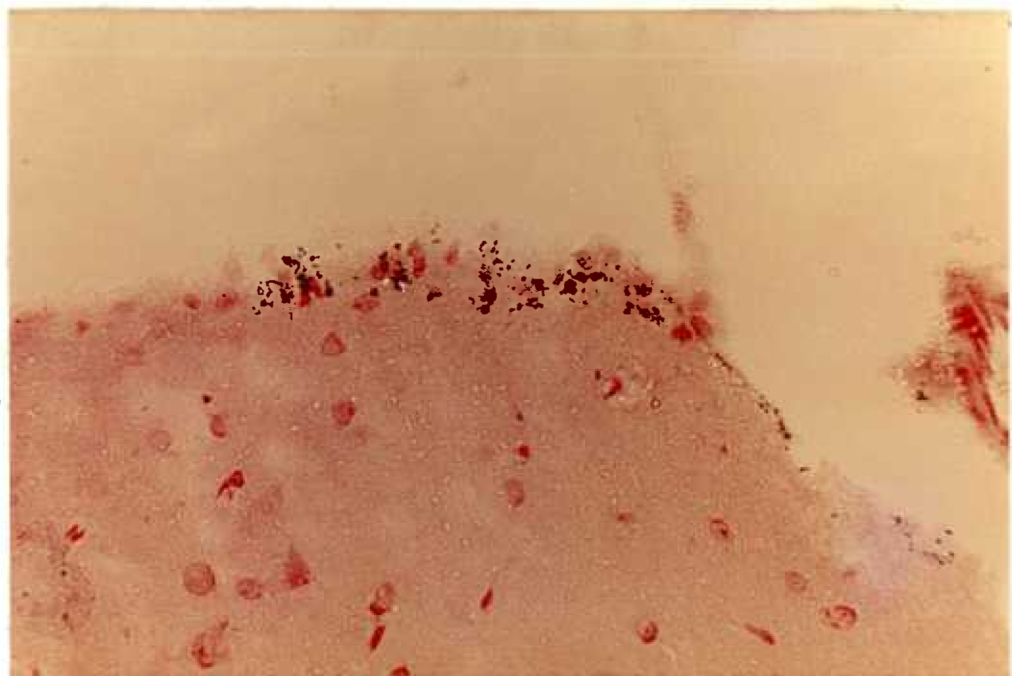

Fig.2-15 Temporal lobe showing localisation of Gram-positive cocci and a mild inflammatory reaction one hour after injection with Str.agalactiae. (Tissue Gram x 460)

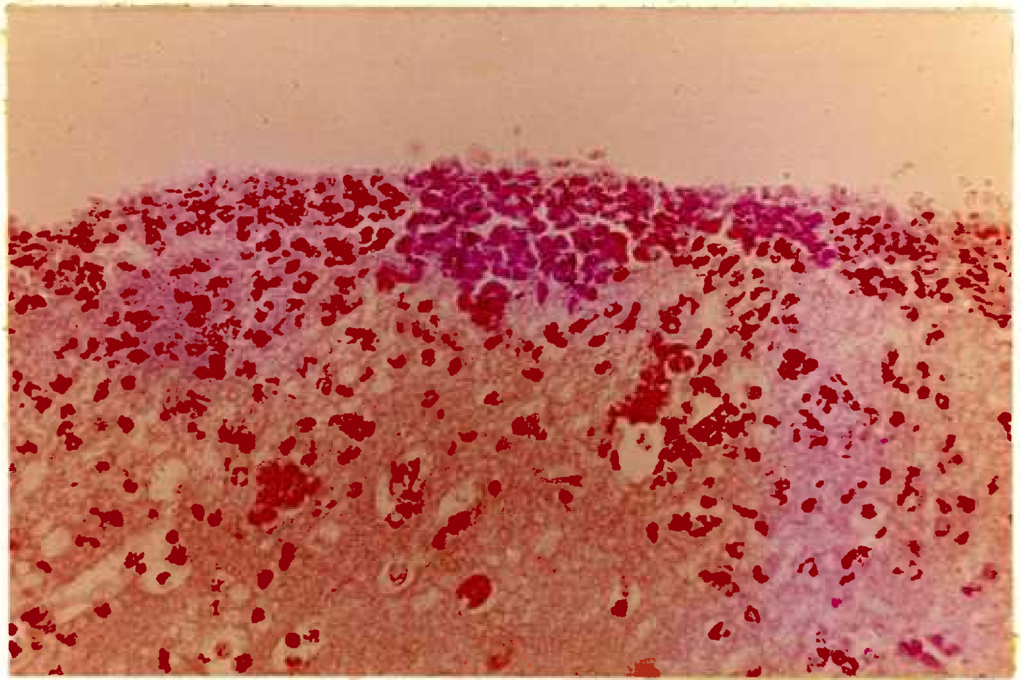

Fig.2-16 Temporal lobe showing intense inflammatory reaction in the meningeal and submeningeal region. Small numbers of Gram-positive cocci can be seen. Six hours after injection with <u>Str.agalactiae</u>. (Tissue Gram x 370)

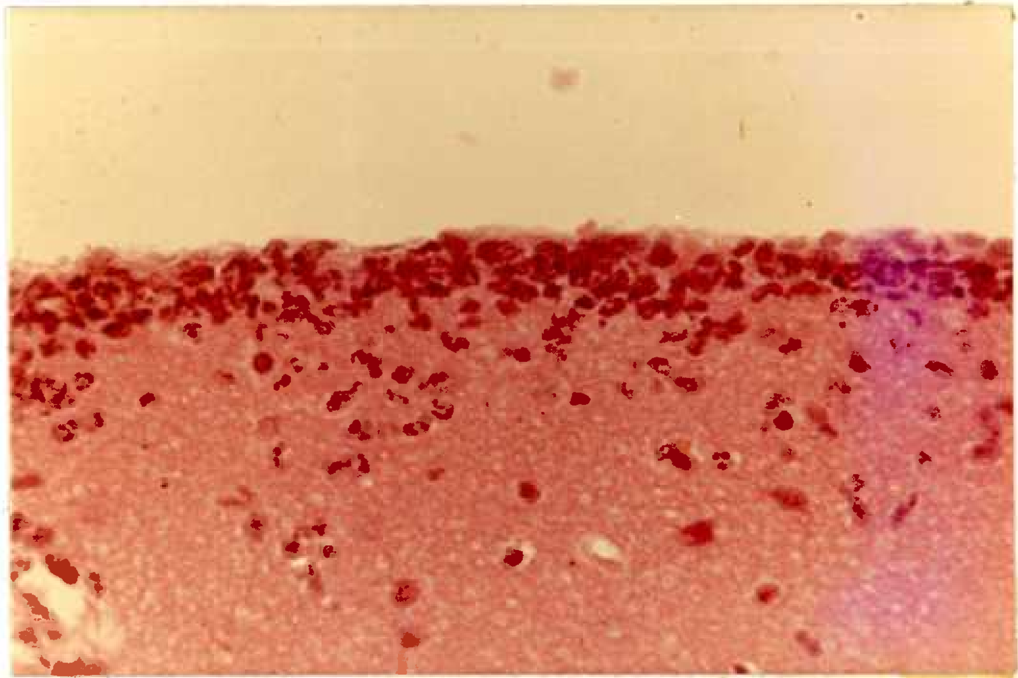

Fig.2-17 Temporal lobe showing a purulent meningitis eighteen hours after injection with <u>Str. agalactiae</u>. (Haematoxylin and eosin x 370)

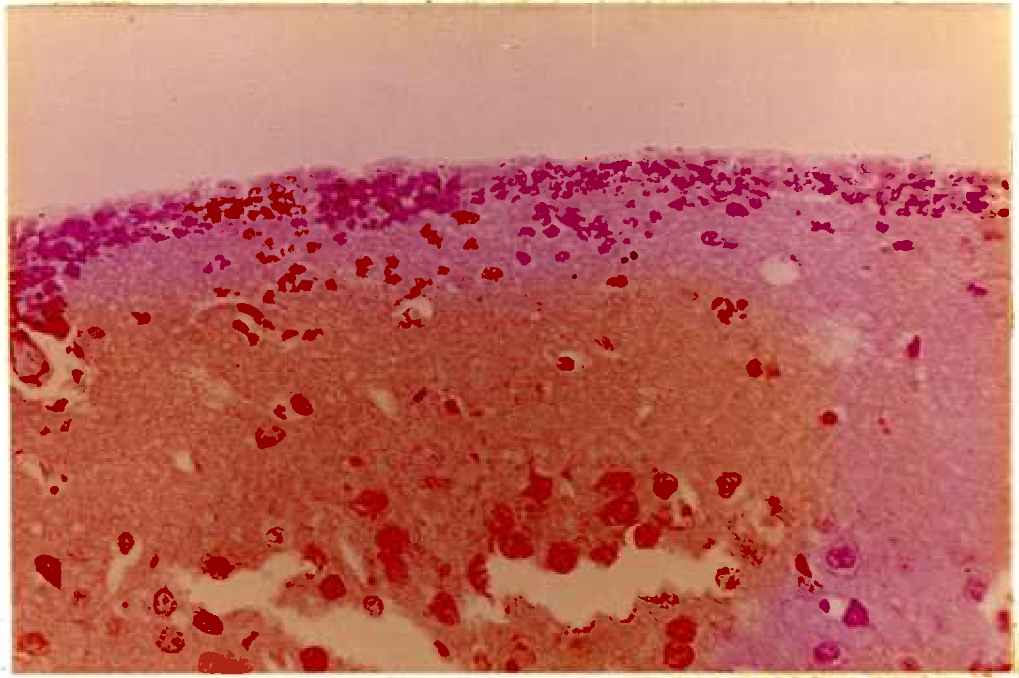

Fig.2-18 From the same brain as Fig.2-17 showing scanty Gram-positive cocci within the purulent exudate. Eighteen hours after injection of Str.agalactiae. (Tissue Gram x 370)

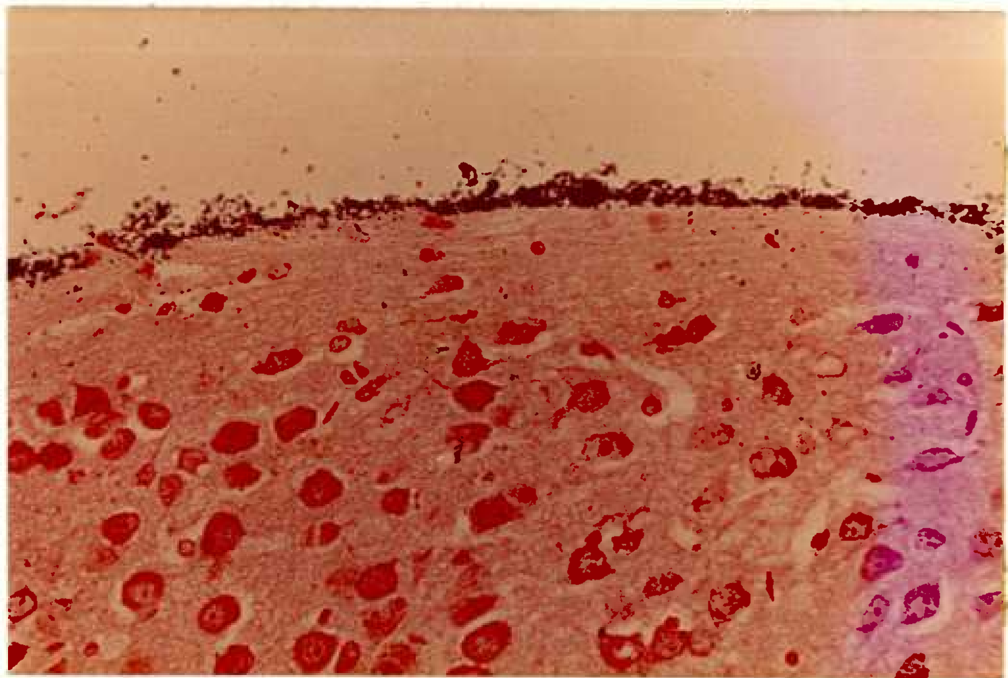

Fig.2-19 Temporal lobe of a mouse which died showing large numbers of Gram-positive cocci in the absence of an inflammatory reaction. Eighteen hours after injection with Str.agalactiae. (Tissue Gram x 370)

Intracerebral injection of Str.mutans produces effects similar to those of Str.agalactiae, with bacteria demonstrable in the injection track, (Figure 2-20) and in adjacent blood vessels (Figure 2-21) within one hour. The appearance of the lesion in Figure 2-20 is due to the core of brain tissue displaced by the inoculating needle. Two hours after injection bacteria are clearly visible in the meninges, an inflammatory reaction has started, and the bacteria are invading the underlying tissue (Figure 2-22). Figure 2-23 shows the inoculation site two hours after injection with Str.mutans. The bacteria are multiplying freely but there does not appear to be any inflammatory reaction. Eighteen hours after injection a necrotic abscess has formed at the inoculation site (Figures 2-24 and 2-25). It contains many bacteria and is surrounded by large numbers of polymorphonuclear leucocytes. As with Str.agalactiae, secondary abscesses are formed in adjacent areas (Figure 2-24) but, with Str.mutans, abscesses are not usually found in remote areas of the brain. The degree of inflammatory response varies with the site of the lesion. Lesions in the white matter are characterised by massive polymorphonuclear infiltration of the infected area with relatively few bacteria (Figure 2-26). In contrast, those forming around the ventricles (Figure 2-27) show very little cellular reaction. The bacteria grow freely in the periventricular tissue and are sometimes seen as compact microcolonies. As with Str.agalactiae, sublethal doses do not result in the formation of circumscribed or definite lesions.

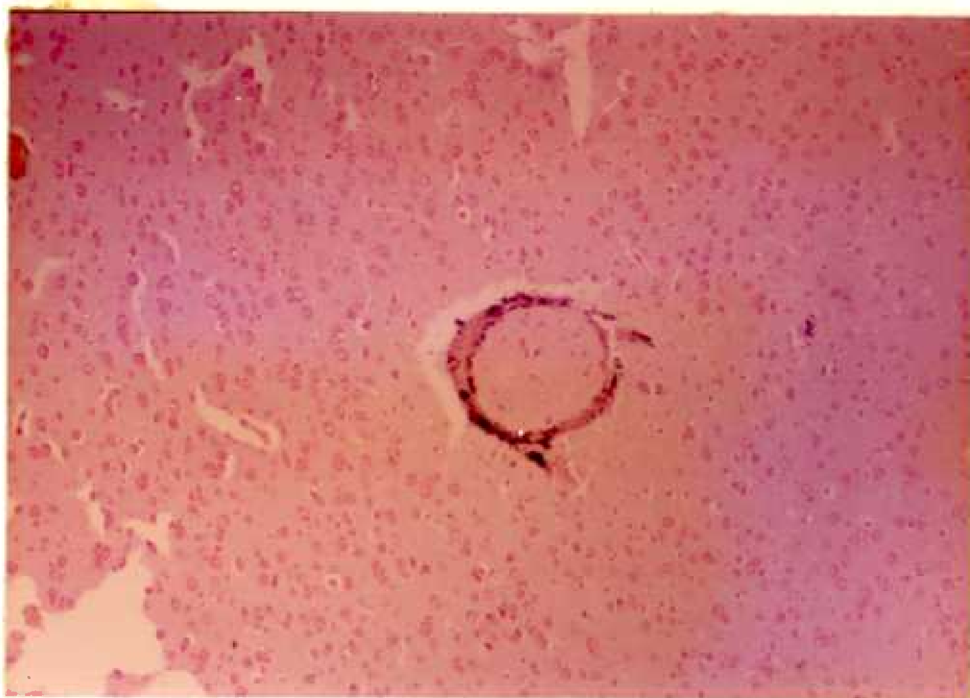

Fig. 2-20 Gram-positive cocci in needle track. The core of displaced brain tissue can be seen. One hour after injection with Str.mutans. (Tissue Gram x 150)

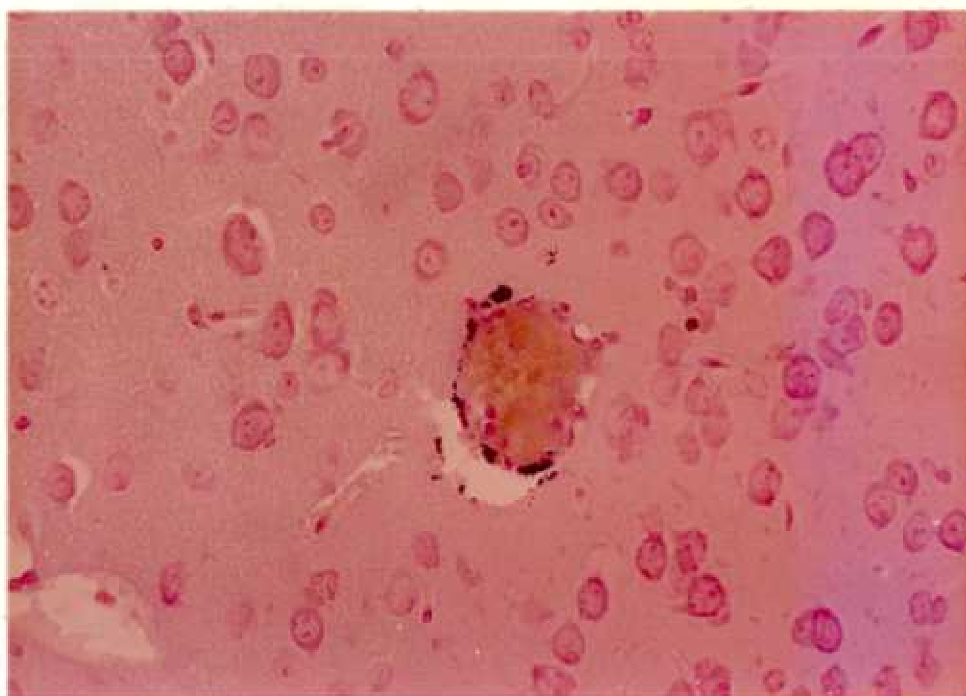

Fig. 2-21 Gram-positive cocci in dilated capillary adjacent to needle track. One hour after injection of Str.mutans. (Tissue Gram x 460)

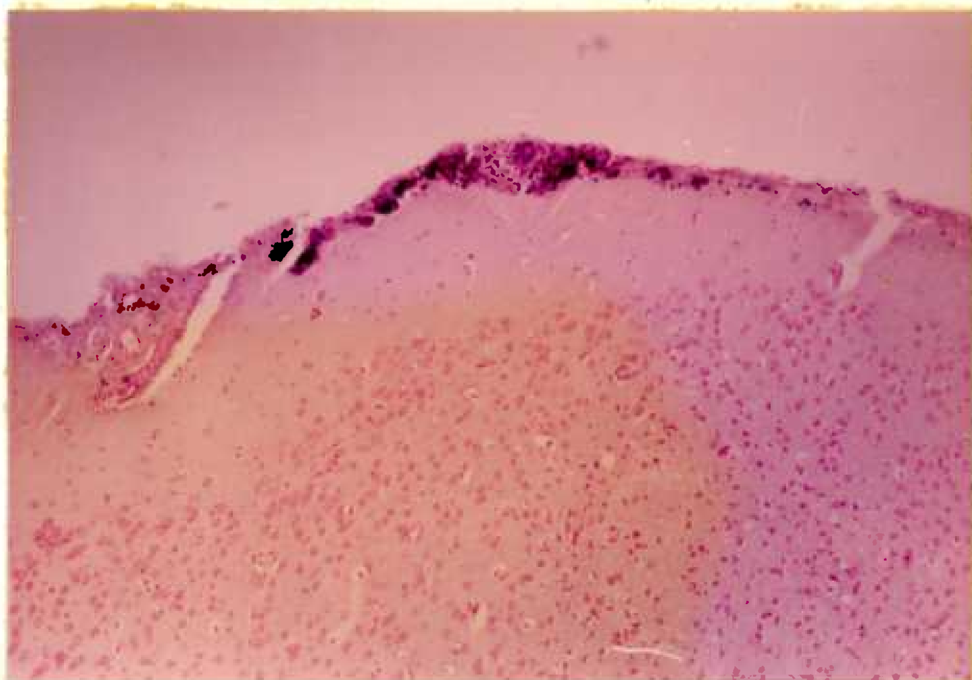

Fig.2-22 Gram-positive cocci multiplying in the meninges in the absence of inflammatory reaction. Two hours after injection of Str.mutans. (Tissue Gram x 120)

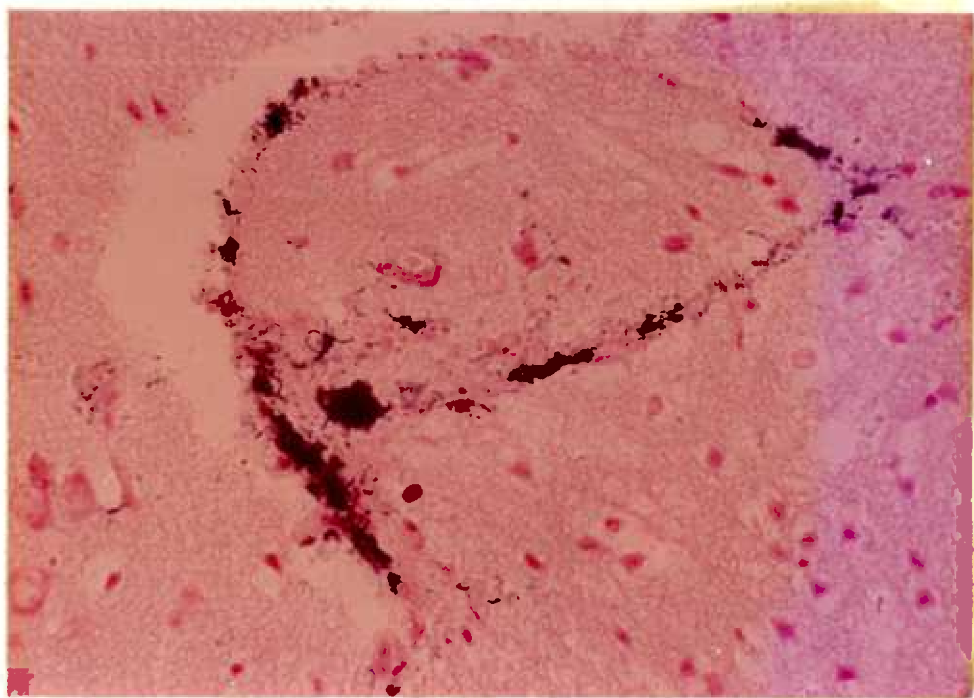

Fig.2-23 Abundant, chaining, Gram-positive cocci in needle track. There is no inflammatory response. Two hours after injection of Str.mutans. (Tissue Gram x 370)

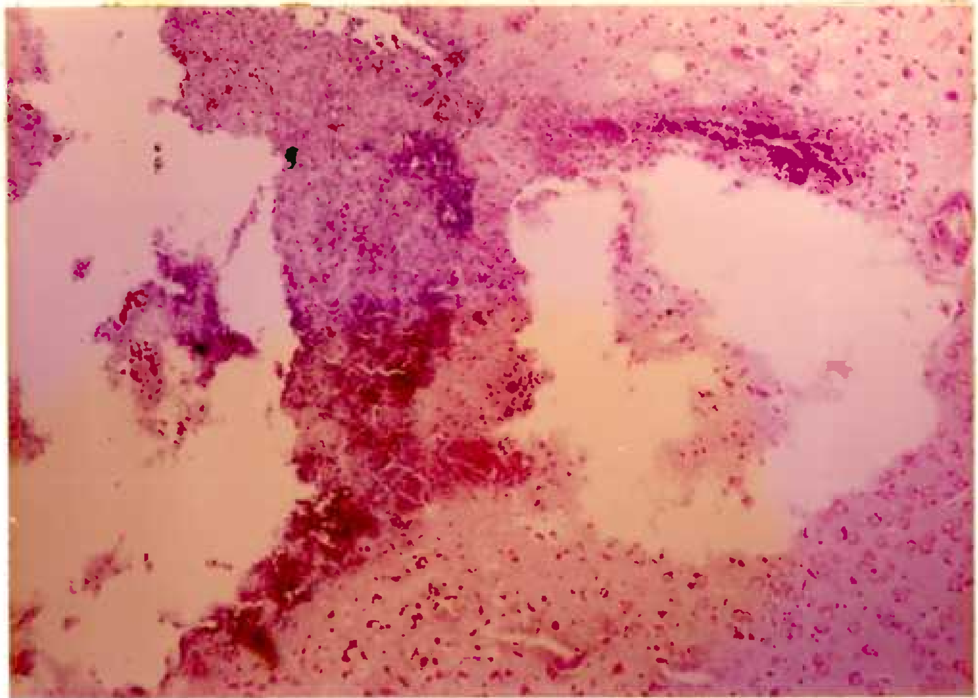

Fig.2-24 Bilocular brain abscess, related to needle track. Abundant Gram-positive cocci abut on the wall of the cavity to the left, and are separated by necrotic inflammatory tissue from the cavity to the right. Eighteen hours after injection of Str.mutans. (Tissue Gram x 100)

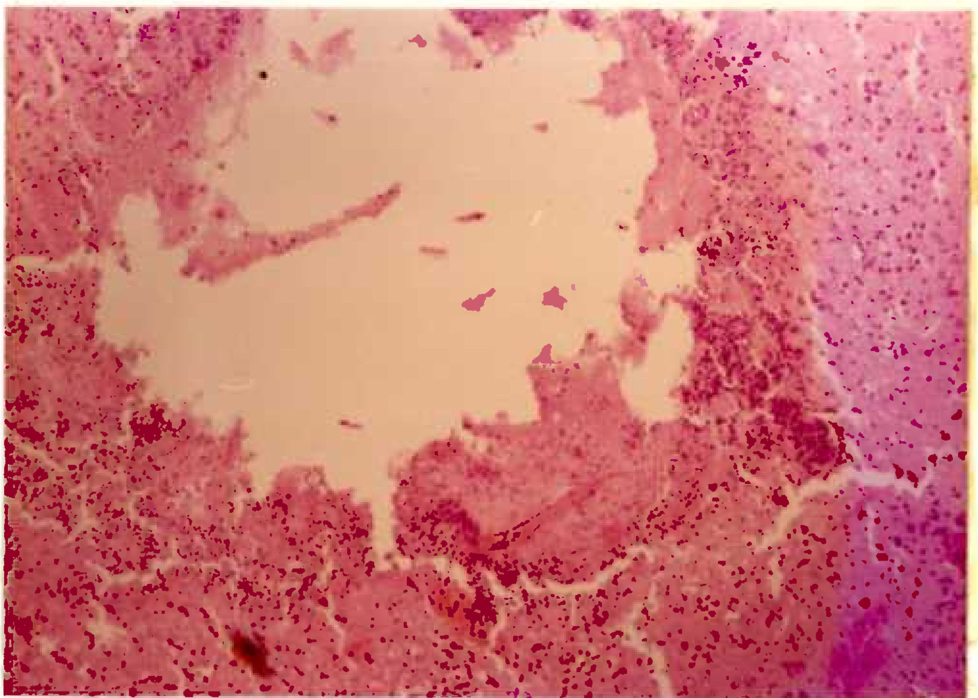

Fig.2-25 Cavitating brain abscess. The wall of the abscess shows dense inflammation. Eighteen hours after injection of Str.mutans. (Haematoxylin and eosin x 100)

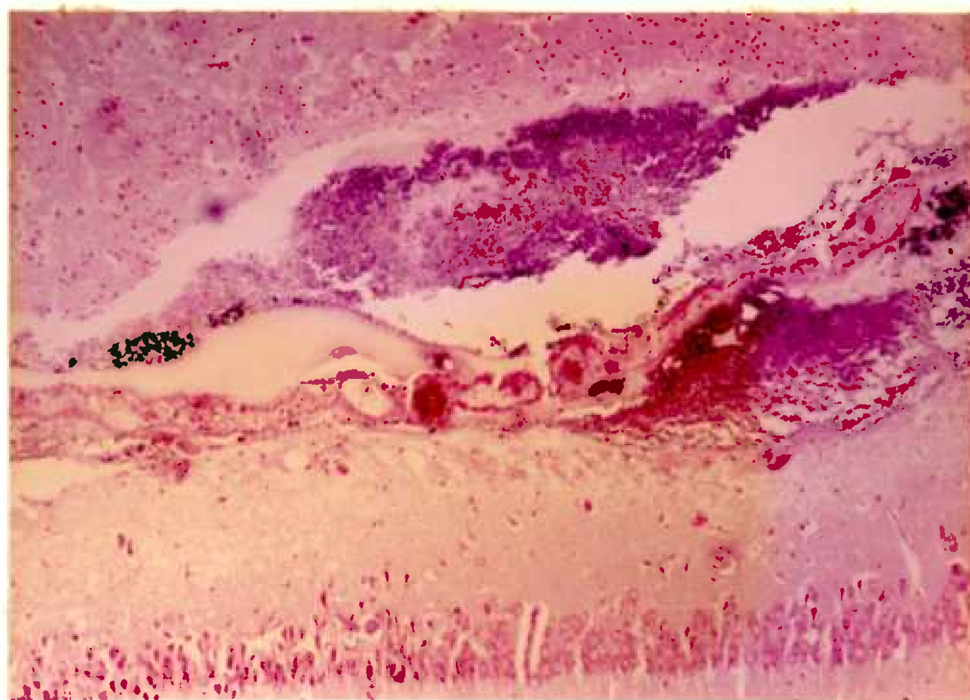

Fig.2-26 Lesion of white matter. Massive polymorphonuclear infiltrate surrounding Gram-positive cocci. Six hours after injection of Str.mutans. (Tissue Gram x 370)

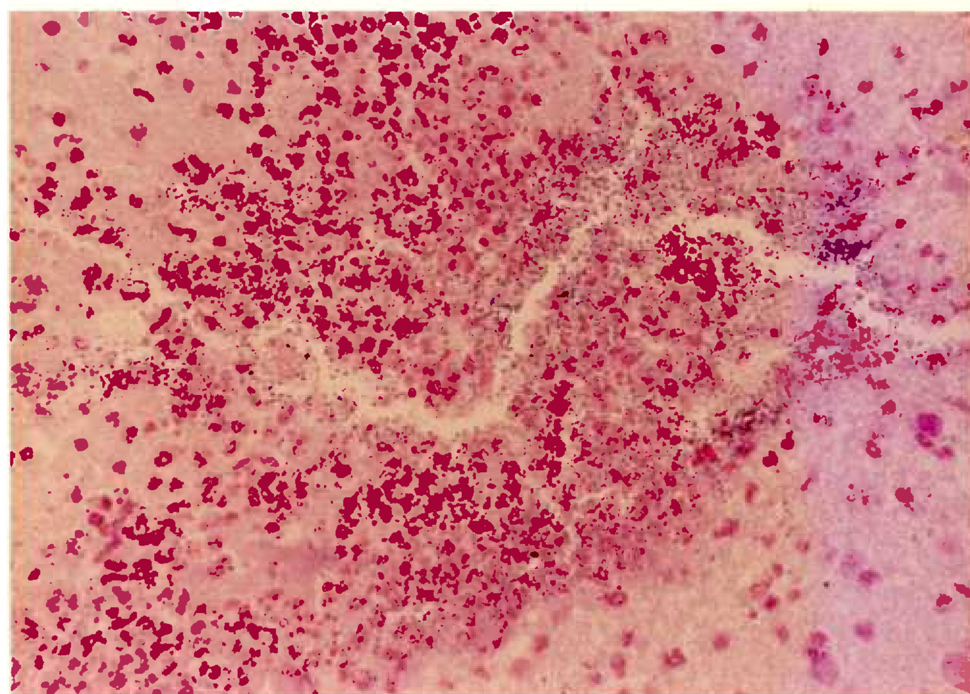

Fig.2-27 Periventricular lesions, consisting entirely of Gram-positive cocci. There is no inflammatory reaction. The ventricle is flattened. Eighteen hours after injection of Str.mutans. (Tissue Gram x 100)

The appearances following inoculation of Str.milleri differ from those caused by the other two organisms. Bacteria are seen at the inoculation site and along the needle track shortly after injection but they disappear rapidly. Two hours after injection the inoculation site shows an inflammatory response (Figure 2-28) but not the large numbers of bacteria seen after injection of the other streptococcal species. After 18 hours it shows aggregates of polymorphonuclear leucocytes (Figure 2-29), surrounding small clusters of bacteria, many of which stain poorly with Gram. The bacteria are more apparent using the staining method of Sowter and McGee (1976) (Figure 2-30). This method probably stains disrupted bacteria since the distribution of the magenta colouration (Gram-positive bacteria) resembles the distribution of bacterial antigen that is demonstrable by the indirect immunofluorescent technique.

A second type of lesion is produced by Str.milleri. Starting as an infected haemorrhage in the submeningeal space with underlying oedema and vacuolation (Figure 2-31), it develops into a haemorrhagic abscess with massive polymorphonuclear infiltration and invasion of the underlying brain tissue (Figure 2-32). Bacteria are just as difficult to demonstrate by Gram's method in this as in the other lesion, although bacterial antigen can be demonstrated using immunofluorescence. Sublethal injection with Str. milleri produces no apparent reaction. Gram-positive cocci can be demonstrated at the inoculation site and in the meninges shortly after injection but they disappear without multiplying appreciably and there is no leucocyte infiltration.

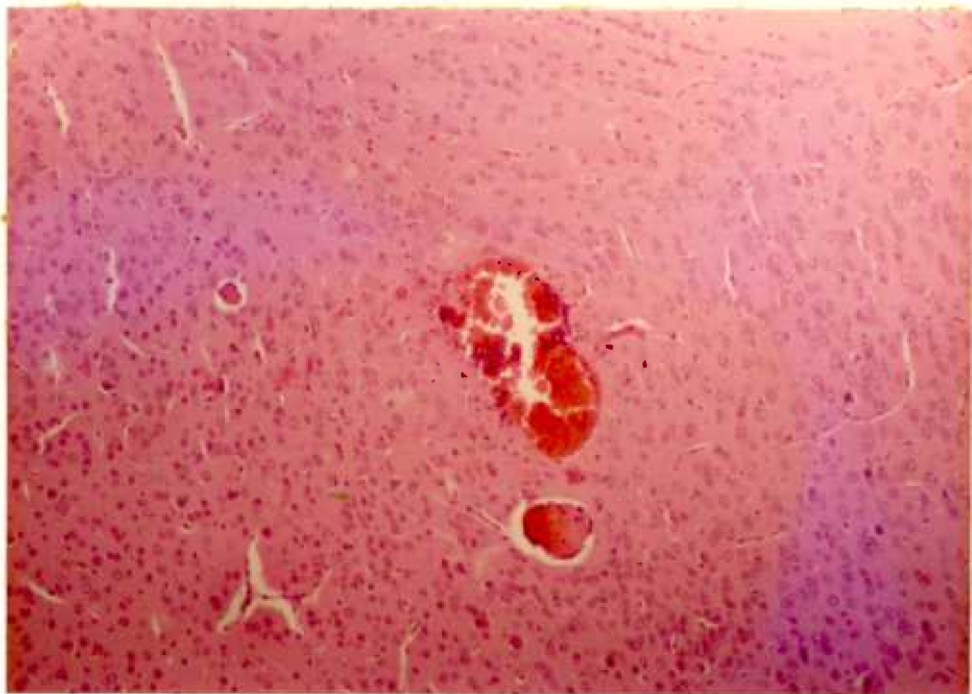

Fig.2-28 Haemorrhagic needle track, with some inflammation. Dilated capillaries are seen in the field. Two hours after injection of Str.milleri. (Haematoxylin and eosin x 120)

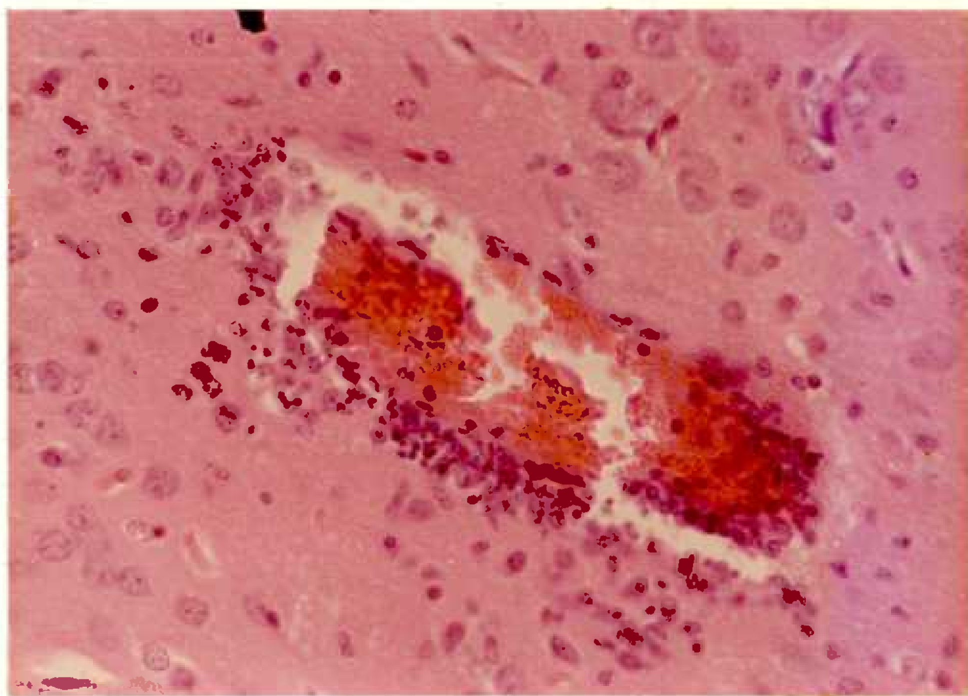

Fig.2-29 Polymorphonuclear infiltration of haemorrhagic needle track. Eighteen hours after injection of Str.milleri. (Haematoxylin and eosin x 460)

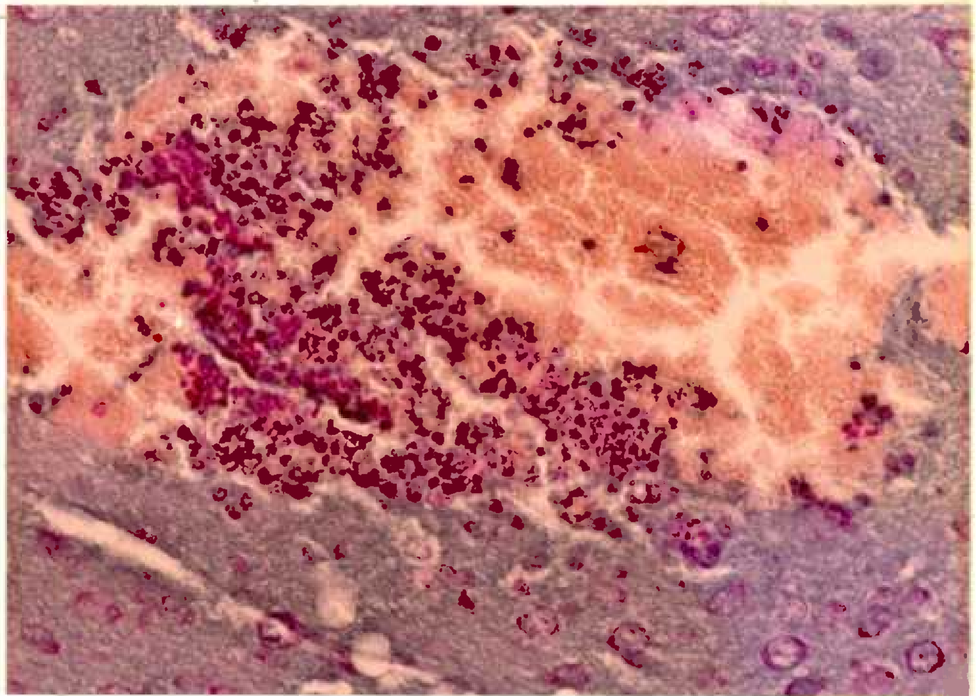

Fig.2-30 From the same animal as Fig.2-29. Magenta-staining (Gram-positive) cocci can be seen within the inflammatory exudate. Eighteen hours after injection of Str.milleri. (Sowter and McGee's stain x 460)

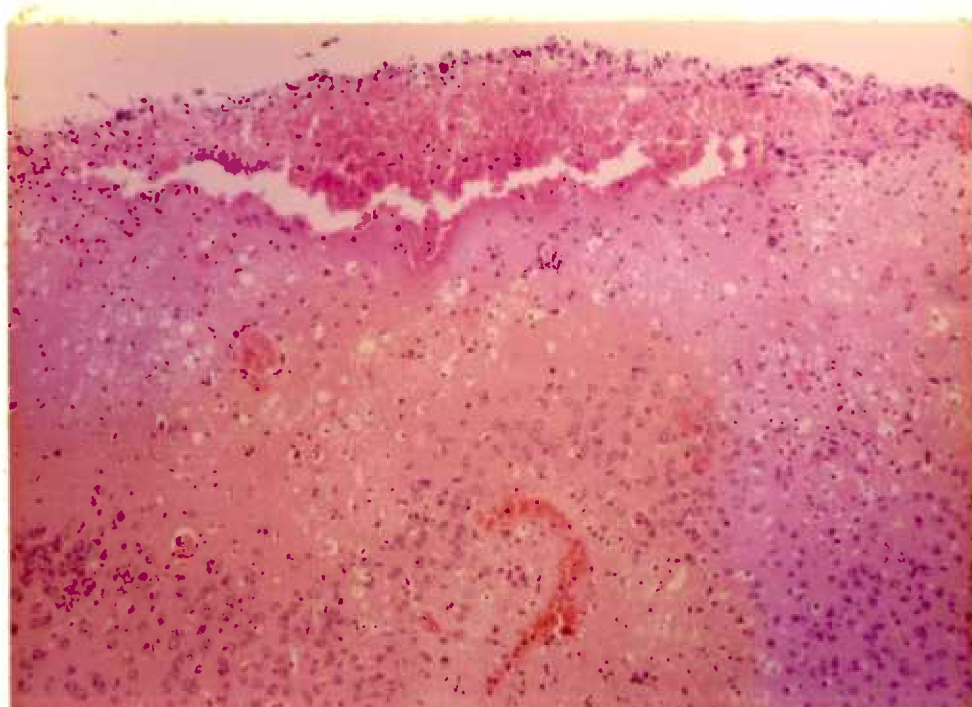

Fig.2-31 Haemorrhage in the submeningeal space. There is oedema and vacuolation in the subjacent brain tissue. Six hours after injection of Str.milleri. (Haematoxylin and eosin x 120)

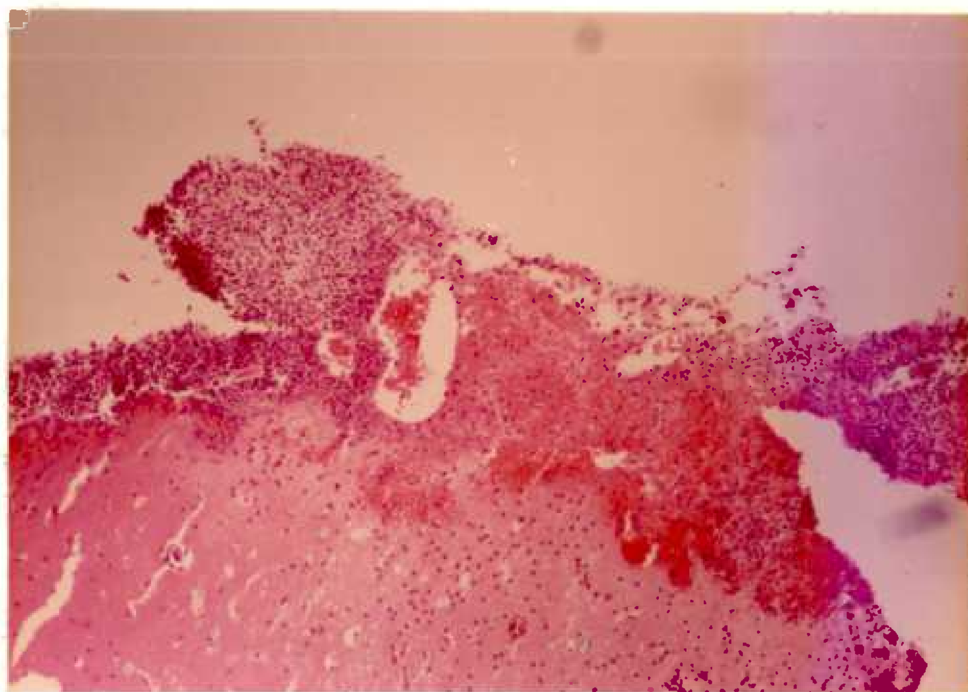

Fig.2-32 Submeningeal inflammation and haemorrhage. There is some inflammatory reaction on the cortex of the brain. Eighteen hours after injection of Str.milleri. (Haematoxylin and eosin x 120)

The histopathology can be summarised briefly:-

1. Str.agalactiae spreads rapidly throughout the meninges within 15 minutes of injection. The bacteria multiply freely during the next hour, and two hours after injection there is a well established meningitis, streptococci can be seen in the Virchow-Robin spaces, and septic infarcts are visible. By 18 hours there is a severe purulent meningitis, with formation of submeningeal abscesses. Abscesses form also in relation to the needle track, and at sites that are apparently anatomically remote from both the meninges and the needle track. The most striking lesion is the meningitis.
2. Str.mutans shows the most marked deep abscess formation, usually within 18 hours and always related to the needle track. Streptococci are abundant within the main abscess and those forming adjacent to it, but there are no lesions elsewhere in the brain. Meningeal lesions are also found.
3. The spread of Str.milleri through the meninges is much slower than Str.agalactiae although it does occur and is apparent in 18-24 hours. The initial meningeal reaction is due to mononuclear cells which are later replaced by polymorphonuclear leucocytes. Pus may form deep to the meninges or at the inoculation site but in both cases there are relatively few streptococci to be seen and the infection shows no signs of spreading to remote parts of the brain. The cellular defences of the mouse appear to be more effective at combating infection with Str.milleri than that of Str.mutans

or *Str.agalactiae*, since the lesions are more circumscribed at all stages of the disease, and there are fewer bacteria in the lesions.

Thus, infection with *Str.agalactiae* characteristically produces a severe meningitis, but also results in deep abscess formation. Infection with *Str.mutans* produces little meningitis; deep abscess formation is a feature, and is more marked than with *Str.agalactiae* but, unlike those occurring with *Str.agalactiae*, the abscesses are always related to the needle track. The lesions produced by *Str.milleri* are the most circumscribed and fewer bacteria are discernible at all stages of the disease. None of the mice killed five or more days after injection showed any discernible histological changes.

SECTION 3 ABSCESS OF THE CENTRAL NERVOUS SYSTEM IN MAN: INVESTIGATION BY THE ENUMERATIVE METHOD

Data pertaining to brain abscess were analysed retrospectively from two sources. The figures of the Registrar General for deaths due to inflammatory disease of the central nervous system for the period 1963-1973 were examined and a more detailed investigation of intracranial and intraspinal abscess occurring at two London neurosurgical units for the period 1951-1973 was made.

MORTALITY FROM INFLAMMATORY DISEASE OF THE CENTRAL NERVOUS SYSTEM IN ENGLAND AND WALES

The Registrar General's figures show that annual mortality due to inflammatory disease of the central nervous system over a ten year period was approximately 600 (Table 3-1). Deaths from specific causes are listed under the following headings: meningitis; phlebitis and thrombophlebitis of intracranial venous sinuses; intracranial and intraspinal abscess; encephalitis, myelitis and encephalomyelitis; and the late effects of intracranial abscess or pyogenic infection. Figure 3-1 represents the number of deaths per year from each of these causes for the period 1963-73. During the period 1964-72 there was a 25% reduction in the total number of deaths from inflammatory disease, which is reflected in each of the major groups. It is not clear from the figures available whether the decrease represents an increased survival time in these conditions or decreasing incidence. Table 3-1 shows the sex distribution of patients who died from inflammatory disease. For the period as a whole, the mortality among men was 20% higher than for women. This difference is reflected in each group with the exception of that of intra-

Table 3-1

DEATHS FROM INFLAMMATORY DISEASE OF THE CNS:
ENGLAND AND WALES, 1963-1973

	Male	Female	Total
1963	371	313	684
1964	394	316	710
1965	359	308	667
1966	340	291	631
1967	373	277	650
1968	343	244	587
1969	332	232	564
1970	328	238	566
1971	303	251	554
1972	290	240	530
1973	283	251	534

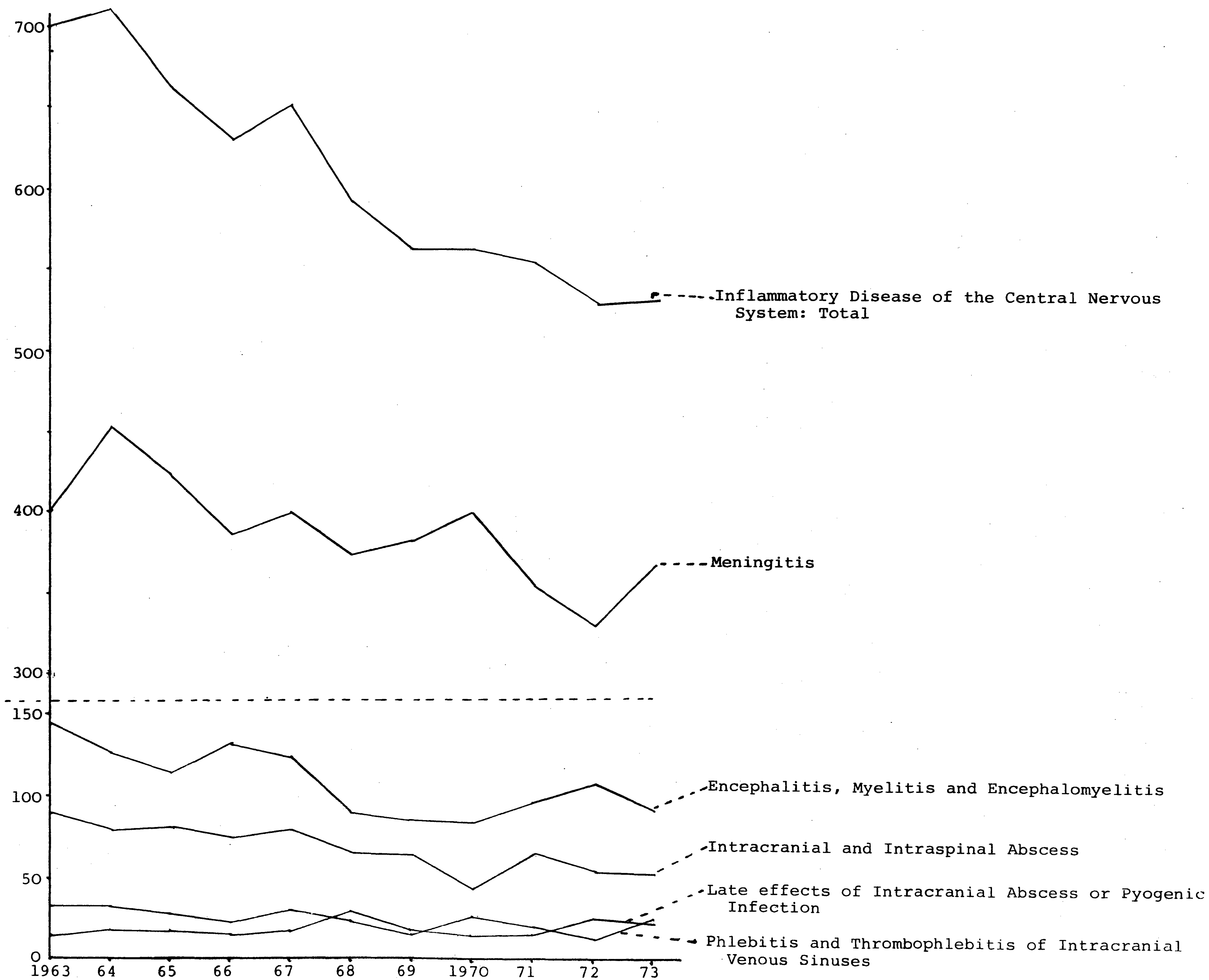

Fig.3-1 DEATHS FROM INFLAMMATORY DISEASE OF THE CENTRAL NERVOUS SYSTEM: REGISTRAR GENERAL'S STATISTICAL REVIEW OF ENGLAND AND WALES - 1963/1973

cranial or intraspinal abscess. In this group, there were approximately twice as many deaths for men as for women. Deaths from intracranial or intraspinal abscess constitute approximately 12% of the total deaths due to inflammatory disease for each of the years studied (Table 3-2). Deaths resulting from the late effects of intracranial abscess or pyogenic infection remained fairly constant during the eleven year period and accounted for approximately 3% of the total deaths (Table 3-3).

When the figures from Table 3-2 and 3-3 are combined it is apparent that not only have the number of deaths from intracranial and intraspinal suppuration decreased during the eleven year period, but also that the period 1963-1968 can be considered separately from the period 1969-1973 (Table 3-4). During the latter period, the number of deaths resulting from an abscess or its late effects was 33% lower than for the previous six years. It is also apparent that deaths from these causes constitute a decreasing percentage of the total deaths from inflammatory disease of the central nervous system falling from 14.6% for the period 1963-1968 to 12.3% for the period 1969-1973.

INTRACRANIAL AND INTRASPINAL ABSCESS AT TWO LONDON NEUROSURGICAL UNITS: 1951-1973

The total number of cases admitted to each hospital during this period was obtained from the hospital records. This information, together with the departmental records of the neurosurgical units was used to trace as many of the clinical notes as were available, which were then analysed.

During the period of the study a total of 257 patients were admitted to the two units, 120 to Centre 1 and 137 to

Centre 2. Unfortunately, like the Registrar General's returns, the hospital index records do not differentiate between cases of brain abscess and those with abscesses elsewhere in the central nervous system. The distribution of the 257 cases over the 23 years of the study, together with the percentage mortality rates, is shown in Table 3-5. It is apparent that during this period there was little decline in the incidence of brain abscess at Centre 1, while at Centre 2 the number of cases admitted declined steadily during the 23 year period. As nothing is known about changes in the referral system, or catchment areas, of the two units during this time, no conclusions can be drawn from the differences in incidence. There were 186 men and 71 women with intracranial and intraspinal abscess, a ratio of 2.6:1. Both Centres experienced a steadily declining mortality rate during the period 1950-1965 (Table 3-6). However, during the next five years, the mortality rate at both Centres rose five-fold, and at both Centres the mortality rates were highest for the period 1966-1968. It would appear that the rate is now dropping again, although it is still significantly higher than the pre-1965 level. For the period as a whole the mortality at Centre 1 (36%) was higher than that at Centre 2 (28%). This was largely due to the differences in mortality rate of women at the two Centres. In Centre 1, 41% of 47 women died, while in Centre 2, $^{11}/44$ (25%) died. Few detailed records from Centre 1 were available prior to 1960, due to a policy of discarding patients' notes after ten years. In addition, many of the notes of patients who died in the period 1960-1973 were also missing.. The clinical notes available from Centre 2 were far more comprehensive, 56% of

the notes were recovered and they constituted a random sample in terms of distribution over the 23 year period, the sex and age of the patients and their mortality. The age distribution of the patients whose notes were studied is shown in Table 3-7. They do not show any association between age and the incidence of brain abscess.

The infections believed to have predisposed these patients to the development of intracranial abscess are shown in Table 3-8. In 21 cases no reference was made to a predisposing cause, and in many instances, it was not clear from the notes whether abscesses were regarded as cryptogenic. The majority of these 21 cases occurred in Centre 2 as did $^{10}/11$ of the lesions which occurred following a respiratory tract infection. The sites of abscess formation in the 111 patients studied is shown in Table 3-9. The preponderance of temporal and frontal lobe abscesses ($^{55}/111$) is to be expected, but there seems to be an unusually large number of patients with parietal and cerebellar abscesses, especially from Centre 2.

Table 3-10 gives details of the micro-organisms isolated from the 111 patients. Samples from 103 patients were cultured and in 53 (51%) the specimens were bacteriologically sterile, even though in half of them (26) bacteria had been seen microscopically. Streptococci constituted 50% ($^{43}/87$) of the bacteria isolated and 20 of these isolates were reported to have been anaerobic streptococci. It was apparent that in many cases the anaerobic nature of these bacteria had not been confirmed.

Anaerobic bacteria constituted a higher proportion of the organisms isolated at Centre 1 (56%) than at Centre 2 (26%).

The cultural findings from Centre 2 are clearly affected by the fact that anaerobic culture of samples of intracranial pus was not a routine procedure during the early years of this study. In contrast to this situation, isolates of Staph.aureus and Esch.coli were far more frequent from Centre 2 than Centre 1. There were eight cases from which samples were either not collected or not cultured.

Table 3-2

DEATHS FROM INFLAMMATORY DISEASE OF THE CNS

ENGLAND AND WALES, 1963-1973

INTRACRANIAL AND INTRASPINAL ABSCESS

	Male	Female	Total	Percentage of deaths from inflammatory disease of CNS
1963	62	27	89	13
1964	47	32	79	11
1965	48	35	83	12
1966	51	25	76	12
1967	54	25	79	12
1968	43	24	67	11
1969	38	27	65	11
1970	34	10	44	8
1971	42	24	66	12
1972	33	23	56	11
1973	32	20	52	10

Table 3-3

DEATHS FROM INFLAMMATORY DISEASE OF THE CNS:

ENGLAND AND WALES, 1963-1973

LATE EFFECTS OF INTRACRANIAL ABSCESS OR

PYOGENIC INFECTIONS

	Male	Female	Total	Percentage of deaths from inflammatory disease of CNS
1963	9	6	15	2
1964	12	6	18	2
1965	11	7	18	3
1966	9	5	14	2
1967	12	6	18	3
1968	22	8	30	5
1969	12	3	15	3
1970	7	5	12	2
1971	9	5	14	2
1972	13	12	25	5
1973	12	12	24	5

Table 3-4

DEATHS FROM INFLAMMATORY DISEASE OF THE CNS:

ENGLAND AND WALES, 1963-1973

INTRACRANIAL AND INTRASPINAL ABSCESS AND

LATE EFFECTS OF INTRACRANIAL SUPPURATION

	Male	Female	Total	Percentage of deaths from inflammatory disease of CNS
1963	71	33	104	15
1964	59	38	97	13
1965	59	42	101	15
1966	60	30	90	14
1967	66	31	97	15
1968	65	32	97	16
1969	50	30	80	14
1970	41	15	56	10
1971	51	29	80	14
1972	46	35	91	16
1973	44	32	76	15

Table 3-5

INCIDENCE OF INTRACRANIAL AND INTRASPINAL ABSCESS AT TWO LONDON NEUROSURGICAL UNITS (1951-73)

	CENTRE 1						CENTRE 2					
	Male		Female		Total		Male		Female		Total	
1951-55	20	(45%)*	8	(50%)	28	(46%)	39	(44%)	16	(13)	55	(35%)
1956-60	17	(36%)	2	(0%)	19	(32%)	19	(5%)	11	(45%)	30	(20%)
1961-65	20	(10%)	8	(12%)	28	(11%)	15	(6%)	9	(11%)	24	(8%)
1966-70	26	(48%)	7	(71%)	33	(52%)	11	(36%)	6	(50%)	17	(41%)
1971-73	10	(30%)	2	(50%)	12	(31%)	9	(44%)	2	(0%)	11	(36%)
	93	(34%)	27	(41%)	120	(36%)	93	(29%)	44	(25%)	137	(28%)

* Percentage Mortality

Table 3-6

RETROSPECTIVE STUDY AT TWO LONDON NEUROSURGICAL UNITS - (1951-1973)

DATA FROM AN ANALYSIS OF CASE NOTES

	CENTRE 1			CENTRE 2		
	Male	Female	Total	Male	Female	Total
1951-55	0	0	0	23 (35%)	6 (33%)	29 (38%)
1956-60	2 (0%)*	0	2 (0%)	11 (18%)	4 (25%)	15 (19%)
1961-65	9 (0%)	4 (0%)	13 (0%)	12 (8%)	8 (0%)	20 (5%)
1966-70	9 (0%)	2 (0%)	11 (0%)	8 (25%)	2 (0%)	10 (20%)
1971-73	7 (16%)	1 (100%)	8 (25%)	2 (50%)	1 (0%)	3 (33%)
	27 (4%)	7 (16)	34 (6%)	56 (28%)	21 (15%)	77 (23%)

* Percentage Mortality

Table 3-7

RETROSPECTIVE STUDY OF CNS ABSCESSES AT TWO LONDON NEUROSURGICAL CENTRES

AGE AND SEX DISTRIBUTION

	Centre 1		Centre 2		Total	
Age (yrs)	M	F	M	F	M	F
0-10	2	3	7	6	9	9
11-20	9	1	9	4	18	5
21-30	2	-	11	2	13	2
31-40	1	-	9	2	10	3
41-50	3	2	12	0	15	3
51-60	4	1	6	4	10	5
> 60	4	-	2	3	6	3
Unknown	2	-	-	-	2	-
	27	7	56	21	83	30

Table 3-8

RETROSPECTIVE STUDY OF BRAIN ABSCESSES AT TWO LONDON NEUROSURGICAL CENTRES

PREDISPOSING CAUSE

	Centre 1	Centre 2	Total
Otitic infection	10	31	41(37%)
Sinusitis	8	7	15(14%)
Trauma - accidental	3	4	7(6%)
- post-operative	3	6	9(8%)
Upper Respiratory Tract Infection	1	10	11(10%)
Meningitis	-	2	2(2%)
Others	4	1	5(5%)
Unknown, or unrecorded	5	16	21(19%)
	34	77	111

Table 3-9

RETROSPECTIVE STUDY OF BRAIN ABSCESSES AT TWO LONDON NEUROSURGICAL CENTRES

SITES OF INFECTION

	Centre 1	Centre 2
Frontal	9	15
Temporal	8	23
Parietal	5	11
Occipital	-	4
Subdural	9	6
Cerebral	1	1
Cerebellar	2	8
Others	-	5
Unrecorded	-	4
	34	77

Table 3-10

RETROSPECTIVE STUDY OF BRAIN ABSCESSES AT TWO LONDON NEUROSURGICAL CENTRES

DISTRIBUTION OF INFECTING ORGANISMS

	Centre 1 (34 patients)	Centre 2 (77 patients)	Total (111 patients)
Anaerobic streptococci	9	11	20
Other streptococci	4	19	23
Bacteroides group	5	5	10
Proteus spp.	5	5	10
E.coli	-	5	5
Staph.aureus	2	11	13
Others	-	6	6
Total isolates:	25	62	87
Sterile samples (film negative)	9	18	27
Sterile samples (film positive)	4	22	26
Not cultured/no specimen	3	5	8

SECTION 4 PROSPECTIVE STUDY OF 50 PATIENTS WITH INTRACRANIAL OR INTRASPINAL ABSCESS : MATERIALS AND METHODS.

ORGANISATION AND GENERAL PLAN OF THE STUDY

Surgeons at neurosurgical centres in England and Wales were invited to join in a multicentre prospective study of abscess of the central nervous system. The microbiological colleagues of those who accepted the invitation were informed of the study and their co-operation was sought. Results obtained during the study were passed to the consultant microbiologist as well as to the appropriate surgeon, thus avoiding embarrassment that might be caused by conflicting reports.

Specimens were sent by neurosurgeons from the following centres:-

Atkinson Morley's Hospital	London
Frenchay Hospital	Bristol
Regional Centre for Neurosurgery	Romford
St.Bartholomew's Hospital	London
Walton Hospital	Liverpool
Wessex Neurological Centre	Southampton

Collection and Despatch of Samples

The participating centres were issued with bright orange boxes 14" x 14" x 10" (Fig.4-1) which contained the following:

1. Proforma and instruction sheet

The box contained a short proforma (Fig. 4-2) for completion by the surgeon together with an instruction sheet giving details on the use of the coldpack, the packaging of specimens and the transport arrangements for specimens from the hospital concerned. An addressed label was also included.

Fig.4-1 The specimen collection box showing the collection kits for pus and CSF, blood culture and swabs together with an instruction sheet and proforma.

STUDY ON INTRACRANIAL SUPPURATION

HOSPITAL:

PATIENT'S NAME:

PATIENT'S HOSPITAL NUMBER AGE: M/F

DATE OF OPERATION:

NAME OF SURGEON:

SITE OF ABSCESS:

SPECIMENS COLLECTED (please tick):
a) Pus
b) CSF
c) Blood culture
d) Clotted blood
e) Nose and throat swabs
f) Ear swabs

HAS PATIENT ALREADY BEEN TREATED WITH ANTIBIOTICS?: YES/NO

IF SO, WHICH? (please tick):
Penicillin
Streptomycin
Cephaloridine
Sulphonamides
Chloramphenicol
Others (please specify)

WHICH ANTIBIOTICS WILL BE USED TO TREAT THE PATIENT NOW?:

a) local dose b) Systemic dose

Penicillin ______________________________

Streptomycin ______________________________

Cephaloridine ______________________________

Sulphonamides ______________________________

Chloramphenicol ______________________________

Others (please specify) ______________________________

OTHER RELEVANT INFORMATION

YOUR CITY LINK TELEPHONE NUMBER:-

Fig.4-2 PROFORMA FOR THE PROSPECTIVE STUDY

2. A coldpack

This contained a 'Freezella' sachet (Raven Scientific Ltd.) and was designed so that samples of pus and CSF could be transported at 4°C. The empty coldpack was kept in the ice box of the neurosurgical theatre refrigerator until required. The coldpack contained three sterile universal bottles. Two of these labelled 'pus' and 'CSF' were empty, the third labelled 'pus' contained 15 ml Brewer's thioglycollate medium to which had been added 0.25 ml β-lactamase solution (Whatman Laboratories).

3. Blood collection set

This consisted of two bottles of digest broth (50 ml) to which 0.1% dextrose and p-amino benzoic acid had been added (Southern Group Laboratories), one bottle of Brewer's thioglycollate medium (50 ml) (Southern Group Laboratories) and a sterile universal bottle for clotted blood. β-lactamase solution (0.25 ml) was added to the blood culture media immediately prior to despatch.

4. Stuart's transport medium

Four bijou bottles containing Stuart's transport medium (6 ml), together with sterile cottonwool swabs, were included for the collection of nose, throat and ear swabs (2).

The boxes were packed with polystyrene chips for insulation. It had been shown previously that under the conditions used for transportation the coldpack would maintain specimens at 4°C for 24 hours.

Transport of Specimens

Samples from neurosurgical centres in the Greater London area were transported to the examining laboratory by mini-cab. The more distant centres were served by City-Link who collected

the orange box, despatched it by rail and delivered it to the examining laboratory. The system worked well although it was restricted by the fact that while the City-Link, London Office, would operate a twenty-four hour delivery service, the despatch offices only operated from 7am to 7pm Monday to Saturday. Despite these restrictions, and with one exception, all specimens reached the examining laboratory within eight hours of collection.

Clinical Reports

A Gram-stained smear was examined immediately the samples arrived and the results telephoned to the neurosurgeon. This was usually followed 10-18 hours later by a provisional report of the infecting organism, its sensitivity to a range of drugs and the results of assay of antimicrobial drugs present in the pus, blood and CSF. These results were telephoned and a written report followed when the identity of the infecting organism(s) and the minimum inhibitory concentration (MIC) of drugs being used for treatment had been determined.

Interim reports, giving cumulative details of all the cases studied and the results obtained, were issued every six months to all centres participating in the study. Case summaries, postmortem reports and other information were provided by the neurosurgeons on completion of the study.

BACTERIOLOGICAL INVESTIGATIONS

Examination of Specimens

Having been informed by telephone that samples were about to be despatched, the examining laboratory prepared sets of appropriate antibiotic assay standards in horse serum which

were stored at 4°C. Antibiotic assays on serum, CSF and pus were put up immediately the report on the Gram-stained smear had been telephoned.

Specimens of pus (in Brewer's medium) and of CSF, and ear, nose and throat swabs were cultured immediately on receipt.

The media used were :-

Medium	Incubation
Horse blood agar	- incubated aerobically
" " "	- " anaerobically
Crystal violet blood agar	- " anaerobically
Nalidixic acid blood agar	- " anaerobically
Neomycin blood agar	- " anaerobically
Cysteine hydrochloride blood agar	- " anaerobically
Chocolated blood agar	- incubated in 10% carbon dioxide
MacConkey agar	- incubated aerobically

All the plates were incubated for up to seven days.

Direct antibiotic sensitivity tests using Mast and Oxoid discs, were also put up on chocolated blood agar plates which were incubated anaerobically and in 10% carbon dioxide.

The Brewer's medium containing pus was incubated at 37°C and subcultured to the media listed above again after 24 and 48 hours.

The blood culture media were incubated at 37°C and were subcultured after 1, 2 and 7 days to two blood agar plates, for incubation aerobically and anaerobically, and to chocolated blood agar, for incubation in 10% carbon dioxide.

Following incubation, all isolates from samples of pus, CSF and blood were identified, and the minimum inhibitory concentration of the antibiotics being used in treatment were determined for all organisms isolated from pus. Cultures

from the nose, throat and ears were examined routinely only for organisms present in samples of pus, CSF or blood from the same patient, although the unassociated presence of certain organisms, for example, Staph.aureus and Pseudomonas aeruginosa was recorded.

Identification of Bacteria

Routine bacteriological procedures based on the methods and nomenclature of Cowan and Steel (1965) were used to identify aerobic isolates other than the green streptococci. Anaerobic organisms were identified using the API system with the API 20 A test kit. The API 20 A system was also used for some of the streptococcal isolates to establish its value in identifying these organisms. The identifying methods used for the green and indifferent streptococci were, in general, those described by Colman (1970). The organisms were classified according to the scheme proposed by Colman (1970) and Colman and Williams (1972). In cases of doubt the organisms were not allocated to a specific group.

Anaerobic Procedure

The anaerobic system used throughout this study consisted of Mackintosh and Fildes jars with three catalysts (Watt et al. 1973). The catalysts were changed weekly and were dried before use. Gaspacks (BBL) were used to provide the anaerobic environment and Pseudomonas aeruginosa on nutrient agar and an anaerobic indicator strip (BBL) were used as anaerobiosis controls. A carbon dioxide (10%) environment was produced by Gaspacks in anaerobic jars from which the catalyst had been removed.

Culture Media - General

The basic medium used throughout this study was 6% horse blood agar prepared from columbia blood agar base (Oxoid), according to the manufacturer's instructions. In addition, blood agar plates were prepared which contained either 0.004% crystal violet, 0.004% nalidixic acid (Bayer), 0.01% neomycin base (Upjohn) or 0.03% cysteine hydrochloride. The additives for these media were dissolved in water and sterilised by membrane filtration prior to storage at 4°C. Chocolated blood agar was prepared by heating the cooled blood medium to 80°C for 10 minutes, with thorough gentle mixing. The medium was again cooled to 50°C, mixed, and poured. MacConkey agar and Diagnostic Sensitivity Test agar (DST) were prepared from dehydrated ingredients (Oxoid). Blood culture broth (digest broth with 0.1% glucose), Brewer's medium and Stuart's transport medium were purchased ready for use from Southern Group Laboratories.

The broth medium, used routinely for the growth of aerobic and microaerophilic organisms, consisted of:-

Brain heart infusion (Oxoid)	37.0g
Yeast extract (Oxoid)	5.0g
Glucose (Oxoid)	5.0g
Water	1 litre

The broth medium for gas liquid chromatography contained:

Sodium thiosulphate	0.05g
Sodium formaldehyde sulphoxylate	0.03g
Peptone (Oxoid)	1.00g
Yeast extract (Oxoid)	1.00g
Sodium chloride	0.25g

Glucose (Oxoid)	1.00g
L-cysteine hydrochloride	0.05g
Vit.K/Hemin (Sigma)	1.00g

Distilled water to 100 ml

The pH of the medium was adjusted to 7.0 and it was sterilised at 115°C for 15 minutes.

<u>Cultural Methods Used for Characterising Streptococci</u>

1. <u>Salt broth</u>

Nutrient broth (Oxoid - No.2)		100 ml
Sodium chloride	either	3.5g
	or	6.0g
Glucose		0.5g

The medium was bottled in 3 ml aliquots and sterilised at 121°C for 15 minutes.

The final salt concentrations were 4.0% and 6.5%.

<u>Test</u>: Add 1 drop of an overnight broth culture to the two salt media and to a control broth (with no added salt). Incubate at 37°C for seven days. Turbidity of the broth indicates a positive result.

2. <u>Arginine hydrolysis</u>

The ability of organisms to hydrolyse arginine and liberate ammonia was tested by growing them for seven days in the following medium (Niven et al.1942):-

Yeast extract powder (Oxoid)	5.0g
Tryptone (Oxoid)	5.0g
Di-potassium hydrogen phosphate	2.0g
Glucose	0.5g
L-arginine monohydrochloride	3.0g
Distilled water	to 1 litre

The ingredients were dissolved and the medium adjusted to pH 7.0 and sterilised at 115°C for 15 minutes.

Test: Reagent A 0.106 M.Phenol
0.17 mM.Sodium nitroprusside
in water

Reagent B 11 mM Sodium hypochlorite
0.125 N.Sodium hydroxide
in water

Free ammonia was detected by adding 1 ml of a 1/10 dilution of culture medium in water to a freshly prepared mixture of 1 ml reagent A and 1 ml reagent B.

A positive reaction was demonstrated by the production of a blue colour. Arginine positive and negative organisms and an uninoculated broth were included as controls.

3. Acetylmethylcarbinol production from glucose - the Voges-Proskauer reaction

The following medium was used:-

Tryptone (Oxoid)		10.0g
Yeast extract powder (Oxoid)		5.0g
Di-potassium hydrogen phosphate		5.0g
Glucose		5.0g
Distilled water	to	1 litre

The ingredients were dissolved and the medium adjusted to pH 7.3 with 1N hydrochloric acid. It was then dispensed into 4 ml aliquots and sterilised at 115°C for 15 minutes.

Test: The formation of acetylmethylcarbinol was detected by mixing 1 ml 6% α-naphthol in ethanol and 0.4 ml 40% potassium hydroxide in water with 2 ml of a 4 day broth culture. A positive reaction was indicated by the development, at room temperature, of a pink/red colour within 15 minutes.

4. <u>Production of acid from fermentable substrate</u>

Serum peptone water sugar medium was prepared aseptically as follows:-

Sterile horse serum	20 ml
Sterile peptone water	70 ml
10% substrate in 1% peptone water (Seitz filtered)	10 ml
0.2% Phenol red (sterile)	1 ml

The following substrates were used:- glucose, mannitol, arabinose, lactose, sucrose, trehalose, raffinose, salicin, inositol and sorbitol.

Cultures were incubated in 10% carbon dioxide at 37°C and were examined after one, two and seven days incubation, for a yellow to red colour change.

Peptone water was prepared as follows:-

Peptone	10g
Sodium chloride	5g
Distilled water	1 litre

The ingredients were dissolved, heated to boiling and cooled. The medium was adjusted to pH 7.3 and sterilised in 70 ml aliquots at 121°C for 15 minutes.

5. <u>Aesculin Hydrolysis</u>

Aesculin agar (Cowan and Steel 1965) was prepared as follows:-

Aesculin	1.0g
Ferric citrate	0.5g
Agar	20.0g
Peptone water	1 litre

The aesculin and ferric citrate were dissolved in half the peptone water and the agar in the other half. The two solutions were mixed, dispensed in 3 ml aliquots in bijou

bottles and autoclaved at 115°C for 10 minutes. The medium was allowed to set in an sloped position.

Test: Slopes were inoculated with test and control organisms and incubated at 37°C for seven days. A positive result was indicated by blackening of the medium.

6. Polysaccharides from sucrose

Medium

Broth base

Tryptone (Oxoid)	14g
Yeast extract powder (Oxoid)	5g
Sodium acetate	20g
Distilled water	1 litre

at pH 7.6.

Dispense into 100 ml aliquots and sterilise at 121°C for 10 minutes. Add 8.5g sucrose (glucose for controls) to 100 ml broth base, dissolve and sterilise by filtration. Add 1 ml aquous potassium carbonate solution (5.5%), previously sterilised at 121°C for 10 minutes, and dispense aseptically in 20 ml volumes into McCartney bottles.

Method

Inoculate each strain under test to one bottle of glucose medium and one of sucrose medium. Incubate for 5 days at 37°C. After incubation centrifuge the cultures and dilute 0.5 ml supernatant with 4.5 ml of 10% sodium acetate in McCartney bottles. Prepare two bottles containing sucrose supernatant and one containing glucose supernatant. Add 1.2 volumes (6 ml) of absolute alcohol to one of the sucrose bottles and 2.5 volumes (12.5 ml) absolute alcohol to the other two bottles of diluted culture fluid. Mix well and leave on the bench.

Results

The formation of a precipitate in either or both of the sucrose bottles indicates the formation of levan or dextran. The absence of a precipitate in the culture fluid containing glucose indicates that the precipitated material has been formed from sucrose.

Controls

	Glucose	Sucrose Broth	
	Control broth	1.2 vol. Abs.alcohol	2.5 vol. abs.alcohol
Levan production (*Str.salivarius*)	-	-	+
Dextran production (*Str.sanguis*)	-	+	+

Str.bovis (NCTC 8177) was used to control each batch of medium. This organism produces large amounts of precipitate in both concentrations of absolute alcohol as well as characteristic gelling of the sucrose broth during growth.

7. Catalase production

Strains to be tested were grown on infusion agar containing 5% horse serum (Colman 1970). Drops of 3% (10 volumes) hydrogen peroxide were placed on individual colonies and examined for the appearance of bubbles within one minute.

8. Nitrofurazone sensitivity

Blood agar plates were inoculated with overnight broth cultures of the test and control organisms using sterile swabs. Two blotting paper discs containing 10ug nitrofurazone were added to each plate. Following overnight incubation at 37°C the plates were examined for zones of inhibition. A zone of 9mm or more was regarded as indicative of sensitivity (Colman 1970).

9. Growth on media containing bile salts

Dehydrated ox-gall (Difco) was added to nutrient agar at concentrations of 4.2% and 1.05%. The medium was autoclaved in 100 ml volumes at 115°C for 10 minutes and cooled to 45°C, 5 ml horse serum was then added. The medium was poured into petri dishes containing blood agar, one half of which had previously been removed. The medium was used on the day of preparation.

Test: Strains were tested by passing a loopful of broth culture first across the blood agar and then across the bile agar. Following incubation for 48 hours positive results were recorded for those strains which grew on both halves of the plate. All other results were recorded as negative.

10. Hyaluronidase production

Hyaluronidase production by clinical isolates was detected by means of a streptococcus which produced hyaluronic acid-containing capsules. (Lancefield group C strain D 181, kindly provided by the Cross Infection Reference Laboratory, Colindale).

Plates of brain heart infusion agar (Oxoid) containing 5% horse blood were prepared and streak inoculated with strain D 181. Test strains were inoculated in the same way at right angles to the indicator. Control strains which produced hyaluronidase rendered the indicator strain non-mucoid for a distance of 5-10mm either side of the test strain. Non-producers had no apparent effect on the indicator organism. *Str.pneumoniae* and *Str.agalactiae* were used as positive controls.

SEROLOGICAL INVESTIGATION OF STREPTOCOCCI

Production of Antisera

Antisera to the following strains were prepared in rabbits:-

Streptococcus milleri	O'Neill (a clinical isolate)
" "	NCTC 10708
" "	NCTC 8037
" "	Ottens and Winkler type O I
" "	Ottens and Winkler type O II
" "	Ottens and Winkler type O III
" "	Ottens and Winkler type O IV
Streptococcus agalactiae	NCTC 9993
Streptococcus mutans	NCTC 10449

The Ottens and Winkler type strains which lack a Lancefield group antigen were provided by Dr. M.T. Parker, Cross Infection Reference Laboratory, Colindale.

Strains were grown for 48 hours (24 hours for *Str. agalactiae*) in brain heart infusion broth (Oxoid) containing 0.5% added glucose sterilised at 121°C for 15 minutes (Ottens and Winkler 1962). The culture was centrifuged and the deposit washed three times in normal physiological saline (NPS). The final deposit was resuspended in NPS to $^1/5$ the original volume and heated at 60°C in a waterbath for 30 minutes. The concentrated antigen was stored at 4°C for use. Rabbits weighing 2-3 kilograms were injected into the marginal vein of the ear with a $^1/10$ dilution of antigen in NPS. Doses of 0.5 ml were administered on three consecutive days followed by six daily doses of 1.0 ml. Five days after the last injection blood was taken and tested for precipitins. If precipitins were present 30 ml blood was removed. When the precipitin reaction was weak or negative rabbits were given three further injections of 1.0 ml and tested again

five days later. Four weeks after being bled rabbits received 1.0 ml of the concentrated antigen on two consecutive days and were again bled five days later.

Absorption of Antisera

Cross-reactions between heterologous strains of streptococci could usually be diluted out. When this was not possible the antisera were absorbed. The heterologous strain was grown for 48 hours in 50 ml brain heart infusion broth (Oxoid) with 0.5% added dextrose. The growth was harvested by centrifugation, washed and collected into a conical centrifuge tube. Saline was added to give a volume of 0.5 ml, and to this was added 0.5 ml antiserum. After thorough mixing the stoppered tube was incubated at 37°C for 30 minutes. The mixture was centrifuged and the resultant supernatant recentrifuged. The final supernatant was preserved with $^1/10,000$ Merthiolate (Eli Lilly) and stored at -20°C.

The antisera produced all reacted with their homologous antigens at titres in excess of $^1/64$. There was cross reaction between the four Ottens and Winkler type sera when they were used neat. At a dilution of $^1/10$ the type sera of OI, OII and OIII were specific but type serum OIV still cross reacted with type OII. It was, therefore, absorbed, after which it gave specific reactions. The antisera to Str. milleri (O'Neill), Str.milleri (NCTC 10708) and Str. milleri (NCTC 8037) all reacted specifically with the Ottens and Winkler type OIII antigen.

Extraction Methods for Streptococcal Type and Group Antigen

1. Lancefield method

A 48 hour culture of Todd-Hewitt Broth (50 ml) (Oxoid), pH 7.3 was centrifuged and 0.4 ml hydrochloric acid (0.1N)

added to the deposit. The suspension was boiled for 10 minutes in a waterbath, cooled and neutralised with 0.1N sodium hydroxide using phenol red as the indicator. The suspension was centrifuged and the supernatant retained as the antigen.

2. Pronase method

The organism was grown as before and 0.5 ml pronase solution (25 mg/ml) (Koch-Light) was added to the centrifuged deposit. The mixture was incubated at 37°C for two hours and then autoclaved at 121°C for 15 minutes. Following centrifugation the supernatant was retained as antigen. (Edwards and Larson 1973).

3. Maxted's method

Enzyme was extracted from Streptomyces albus by the method of Maxted (1948), tested, and stored in 2 ml aliquots at -20°C for use. Strains of streptococci were grown as above. Following centrifugation some of the deposit was added to 2 ml enzyme solution. The mixture was incubated in a waterbath at 50°C for two hours or until it cleared. Following centrifugation the supernatant was used as antigen.

Antigens were prepared from clinical isolates of streptococci by the three methods described and were tested against antisera to Lancefield's groups A, C, D, F and G (Burroughs Wellcome). Antigens from isolates of Str.milleri were also tested against antisera to Ottens and Winkler types OI - OIV.

Counter-Current Immunoelectrophoresis (CIE)

Following preliminary investigations it was found more convenient to use CIE for serological studies and this was used for all serological investigations on clinical isolates

and specimens.

CIE was used with a number of antigen, antibody and pH variables. The method found to be most satisfactory was as follows:-

1. Molten agarose (1%) in 0.05M barbitone buffer pH 8.6 was cooled and 7.5 ml pipetted onto 83 x 83 mm glass slides.
2. Two rows of holes 4 mm in diameter were cut in the agarose. The distance between the rows was 6 mm and between holes in the same row the distance was 10 mm.
3. Streptococcal antigens were placed in one row and antisera in the other.
4. A Shandon electrophoresis tray was used in which the reservoirs were filled with 0.05M barbitone buffer pH 8.6. The agarose slide was placed in the tray so that the antibody containing wells were nearest to the cathode. Blotting paper wicks were used to complete the circuit. A constant current of 1.3 mA/cm was passed for one hour at room temperature.
5. The plate was examined for lines of precipitation.

GAS LIQUID CHROMATOGRAPHY

Samples of pus, cerebrospinal fluid and broth cultures were passed through a gas chromatograph to determine the presence of short and long chain fatty acids. The system used was a Pye series 104, model 64, gas chromatograph with a glass column 5ft long and 4 mm internal diameter. The packing material was Chromosorb 101, mesh 80/100 (Field Instruments Ltd.) which was not coated. Using this column it was not necessary to extract or esterify the specimens. Samples of one µl of crude material were injected directly onto a precolumn packed with the same material. The injection port

was maintained at a temperature of 200°C while the flame ionisation detector was at 240°C. The carrier gas was nitrogen, at a flow rate of 40 ml per minute, and the machine was run at an attenuation of 2×10^{-2}. The results were traced on to a linear chart recorder.

A standard solution of fatty acids was prepared in water and contained the following:-

acetic acid	5 µmols/ml
propionic acid	"
isobutyric acid	"
butyric acid	"
isovaleric acid	"
valeric acid	"
lactic acid	20 µmols/ml
isocaproic acid	"
caproic acid	"
succinic acid	"

The culture medium used in cases where organisms were grown before being chromatographed was as follows:-

Sodium thiosulphate	0.5g
Sodium formaldehyde sulphoxylate	0.3g
Peptone	10.0g
Yeast extract	10.0g
Sodium chloride	2.5g
Glucose	10.0g
L-cysteine hydrochloride	0.5g
Vitamin K/Hemin *(Sigma)	10.0g
Distilled water	1 litre

*Prepared as a solution and sterilised separately by Seitz filtration.

PRESERVATION OF CLINICAL ISOLATES

All clinical isolates were preserved in liquid nitrogen in a growth medium to which had been added 16% glycerol and 5% horse blood. The basal medium used for anaerobic bacteria was that used for G.L.C. - see above. For aerobic and microaerophilic bacteria the basal medium was the brain heart infusion broth described previously. Cultures were grown for 24-48 hours, dispensed in 0.5 ml aliquots into three screw top polypropylene tubes and immediately frozen in liquid nitrogen vapour. All of the cultures preserved in this way survived well.

ANTIBIOTIC SENSITIVITY TESTING AND ASSAY

ANTIBIOTIC SENSITIVITY TEST

Test strains were suspended in peptone water so as to make the suspension faintly opalescent. A sterile swab, having been dipped into the suspension and drained, was spread evenly over the surface of the agar and appropriate antibiotic discs (Oxoid and Mast) added. The plate was incubated without prediffusion, in an appropriate atmosphere.

The following control organisms were included:- *Escherichia coli* (NCTC 10418), *Str.milleri* (NCTC 10708), *Staph.aureus* (NCTC 6571) and a sensitive strain of *Bacteroides melaninogenicus*.

For fastidious organisms the medium used for sensitivity testing was chocolated blood agar; for other organisms DST (Oxoid) agar was used. An organism was reported as sensitive to the antibiotic under test when the zone of inhibition was equal to or larger than that around the control.

The chemotherapeutic agents tested were as follows:-

Penicillin	2 units/disc
Ampicillin	10 mcg/disc
Cephaloridine	5 mcg/disc
Cotrimoxazole	25 mcg/disc
Sulphonamide	100 mcg/disc
Streptomycin	10 mcg/disc
Chloramphenicol	10 mcg/disc
Clindamycin	2 mcg/disc
Erythromycin	10 mcg/disc
Metronidazole	5 mcg/disc
Tetracycline	25 mcg/disc

Sensitivities to other antimicrobial drugs were tested

as required.

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (M.I.C.)

The M.I.C. of an antibiotic was determined by use of serum indicator broth (Stokes 1968, p 294).

The medium was prepared from the following ingredients which were presterilised and aseptically mixed:-

Nutrient broth	60 ml
10% glucose	15 ml
Saturated aquous phenol red	10 ml
Horse serum	25 ml

Sterility was checked by incubation at 37°C overnight. The medium was stored at 4°C.

To determine the M.I.C. of an antibiotic a two row double dilution method was used, the first row containing $^2/3$ the antibiotic concentration of tubes in the second row.

The inoculum used was 10^3 - 10^4 colony forming units per tube. The antibiotic dilutions were prepared from concentrated standards kept at -20°C. Organisms of known M.I.C. were included as controls.

The M.I.C. of antibiotic was read as the lowest concentration of drug which did not show a colour change after 24 hours incubation at 37°C in an appropriate atmosphere.

To determine the minimum bactericidal concentration (M.B.C.) of an antibiotic all tubes showing no colour change were subcultured to blood agar, and incubated at 37°C for 48 hours. The M.B.C. was the lowest concentration of antibiotic to give ten colonies or less from a 5mm loop inoculum.

ASSAY OF ANTIMICROBIAL DRUGS IN PUS, SERUM AND CEREBROSPINAL FLUID

This part of the study was undertaken to investigate the factors affecting the assay of antimicrobial drugs in pus and to devise an accurate, straightforward procedure for the assay of antibiotics in pus preliminary to a prospective study of the microbiology and treatment of brain abscess in man.

MATERIALS AND METHODS

Assay of Antimicrobial Drugs

Assay media

Antimicrobial drugs were assayed by the agar plate diffusion technique and the assay conditions used for each antibiotic are shown in Table 4-1.

The assay medium used routinely was Oxoid DST agar which contains:-

Proteose peptone	10.0g
Veal infusion solids	10.0g
Dextrose	2.0g
Sodium chloride	3.0g
Di-sodium phosphate	2.0g
Sodium acetate	1.0g
Adenine sulphate	0.01g
Guanine hydrochloride	0.01g
Uracil	0.01g
Xanthine	0.01g
Aneurine	0.00002g
Agar	12.0g
Distilled water	1 litre

Table 4-1

ASSAY SYSTEMS FOR DIFFERENT ANTIMICROBIAL AGENTS

Agent	Indicator Organism	Medium	pH
Penicillin) Ceporin)	Staphylococcus aureus (Oxford strain) NCTC 6571	DST agar	6.8
Cloxacillin	Penicillin resistant Staph.aureus	DST agar	6.8
Ampicillin	Esch.coli NCTC 10418 Staphylococcus aureus (Oxford strain) NCTC 6571	DST agar	6.8
Fusidic acid	C.xerosis FF VIII	Fusidic acid	6.0
Chloramphenicol	Esch.coli NCTC 10418 M.lutea	DST agar	6.8
Streptomycin	Esch.coli NCTC 10418	DST agar	7.8
Clindamycin	Staphylococcus aureus (Oxford strain) NCTC 6571	DST agar	7.8

The medium was dissolved by heat and the pH adjusted. It was autoclaved at 115°C for 15 minutes in 60 ml aliquots.

The medium used for the assay of fusidic acid was that recommended by Leo Laboratories and consisted of:-

Vitamin-free casamino acids (Difco)	15.0g
Yeast extract (Difco)	5.0g
L-cysteine	0.05g
Sodium chloride	2.5g
Dextrose	1.0g
Agar (Difco)	24.0g
Distilled water	1 litre

The ingredients were heated to dissolve and the pH was adjusted to 6.2. After sterilising at 121°C for 20 minutes, the final pH was 6.0 $\pm$ 0.1.

Assay procedure

Sixty millilitres of the appropriate assay medium cooled to 50°C were poured into a 6" diameter petri dish (Sterilin) on a levelling table. When the medium had set the plate was labelled and dried at 37°C for 30-40 minutes. A suspension of indicator organisms, consisting of a log-phase culture diluted to faint opacity and then further diluted $^{1}/100$ in nutrient broth, was used to flood the plate. Excess broth was removed and the plate was again dried, before being kept at 4°C for use within four days. Before use, wells were cut with a No. 2 (6 mm diameter) or No. 3 (8 mm diameter) sterile cork borer. The wells were filled at random with test and standard solutions in duplicate. The plate was allowed to prediffuse at 4°C for one hour before being incubated at 37°C overnight or at 30°C for assay of cloxacillin. Zones of

inhibition were read with calipers. The zone diameters of the standards were plotted against the log of their concentration. The concentrations in the test fluids were determined from the line of best fit drawn through the standard points.

Assay of Antibiotic Mixtures

Four procedures were used to assay mixtures of antimicrobial drugs.

1. Use of resistant indicator organisms.

Klebsiella aerogenes (NCTC 10896) which, if carefully maintained, is resistant to all the common antimicrobial drugs except the aminoglycosides, was used for the assay of gentamicin, kanamycin and streptomycin.

Esch.coli (NCTC 10418) was used for the assay of ampicillin in the presence of penicillin.

Corynebacterium xerosis (FFM V - Leo Laboratories) is selectively resistant to fusidic acid, clindamycin and chloramphenicol but otherwise sensitive to antibiotics in general use. It was used to assay penicillins and tetracyclines in the presence of agents named above. *C.xerosis* (FFZN6 IV - Leo Laboratories) which is resistant to fusidic acid but otherwise fully sensitive was used to assay clindamycin in the presence of fusidic acid. *C.xerosis* (FF VIII - Leo Laboratories) is a fully sensitive strain which was used as a control for assays requiring the use of the other two corynebacteria.

2. Inactivation of antimicrobial drugs

The following inactivators were used:-

i) beta-lactamase I for the inactivation of penicillin and ampicillin;

ii) beta-lactamase I and II for the inactivation of all

the penicillins and cephalosporins (Waterworth 1973);

iii) p-aminobenzoic acid for the inactivation of sulphonamides;

iv) thymidine for the inactivation of trimethoprim.

Beta-lactamase I was obtained by incubating penicillinase solution (Burroughs Wellcome) at 37°C until all the beta-lactamase II which was present had been inactivated, as determined by its controlled effect on a standard concentration of cloxacillin. Beta-lactamases I and II were purchased as a mixture from Whatman Laboratories in a freeze-dried form. When reconstituted the solution was moderately stable at -20°C.

3. pH control of the assay system

The antimicrobial effect of many chemotherapeutic agents is affected by the pH of the assay system. The activity of the aminoglycosides, erythromycin and lincomycin is reduced at acid pH while that of fusidic acid and the tetracyclines is reduced if the pH is above 7.0. The pH in the assay system was adjusted to reduce the activity of one agent when assaying another in a mixture.

4. Differential diffusion rates

The differences in the rates of diffusion between one drug and another is such that it is possible to assay a more mobile compound, for example, penicillin, in the presence of a less mobile one, for example, gentamicin.

In most instances, combinations of the methods described above were used to assay mixtures of antimicrobial drugs. A wide range of controls and standards was required but no attempt was made to determine synergistic or antagonistic

activity. 'In-use' controls, to confirm the accuracy and sensitivity of the assay methods and to determine the stability of antibiotics in biological fluids were included. They consisted of graded quantities of antimicrobial drug added to measured volumes of sterile antibiotic-free pus and serum.

Preparation and Stability of Antibiotic Standards

Stock standards were prepared from pure antimicrobial agents of known potency which were stored in a dessicator at 4°C. Accurately weighed amounts were dissolved in sterile distilled water unless recommended otherwise by the manufacturer. Liquid standards of gentamicin and fusidic acid of known potency were obtained from the manufacturers.

The stock standard solutions were used to prepare working standards. Four or five concentrations of drug were prepared, to cover the biological range, in a fluid comparable to that being assayed. The working standards were prepared volumetrically, and not by doubling dilution, from the stock solution. After preparation all standards were stored at -20°C for use. Stable agents, for example, chloramphenicol and the aminoglycosides, retained their activity for long periods but the penicillins and other unstable compounds had, usually, to be made up freshly for each assay.

Working standards for penicillin and cloxacillin, two of the more unstable antimicrobial compounds, were prepared in saline and serum in the range 1.0 - 10.0 mg/l and were stored at -20°C for one week. Fresh standards were prepared and these, together with the stored standards were assayed

simultaneously in triplicate.

The results for penicillin are shown in Table 4-2 and Figures 4-3 and 4-4. At a concentration of 5 mg/l there was no appreciable decay of penicillin either in serum or in saline. Below this level, the percentage decrease in activity was greater as the amount of active agent in the sample decreased. Similar experiments carried out on concentrated solutions of penicillin (30 g/l) stored at -20°C for one week showed that these also were stable provided the solution was frozen immediately after preparation.

The results for cloxacillin (Table 4-3, Fig. 4-5) were somewhat different. There was no loss of activity in saline. In serum, however, there was a significant loss of activity during storage for one week at -20°C. This loss was constant within the range 1.0 to 10.0 mg/l, the stored samples showing 30-33% less activity than those freshly prepared.

In further experiments it was shown that penicillin in serum or saline at a concentration of 3 mg/l decayed by 16% during overnight storage at -20°C, as compared to a concentrated solution (30 g/l) stored in the same way. It was shown subsequently that this decay was directly proportional to the time that the samples spent at room temperature and that it could be reduced to an insignificant level if samples were deep frozen immediately after preparation.

THE ESTABLISHMENT OF AN ANTIBIOTIC ASSAY METHOD FOR PUS

Liquefaction of Mucoid Samples of Pus

Many samples of pus are so viscous that accurate volumetric manipulations and, therefore, assays cannot be performed.

Table 4-2

DECAY OF PENICILLIN IN SALINE AND SERUM

Penicillin Conc (mg/l)	Saline Standards		Serum Standards	
	Stored at $-20^{o}C$ for one week	Freshly prepared	Stored at $-20^{o}C$ for one week	Freshly prepared
5.0	32.5*	32.5	30.3	30.5
3.5	29.2	30.0	27.5	28.2
2.5	24.5	28.0	23.0	26.2
1.3	19.0	24.0	16.8	21.5
1.0	15.8	22.5	13.8	19.8

*Mean zone diameter (mm)

Table 4-3

DECAY OF CLOXACILLIN IN SALINE AND SERUM

Cloxacillin Conc (mg/l)	Saline Standards		Serum Standards	
	Stored at $-20^{o}C$ for one week	Freshly prepared	Stored at $-20^{o}C$ for one week	Freshly prepared
10.0	28.7*	28.0	25.8	27.3
7.5	27.0	26.5	24.0	25.8
5.0	24.0	24.0	21.3	22.8
2.0	18.8	18.8	14.0	16.5
1.0	13.8	14.0	-	10.5

*Mean zone diameter (mm)

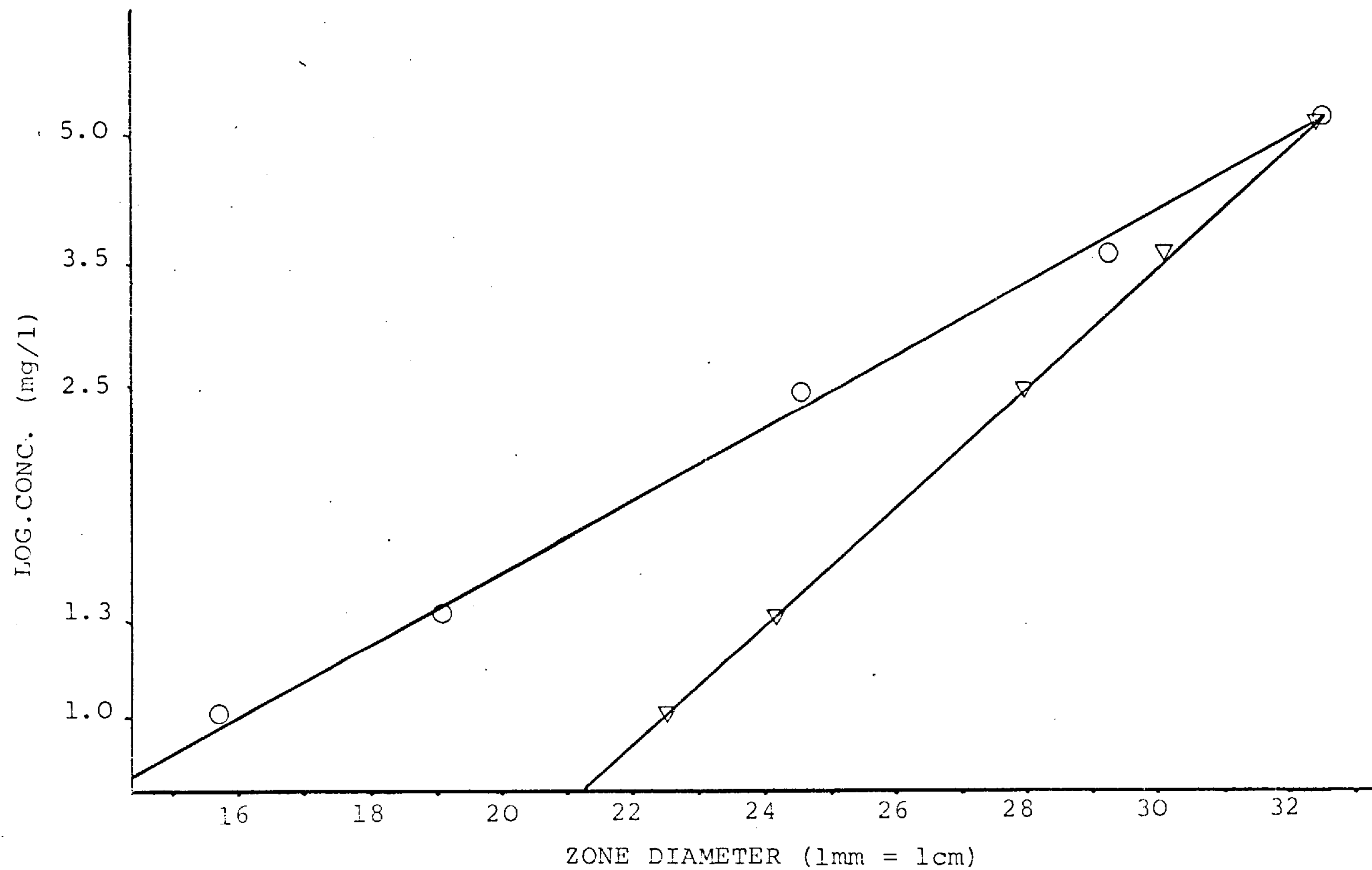

Figure 4-3

DECAY OF PENICILLIN IN SALINE

○ = stored standards ▽ = fresh standards

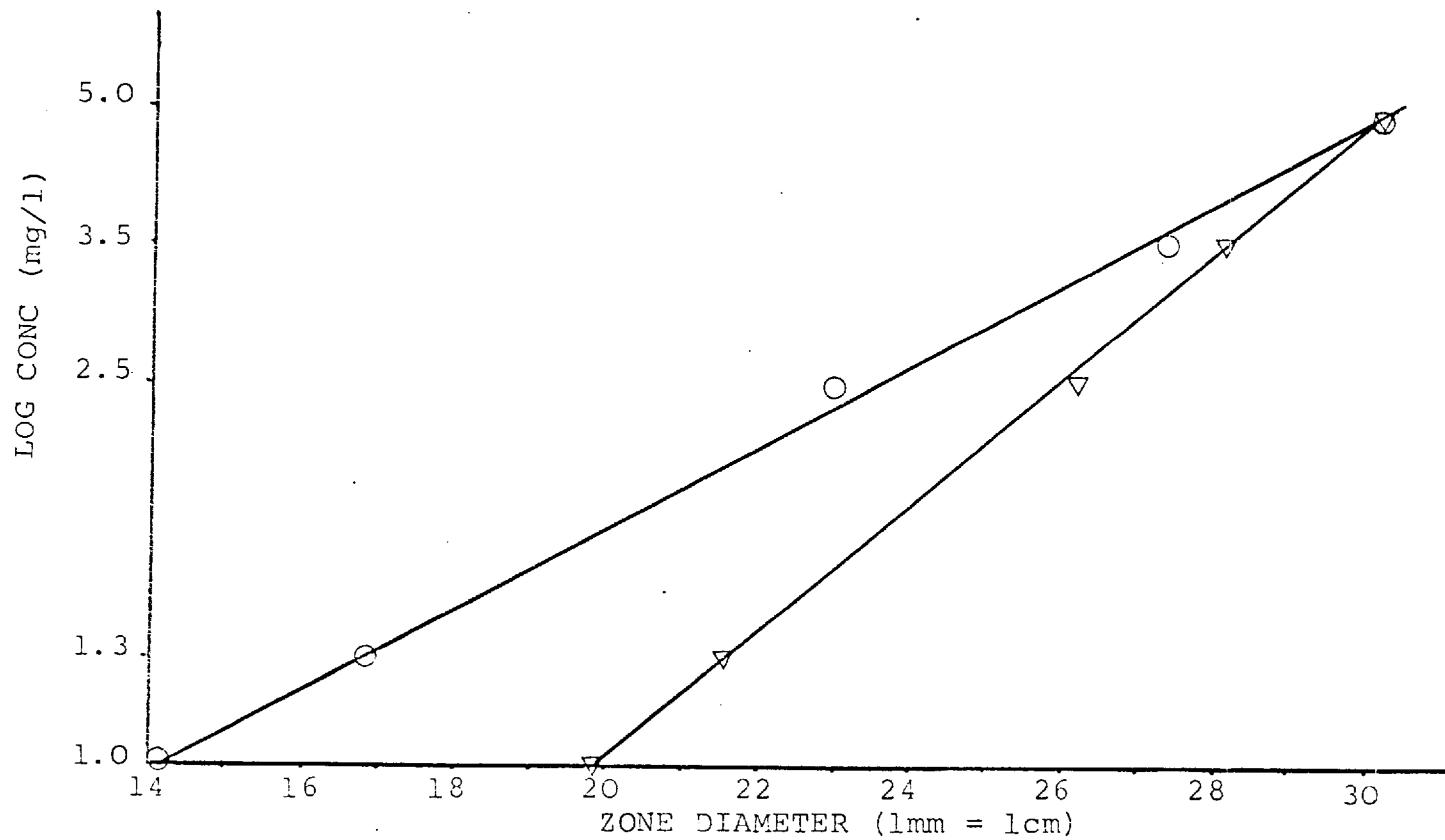

Figure 4-4

DECAY OF PENICILLIN IN SERUM

○ = stored standards ▽ = fresh standards

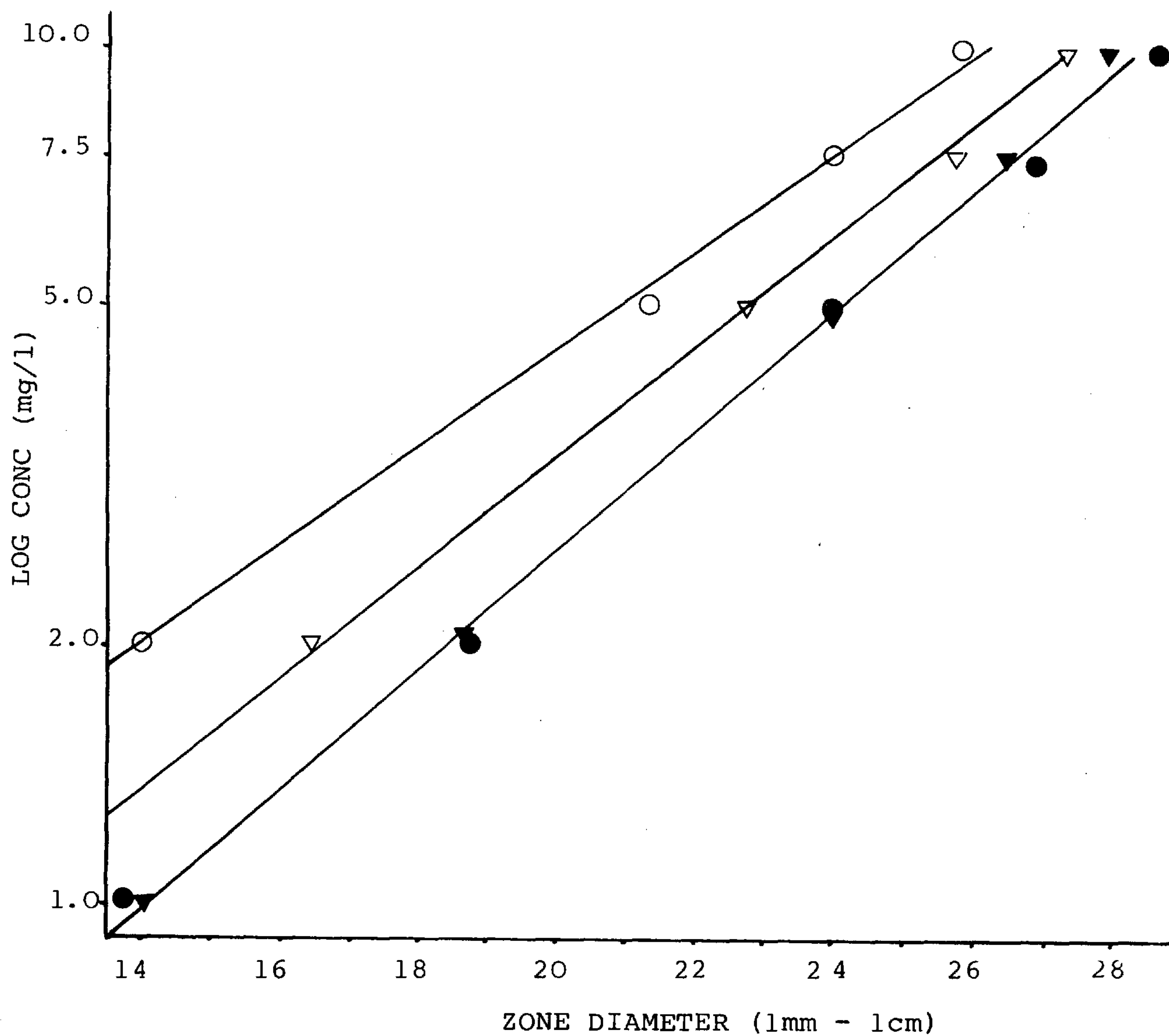

Figure 4-5

DECAY OF CLOXACILLIN IN SALINE AND SERUM

● = stored saline standards ▼ = fresh saline standards

○ = stored serum standards ▽ = fresh serum standards

Experiments were carried out to determine whether liquifying agents could be used without adverse effect on any antibiotic present. Those tested were pancreatin (Oxoid), one tablet dissolved in 25 ml sterile water, dithiothreitol (Clelands reagent) (Koch-Light), at concentrations between 0.3 and 10.0% in sterile water, and N-acetyl-l-cysteine (2% in water pH 7.0). Their liquefying activity for five pus samples was determined by adding approximately 1g mucoid pus to 2 ml aliquots of each reagent and incubating the mixture at 37°C for up to four hours, agitating it at intervals.

High concentrations of Clelands reagent (10%) produced partial digestion in this time, while the lower concentrations did not. N-acetyl-l-cysteine digested most samples within three hours but very dense material required four hours incubation. Pancreatin liquefied all the samples within 30 minutes, retaining its activity even if diluted one in four.

Effect of Pancreatin on Antibiotics in Saline

Pancreatin digested pus rapidly, but its effect on the antibiotics to be assayed was unknown. The effect of pancreatin on the activity of penicillin was determined as follows:-

A 60 mg per litre standard was prepared in phosphate buffered saline (PBS) and was used to prepare a range of dilutions in:-

a) PBS - 2 sets;

b) equal parts PBS and pancreatin solution.

The plate assay system used employed *Staphylococcus aureus* (NCTC 6571) as indicator organism and agar wells 6 mm in diameter. The penicillin standards in pancreatin and one set of the standards in PBS were incubated at 37°C for one hour while the other PBS standards were kept at 4°C. Next, the

three sets of standards were assayed in triplicate, being allowed to prediffuse for one hour at 4°C, before overnight incubation. The results are shown in Table 4-4 and Figure 4-6. There was no appreciable loss of activity of the saline standards. The addition of pancreatin produced larger zones of inhibition and appeared to enhance the activity of penicillin, but wells containing pancreatin only showed zones of altered bacterial growth around them. The addition of 1 ml pancreatin solution to the assay medium before pouring the plate produced a uniform inoculum and eliminated the apparent difference in penicillin activity (Table 4-5, Fig.4-7). Sterile pancreatin solution (1 ml) was therefore added to the assay medium if samples which had been treated with pancreatin were to be tested. The experiment was repeated for ampicillin, cephaloridine, chloramphenicol, tetracycline, fusidic acid, gentamicin and streptomycin, and in every case incubation of the standards in the presence of pancreatin had no effect on the activity of the antimicrobial agent.

The situation with cloxacillin, however, appeared to be different (Table 4-6, Fig. 4-8) and indicated that there was some loss of activity in the presence of pancreatin. Figure 4-8 shows that the effect is uniform and it was found that samples treated with pancreatin could be accurately assayed if the working standards were also prepared in pancreatin solution at the same concentration.

Effect of Pancreatin in the Binding of Antibiotic to Protein

While pancreatin may have no effect on the antibiotic itself, it is possible that the proteolytic action of such an agent might affect the degree to which an antibiotic is bound to

Table 4-4

EFFECT OF PANCREATIN ON PENICILLIN IN SALINE

Conc of Penicillin (mg/l)	Penicillin (4°C) zone diameter (mm)	Penicillin (37°C) zone diameter (mm)	Penicillin + Pancreatin (37°C) zone diameter (mm)
6.4	35.0	35.0	35.5
3.2	30.5	30.5	30.5
1.6	26.0	25.0	26.5
0.8	20.0	20.0	21.5
0.4	15.0	14.5	15.0

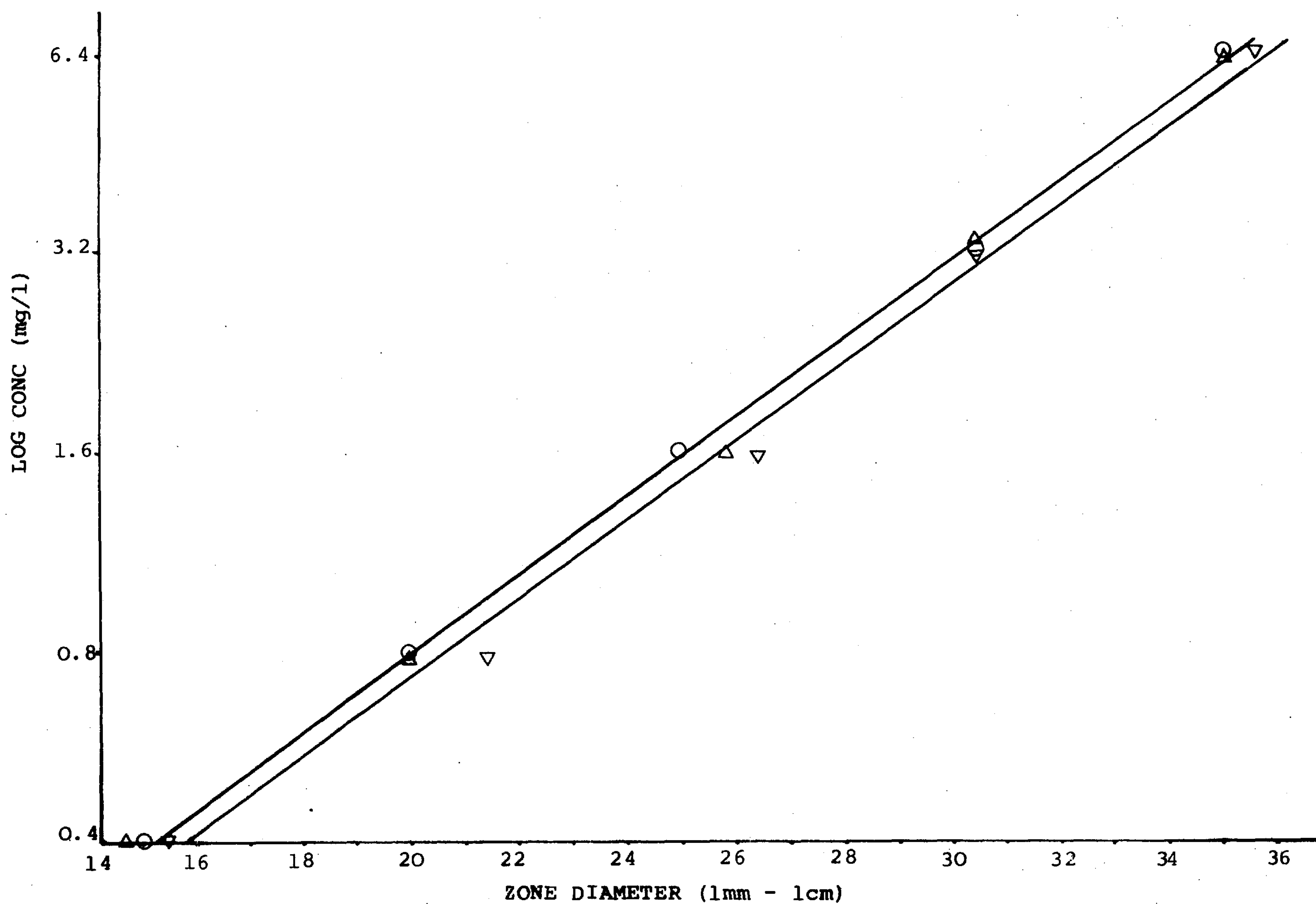

Figure 4-6

EFFECT OF PANCREATIN ON THE ACTIVITY OF PENICILLIN IN SALINE

○ = saline standards at 4°C △ = saline standards at 37°C
▽ = saline standards + pancreatin at 37°C

Table 4-5

PENICILLIN ACTIVITY IN PANCREATIN AND SALINE

Conc of Penicillin (mg/l)	Activity in Saline	Activity in Pancreatin
5.0	28.3*	28.0
3.5	25.6	25.3
2.5	23.5	23.8
1.3	19.0	18.5
1.0	16.5	17.0

* Average zone sizes in mm

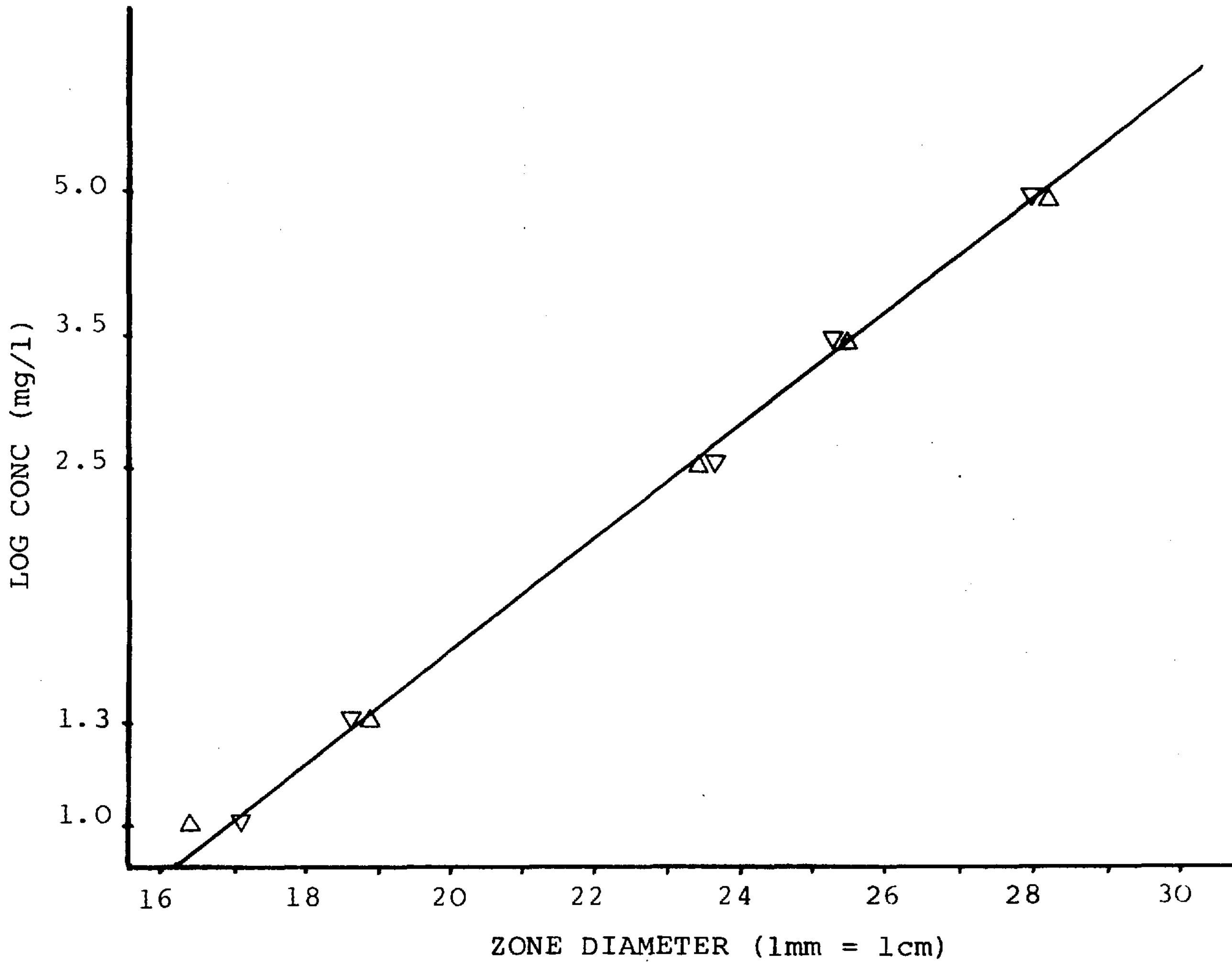

Figure 4-7

EFFECT OF PANCREATIN IN THE ASSAY MEDIUM ON THE ACTIVITY OF PENICILLIN IN SALINE WITH AND WITHOUT ADDED PANCREATIN

△ = saline standards at 37°C

▽ = saline standards + pancreatin at 37°C

Table 4-6

CLOXACILLIN ACTIVITY IN PANCREATIN AND SALINE

Conc. of Cloxacillin (mg/l)	Activity in Saline	Activity in Pancreatin
20.0	29.5*	28.8
15.0	28.0	27.0
10.0	26.0	24.8
7.5	24.0	23.0
5.0	22.0	20.5
2.5	18.0	16.0
1.2	13.5	11.0

*Average zone sizes in mm

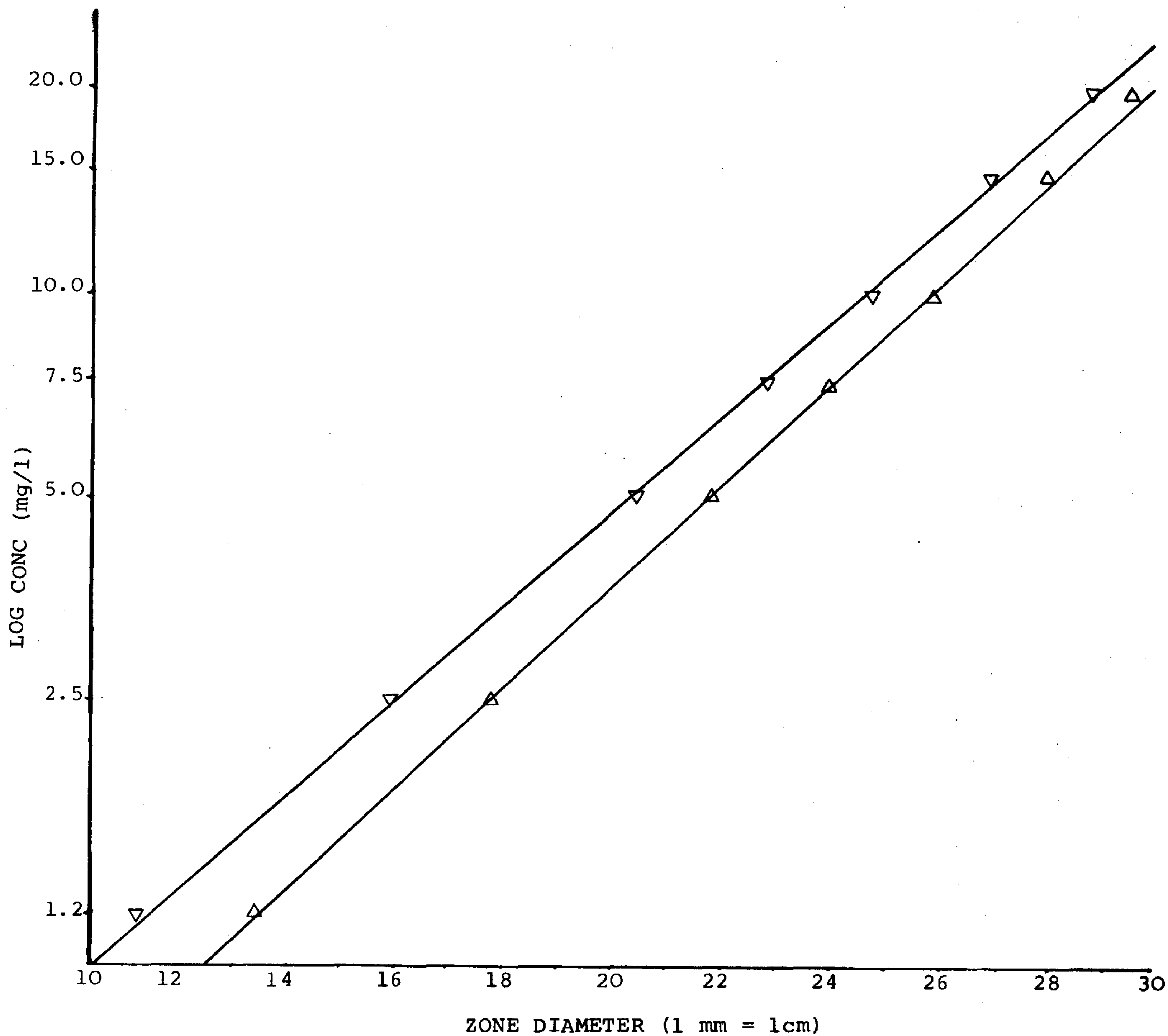

Figure 4-8
EFFECT OF PANCREATIN ON THE ACTIVITY OF
CLOXACILLIN IN SALINE

△ = saline standards at 37°C
▽ = saline standards + pancreatin at 37°C

protein. To investigate this possibility experiments were carried out using chloramphenicol, penicillin, cloxacillin, and fusidic acid, which are protein bound in serum in the range 40-95% (Garrod et al.1973). It has been suggested that some of them, eg. fusidic acid, are reversibly bound to protein.

Samples containing 20 mg/l fusidic acid either in serum or in saline were prepared and were added to equal volumes of :-

a) pancreatin solution

b) saline

c) serum.

The six test solutions were incubated at 37°C for 30 minutes and assayed against a set of fusidic acid standards in the range 0.16 - 10.00 mg/l. Fusidic acid assay medium was used with C.xerosis (FF VIII) as indicator organism.

Although the semi-log plot of the zone sizes against the concentrations of the standards was satisfactory (Fig. 4-9), the assays on the six samples were too variable for meaningful conclusions to be drawn. When diluted in saline, the serum-containing samples gave erroneous results, probably due to reversal of the protein binding effect.

To overcome this difficulty, standards were prepared in the range 0.16 - 10.00 mg/l in serum and saline respectively and 0.5 ml aliquots of each standard solution were added to equal volumes of saline, serum and pancreatin solution. After incubation at 37°C for 30 minutes, each mixture was assayed using the fusidic acid system described previously. The results are shown in Table 4-7, Figure 4-10. Differences between the saline standards plus saline and the serum standards plus serum confirmed that fusidic acid is 95% protein bound in neat serum. In 50% serum the binding dropped to

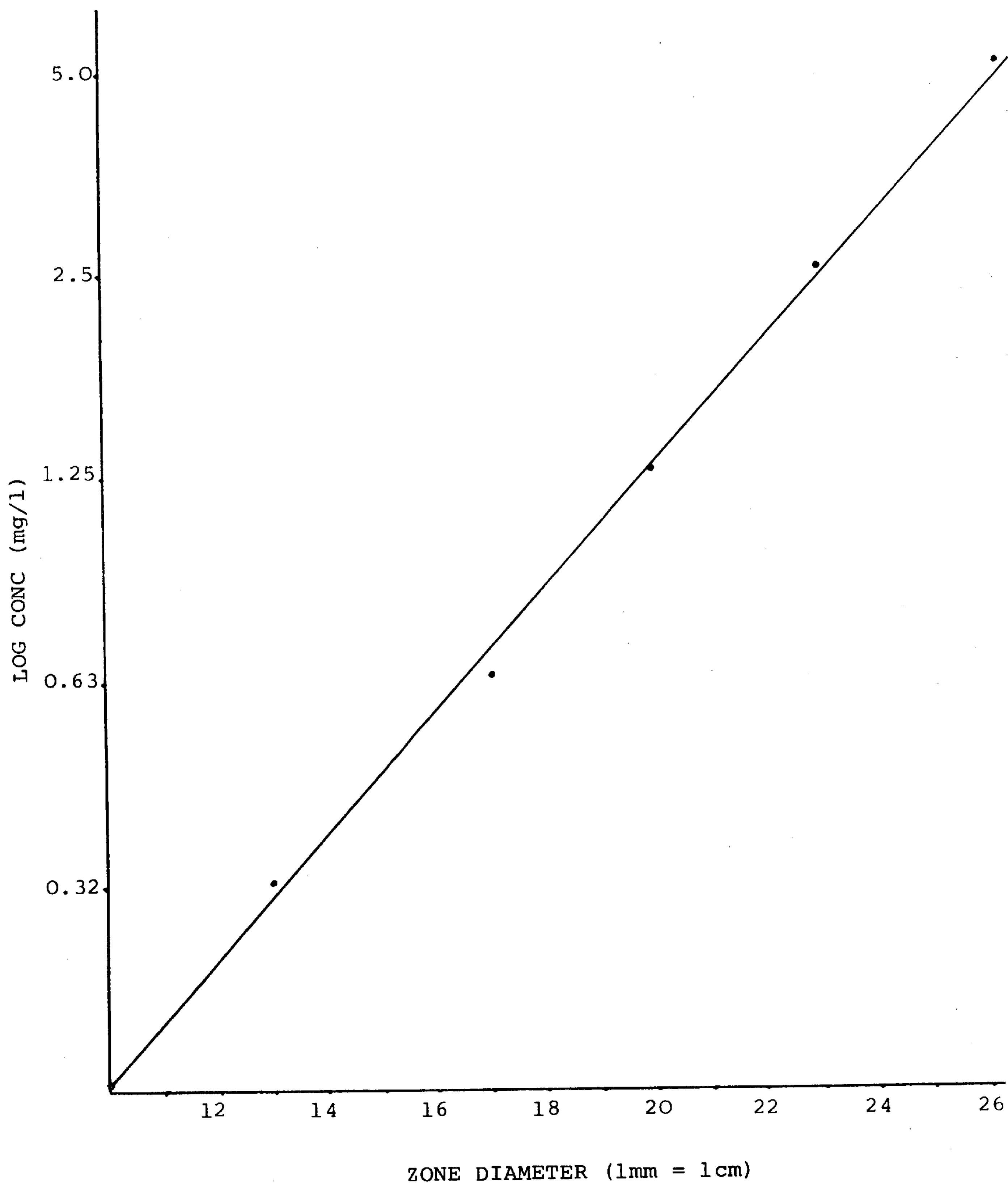

Figure 4-9
SEMI-LOG PLOT OF FUSIDIC ACID IN SERUM

Table 4-7

EFFECT OF SERUM AND PANCREATIN ON FUSIDIC ACID

Fusidic Acid	Serum Standards			Saline Standards		
Conc. (mg/l)	+ serum	+ saline	+ pancreatin	+ serum	+ saline	+ pancreatin
5.0	26.0*	27.0	30.5	27.0	37.0	37.0
2.50	21.0	23.5	26.5	24.0	33.5	33.5
1.25	19.0	20.0	24.0	20.5	30.0	30.5
0.63	15.0	17.0	20.5	17.0	27.5	27.5
0.32	12.0	13.5	18.0	13.0	24.5	25.0
0.16	-	11.0	15.5	11.0	21.0	21.5
0.08	-	-	11.5	-	18.5	18.5

* Average zone sizes in mm

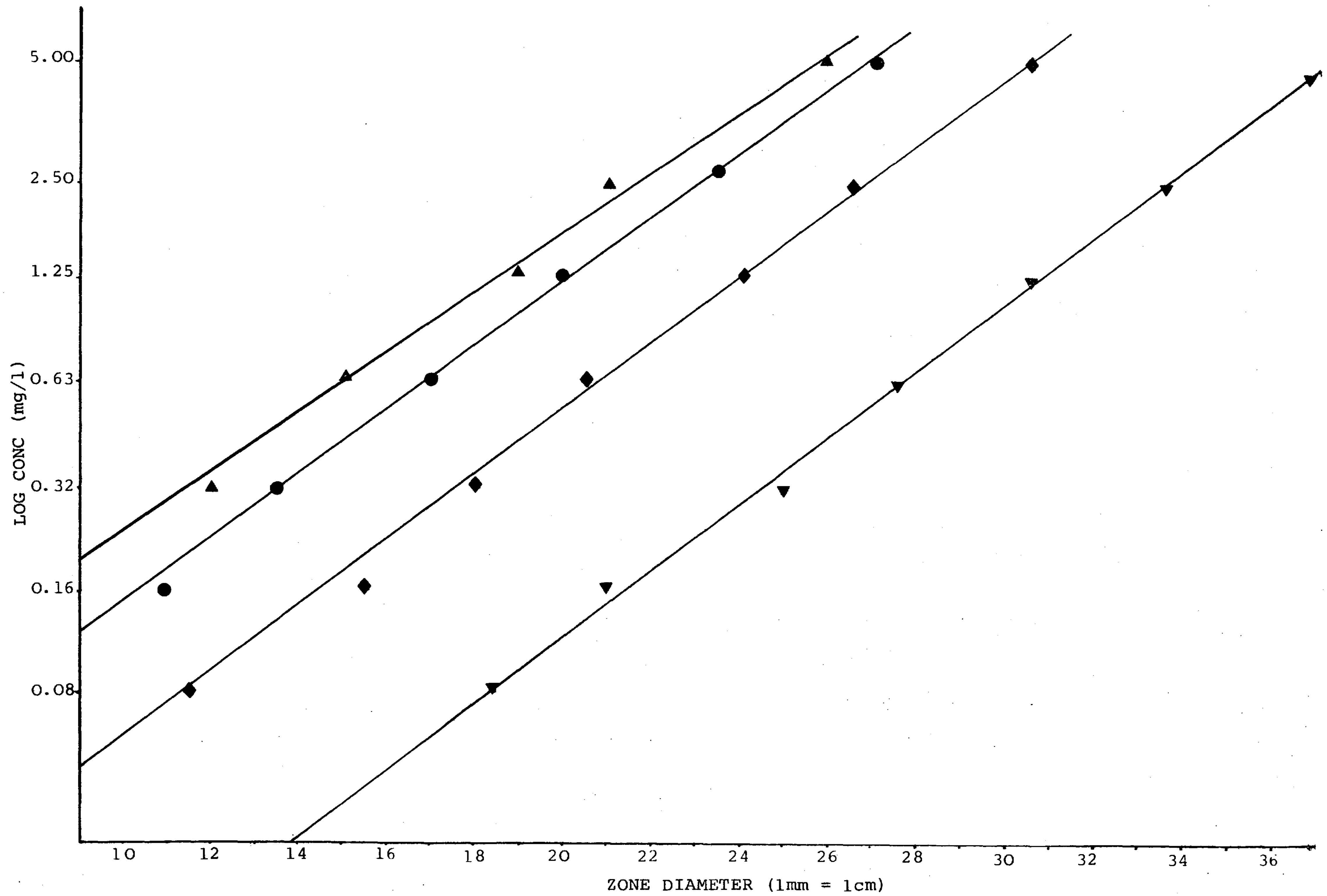

Figure 4-10

EFFECT OF SERUM WITH AND WITHOUT PANCREATIN ON THE ACTIVITY OF FUSIDIC ACID

▼ = standards in saline ▲ = standards in serum
● = standards in 50% serum ◆ = standards in 50% serum/pancreatin

approximately 90%. Treatment with pancreatin reduced the protein binding of fusidic acid in serum to approximately 75% (ie. a reduction of 20%). This was due to the effect of pancreatin on serum protein since saline standards with and without pancreatin gave the same results (Table 4-7). Thus pancreatin would appear to reduce the degree to which fusidic acid is protein bound.

The procedure described above was applied to cloxacillin using DST (Oxoid) agar - pH 7.2, with the Oxford staphylococcus as indicator organism and an incubation temperature of 30°C. The results are shown in Table 4-8 and Figure 4-11 and indicate that approximately 40% of cloxacillin is bound to protein. Dilution with saline of serum containing cloxacillin reduced the degree of protein binding. However, the addition of pancreatin to serum containing cloxacillin did not further reduce the amount of protein binding, as was the case with fusidic acid. Although the addition of pancreatin to cloxacillin in saline reduced the antibacterial activity of this drug (Fig. 4-8) the same effect was not produced by the addition of pancreatin to cloxacillin in serum (Fig. 4-11). Thus, in the presence of serum, pancreatin appeared to have no more effect than saline on the activity of cloxacillin.

The results obtained using penicillin are shown in Table 4-9 and Figure 4-12. Approximately 30% of penicillin was bound to protein in serum but the addition of pancreatin had little effect, since the same reduction in protein binding (50%) was produced by equal dilution in saline or pancreatin solution. The experiments were repeated using chloramphenicol and similar results were obtained.

Table 4-8

EFFECT OF SERUM AND PANCREATIN ON CLOXACILLIN

Cloxacillin	Serum Standards			Saline Standards		
Conc. (mg/l)	+ saline	+ serum	+ pancreatin	+ saline	+ serum	+ pancreatin
20.0	28.0*	26.3	27.8	29.5	27.8	28.8
15.0	26.0	24.5	26.0	28.0	26.0	26.8
10.0	23.8	22.3	24.0	26.0	24.0	24.8
7.5	22.3	21.8	22.0	24.3	22.7	23.8
5.0	18.3	17.5	18.5	23.0	19.2	21.3
2.5	14.5	12.5	14.5	17.8	14.5	15.5
1.2	10.0	8.0	9.5	13.0	10.0	10.5

* Average zone sizes in mm

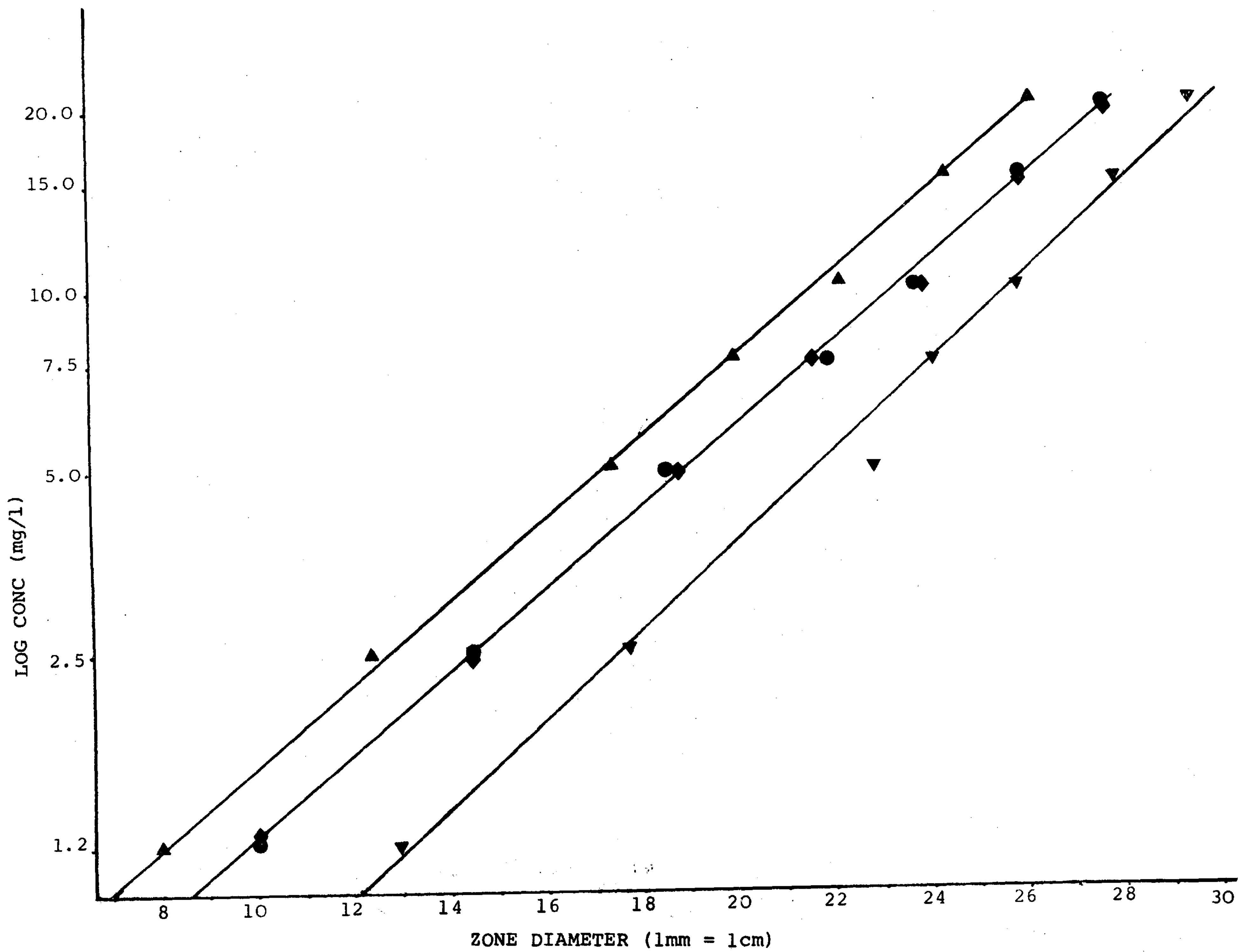

Figure 4-11

EFFECT OF SERUM WITH AND WITHOUT PANCREATIN ON THE ACTIVITY OF CLOXACILLIN

▼ = standards in saline ▲ = standards in serum

● = standards in 50% serum ◆ = standards in 50% serum/pancreatin

Table 4-9

EFFECT OF SERUM AND PANCREATIN ON PENICILLIN

Penicillin	Serum Standards			Saline Standards		
Conc. (mg/l)	+ saline	+ serum	+ pancreatin	+ saline	+ serum	+ pancreatin
5.0	27.5	27.5	27.5	28.3	27.8	28.0
3.5	24.7	24.3	24.5	25.6	25.0	25.0
2.5	22.0	21.0	22.3	23.5	23.0	23.8
1.3	16.8	16.0	16.7	19.0	17.5	18.0
1.0	14.8	14.0	14.5	16.5	16.0	16.5

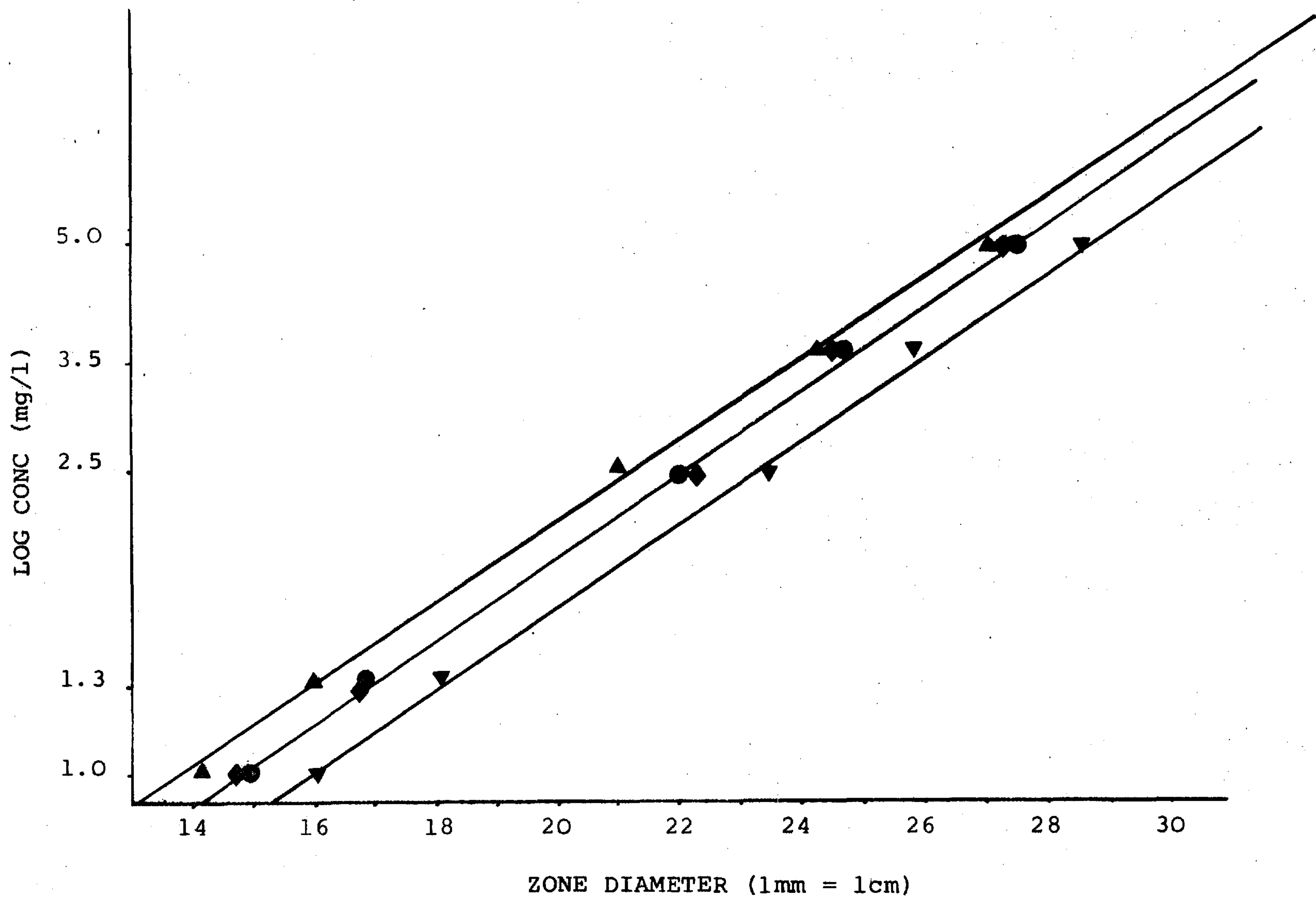

Figure 4-12

EFFECT OF SERUM WITH AND WITHOUT PANCREATIN ON THE ACTIVITY OF PENICILLIN

▼ = standards in saline ▲ = standards in serum

● = standards in 50% serum ◆ = standards in 50% serum/pancreatin

The experiments show that pancreatin solution can be used satisfactorily to liquefy mucoid samples of pus prior to antibiotic assay. The addition of pancreatin solution to the assay medium prior to pouring eliminates any enhancing effect on the growth of the indicator organism produced by pancreatin/pus mixtures in the wells.

Pancreatin has no effect on the activity of the common antimicrobial drugs used in the treatment of brain abscess, and with the exception of fusidic acid, it has no effect on the degree to which these drugs are bound to protein.

ASSAY OF ANTIBIOTICS ADDED TO ANTIBIOTIC-FREE PUS

Preliminary Examination of Pus

Eight samples of liquid pus from patients who were reported not to have received antimicrobial drugs, were collected and examined microbiologically for the presence of chemotherapeutic agents. Wells 6 mm in diameter were cut in DST agar, pH 7.2 which had been seeded with *Staph. aureus* (NCTC 6571). The wells were filled with pus samples and, following prediffusion for one hour at 4°C and overnight incubation at 37°C, the plates were examined for zones of inhibition. Antibiotic -free human serum was used as a control. Only those samples showing no antibacterial activity were used to determine whether antibiotics added in known concentrations could be assayed accurately.

Addition of Antibiotic and Assay

1 ml of saline containing 30 µg penicillin was mixed with 4 ml aliquots of pus, serum and saline. The samples were stored at -20°C overnight after which two 2 ml aliquots

were removed. Two ml pancreatin solution was added to one and 2 ml saline to the other. All were incubated at 37°C for 30 minutes and assayed against penicillin standards prepared in serum using DST agar at pH 7.2 seeded with the Oxford staphylococcus.

Results

The results of the first experiment, using pus from a single patient (sample 1) are shown in Table 4-10. They confirm the earlier findings that the addition of pancreatin to solutions of penicillin in serum or saline has no effect on antibacterial activity. In contrast to the results with saline and serum, the sample of pus tested showed a 95% loss of activity during 18 hours storage at -20°C and while pancreatin had no effect on penicillin in serum or saline, it doubled the activity in the sample of pus producing an increase in zone diameter of 4.6 mm. The experiment was repeated twice and gave identical results. The inactivation of penicillin by the sample of pus occurred fairly rapidly since although, for the repeat experiments, the samples were tested immediately after preparation, the results were the same. Other samples were tested and no inactivation of penicillin could be demonstrated (Table 4-11, pus samples 3 and 4).

The experiments were repeated with ampicillin, cloxacillin (incubated at 30°C), fusidic acid and chloramphenicol using six pus samples which did not show activity against penicillin. In each case the antibiotic activity in the pus sample was the same as that in the serum control and the activity in pus samples treated with pancreatin was the same as that in serum with added pancreatin.

Table 4-10

EFFECT OF PUS (SAMPLE 1) WITH AND WITHOUT PANCREATIN ON THE ACTIVITY OF PENICILLIN.

	Mean zone size (mm)	Penicillin concentration (mg/l)
6 mg/l Penicillin in saline		
+ saline 1:1	31.0	2.5
+ pancreatin 1:1	30.5	2.3
6 mg/l Penicillin in serum		
+ saline 1:1	31.0	2.5
+ pancreatin 1:1	31.1	2.5
6 mg/l Penicillin in pus		
+ saline 1:1	13.8	0.1
+ pancreatin 1:1	18.4	0.3

Table 4-11

EFFECT OF PUS (SAMPLES 1, 3 and 4) WITH AND WITHOUT PANCREATIN ON THE ACTIVITY OF PENICILLIN.

	Mean zone size (mm)	Penicillin concentration (mg/l)
6 mg/l Penicillin in serum		
+ saline	29.0	3.0
+ pancreatin	29.0	3.0
6 mg/l Penicillin in pus 1		
+ saline	16.8	0.3
+ pancreatin	20.0	0.6
6 mg/l Penicillin in pus 3		
+ saline	28.8	3.0
+ pancreatin	28.8	3.0
6 mg/l Penicillin in pus 4		
+ saline	29.0	3.0
+ pancreatin	29.0	3.0

It was concluded that pancreatin had no effect on the activity of antimicrobial drugs in the majority of pus samples and that this agent could therefore be used satisfactorily to liquefy mucoid samples of pus prior to assay. The addition of pancreatin solution to antibiotic standards in horse serum as well as to the sample of pus under investigation provided an accurate means by which antibiotics could be assayed in these specimens.

INVESTIGATION OF THE PENICILLIN INACTIVATION PHENOMENON

Twenty-two samples of pus from intracranial and other sites were examined for their ability to inactivate penicillin. Four out of the 22 samples tested had this ability. The possible causes for this high degree of penicillin inactivation could be:-

a) A high protein content of the pus resulting in increased protein binding.

b) A low pH of the pus resulting in destruction of the drug.

c) Penicillinase produced by the infecting organism.

d) Other causes.

Protein Estimation

The protein content of the 22 pus samples was determined by the salicylsulphonic acid method (Varley 1954 p.462). All samples had a protein content in the range 700 - 8,000 mg/dl. The samples showing penicillin inactivation did not have higher protein contents than the others nor were the protein levels in the pus samples significantly higher than those found in normal human serum.

pH Effect

The pH values of the 22 samples of pus were all in the range 6.0 - 7.3 as determined by a Pye Unicam 290 pH meter. Therefore, acidity could not be responsible for the penicillin inactivation.

Demonstration of Beta-Lactamase Activity by Colorimetric Methods

Attempts were made to demonstrate beta-lactamase activity in the pus samples by the iodometric techniques of Perret (1954) and Sykes and Nordstrom (1972), and the electron shift method of O'Callaghan et al. (1972).

Iodometric method of Perret

This was used as a screening procedure. To 50 ml of 0.0014M penicillin in 0.2M phosphate buffer - pH 6.5, was added 2 ml of 2% sodium starch gluconate and 0.01M iodine dropwise until a faint blue colour persisted for at least three minutes. A further 6 ml iodine solution was then added.

2.5 ml of the above reagent, freshly prepared, was mixed with 0.5 ml of sample and examined over a period of 15 minutes at room temperature for decolorisation. The product of activity and time is constant (plus or minus 10%) under these conditions and decolorisation in 0.5 - 15.0 minutes represents a penicillinase activity of between 60 and 2 unit doses (U.D.). One unit dose of penicillinase activity is that amount of enzyme which will destroy 1 μ mol penicillin per hour under standard conditions.

Iodometric method of Sykes and Nordstrom

Reagents: Solution A Hydrolysed starch (0.2%) in 0.1M potassium phosphate buffer - pH 5.9. The starch was dissolved by gentle boiling for two to three minutes.

Solution B Iodine (0.08M) in 3.2M potassium iodide dissolved in potassium phosphate buffer - pH 5.9.

Starch-iodine solution was prepared by adding 0.15 ml solution B to 100 ml solution A.

Substrate: The substrate used was 1.0 mM penicillin dissolved in 0.1M phosphate buffer - pH 5.9.

Test: To 1 ml starch iodine solution in 1cm cuvette was added 1 ml substrate and 0.9 ml phosphate buffer - pH 5.9. The cuvette was placed in a spectrophotometer at 30°C for five minutes. At the end of this time 0.1 ml enzyme solution was added and the absorbance measured at 620nm at 10 minute intervals for 40 minutes. The initial absorbance of the enzyme-free mixture was 1.20.

For this test, one unit of beta-lactamase was taken as the amount of enzyme that hydrolysed benzyl penicillin at the rate of 1 μ mol per minute at 30°C. It is claimed that the method will detect 0.001 units enzyme activity in 3 ml of reaction mixture.

Standards: A standard solution of β-lactamase (Whatman) containing approximately 100 units/ml was prepared in 0.1M phosphate buffer. Dilutions of this solution were used to prepare standard curves.

Electron shift method of O'Callaghan et al.

Chromogenic cephalosporin solution (87/312) was supplied by Glaxo Research Laboratories, Greenford. The test procedure used was as follows:-

Samples of pus were mixed with an equal volume of saline. Two drops of 87/312 solution were added and the mixture was examined after 5, 15 and 30 minutes in a 37°C waterbath. Broth cultures of known β-lactamase producers were used as controls. Production of a red colour in the solution indicated the presence of β-lactamase.

Results

Perret's method did not work satisfactorily. The volume of pus required was large (0.5 ml) and resulted in gross discoloration of the reagent mixture. The control tubes, which contained pus without any added penicillin decolorised the iodine solution at such a rate that no difference could be observed between the pus sample under test, that is, with added penicillin, and the control. The method of Sykes and Nordstrom requires a smaller volume of pus (0.1 ml) and is reported to be more sensitive, detecting 0.001 units β-lactamase. It worked well with the commercial β-lactamase used as a control, producing curves similar to those reported (Fig. 4-13), but was unsatisfactory when pus was used. Neat pus decolorised the iodine solution in the absence of added penicillin while pus diluted to $^{1}/10$ and $^{1}/20$ possessed no detectable enzyme. In addition to the iodometric methods which demonstrate the presence of β-lactamase by the production of 6-amino penicilloic acid from penicillin, attempts were made to demonstrate the enzymes by the electron shift method of O'Callaghan et al. which uses a chromogenic cephalosporin. The presence of β-lactamase is indicated by a red coloration and therefore blood stained samples could not be tested by this method. Other samples showed no activity in neat specimens or in those diluted $^{1}/10$.

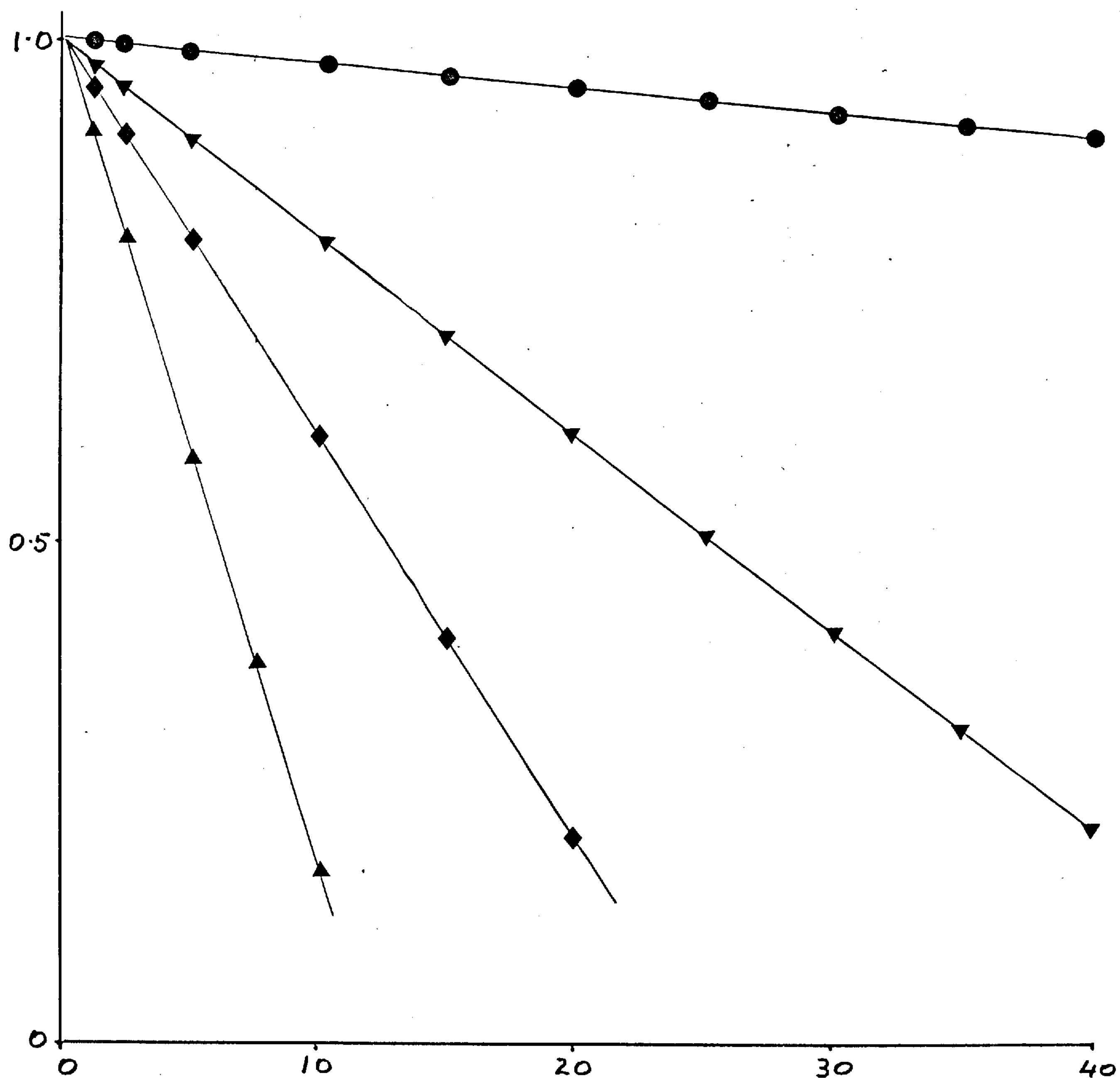

Fig. 4-13 MICROIODOMETRIC ASSAY OF B-LACTAMASE WITH PENICILLIN G (1mM) AS SUBSTRATE. STANDARD CURVES PRODUCED BY DILUTIONS OF B-LACTAMASE SOLUTION CONTAINING APPROXIMATELY 100 UNITS/ML.

▲ = 10^{-3} dilution ◆ = 5×10^{-3} dilution

▼ = 10^{-4} dilution ● = control

Culture of the four samples able to neutralise penicillin activity showed that two of them contained organisms of the Bacteroides group while one of the others contained Esch.coli. The fourth sample from a case of pelvic sepsis had been reported as bacteriologically sterile.

Demonstration of Penicillinase Activity by Microbiological Assay

1. Attempts were made to demonstrate whether pus would neutralise the antibacterial activity of penicillin against Staph.aureus (NCTC 6571) in an agar diffusion system. Sensitivity medium (Oxoid DST) pH 7.2 was flooded with a diluted broth suspension of Staph. aureus and dried. Wells 6mm in diameter were cut 3cm, 2cm and 1cm distant from a 2 µg penicillin disc (Mast), and were filled with pus or with commercial β-lactamase solution in various dilutions. After prediffusion for 30 minutes at 4°C the plate was incubated overnight, and the zone diameters of test and control were compared.

   The penicillinase solutions neutralised the antibacterial effect of the penicillin, but no such effect was demonstrable by the pus samples, indicating that the penicillin-inactivating activity did not lie in a diffusible fraction. It seemed that such activity must be intrinsic to the solid portion of the pus.

2. Two liquid samples of pus were centrifuged at 5000 rpm for 30 minutes (BTL bench centrifuge, head radius 6.5cm). An aliquot of the supernatant from one sample was passed through a 22 nm membrane filter, but the

second supernatant was too viscous to pass. The sediments (2) and supernatants (3) were tested for their ability to inactivate penicillin using the method described previously. Table 4-12 shows that the inactivating property lies in some solid fraction of the pus (sample 14). There was little inactivation by the clear supernatant after centrifugation and, following removal of all particulate matter by filtration, the effect was lost completely. In pus sample 1, the inactivating property lies in a solid fraction which, because of the viscous nature of the sample, was not deposited on centrifugation. Cephaloridine also was shown, in a further experiment, to be inactivated by the solid fraction of the pus.

3. Three of the four pus samples that inactivated penicillin rapidly were tested against ampicillin, cephaloridine, chloramphenicol, fusidic acid and streptomycin, these being added to aliquots of pus to give the final concentrations shown in Table 4-13. Standard concentrations of antibiotic were prepared in horse serum and treated in the same way as the tests. Test and standard solutions were prepared, stored at 4°C for two hours and assayed. Following subsequent storage at -20°C for 72 hours they were again assayed.

Penicillin, ampicillin and cephaloridine were destroyed. In two of the samples (1 and 6) destruction was rapid and was completed, or nearly so, within two hours. In sample 7 the inactivating effect was progressive being significantly lower at 2 hours than at 72 hours.

Table 4-12

PENICILLIN INACTIVATION BY TWO SAMPLES OF PUS
containing 6mg/l

	Mean zone size (mm)	Residual Penicillin activity (mg/l)	Inactivation
Pus Sample 14:			
Deposit	28.3	2.4	60%
Unfiltered supernatant	31.2	5.0	16%
Filtered supernatant	32.0	6.0	0%
Pus Sample 1:			
Deposit	21.8	0.5	> 90%
Unfiltered supernatant	22.0	0.5	> 90%

Table 4-13

ACTIVITY OF ANTIMICROBIAL DRUGS ADDED TO THREE SAMPLES OF PUS

	Concentration (mg/l)	Time (hrs) since mixing	Loss of activity (%) Sample 1	6	7
Penicillin	5.0	2	> 95	74	58
		72	> 95	83	83
Ampicillin	5.0	2	90	58	72
		72	>95	58	86
Cephaloridine	5.0	2	88	88	20
		72	> 95	88	56
Streptomycin	7.5	2	0	0	0
		72	0	0	0
Fusidic Acid	1.0	2	0	0	0
		72	0	0	0
Chloramphenicol	8.5	2	0	40	0
		72	0	40	0

The activity of streptomycin and fusidic acid was unimpaired. Sample 6 apparently destroyed chloramphenicol but there was insufficient material to investigate this effect further. The penicillin inactivating properties of the two remaining samples were still demonstrable after storage of the pus for six months at -20°C.

Method used for Assay of Antimicrobial Drugs in Pus from Abscesses of the Central Nervous System

The concentration of antimicrobial drugs in samples of cerebral pus was assayed using the technique described previously but with the following modifications.

1. Avoidance of falsely low readings due to decay of antibiotics in transit.

Serum standards for the anticipated assay were prepared as soon as collection of a sample was notified. They were stored at 4°C until the assay was performed. Thus standards and samples were maintained at the same temperature for the same time.

2. Separation and assay of serum

Samples of serum for assay were separated from the blood clot immediately on arrival and maintained at 4°C.

3. Liquefaction of pus

Mucoid samples were liquefied by adding 1 ml pancreatin solution to approximately 1 ml pus and incubating the mixture at 37°C for 30 minutes. An accurate volumetric measurement of the pus was made at the end of this time by the method of subtraction.

Samples of pus which required pancreatin treatment were assayed in agar to which 1 ml pancreatin solution (membrane

filtered) had been added prior to pouring.

4. Treatment of standards

When pus samples required pancreatin treatment 1 ml aliquots were removed from each of the serum antibiotic standards prepared previously. An equal volume of pancreatin was added to each aliquot and standards and test solutions were incubated together. The untreated standards were used to determine the concentration of antibiotic in serum and/or CSF samples.

5. Inactivation of other antibiotics likely to be present

Since it had been found that many samples contained antimicrobial drugs other than those stated, samples were treated with combinations of β-lactamase I, β-lactamase I and II, p-aminobenzoic acid and thymidine. Standards of the drug being assayed were similarly treated.

SECTION 5 PROSPECTIVE STUDY OF 50 PATIENTS WITH INTRACRANIAL OR INTRASPINAL ABSCESS : RESULTS

There were fifty patients in the prospective study of intracranial and intraspinal sepsis. The geographical location of the six centres participating and the numbers of patients from each are shown in Figure 5-1. The large numbers from two of the London centres reflect easier access to the examining laboratory rather than any real difference in distribution of brain abscess. In addition, all the cases of post-operative infection came from these centres. Table 5-1 shows that male:female ratio was 3:2 and that the cases occurred in all age groups.

The patients were divided into four groups according to the nature of their infection and its site, as shown in Table 5-2. There were 35 patients who had a defined brain abscess from which pus was aspirated; some of these patients also had extra- or subdural empyemata. These cases are considered separately from a group of eight patients in whom suppuration was extracerebral. There were six patients who developed intracranial sepsis following neurosurgery. In one case an extradural abscess developed, in two, infection progressed to parenchymal abscess formation within three weeks, and in three, the infection did not lie deep to the cranium (Table 5-2). In one of the former the abscess was found only at postmortem; its extent can be seen in Figure 5-2. The six patients with post-operative infection are included in the appropriate categories of Table 5-2. There were three cases of spinal abscess, all extradural.

Fig. 5-1

NEUROSURGICAL CENTRES PARTICIPATING IN THE PROSPECTIVE STUDY

AMH - Atkinson Morley's Hospital, London

FH - Frenchay Hospital, Bristol

OCH - Oldchurch Hospital, Romford

SBH - St.Bartholomew's Hospital, London

WNC - Wessex Neurological Centre, Southampton

WH - Walton Hospital, Liverpool

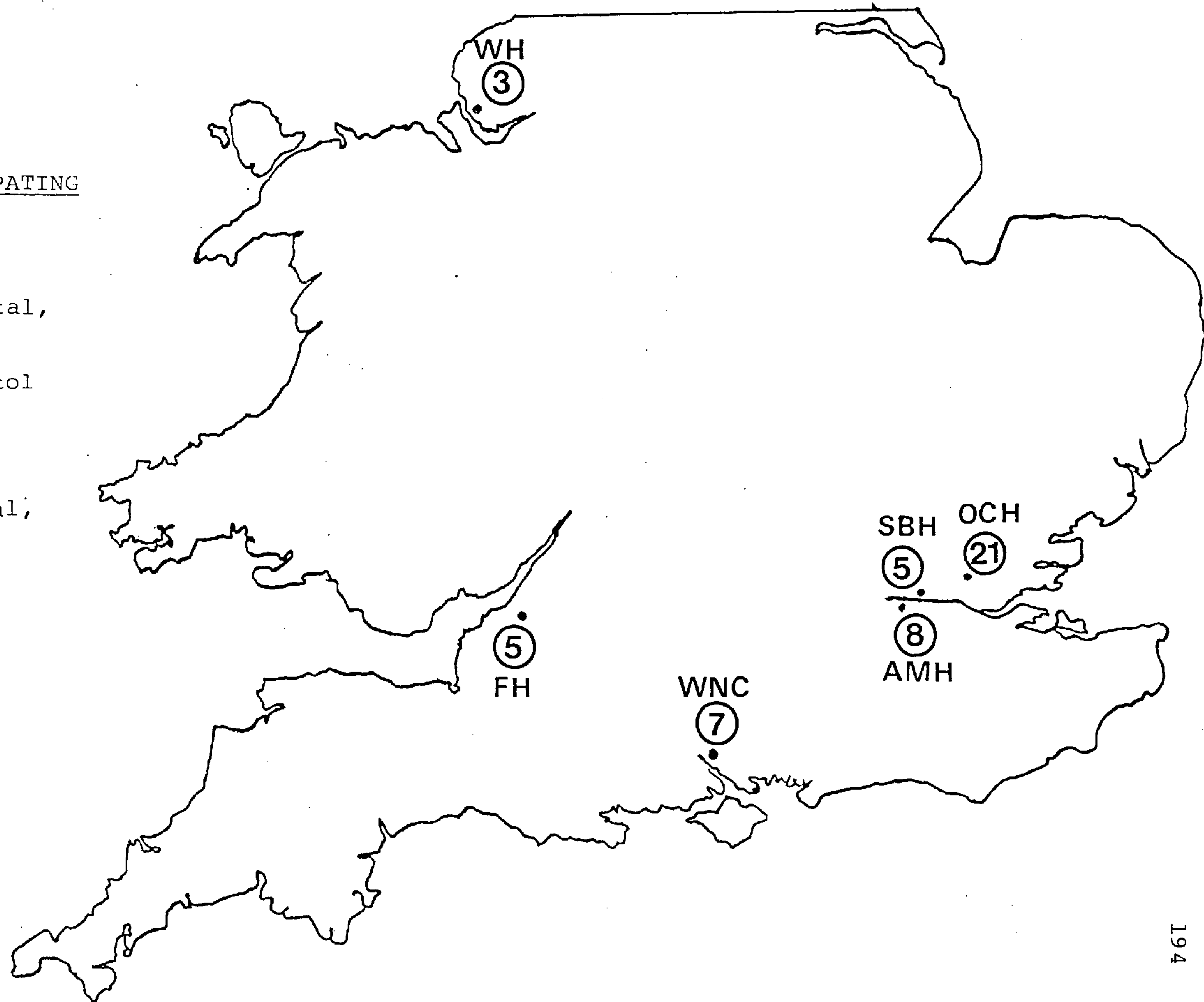

Table 5-1

SEX AND AGE DISTRIBUTION OF 50 PATIENTS STUDIED

M : F ratio 3 : 2

	Male	Female
0-10	3	3
11-20	7	1
21-30	2	4
31-40	3	2
41-50	7	2
51-60	5	4
> 60	4	3
	31	19

Table 5-2

49 CASES OF INTRACRANIAL AND INTRASPINAL SUPPURATION

Parenchymatous abscess of the brain (otogenous, haematogenous, metastatic, cryptogenic)	35	(7)
Extradural or subdural abscess of the brain	8	(3)
Spinal extradural abscess	3	(1)
Post-operative extradural wound infection	3	(0)

Number of deaths in brackets.

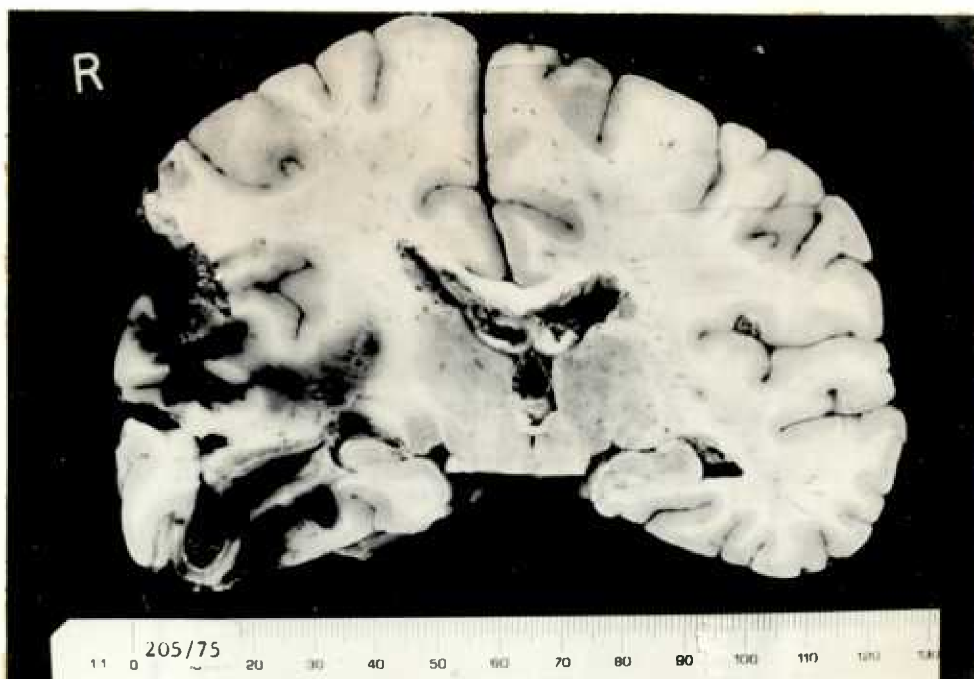

Fig.5-2 Coronal cut of brain (Case 2) showing a temporal abscess in the swollen right hemisphere.

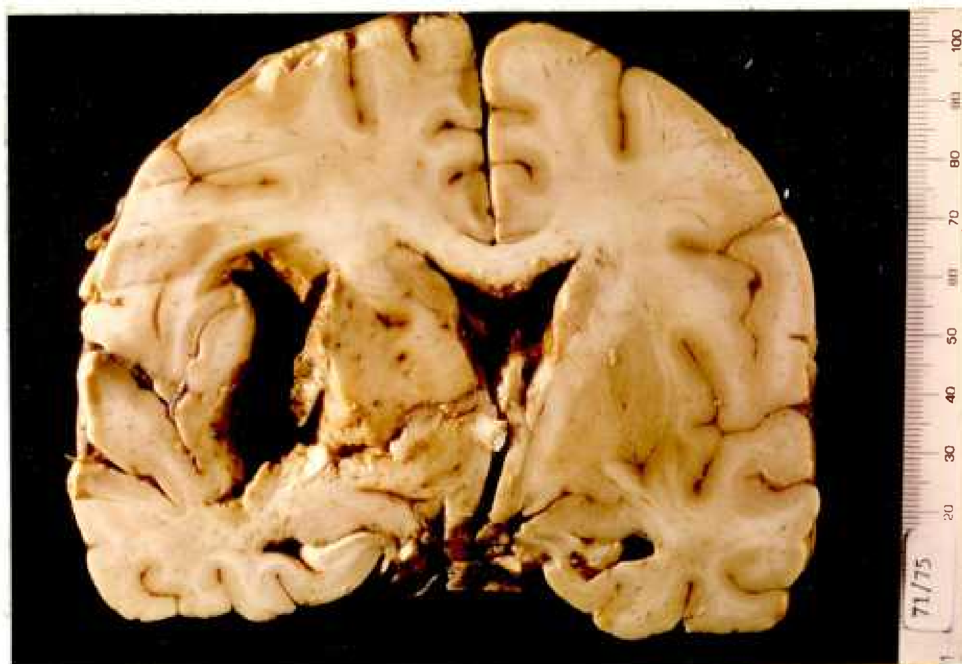

Fig.5-3 Brain section from Case 6 showing a large abscess cavity involving the basal ganglia of the right hemisphere.

The remaining case, not included in Table 5-2, was of a 59 year old man who sustained a penetrating wound through the right orbit into the thalamus, followed by hydrocephalus. Eleven weeks later thick fluid was aspirated from an encapsulated area in the right frontal lobe which had appeared as an abscess radiographically. The fluid contained large amounts of non-septate fungal mycelium but very few leucocytes, and motile "zoospores" were seen in the cerebrospinal fluid. No fungus could be isolated. Because of the non-suppurative nature of this lesion, the case has been excluded from the general discussion and is detailed separately (Appendix B p 245)

Table 5-3 shows the antecedent infections and conditions associated with the cases. The majority ($^{30}/49$) occurred following otitis, sinusitis or cerebral trauma, whether surgical or accidental. There were ten cases in which there was no associated lesion.

None of the 49 primary pus samples was bacteriologically sterile. The liquid sample from the fiftieth case was not pus and consisted of fungal elements with only scanty leucocytes.

There were 69 isolates from 66 samples of pus obtained from the 49 patients studied (Table 5-4). Streptococci were isolated most frequently, occurring in 36 (72%) patients; samples from 20 patients (40%) contained Str.milleri. The specimen from one patient contained a mixed streptococcal population. Staph.aureus was isolated from 24% patients; organisms belonging to the bacteroides group were found in 22% patients and Enterobacteriaceae were found in 16% patients.

Mixed bacterial populations were found in fourteen patients (29%) (Table 5-5). The majority ($^{12}/14$) occurred in patients with brain abscess and in $^{7}/12$ cases were associated

Table 5-3

49 CASES OF INTRACRANIAL AND INTRASPINAL SUPPURATION:

PREDISPOSING CAUSE

Predisposing cause	Cases
Sinusitis/Influenza	12
Otitis	9
Congenital Heart Defect	3
Metastatic (miscellaneous)	6
Post-operative infection (neurosurgery)	6
Head injury	2
Penetrating wounds	1
Cryptogenic	10
	49

Table 5-4

BACTERIA ISOLATED FROM 49 CASES OF INTRACRANIAL AND

INTRASPINAL SUPPURATION

Bacteria	Number
Streptococcus milleri	20
" species	6
" faecalis	2
" faecium	1
" mutans	1
" mitior	1
" Lancefield group C	2
" pneumoniae	1
Staphylococcus aureus	12
Proteus mirabilis	4
" vulgaris	3
Klebsiella aerogenes	1
Bacteroides fragilis group	7
Fusobacterium sp.	3
Eikonella corrodens	1
Peptostreptococcus anaerobius	3
Haemophilus aphrophilus	1
	69

Table 5-5

MICRO-ORGANISMS ISOLATED FROM 49 CASES OF INTRACRANIAL AND INTRASPINAL SUPPURATION

	Pure Culture	Mixed Culture
Brain abscess (35)	23	12
Extradural and subdural abscess (8)	6	2
Spinal abscess (3)	3	0
Post-operative wound infection (3)	3	0

Table 5-6

MICROBES IN 49 CASES OF INTRACRANIAL OR INTRASPINAL SUPPURATION

	Streptococci	Staphylococci	Others
Brain abscess (35)	27 (79%)*	6**	19
Extradural or subdural abscess (8)	9 (100%)	1+	1
Post-operative infection (3)	0	3 (100%)	0
Spinal abscess (3)	1	2 (66%)	0

* percentage of patients infected

** 4/6 post-operative or post-traumatic infection

\+ post-operative infection

with temporal lobe abscesses. In two cases, one with a subdural abscess and the other with a cerebral abscess, a mixed infection arose following neurosurgery; both patients came from the same unit and were infected with Staph. aureus and a β-haemolytic streptococcus belonging to Lancefield group C. Thirty-four percent of patients with brain abscess were infected by more than one microbe. A patient with a subdural abscess yielding a mixed bacterial population also had a positive blood culture (Case 11, Appendix A p 244)

The distribution of streptococci and staphylococci in four clinical categories is shown in Table 5-6. Streptococci were isolated from $^{27}/35$ (79%) patients with brain abscess and from all the patients with a sub or extradural abscess. Str. milleri was cultured from fourteen (50%) of patients with streptococcal brain abscess and from six (75%) of patients with sub or extradural abscess due to streptococci.

Staph. aureus, on the other hand, was an unusual isolate unless the abscess had arisen following accidental or surgical trauma, but occurred in two of three cases of spinal abscess. Eight of the ten intracranial lesions from which Staph. aureus was isolated were traumatic in origin.

Among the 35 cases of brain abscess there were 19 men and 16 women, a ratio of 5:4. This is not significantly different from the 3:2 ratio found in the group as a whole. The age distribution of these patients was also unremarkable (Table 5-7).

The sites at which the abscesses were found are shown in Table 5-8 and the relationship between the site of infection and the predisposing cause is shown in Table 5-9.

Table 5-7

35 CASES OF BRAIN ABSCESS: AGE AND SEX DISTRIBUTION

M : F ratio 5 : 4

Age	Male	Female
0-10 yrs	3	2
11-20	2	1
21-30	2	4
31-40	3	2
41-50	5	1
51-60	1	4
> 60	3	2
	19	16

Table 5-8

35 CASES OF BRAIN ABSCESS: SITES OF INFECTION

Temporal lobe	13
Frontal lobe	11
Parietal lobe	2
Occipital lobe	2
Cerebral	6
Cerebellar	1
	35

Table 5-9

35 CASES OF BRAIN ABSCESS:
ABSCESS SITE IN RELATION TO ANTECEDENT HISTORY

Cause	Temporal lobe	Frontal lobe	Parietal lobe	Occipital lobe	Cerebellum	Cerebral
Sinusitis	-	4	-	-	-	-
Influenza	1	2	-	-	-	-
Otitis	9	-	-	-	-	-
Metastatic Empyema	-	1	-	-	-	1
Abdominal Surgery	-	-	-	1	-	-
Dental Surgery	-	-	1	-	-	-
Recurrent abscess	-	1	-	-	-	-
Congenital Heart Disease	1	-	1	-	-	1
Head Injury	-	1	-	-	-	-
Penetrating Head Wound	-	1	-	-	-	-
Post-operative infection (neurosurgery)	-	-	-	1	-	1
Cryptogenic	2	1	-	-	1	3
	13	11	2	2	1	6

Abscess formation in the temporal lobe is associated with otitis while frontal lobe infection is associated with sinusitis. Metastatic, haematogenous and post-traumatic abscesses do not show any marked associations and are found in different sites. In this group of 35 patients there were seven (20%) with no antecedent medical history relevent to intracranial suppuration.

It is clear that a variety of organisms are responsible for temporal lobe abscess (Table 5-10) reflecting the wide range of bacteria found in the aural canal, especially in cases of chronic otitis media. With one exception, a woman who developed an occipital lobe abscess following extensive bowel surgery, all the isolates of Proteus were found in this group. Organisms of the bacteroides group were isolated frequently from patients with abscess of the temporal lobe, while Str.milleri and Staph.aureus were rarely found. Pus from one patient, a women of 55 who developed a temporal lobe abcess following an influenza-like illness, grew Haemophilus aphrophilus. Str.milleri was the predominant isolate in brain abscess other than temporal lobe abscess, being present in more than half of the cases. Brain abscess due to infection with Staph.aureus was associated with previous trauma in four cases out of six.

In $^{10}/_{35}$ cases the lesion was demonstrably encapsulated and eight were excised. Three abscesses which did not have an apparent capsule were also excised. There were seven patients who had multiple abscesses. Seven of the patients with brain abscess died during the course of the study (see below), but there was no correlation in this small study between mortality and excision of the abscess, encapsulation

Table 5-10

SITES OF ISOLATION OF VARIOUS MICROBES IN 35 CASES OF BRAIN ABSCESS

	Temporal lobe	Frontal lobe	Parietal lobe	Occipital lobe	Cerebellum	Cerebral
Number of cases	13	11	2	2	1	6
Str.milleri	2	7	1	1	-	3
Str.sp.	3	2	-	-	-	-
Str.pneumoniae	1	-	-	-	-	-
Str.faecalis/faedum	1	1	1	-	-	-
Peptostreptococcus sp.	2	-	-	-	-	-
Str.mutans	-	-	-	-	-	1
Str.Lancefield gp.C	-	-	-	-	-	1
Staphylococcus aureus	1	2	-	-	1	2
Bacteroides group	6	3	-	1	-	-
Proteus sp.	6	-	-	1	-	-
K.aerogenes	1	-	-	-	-	-
H.aphrophilus	-	1	-	-	-	-

or multiplicity of abscesses.

Table 5-11 shows that sub and extradural suppuration was also due predominantly to Str.milleri (6/8 cases) while staphylococci were only isolated from spinal abscess or where infection followed trauma. With the exception of one case of post-operative infection all the patients with sub or extradural suppuration were men.

TREATMENT

Sensitivity of infecting organisms

Antibiotic sensitivity tests carried out on all 69 isolates showed that 28 of them, from 26 patients (53%) were resistant to penicillin. In the majority of cases the patients were receiving multiple antibiotic therapy and the infecting organism was sensitive to one of the other antibiotics being used. In six cases, however, the patients received antibiotics that were inappropriate for treatment of their infecting organism as judged by in vitro sensitivity testing using discs of standard concentration. Five of these, who were treated initially only with penicillin, were found to be infected with strains of Staph. aureus which were resistant to penicillin. In the sixth case, K.aerogenes was isolated from the repeated aspiration of a patient treated with penicillin for an abscess from which Str.milleri and fusobacteria had previously been isolated. Str.milleri was not isolated from the second specimen and, therefore, treatment was changed to gentamicin. Four weeks later Str.milleri was again isolated from the abscess.

The minimum inhibitory concentration (MIC) of the drug being used in treatment was determined for each of the microbes isolated from the abscess. In no case was the MIC

Table 5-11

14 CASES OF INTRACRANIAL AND INTRASPINAL SUPPURATION:

MICRO-ORGANISMS ISOLATED

Organism	Site of Infection		Post-operative Wound
	Spinal (3)	Sub/Extradural (8)	Infection (3)
Str.milleri	-	6	-
Str.sp.	1	-	-
Str.mitior	-	1	-
β-haemolytic streptococci	-	1*	-
Staph.aureus	2	1*	3
Bacteroides sp.	-	1	-
Peptostreptococcus sp.	-	1	-
Proteus sp.	-	-	-

* Post operative infection (1 patient)

higher than that expected for sensitive bacteria of the same species or group. No allowance was made in the determination of antibiotic sensitivity for the high concentrations of drug which might exist within a brain abscess cavity following direct instillation. This was because of lack of information on the activity of antimicrobial drugs under such conditions.

The antibiotics used for the primary treatment of $^{33}/35$ cases of brain abscess are shown in Table 5-12. The figures show the treatment initiated or continued by the neurosurgeons and do not include that previously undertaken by the patients' general practitioners or in the referring hospital. Thirteen patients received treatment with a single antibiotic, used speculatively in twelve. Four patients treated with penicillin were found subsequently to be infected with bacteria resistant to penicillin. In the patient treated only with cephaloridine, sensitive bacteria had been isolated from the ear pre-operatively and this was used as a guide for initial therapy. Attempts were made to treat one patient conservatively with penicillin and cotrimoxazole. This was unsuccessful and the abscess had to be aspirated on five occasions. Culture of the pus yielded a mixed growth of P.vulgaris and a streptococcus sensitive to penicillin. Both organisms were sensitive to cotrimoxazole.

The sensitivity of 66 isolates to the antimicrobial drugs used most often in the treatment of intracranial and intraspinal suppuration is shown in Table 5-13. The results are expressed as the percentage of isolates found to be fully sensitive when compared to control organisms, using

Table 5-12

ANTIBIOTIC TREATMENT IN 33 CASES OF BRAIN ABSCESS

Drug	Number of patients receiving the drug	
	Alone	In Combined Therapy
Penicillin	11	13
Ampicillin	0	2
Cephaloridine	1	6
Cloxacillin	1	1
Sulphonamide/ Cotrimoxazole	0	7
Chloramphenicol	0	3
Streptomycin	0	2
Gentamicin	0	2
Kanamycin	0	1
Others	0	2
No antibiotic treatment	2	

Table 5-13

46 CASES OF INTRACRANIAL AND INTRASPINAL SUPPURATION:

PERCENTAGE SENSITIVITY OF MICROBES ISOLATED TO MAJOR ANTIBIOTICS USED IN TREATMENT

	Penicillin	Chloramphenicol	Cephaloridine	Cotrimoxazole	Amino-Glycosides*
Streptococcus sp. (37)	94	100	94	96	0
Staphylococcus sp. (9)	0	100	100	100	100
Enterobacteriaceae (8)	0	88	25	88	100
Bacteroides Group (11)	36	100	66	100	0
H. aphrophilus (1)	100	100	100	100	100
Percentage of patients with sensitive organisms	50	98	74	96	27

* Gentamicin, Streptomycin, Kanamycin

standard discs and a plate diffusion method. The Table shows that 50% of patients with brain abscess were infected with organisms which were inherently resistant to penicillin.

Mortality

During the course of the study eleven of the 49 patients, three females and eight males, died (Table 5-14). The overall mortality of patients with intracranial abscesses was 23% (10/43) and the treated mortality 19% (8/43). Treated mortality means death during the course of the study in patients who underwent any form of neurosurgical treatment. There were two patients whose abscesses were diagnosed at post-mortem or who died before any treatment could be initiated. Their deaths, with the others, constitute the overall mortality. Table 5-15 gives details of the patients who died, together with the infecting organisms, antibiotic treatment, and the interval between neurosurgical treatment and death. Two of the patients treated only with penicillin were infected with penicillin resistant organisms. The distribution of mixed infections in those who died (4/11) is no higher than that found in the total population (13/49). The age distribution among the patients who died is also the same as for the whole population. However, the male:female ratio was 2.7:1 in the patients who died, compared to 1.5:1 overall.

There is wide variation in the survival time. In two cases the abscesses were found at post-mortem; five cases died within three weeks of operation and the remaining three survived for ten weeks to six months.

Table 5-14

INTRACRANIAL AND INTRASPINAL SUPPURATION:

MORTALITY RATES

	Brain abscess	Sub and Extra dural abscess	Spinal abscess	Post-operative infection
No. of patients	35	8	3	3
Overall mortality	7 (20%)	3 (38%)	1	0
Treated mortality	5 (14%)	3 (38%)	1	0

Table 5-15

INTRACRANIAL AND INTRASPINAL SUPPURATION

PATIENTS WHO DIED

Case	Sex	Age	Site of Abscess	Predisposing cause	Organisms isolated	Antibiotic Treatment Following Drainage	Survival Time after operation
1	F	30	Temporal	CHD++	Str.milleri	Penicillin	3 weeks
2	F	54	Temporal	Otitis	Bacteroides+ Str.species	Penicillin	3 days
3	M	48	Temporal	Otitis	Proteus sp+ Str.species	Penicillin	3 days
4	M	47	Cerebral	Cryptogenic	Str.milleri	None	- **
5	M	9	Parietal	CHD++	Str.milleri	Penicillin	6 months
6	F	60	Cerebral	Post operative	Staph.aureus+ BHS Gp.C	None	- **
7	M	53	Subdural	Sinusitis	Str.milleri	Chloramphenicol Ampicillin Cloxacillin	3 days
8	M	57	Spinal	? Chest Infection	Str.species	Penicillin Ceporin	3 months
9	M	47	Subdural	Cryptogenic	Str.milleri	Penicillin	7 days
10	M	26	Temporal	Otitis	Bacteroides*	Sulphonamides Ampiclox	10 weeks
11	M	16	Subdural	Sinusitis	Str.milleri Peptostreptococcus Bacteroides+	Penicillin Gentamicin Lincomycin	12 days

++ Cyanotic Heart Disease   + Penicillin resistant   ** Diagnosed at postmortem   * 2nd tap

Brief clinical details of the patients who died are contained in Appendix A (p 236). It may be considered that some of these cases, for example, the two patients with cyanotic heart disease should not be included in the mortality figures, since their deaths were due primarily to their heart conditions and not directly to the brain abscess. This point is elaborated in the discussion.

Bacteriological Results on Patients with Intracranial or Intraspinal Sepsis

Viable bacteria were isolated from samples of pus from all 49 patients with intracranial or intraspinal suppuration at the first operation and from $^{10}/17$ samples taken at subsequent aspiration (Table 5-4). The isolation of non-fastidious organisms posed few problems, but there were difficulties with many of the streptococci and anaerobic Gram-negative rods especially when they were mixed with proteus. Most of the isolates subsequently identified as Str.milleri grew poorly, if at all, on primary blood agar plate cultures incubated aerobically, although they all grew well anaerobically. Visible growth of $^{14}/20$ isolates was obtained from primary cultures incubated in 10% carbon dioxide but only after 48 - 72 hours incubation. The aerotolerance of these strains increased with subculture. Six isolates of Str.milleri were strictly anaerobic on primary culture, three of these remained so through all subsequent manipulations while the remaining three were able to grow in 10% carbon dioxide following subculture. Seventeen of the isolates of Str. milleri were therefore considered to be microaerophiles,

while the remaining three were recognised as anaerobes. Their biochemical reactions, together with those of the other green streptococci isolated, are shown in Table 5-16. There were no marked differences in the reactions of micro-aerophilic and anaerobic strains of Str.milleri.

The six isolates of unspeciated streptococci (Table 5-4) include two which were mixed with proteus in the primary culture and which died before they could be separated, and four whose biochemical characteristics were distinct from those of the named species. However, none of these isolates grew on bile or salt media nor did they produce polysaccharides from sucrose or ferment mannite. None of these isolates were Voges-Proskauer positive. Many isolates of Str.milleri caused alpha-haemolytic changes on blood agar, which were more pronounced anaerobically than aerobically. None caused beta-haemolysis. The three isolates referred to as peptostreptococci were Gram-positive chaining cocci which did not grow after seven days' incubation in 10% carbon dioxide. They were tested by the API 20 test anaerobic system and gave the reactions of P.anaerobius. Because of the similarities between Str.milleri (Colman 1970) and the organism identifiable as P.intermedius using the API system, 30 clinical isolates of Str.milleri, including the three anaerobic strains, were tested by the API system together with six standard strains of Str.milleri supplied by the Cross Infection Reference Laboratory, Colindale. All gave reactions, following anaerobic incubation, which, according to the API system, characterise P.intermedius. Although

Table 5-16

BIOCHEMICAL REACTIONS OF STREPTOCOCCI ISOLATED FROM CASES OF INTRACRANIAL SEPSIS (Percentage Positive).

	Str. milleri	Str. mitior	Str. mutans	Str. faecalis	Str. faecium
No. of strains	20	1	1	2	1
Growth in -					
10% Bile	20	0	0	100	100
40% Bile	10	0	0	100	100
Growth in -					
4.0% NaCl	5	0	0	100	100
6.5% NaCl	0	0	0	100	100
Hydrolysis of -					
Aesculin	90	0	100	100	100
Arginine	85	0	0	100	0
V.P.test	100	0	100	50	0
Polysac from -					
Sucrose	0	0	Dextran	0	0
Acid in -					
Lactose	95	0	100	100	100
Salicin	85	100	100	100	100
Sucrose	90	100	100	50	100
Mannitol	0	0	100	100	100
Inositol	0	0	100	0	0
Sorbitol	0	0	100	100	100
Raffinose	5	0	100	0	0
Trehalose	95	100	100	100	100
Arabinose	0	0	0	0	100
Production of -					
Hyaluronidase	0	0	0	0	0

there are microaerophilic and anaerobic variants of Str. milleri it would appear that these cannot easily be separated biochemically. The term P.intermedius, if it is to be used at all, can only apply to the strictly anaerobic variants of Str.milleri.

Clinical isolates of Str.milleri were difficult to test for sensitivity to nitrofurazone. Stock strains gave reliable and reproducible results, but the results on clinical isolates were often equivocal, and the test was, therefore, not used in the identification of these organisms.

Isolates of bacteroides and fusobacteria were also identified by the API anaerobic system. There were six isolates of B.fragilis ssp fragilis and one of subspecies thetaiotaomicron. Three isolates were identified as belonging to the genus Fusobacterium.

The single isolate of E.corrodens and the various enterobacteriaceae were identified using the criteria of Cowan and Steel (1965). The organism named as Haemophilus aphrophilus conformed to the description in Cowan and Steel (1975) although it did not satisfy the taxonomic requirements for inclusion in the genus Haemophilus. The twelve strains of Staph.aureus isolated were all of different phage types.

Further Bacteriological Investigations

In addition to the 66 pus samples received, there were 38 blood cultures, 160 swabs, three CSF samples and an antral washout. The bacteriological results on these specimens are shown in Table 5-17. Only those organisms which were present in the pus sample were sought in the swabs. Five of the eleven patients whose abscesses contained Staph.

Table 5-17

BACTERIOLOGICAL RESULTS ON OTHER SPECIMENS

	Number of Patients	Patients with Positive Cultures	Staph. aureus	Proteus sp.	Str.milleri/ Bacteroides	Str.milleri
Blood culture (38)*	38	3	2	-	1	-
Nose and Throat swab (84)	34	7	6	-	-	1
Ear swabs (76)	31	5	-	5	-	-
Cerebrospinal fluid (3)	3	2	2	-	-	-
Antral washout (1)	1	1	-	-	-	1

* Number of specimens examined

aureus had organisms of the same phage type in other sites. Two patients with frontal abscesses harboured Staph.aureus in their noses and/or throats and one of them, a child who had sustained a penetrating wound through the orbit, also had staphylococci in the cerebrospinal fluid. Two of the patients with staphylococcal spinal abscesses had positive nasal and CSF or blood cultures. The fifth patient, who had a post-operative wound infection, had a positive blood culture. The third positive blood culture contained a mixture of Str.milleri and B.fragilis, organisms which were also present in the subdural abscess. Cultures from the ears of five of the seven patients infected with proteus grew organisms of the same species. All five patients had temporal lobe abscesses. Attempts to isolate Str.milleri from nose, throat and ear swabs were, with one exception, unsuccessful. In this single case, small numbers of Str. milleri were isolated from the throat of a patient whose abscess did not contain this organism. It was, however, isolated from the antral washout of a patient with a Str. milleri subdural abscess over the frontal lobe which followed an attack of sinusitis.

The small number of blood cultures taken pre-operatively were, with one exception, all sterile. The exception was a patient with a spinal abscess due to Staph.aureus, whose blood culture before and after operation contained organisms of the same phage type.

Twelve of the 35 patients with brain abscess had pre-operative lumbar punctures. In ten, the CSF protein was

raised but in only one was the CSF sugar level below normal limits. The number of leucocytes varied considerably. Two patients had fewer than five leucocytes/mm^3, three had between 5-10 cells/mm^3, and the remaining seven had from 20-2000 cells/mm^3. In no case was the CSF culture reported to have been positive.

Antibiotic Sensitivity

The antibiotic sensitivity patterns of the 69 isolates are shown in Table 5-18. None of the isolates of Str.milleri or of the unspeciated streptococci were resistant to penicillin, but penicillin resistance was demonstrated in two of the remaining streptococci, in $^{7}/11$ isolates of bacteroides and, in all isolates of staphylococcus and Eneterobacteriaeceae. Thus, $^{29}/69$ isolates from 26 patients were penicillin resistant. In addition to their sensitivity to penicillin the streptococci were equally sensitive to other antimicrobial drugs used in therapy of brain abscess, only two isolates being resistant to cephaloridine and only one to tetracycline. Chloramphenicol was active against all but one of the bacteria isolated.

The minimum inhibitory concentration of penicillin was determined for all penicillin sensitive isolates. Strains of Str.milleri had an MIC of 0.05 mg/l or less, and in 14 of 20 isolates the MIC was 0.01 mg/l. MICs to other antimicrobial drugs were performed according to the antibiotic treatment being given or proposed. In no case did the MIC obtained suggest that the isolate was more resistant to the drug under test than other sensitive strains of the same species or group.

Table 5-18

PERCENTAGE SENSITIVITY OF BACTERIA ISOLATED FROM SAMPLES OF INTRACRANIAL AND INTRASPINAL PUS

Organism	Penicillin/ Ampicillin	Chloramphenicol	Cephaloridine	Cotrimoxazole	Tetracycline	Amino-glycosides
Str.milleri (20)	100	100	100	100	100	0
Str.species (6)	100	100	100	100	100	0
Str.faecalis/ faecium(3)	66	100	66	100	100	0
Str.mutans (1)	0	100	0	0	0	0
Str.mitior (1)	100	100	100	100	100	0
Str.Lancefield Gp.C (2)	100	100	100	100	100	0
Str.pneumoniae (1)	100	100	100	100	100	0
Staph.aureus (12)	0	100	100	100	83	100
Proteus mirabilis (4)	100[+]	100	50	100	0	100
P.vulgaris (3)	0[+]	100	0	100	0	100
K.aerogenes (1)	0[+]	0	0	0	0	100
Bacteroides fragilis (7)	0	100	57	100	100	0
Fusobacteria (3)	100	100	100	100	100	0
E.corrodens (1)	100	100	100	100	100	100
Peptostreptococcus sp (3)	100	100	100	100	100	0
H.aphrophilus (1).	100	100	100	100	100	100

[+] Sensitivity to ampicillin

Tests of the minimum bactericidal concentration (MBC) of an antibiotic showed that, for the streptococci tested, the MBC was between 2 and 4 times the MIC. No attempt was made to estimate the antimicrobial activity of the pus sample against the infecting organism since it had already been shown that antibiotic activity in pus, especially the activity of penicillin, decays rapidly. Since isolates took at least 48 hours to grow in pure culture it was felt that the results of such a test would be misleading.

Antibiotic Content of Pus and Serum Samples

One hundred and twenty-five antibiotic assays were carried out on samples of pus, CSF and serum. In the majority of cases, although it was known which antibiotics the patient had received, there was no detailed information relating to the time at which the last dose had been given.

The results of assays on twenty-six patients receiving penicillin are shown in Table 5-19. There were four patients whose serum contained penicillin at levels between 0.8 and 2.2 mg/l and whose corresponding pus samples were free from detectable penicillin. In only one case was the infecting organism a recognised β-lactamase producer. Similarly, there were five patients whose pus samples contained penicillin at a level of between 0.7 and 2.5 mg/l and whose corresponding serum samples were free of detectable penicillin. None of these patients had bacteraemia nor did their intracranial lesions contain β-lactamase producers. The assay results (Table 5-19) suggest that the drug persists longer in pus than in serum, since some pus samples contained penicillin in the absence of any detectable circulating drug. In

Table 5-19

CONCENTRATIONS OF PENICILLIN IN INTRACRANIAL PUS AND SERUM

Dose (megaunits/24 hrs)	Route	Pus (mg/l)	Serum (mg/l)
1.6	oral	0.7	0.0
4.0	NK	1.0	NS
4.0	NK	1.0	1.3
4.0	NK	2.5	0.0
4.0*●	IV	10.0	2.4
6.0	IM	4.0	NS
6.4	NK	0.5	0.5
6.4	NK	0.5	1.0
8.0	NK	0.0	2.0
8.0	IV	1.0	0.0
12.0*	IV	7.0+	10.0
12.0*	IV	5.0+	NS
12.0*	IV	6.0+	7.0
12.0	IV	1.0	NS
24.0	NK	0.0	2.2
24.0	NK	0.0	1.3
24.0	NK	7.0	13.0
24.0	IV	1.0	0.0
24.0*	IV	1.9	7.1
24.0	IV	NS	0.2
40.0	IV	11.0	NS
NK	NK	0.0	0.8
NK	NK	1.0	0.0
NK	NK	1.0	2.5
NK	NK	2.0	2.5
NK	NK	NS	10.0

NS = No specimen

NK = Not known

* = Timed dose

\+ = Blood stained specimen

● = Subdural abscess

addition to these 26 patients there were ten who were reported to be receiving penicillin at dosage regimens of between 4 and 24 megaunits per 24 hours but for whom assays on both pus and serum proved negative.

The results show that, even allowing for the possibility that doses may not have been given during the operative pre-medication period, there is considerable variation in the efficiency with which penicillin enters an abscess cavity. This is borne out by the results of assays carried out on specimens collected at timed intervals after the last injection (Table 5-20). All the samples specified in the Table were collected at the same neurosurgical unit and transported under identical conditions. The results indicate that there is considerable variation in individuals with regard to the level of penicillin in serum. The peak level of penicillin in intracranial pus seems to occur later and to be at a lower level than the peak serum level.

No correlation can be established between the dose administered and the concentration of active drug in intracranial pus on the results obtained, although it is apparent that dosage regimens of four megaunits per day or less fail to produce adequate concentrations of active drug within an abscess cavity as judged by clinical response. Serum levels of penicillin may prove a useful indication of drug concentration in intracranial pus since in the majority of cases where the serum level was in excess of 1 mg/l there was also detectable drug in the pus sample.

The results of assay of other β-lactam antibiotics

Table 5-20

LEVELS OF ANTIMICROBIAL DRUGS IN INTRACRANIAL PUS AND SERUM FOLLOWING TIMED INJECTIONS IN 4 PATIENTS

Drug	Dose	Route	Time (hrs) since last injection.	Level in PUS (mg/l)	Level in SERUM (mg/l)
Penicillin	4 m.u	IV	2.0	1.9	7.1
	4 m.u	IV	2.5	10.0	2.4
	3 m.u	IV	5.0	7.0	10.0
	3 m.u	IV	5.0	5.0	NS
	3 m.u	IV	5.0	6.0	7.0
Cephaloridine	1.0 g	IM	5.0	0.7	1.5
	1.0 g	IM	5.0	1.0	NS
Chloramphenicol	1.2 g	IM	1.0	>10.0	>10.0

NS = No specimen

are shown in Table 5-21. The number of cases in each of the three groups is small but, nevertheless, the penetration of ampicillin, cloxacillin and cephaloridine appears to be erratic. Details of dosage and route of administration were not available for these patients and, therefore, it is difficult to draw any conclusions from the results. If the patients were receiving standard treatment regimens then it would seem that the three agents penetrate intracranial pus less effectively than penicillin. Assay results on a further five patients who were, reportedly, receiving these drugs were negative for both serum and pus samples.

The results of assays for the non-β-lactam antibiotics are shown in Table 5-22. Although the number of patients who received the drugs is small it is apparent that gentamicin penetrates poorly. Chloramphenicol, fusidic acid and lincomycin, on the samples tested, all penetrated well. No attempt was made to differentiate between antibiotic which was free in the abscess cavity and that which was due to contamination with blood. However, Tables 5-19 and 5-21 show that many of the highest levels found in pus samples occurred in specimens which were heavily bloodstained.

It did not prove possible to draw any conclusions on the adequacy of treatment in terms of the level of active agent at the site of infection. There was considerable variation in the amount of drug prescribed. Penicillin dosage ranged from 1.6 megaunits per day orally to 40 megaunits per day I.V., the majority of patients receiving between 4 and 24 megaunits by the intravenous or the intramuscular route.

Table 5-21

CONCENTRATIONS OF OTHER β-LACTAM ANTIBIOTICS IN INTRACRANIAL PUS AND SERUM IN EIGHT PATIENTS

Drug	PUS (mg/l)	SERUM (mg/l)
Ampicillin	3.5+	6.0
	< 0.5	10.0
	0.5	0.5
	1.1	2.5
	1.0	2.5
Cloxacillin	2.0+	2.0
	0.5	2.7
	< 0.5	10.0
	2.0+	2.0
	< 0.5	7.5
Cephaloridine	1.5	1.5
	0.1	0.0
	0.7*	1.5
	1.0*	NS
	4.0+	5.0

NS = No specimen
* = Timed specimen
\+ = Heavily bloodstained

Table 5-22

CONCENTRATIONS OF NON β-LACTAM ANTIBIOTICS IN INTRACRANIAL PUS AND SERUM IN NINE PATIENTS

Drug (Daily dose)	Route	PUS (mg/l)	SERUM (mg/l)
Gentamicin			
160 mg	IM	< 1.0	1.5
240 mg	IM	< 0.5	1.0
240 mg	IM	1.0	1.0
240 mg	IM	0.0	0.3
NK	IM	< 0.5	4.5
Chloramphenicol			
2.4 g	IV	< 5.0	13.0
0.12 g	topically	6.0	NS
7.2 g	IM	>10.0	>10.0
Fusidic Acid			
1.5 g	NK	6.2	6.2
NK	NK	>10.0	NS
Lincomycin			
NK	NK	4.0	8.0

NS = No specimen
NK = Not known

Higher serum and pus levels were obtained in those on the higher dosage regimens. There were two patients, one being treated with cephaloridine, the other with ampicillin, who improved clinically after the dosage of antibiotics was increased. Both had previously low levels of antibiotics in pus and in serum.

Two of the ten patients with encapsulated abscesses had adequate serum levels of antibiotic but no detectable drug in the pus sample. The remaining eight patients either had not received antibiotic or had undetectable levels of the drug in serum and pus before excision of the abscess. No detectable drug was found in samples of CSF received from three patients being treated with penicillin. In one patient, assays on the corresponding samples of pus and serum were also negative but, in the other two, there were detectable levels in the serum (between 1 and 3 mg/l), but none in the pus samples.

Serological Results

All the isolates of Str.milleri reacted with Lancefield group F antiserum and with the Ottens and Winkler type O III serum but not with any of the other antisera. Since the Ottens and Winkler strain used to produce the serum lacked a Lancefield group antigen, the reaction was adjudged to be specific for the type O III antigen. Serum from patients was tested also against the homologous antigen and against the crude untreated sample of pus. In the majority of cases serum from patients contained precipitating antibodies to the infecting organism. The reacting sera also produced precipitin lines when run against the corresponding pus sample,

demonstrating the presence of antigen therein. No attempt was made to determine whether the circulating antibody was directed against a streptococcal group antigen, the Lancefield group F antigen, the Ottens and Winkler type O III antigen or the unspecified antigen referred to by Colman (1970).

Serum from a patient whose pus contained Str.milleri only was selected and was used to screen a number of pus samples. It reacted strongly with its homologous antigen, and with Lancefield group F and Ottens and Winkler type O III antigens. The antigen produced from the infecting organism gave strong reactions with Lancefield group F and Ottens and Winkler type O III antisera. The selected serum was tested against a number of streptococcal antigens and pus samples infected with the microbe from which they had derived. Heavy precipitin lines were produced against this serum by Str.milleri antigen whether as an extract or in pus. Weak lines, which disappeared on dilution of the serum, were produced by other streptococci while pus samples containing organisms other than streptococci failed to produce precipitin lines. These results indicate that the serum contained specific group and type antibodies in high titre but only low levels of non-specific streptococcal antibody. Using the serum selected as an indicator, Str.milleri antigen was detected in three samples of pus within 1½ hours of receipt of the specimen.

The three isolates of faecal type streptococci all reacted specifically with the Lancefield group D antiserum.

Gas Liquid Chromatography

Forty samples of intracranial pus, six of pus in Brewer's medium which had been incubated for 48 hours and three of cerebrospinal fluid were examined chromatographically. Table 5-23 shows the fatty acids detected in liquid pus samples from which aerobic bacteria only were isolated while Table 5-24 shows those in samples which contained anaerobic bacteria.

Aerobic bacteria either produce no fatty acids or only those of short chain length, acetic and propionic acids. Anaerobic bacteria are characterised by the production of the longer chain fatty acids, butyric, valeric and caproic. There was a close correlation between the GLC and the cultural results on these specimens. In only one case did the pattern of fatty acids detected indicate the presence of anaerobic bacteria in a sample that yielded aerobes only on culture. This sample consisted of a small amount of pus which was sent in a dry state from Liverpool. The failure to isolate anaerobic bacteria is, therefore, not surprising.

Gas liquid chromatographic analysis cannot be performed unless the specimen is in a liquid state. The pancreatin procedure previously described (p 156) for liquefying samples of pus prior to antibiotic assay was, therefore, investigated. Three samples of liquid pus were each divided into two portions. To one was added an equal volume of pancreatin solution and to the other an equal volume of sterile water. Following incubation at 37°C for thirty minutes, the aliquots were chromatographed and the results are shown in Table 5-25. Pancreatin treatment had no effect on the GLC profiles of pus

Table 5-23

FATTY ACIDS DETECTED BY GLC IN PUS SAMPLES

FROM WHICH AEROBIC BACTERIA ONLY WERE ISOLATED

Fatty Acids	Result of Culture
No acids	Proteus vulgaris*
Acetic, propionic	Str.milleri
" "	P.vulgaris*
" "	Str.milleri
" "	P.mirabilis
" "	No growth*
" "	Str.milleri
" "	No growth*
" "	Str.milleri
" "	Str.milleri
No acids	Streptococcus sp
	P.vulgaris
Acetic, propionic	Str.milleri
" "	Str.milleri
" "	Str.faecalis
" "	Haemophilus aphrophilus
" "	Str.milleri, Staph.aureus
Acetic, propionic, butyric, isovaleric	Str.milleri

* These specimens were from second intracranial aspirations

Table 5-24

FATTY ACIDS DETECTED BY GLC OF PUS SAMPLES FROM WHICH ANAEROBIC BACTERIA WERE ISOLATED

Fatty Acids	Result of Culture
Acetic, propionic, isobutyric, butyric, isovaleric	Bacteroides sp. Streptococcus sp.
Acetic, propionic, isobutyric, butyric, isovaleric	Bacteroides sp. Str.milleri
Acetic, propionic, isobutyric, butyric, isovaleric	Peptostreptococcus sp. Proteus mirabilis
Acetic, propionic, butyric, isovaleric, valeric, isocaproic	Bacteroides sp. Proteus vulgaris
Acetic, propionic, isobutyric, butyric, isovaleric	Peptostreptococcus sp. Proteus vulgaris
Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	Bacteroides sp. Peptostreptococcus sp. Proteus mirabilis

Table 5-25

THE EFFECT OF PANCREATIN ON THE GLC OF 3 PUS SAMPLES

Specimen	Fatty Acids	Culture Results
Pus A + pancreatin	Acetic, propionic, butyric, isovaleric	Bacteroides sp.
Pus A + water	Acetic, propionic, butyric, isovaleric	
Pus B + pancreatin	Acetic, propionic	Str.milleri
Pus B + water	Acetic, propionic	
Pus C + pancreatin	Acetic, propionic	P.vulgaris
Pus C + water	Acetic, propionic	
Water + pancreatin (control)	Acetic	

samples for short chain fatty acids, and mucoid specimens could be liquefied satisfactorily in this way. Eight mucoid samples of pus were treated in this way. There was complete correlation between chromatographic and cultural results (Table 5-26).

Three untreated samples of pus, analysed by gas liquid chromatography were analysed again following 48 hours incubation at 37°C in Brewer's medium. The results are shown in Table 5-27 and would suggest that, where the number of anaerobic bacteria present is high, there is no difference in the fatty acids detected in crude pus or in broth culture. If the number of anaerobic bacteria present is small then GLC of incubated cultures may give more reliable results. Table 5-28 illustrates the potential application of GLC in monitoring the course of intracranial suppuration. Eradication of anaerobic bacteria from the abscess is accompanied by rapid disappearance of the longer chained fatty acids from the GLC profile.

The results of GLC on three samples of cerebrospinal fluid and on pus drawn from the same patients are shown in Table 5-29. All of these samples contained acetic and propionic acids only as did two samples of physiologically normal CSF collected from patients with pyrexia of unknown origin. From these results it is, therefore, impossible to know whether or not fatty acids from intracranial abscess pass into the CSF.

Table 5-26

FATTY ACIDS DETECTED BY GLC OF MUCOID PUS SAMPLES

FOLLOWING TREATMENT WITH PANCREATIN

Fatty Acids	Results of Culture
Acetic, propionic, butyric, isovaleric, valeric	Bacteroides sp. Str. faecium Proteus mirabilis
Acetic, propionic, butyric, isobutyric, valeric	Bacteroides sp. Str.milleri
Acetic, propionic, butyric	Fusobacterium
Acetic, propionic	Str.milleri
Acetic, propionic	Str.milleri
Acetic propionic	Str.sp.
No acids	Staph.aureus
No acids	Staph.aureus

Table 5-27

GLC ON PAIRED SAMPLES OF PUS AND BROTH CULTURE

Sample	Fatty Acids	Culture Results
Pus	Acetic, propionic	P.mirabilis
Broth	Acetic, propionic, isobutyric, butyric, isovaleric, valeric isocaproic	Bacteroides sp. Peptostreptococcus P.mirabilis
Pus	No acids	Proteus vulgaris
Broth	Acetic, propionic,	Streptococcus sp.
Pus	Acetic, propionic	P.vulgaris
Broth	Acetic, propionic	P.vulgaris

Table 5-28

CHANGES IN FATTY ACID PROFILE DURING INFECTION

Patient	Pus Samples	Fatty Acids	Culture Results
1	1 - 3	Acetic, propionic, butyric	Bacteroides sp. Str.milleri
	4 - 7	Acetic, propionic	Str.milleri
	8	Acetic, propionic	No bacterial growth
2	1	Acetic, propionic, isobutyric, isovaleric	Peptostreptococcus sp. Proteus vulgaris
	2	No acids	Proteus vulgaris
3	1	Acetic, propionic, isobutyric,butyric isovaleric	Bacteroides sp. Proteus vulgaris
	2	Acetic, propionic	Proteus vulgaris
	3.	Acetic, propionic	Proteus vulgaris

Table 5-29

GLC ON SAMPLES OF PUS AND CSF FROM THREE PATIENTS

Specimen	Fatty Acids	Culture Result
Pus	acetic, propionic	Str.faecalis
CSF	acetic, propionic	Str.faecalis
Pus	acetic, propionic	No growth - 2nd tap
CSF	acetic, propionic	No growth
Pus	acetic, propionic	Staph.aureus, Str.milleri
CSF	acetic, propionic	Staph.aureus

APPENDIX A

CASE HISTORIES OF ELEVEN PATIENTS DYING OF INTRACRANIAL OR INTRASPINAL ABSCESS

Eleven of the 46 patients (24%) are known to have died during the course of the study. An examination of the clinical records, however, reveals that death was not always due to the failure of treatment or directly to the infection. details of those who died are given below :

Case 1

A 30 year old woman suffering from congenital cyanotic heart disease was admitted from another hospital with pyrexia, severe photophobia and neck stiffness. On admission she was found to be cyanosed, drowsy and severely dysphasic. An EMI scan revealed a low density area in the left temporo-frontal region, suggestive of an abscess. Aspiration through a left temporal burr-hole released 20ml of fluid pus from which *Str.milleri* was isolated. Pre-operatively she had been treated with penicillin (40 mega units per day), and the pus sample was found to contain 11 mg/l penicillin. Post-operatively she made a good recovery, with improvement in the right upper motor neurone facial weakness and the dysarthria. Within three weeks she was able to walk round the ward, her abscess cavity had decreased in size, and she was receiving oral penicillin only. She collapsed suddenly, had a cardiac arrest and died. The cause of death was recorded as congenital heart disease complicated by a cerebral abscess.

Case 2

A 54 year old woman, with a four year history of right sided earache and facial pain, was admitted to hospital following a two week period of nausea, vomiting and disturbed balance. On examination, she had right otitis media with no swelling of the optic discs. The visual fields were full but she had a mild hemiparesis. A carotid angiogram revealed a large space-occupying lesion in the right temporal lobe. This was aspirated via a burr-hole on the first day and 28 ml of pus was released. She was retapped on the following day, and a further 10 ml of pus were aspirated, from which *B.fragilis* and a streptococcus were isolated. In spite of treatment the patient's condition continued to deteriorate and she died. At post mortem multiple abscesses were found in the temporal lobe.(Figure 5-2.)

Case 3

A 48 year old man with long standing otitis media was admitted to hospital for mastoidectomy, because of increasing headaches and giddiness. On the day of operation he became drowsy and was referred to the Neurosurgical Unit. A burr-hole was made and 15 ml of foul pus was released. After an initial improvement the patient rapidly deteriorated into coma with fixed pupils and had to be maintained on a ventilator. A retap through the existing burr-hole produced necrotic brain only. An angiogram revealed a large avascular mass in the temporal region but attempts to aspirate it were unsuccessful. The patient's condition did not improve and he developed severe diabetes insipidus. A craniotomy was performed and part of the temporal lobe containing an indurated mass the size of a golf ball was removed. The centre of the mass consisted of blood clot.

Following operation the patient showed severe medullary dysfunction and was unable to maintain spontaneous respiration. He died twenty-four hours later. Culture of the pus sample produced P.mirabilis indistinguishable from an organism present in the ear, a streptococcus and diphtheroids. Post mortem examination was not performed.

Case 4

A 47 year old man was transferred to the Neurosurgical Unit, from another hospital, with acute paraplegia. He had a history of being unwell for about three weeks, with progressive weakness of the lower limbs. On admission he was conscious and orientated. He had spastic weakness of the lower limbs and the tendon reflexes were absent. Both plantar responses were extensor. There was blunting to pin prick stimuli up to the level of the umbilicus. X-ray of the dorsal spine showed marked spondylotic changes at the level of D8 and D9. A myelogram showed no obstruction in the spinal canal. The day after admission the patient collapsed and died in spite of all resuscitative measures. Post mortem examination showed multiple cerebral abscesses with purulent meningitis from which Str.milleri was isolated in pure culture.

Case 5

A 9 year old boy with Fallot's tetralogy, who had had a left parietal abscess 4 years previously, was admitted with a 24 hour history of right frontal headaches, followed by nausea and vomiting. On admission he was found to be cyanosed, with a mild pyrexia and a left systolic murmur over the sternum. He was conscious and orientated with no papilloedema. He had a left hemiparesis, with extensor plantars and diminished sensation to pin prick on the left side. An EMI scan showed

an area of diminished density in the right hemisphere, and a repeat scan one week later showed a large abscess in this region. A right parietal burr-hole was made and through it 50 ml of pus was aspirated from a large thick-walled abscess. Culture of the pus yielded a pure growth of Str.milleri. Post-operatively he made good progress with almost immediate recovery from his hemiparesis. The abscess was aspirated on a number of occasions and penicillin was instilled. He improved and became ambulant and apyrexial, and the pyogram showed shrinkage in the abscess cavity. He was discharged home. Three months later while undergoing elective surgery for his heart condition the patient died.

Case 6

A 60 year old woman suddenly lost consciousness following a two week history of generalised headaches, neck stiffness and aches and pains. On admission, a diagnosis of sub-arachnoid haemorrhage was made and she was transferred to the Neurosurgical Unit, where she was found to be conscious and rational. There was marked neck stiffness and the left plantar response was extensor. A bilateral carotid angiogram revealed a large aneurysm arising at the bifurcation of the right internal carotid artery. The aneurysm was dissected out and a clip inserted. Post-operatively the patient made a good recovery and was conscious and alert with minimal hemiparesis. Ten days after the operation she complained of headache and was found to have a left hemiparesis. There was no indication of haemorrhage at the operation site. She improved gradually but suddenly lost consciousness on the tenth day. An emergency carotid angiogram was undertaken but blood failed to flow

past the syphon of the carotid artery. In the evening of the same day the patient died. At post mortem she was found to have meningitis and a right-sided cerebral abscess (Figure 5-3). The pus sample was cultured and grew Staph. aureus and a Lancefield group C β-haemolytic streptococcus.

Case 7

A 53 year old man was admitted with a six week history of pain over the sinuses, associated with a purulent and sometimes bloody nasal discharge which had not responded to antibiotics. His condition deteriorated and he was admitted to hospital in a drowsy unco-operative state with a temperature of 40°C. A lumbar puncture revealed an excess of leucocytes and raised protein concentration but the culture was sterile. While in hospital he developed a right-sided weakness and had a series of focal epileptic attacks affecting the right side. He was referred to a neurosurgical unit and was found, on admission, to be unconscious with a right-sided flaccid hemiplegia and marked neck stiffness. The pupils were equal and unreactive, the fundi normal and both plantar responses were extensor. A left carotid angiogram was carried out which revealed the presence of a subdural effusion with marked swelling of the left cerebral hemisphere. At operation an extensive left-sided subdural abscess was found. Approximately 25ml thick pus was aspirated from which Str.milleri was isolated. In spite of treatment with large doses of antibiotics his condition showed very little improvement postoperatively and he continued to have right-sided fits. He died without regaining consciousness two days later. No post mortem examination was performed.

Case 8

A 57 year old man was admitted with a two week history of left sided chest pain which later extended to the middle of the back and which did not respond to ampicillin. On the day of admission his legs went weak and he fell to the floor. On examination he was found to have a flaccid paraparesis of the lower limbs. Vibration and position senses of the lower limbs were normal. The reflexes were increased and the plantar responses were extensor. Plain X-rays of the spine showed no abnormality but myelography revealed a complete block at the level of T8. A laminectomy was performed from T7 to T9 and an extradural abscess was found in the spinal canal from which streptococci were isolated. He was treated with ampicillin and cephaloridine. Post-operatively he made poor progress although the wound closed satisfactorily. Initially his temperature rose to 40°C but settled on treatment. At this stage he was found to be blind in one eye, probably due to a thrombosis of the central retinal artery. He gained a little movement of his lower limbs but could not move his hips or knees. Three weeks after the operation he had profuse melaena and vomited a little blood. He went into a state of surgical shock which was corrected by massive blood transfusions. Over the next three weeks he improved gradually, but then complained of shortness of breath and vomited coffee-coloured material. His blood urea was 365 mg/dl and his blood sugar 920 mg/dl. In spite of treatment his breathlessness worsened until he lapsed into unconsciousness and died. Two electrocardiograms were done before death. The first showed myocardial insufficiency and the second a posterior myocardial infarct. A coroner's post mortem was performed butthe report

was not available.

Case 9

A 47 year old man was admitted to hospital following a grand mal fit. Ten days prior to admission he had hit his head but had not lost consciousness. Following this accident he had felt unwell and feverish and had developed a puffy swelling on the forehead which slowly increased in size. On admission he was found to be delirious and incontinent of urine. He was pyrexial (40°C) with a tachycardia of 130. He had a large boggy swelling over the right frontal bone with right periorbital oedema. The reflexes were symmetrical and there was an extensor plantar response on the right. He continued to deteriorate during his stay in hospital and three days before his transfer to the Neurosurgical Unit he developed neurological signs on the left side. On transfer, he was found to be drowsy although responding to the spoken word. The pupils were equal and reacting. There was marked neck stiffness and a "Potts puffy tumour" in the right frontal region. There was a left central facial weakness and a left hemiparesis. Touch and pin prick sensations were present bilaterally and the reflexes were symmetrically increased in the arms. The plantar responses were flexor. This very ill toxic patient was pyrexial (40°C) had a pulse of 130 and blood pressure of 190/100. The only abnormal laboratory finding was a leucocytosis of 27,500/mm^3. Two EEGs were performed. The first showed a posterior frontal right sided abnormality and the second a right parietal abnormality which was not typical of an acute cerebral abscess. A right carotid angiogram showed a shift of the middle cerebral artery across the mid-line which suggested a marked swelling of the hemisphere.

At operation a subgaleal abscess was removed together with a quantity of necrotic debris. Following exposure of the underlying bone, four burr-holes revealed a pus filled diploe. After craniectomy extra and subdural abscesses were found. The patient was treated with intravenous and intrathecal penicillin. Post-operatively he remained unconscious with a persistent tachycardia and pyrexia. On the third post-operative day he became jaundiced and began to bleed from many sites. Despite intensive treatment he continued to deteriorate and died on the seventh post-operative day. Culture of the pus sample yielded Str.milleri. A coroner's post mortem was performed but the report was not available.

Case 10

A 26 year old man with meningitis following a six month history of right ear infection was transferred because of progressive drowsiness. On admission he was conscious and orientated. There was no papilloedema or visual field defect, but signs of meningitis were present and the right ear was discharging. The cerebrospinal fluid was clear and under normal pressure, but right carotid angiography showed a mass in the temporal lobe. Pus drained from a right temporal burr-hole yielded aerobic and anaerobic streptococci and staphylococci. The abscess cavity was irrigated with antibiotics. Following operation, the patient fell into coma almost immediately. Right temporal craniotomy and decompression was performed with enucleation of the chronic abscess. Culture of this sample yielded a pure growth of Bacteroides sp. He improved immediately.

Radical mastoidectomy with removal of a massive cholesteatoma was performed subsequently. His conscious level fluctuated and he continued to drain heavily infected material. Haematemesis and melaena followed. Despite healing of the head wounds and re-establishment of spontaneous respiration, ventriculitis supervened and he died. There was no post mortem examination but the sequence of events suggested that the abscess had ruptured into the ventricle immediately after the first operation.

Case 11

A boy of 16 was admitted extremely ill and totally aphasic with swelling of the right optic disc and a right hemiplegia. He had had an influenza-like illness two weeks before, followed by pain around the left eye, headaches and photophobia, and had been treated for sinusitis with Septrin. An extensive left sided subdural abscess was drained by multiple burr-holes on the day of admission to a neurosurgical unit. His condition deteriorated and left frontal craniotomy with frontal lobectomy was performed.

He did not improve and died ten days later. Autopsy showed oedema and softening of the whole left hemisphere with pus in both frontal sinuses. Culture of the pus sample yielded Str.milleri, Bacteroides fragilis and Peptostreptococcus sp. and a blood culture, collected pre-operatively, yielded Bacteroides fragilis and Str.milleri.

APPENDIX B

A CASE OF FUNGAL CONTAMINATION OF A PENETRATING WOUND OF THE CRANIUM

Case 12

A 59 year old man sustained a penetrating wound of the right orbit as a result of a motor cycle accident. Radiographic investigations showed that there was a comminuted fracture of the roof of the right orbit with extensive damage and haemorrhage along the line of penetration which extended into the substance of the right thalamus. Haemorrhage was seen extending medially into the caudate nucleus and the right lateral ventricle. During his stay in hospital, the patient developed a considerable hydrocephalus and a ventriculo-peritoneal shunt with a Spitz-Holter valve was used to correct it. Following transfer to another hospital for convalescence the patient was readmitted eleven weeks after his accident for reassessment. An EMI scan carried out at this time showed a marked reduction in the size of the lateral ventricles. There was a dense mass in the lower portion of the right frontal lobe which was displacing the ventricular system to the right. A low density area in the right frontal lobe, which had been seen eight weeks earlier, had become more defined and in the post Conray scans a definite vascular blush was seen outlining a capsule. This feature suggested that a chronic abscess had formed. The area was aspirated via a right frontal burr-hole.

Approximately 10ml of yellowish-green liquid was removed, in which was suspended particulate matter, some of which was

pigmented. Microscopically the specimen contained non-branching, nonseptate hyphae of irregular outline, the ends of which showed features suggestive of budding (Figures 5-4 and 5-5). The hyphae did not appear to be pigmented. In addition there were large pigmented structures. Figure 5-6 shows the Gram stained appearance of this material. The specimen was not pus and there were only a few red and white cells present. Following incubation of the specimen at 37°C for five days the spherical bodies visible in Figure 5-5 appeared to have increased in size (Figure 5-7).

A sample of cerebrospinal fluid taken at the same time contained a few red blood cells but no white cells. There were pigmented aggregates, 20/30µ in diameter, similar to those seen in the aspirate (Figure 5-8). These were apparently composed of smaller round moieties 2-3µ diameter, some of which were actively motile. There were no hyphal elements (Figure 5-9).

Mycological Investigations

In addition to the routine media used in clinical mycology the samples were also inoculated onto:-

a) Chytrid medium (Booth 1971, p 71) which contained:

Cornmeal agar	17g
Glucose	5g
Soluble starch	5g
Peptone	1g
Yeast extract	1g
Distilled water	1 litre

The ingredients were dissolved and sterilised at 121°C for 15 minutes.

Fig.5-4 Wet preparation of liquid from the brain of Case 12 showing dimorphism of structure (x 150).

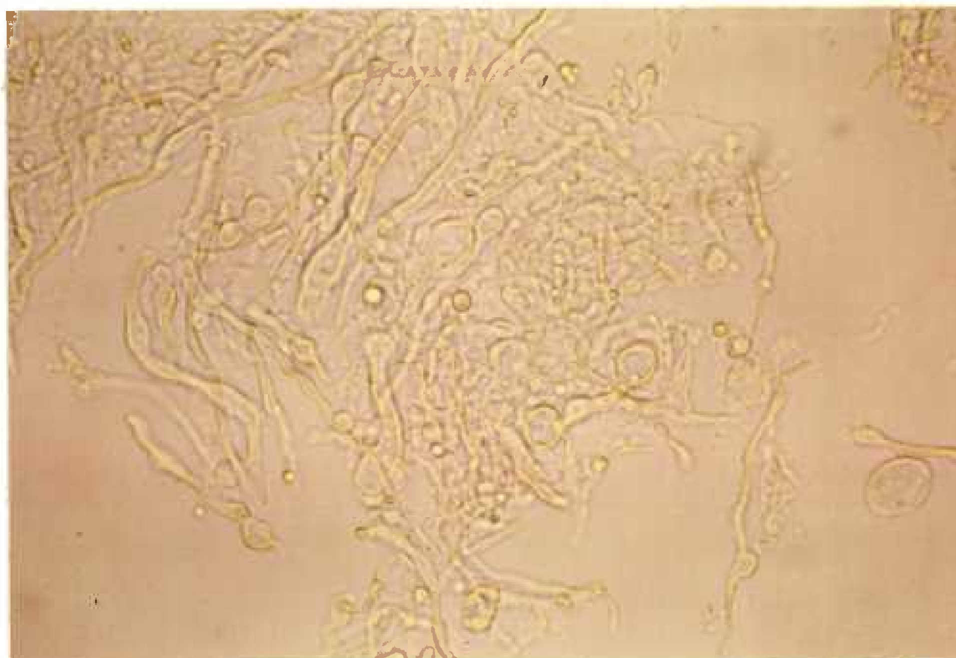

Fig.5-5 A high power view of Fig.5-4. Non-septate non-branching mycelium is seen, with round structures either terminally or free (x 600).

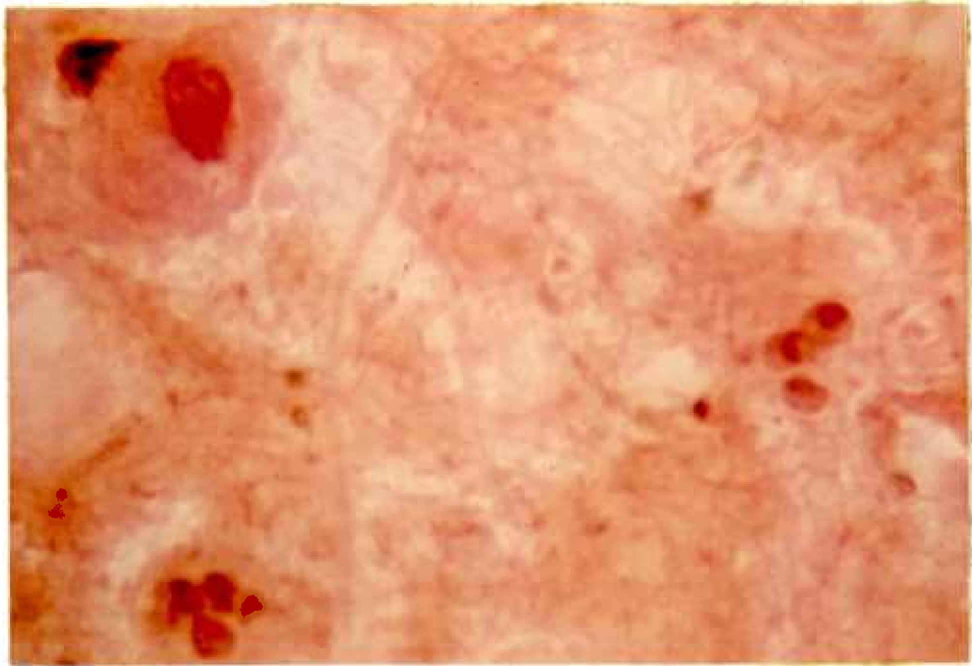

Fig.5-6 Gram film of liquid from the brain of Case 12. It is not pus and faintly staining elongated structures can be seen centrally and to the right (x 800).

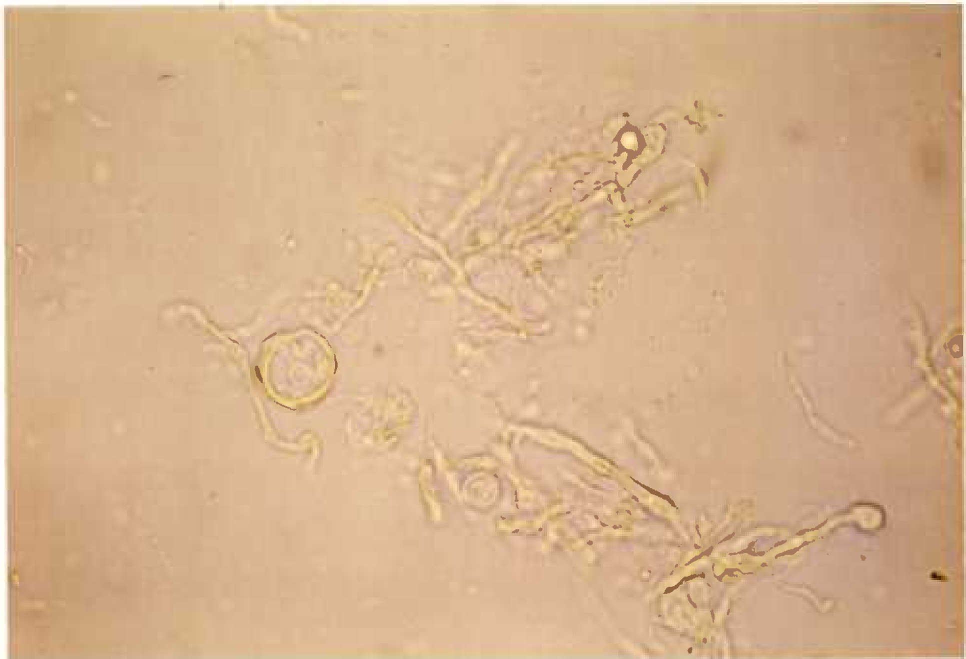

Fig.5-7 A high power view of the fluid from the brain of Case 12 after incubation of the specimen for 5 days at 37°C. The thick walled round structures seem to have increased in diameter. No other changes were observed following incubation (x 600).

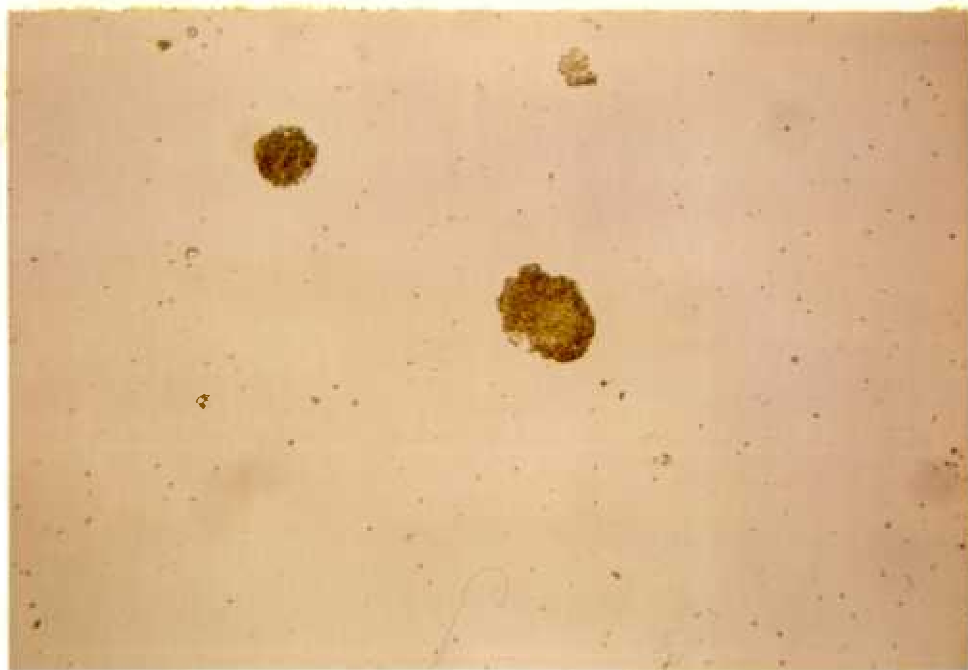

Fig.5-8 Cerebrospinal fluid from Case 12 containing pigmented aggregates (30-50um in diameter). (x 200)

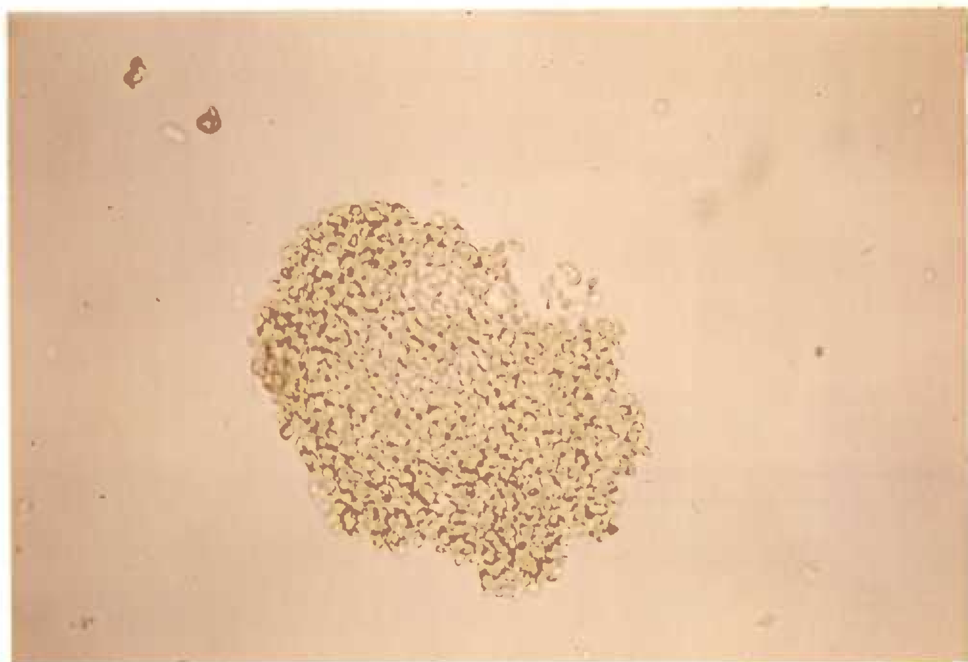

Fig.5-9 A high power view of Fig. 5-8 showing that the aggregates are composed of myriads of smaller structures (2-3 um in diameter). These cells were flagellated and actively motile (x 800).

b) Tap water agar, prepared by adding 2% agar to tap water and autoclaving at 121°C for 15 minutes.

c) Tap water agar with the addition of 0.5% peptone.

Aliquots of untreated specimen were also incubated in bijou bottles.

Cultures were incubated at 4°C, 25°C and 37°C and were maintained for ten weeks with weekly examination. The organisms failed to grow on any of the media although the pus samples incubated at 37°C showed some changes in the large spherical bodies which had been seen previously (Figure 5-9).

Aliquots of the specimens were also sent to Dr.D.W.R. McKenzie, Mycological Reference Laboratory, London W1, Dr. Phyllis Stockdale, Commonwealth Mycological Institute, Kew, Surrey, and to Dr. Nicole Nolard, Brussels, all of whom failed to isolate a viable fungus.

Serological tests on the patient's serum failed to detect antibodies to the Phycomycete, Absidia or Rhizopus groups of fungi.

SECTION 6 DISCUSSION

This study has demonstrated that streptococci are the group of organisms most frequently isolated from brain abscess (80%), a finding in agreement with the reports of McFarlan (1943) and Garfield (1969). Further, the majority of isolates and the most common single species found in the abscesses is a recognisable strain of streptococcus, Str.milleri, substantiating the original hypothesis that Str.milleri has a predilection for abscess formation in the central nervous system.

The criteria used to identify an organism as Str.milleri were more rigid than those of Colman (1970), Parker and Ball (1976) or Poole and Wilson (1976). By using a large number of tests Colman (1970) and Parker and Ball (1976) were able to speciate the green streptococci with a high degree of accuracy and could allocate organisms with discrepant biochemical characters to an appropriate species confidently. Fewer tests were used in this investigation but all the isolates named as Str.milleri produced acetylmethylcarbinol from glucose, and in other respects conformed to the characteristics laid down by Colman (1970). Six isolates were identified only to the level of genus: two died before they could be fully investigated, while the remaining four gave negative results with the Voges-Proskauer test, were slow to grow even after subculture and differed from the expected reactions of Str.milleri in at least one other respect. Slow growth may have been responsible for discrepancies in their biochemical reactions and their failure to react with the Lancefield or Ottens and Winkler antisera. Comparing

their reactions with those reported by Colman (1970), they were found to approximate more closely to those of Str.milleri than of any other species. Nevertheless they were regarded as a separate group.

In agreement with the work of others dealing with fresh clinical isolates of Str.milleri and group F streptococci (Lui 1954, Bateman et al. 1975, Poole and Wilson 1976) the present study demonstrated that primary growth of many isolates of Str.milleri occurred only anaerobically or in the presence of 10% carbon dioxide, the majority of strains showing increased aerotolerance with repeated subculture. There were, however, three strains of streptococci which were, apparently, strict anaerobes, although they had the biochemical characteristics of Str.milleri, were metronidazole resistant and had a GLC profile characteristic of aerobic bacteria.

The problem of establishing the role of anaerobic bacteria in intracranial sepsis is complicated by the fact that the organism described and recognised as Str.milleri (Colman 1970, Colman and Williams 1972), is biochemically indistinguishable from that referred to as Peptostreptococcus intermedius in the Virginia Polytechnic handbook (Holdeman and Moor 1972) and in the API system for the identification of anaerobic bacteria. The fatty acid profile for P.intermedius, determined by gas liquid chromatography, shows that it produces only acetic acid, with or without propionic acid, and none of the longer chain acids. In the present study, all isolates of Str.milleri, irrespective of their atmospheric requirements, produced only the two short chain fatty acids. The results of the present investigation suggest that there are no biochemical differences between the carboxyphilic and the anaerobic strains of Str.milleri. The readiness with which the majority

become more aerotolerant, their resistance to metronidazole and their 'aerobic' GLC profile, raises doubts about the validity of the binomial P.intermedius for these organisms.

All strains of Str.milleri isolated from abscess of the central nervous system possessed the Lancefield group F and the Ottens and Winkler type O III antigens. No studies of the distribution of the Ottens and Winkler subtypes in the British population have been made but the work of Ottens and Winkler (1962) suggests that it is unlikely that a single serotype would predominate. Consideration must, therefore, be given to the suggestion that the predilection of Str.milleri for the central nervous system is confined to organisms which possess the Lancefield group F and the Ottens and Winkler type O III antigens. Although Collis (1944) proposed that the streptococci responsible for brain abscess possessed an invasive factor, this was not investigated in the present work. The mechanisms by which Str.milleri establishes pyogenic lesions in man, and any differences in pathogenicity between streptococci of different Lancefield groups or Ottens and Winkler types have yet to be established.

The first report of the isolation of an organism resembling Str.milleri from a brain abscess was by Wheeler and Foley (1943), who isolated a Lancefield group F streptococcus from the abscess of a child. Thomas (1939) had, previously, reported a fatal case of purulent meningitis due to a minute haemolytic streptococcus belonging to Lancefield group F, and a similar case of meningitis was reported by Koepke (1965). Following the publicity given to the viridans, 'minute' and 'indifferent' streptococci by Colman and Williams

(1972,1973) there have been a number of reports on the role of *Str.milleri* in human infections (Bateman et al.1975, Wort 1975, Parker and Ball 1976, Poole and Wilson 1976, Reid and Davidson 1976). The unpublished records of Dr. M.T.Parker, Cross Infection Reference Laboratory, Colindale, for 1972 (Table 6-1) show that both *Str.milleri* and *Str.agalactiae* are isolated more frequently from infections of the central nervous system than from lesions elsewhere in the body, and that *Str.milleri* is associated with brain abscess while other viridans streptococci are not. These results relate only to strains sent to the Reference Laboratory and in most cases there was very little clinical information. Parker and Ball (1976) found that *Str.milleri* and *Str.agalactiae* were the streptococci most frequently isolated from purulent lesions. They also reported that *Str.milleri* was isolated from 13/16 (80%) of streptococcal brain abscess and that *Str.agalactiae* constituted 46% and *Str.milleri* 12% of streptococci from cases of meningitis. Parker and Ball studied 81 isolates of *Str.milleri* and found that only 20 of them had a detectable Lancefield group antigen. Seven of these belonged to Lancefield group F. In contrast, Poole and Wilson (1976), in their study of 131 isolates of *Str.milleri* from various sites, found that 111 (84%) belonged to Lancefield's groups A, C, F or G and that group F strains occurred most frequently, constituting 45% of all isolates. Differences in technique are insufficient to account for the discrepancies between the findings of Poole and Wilson (1976), this study, and that of Parker and Ball (1976).

Table 6-1

PERCENTAGE DISTRIBUTION OF CERTAIN SPECIES IN 374 PATIENTS WITH STREPTOCOCCAL DISEASE*

	Percentage in total diseased population	Percentage associated with disease of central nervous system
Str.agalactiae	13.37	59.6
Str.milleri	6.15	12.8
Str.mutans	6.68	-

* from the records of Dr. M.T. Parker (1972)

The reports of Bateman et al. (1975) and Reid and Davidson (1976) on liver abscess due to Str.milleri, coupled with the study of Parker and Ball (1976), who found it to be the streptococcus most often associated with purulent lesion in internal organs, indicate that its pathogenic role is not confined to the central nervous system. The lack of comprehensive information on the antigenic constitution of the organisms reported by other workers makes comparison between the present investigation and other studies impossible. It has yet to be shown whether there are any antigenic differences between strains of Str.milleri infecting the central nervous system and those isolated from purulent lesions elsewhere in the body. The status of Str.milleri as a pathogen has been clearly demonstrated in the clinical and experimental findings reported in this study. Wort (1975), who studied 22 isolates of Lancefield group F streptococci, was not so certain of their pathogenic role and concluded that, although many isolates came from purulent lesions, their pathogenicity was in doubt because of the presence of other potential pathogens within the lesions.

The natural habitat of Str.milleri appears to be the mouth and, especially, dental plaque (Mejàre 1975, Mejàre and Edwardsson 1975b, Phillips et al.1976a). The only report of its presence in other parts of the body was that of Wort (1975) who isolated it from the intestine and vagina as well as the upper respiratory tract. Parker and Ball (1976) postulated that the majority of infections due to Str.milleri originated from a primary lesion in the intestine. The association between frontal lobe abscess, Str.milleri and sinusitis or influenza demonstrated in the present study

strongly suggests that the source of infection is in the upper respiratory tract, the organism passing into the brain substance via the paranasal sinuses. Attempts to isolate Str. milleri from the throats of patients with intracranial sepsis were largely unsuccessful, and it was isolated from the throat swab of only one patient. The observation of Mejàre and Edwardsson (1975b) that Str.milleri constituted less than 1% of the streptococci in saliva but 4 - 56% of those in gingival crevices, could account for the low yield from throat swabs.

In this series, three abscesses due to Str.milleri followed dental or abdominal surgery or empyema, suggesting that haematogenous spread may be important, as reported previously by Haymaker (1945). In the experimental situation capillaries are invaded by streptococci early in the genesis of the disease, and Str.milleri, together with Bacteroides sp, was isolated from the blood culture of a patient with brain abscess in the study reported here. A common feature of this and other studies is the low incidence of positive blood cultures in patients with brain abscess, suggesting that if the route of spread is haematogenous, bacteraemia is transitory. Yet, Str.milleri is able to invade the blood stream. Parker and Ball (1976) studied fifteen strains from bacteraemic patients and a further 17 from patients with endocarditis, while positive blood cultures were obtained from two-thirds of the patients with liver abscess due to Str.milleri (Bateman et al.1975). Phillips et al.(1976a) found that five out of 67 patients had bacteraemia due to Str.milleri following dental extraction. Since positive blood cultures are an

unusual finding in patients with intracranial abscess, this suggests that there are mechanisms within the cranial cavity for removing and destroying bacteria.

Meningitis in neonates caused by Str.agalactiae has been widely reported from the United States (Patterson and El Batool Hafeez 1976), and its incidence in other countries seems to be increasing, although it is still low in the United Kingdom. Davies (1976) reported 26 cases of neonatal bacteraemia without meningitis occurring in 16,240 liveborn children at a London teaching hospital during the nine year period 1967-1975, a rate of 1.6/1,000 live births. Seven infections were due to Str.agalactiae, a rate of 0.4/1,000 live births. The organisms usually belonged to Types Ia or II. Parker and Ball (1976) reported on 24 isolates of Str.agalactiae, which had been isolated from cases of neonatal meningitis during a three year period. A further 12 strains had been isolated during the same period from babies with neonatal septicaemia but without meningitis. A bacteriological study was carried out at Queen Charlotte's Maternity Hospital, London between 1967-1973 on 340 neonates that came to postmortem (Hurley and Pryse-Davies pers. comm.). Str.agalactiae was isolated from only two babies (0.6% of the total). In one case there was evidence of widespread infection, including meningitis, and the organism was isolated from the heartblood, bronchus and central nervous system, (Figures 6-1, 6-2 and 6-3). In the second case the organism was isolated from the cerebrospinal fluid and bronchus but not from the heartblood. Str. agalactiae has not been isolated from any baby at postmortem since 1971, but two cases of Str.agalactiae bacteraemia,

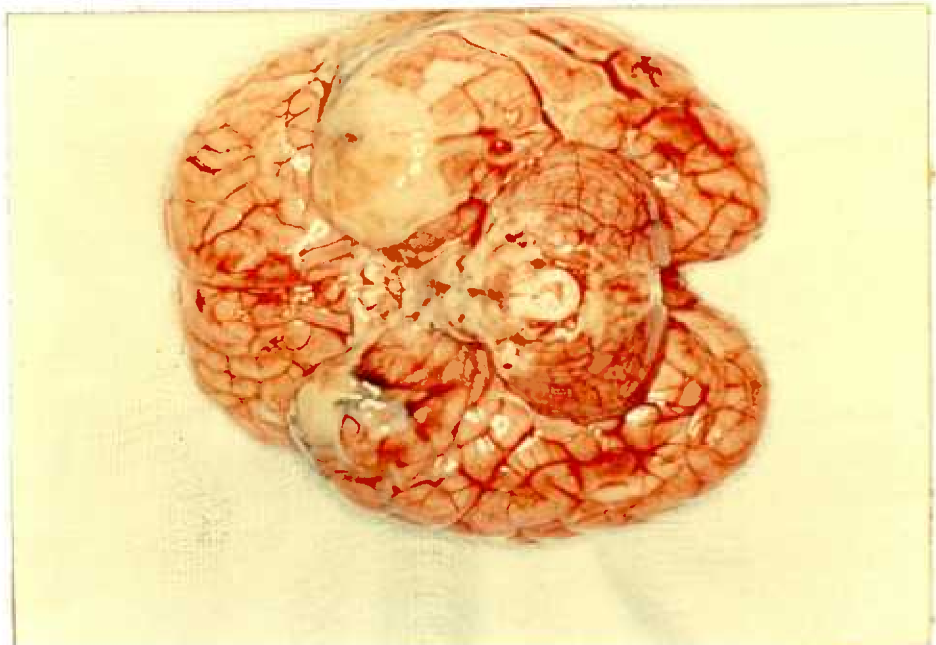

Fig. 6-1 Brain from a case of neonatal meningitis caused by Str.agalactiae showing inflammation of the meninges and localised areas of purulent exudate.

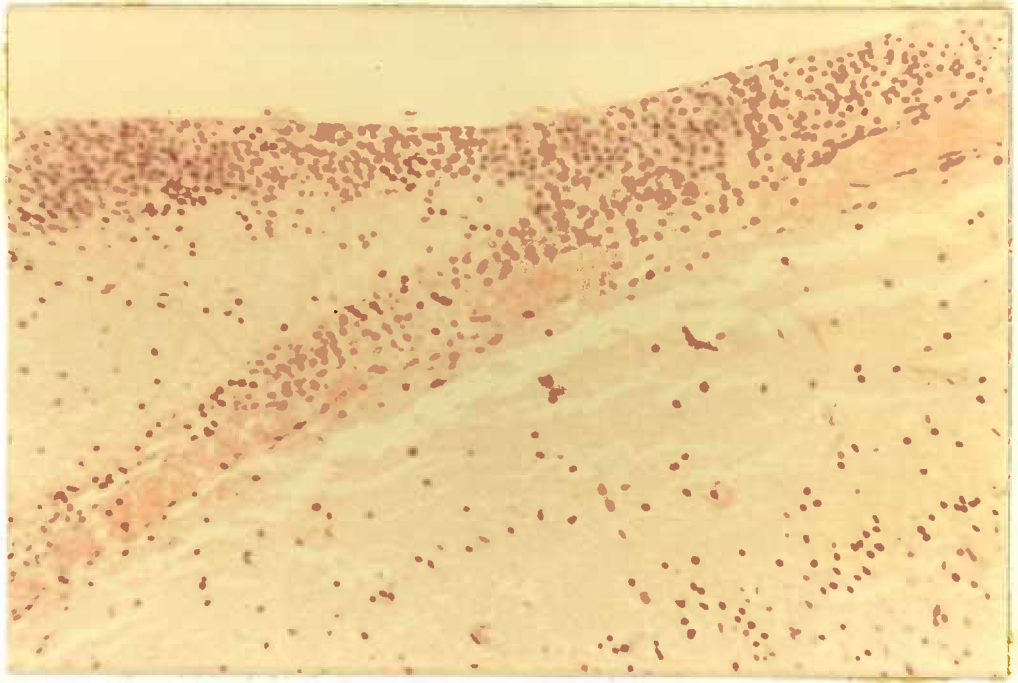

Fig.6-2 Section from the same brain as Fig.6-1 showing intense infiltration of mononuclear cells.
(Haematoxylin and eosin x 100)

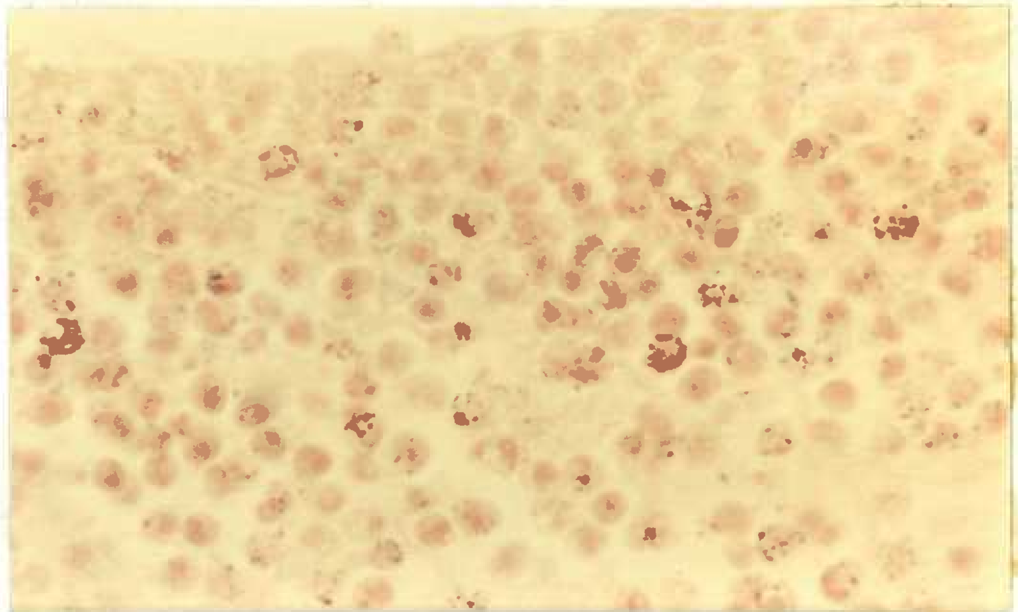

Fig.6-2 Section from the same brain as Fig.6-1 showing mononuclear cells, many of which contain Gram-positive cocci (Tissue Gram x 600)

responding to antibiotic treatment have occurred. The incidence of severe streptococcal disease ascribed to the Lancefield group B streptococcus is, thus, approximately 1:9,000 births at Queen Charlotte's Hospital. The natural habitat of Str.agalactiae is usually considered to be the female lower genital tract. Studies by Franciosi et al.(1973) and Patterson and El Batool Hafeez (1976) however suggest that the faecal reservoir is also of importance.

Meningitis in adults due to Str.agalactiae is rare, and Wilson et al.(1971) found only eight reported cases during the period 1942-1968. Most of these ($^7/_8$) arose either following operation or secondary to infected sites elsewhere in the body. They reported an 80 year old woman who developed meningitis spontaneously and in whom no primary focus of infection could be found. Parker and Ball (1976) reported six cases of meningitis due to Str.agalactiae in patients outside the neonatal period and mentioned 'several cases' of septicaemia occurring in previously healthy adults, some of them men.

Micro-organisms were cultured from pus from the abscesses of all 46 patients studied in this thesis, and none of the primary samples was sterile, in direct contrast to all previous reports. McFarlan (1943), Garfield (1969), Beller et al.(1973), Samson and Clark (1973), Shaw and Russell (1975) and many others reported that pus from brain abscess was frequently sterile, ranging in incidence from 4% (McFarlan 1943) to 62% (Kao 1973). In subdural empyema, the reported incidence of sterility varies from 15% (Hitchcock and Andreadis 1964) to 53% (Anagnostopoulos and Gortvai 1973). The true incidence of sterility is probably higher than reported figures suggest, since a number of workers did not regard as sterile those

samples in which bacteria were seen, but which failed to grow. In the light of this latter observation, a number of workers (Liske and Weikers 1964, Morgan et al.1973, Samson and Clark 1973) have suggested that sterile cultures, which they believe have increased in number since the introduction of antibiotics, are due to the antimicrobial drugs administered before aspiration. This may well be so, if no attempt is made, during culture, to counteract the effect of drugs known to have been administered.

Pus samples collected at antisocial hours pose difficulties (Samson and Clark 1973). Heinemann and Braude (1963) and Bartlett et al. (1976) showed that anaerobic bacteria survive well in samples of pus. This was not so in the present study, where, possibly because of the small volumes of pus used, many of the anaerobic organisms died during overnight storage at room temperature. Inhibitory concentrations of antimicrobial drug may be present within the pus sample, and sensitive aerobic and anaerobic bacteria may be killed unless the specimen is cultured immediately or diluted in a suitable anaerobic culture medium. No published report recommends the inactivation of antimicrobial drugs present in the pus samples at the time of collection as a routine measure, a technological omission that may have contributed to the large numbers of bacteriologically sterile samples recorded. The results obtained in this thesis suggest that anaerobic culture must be undertaken immediately on receipt of the specimen if growth is to be obtained, taking care to inactivate residual drug in the specimen.

Gas liquid chromatography of pus is a useful adjunct to anaerobic culture. The fermentation products resulting

from the growth of anaerobic bacteria in a glucose containing medium are different from those of aerobic bacteria (Holdeman and Moor 1972). Demonstration of the role of non-sporing anaerobic bacteria as pathogens in suppurative lesions and the difficulties associated with their rapid isolation and identification has caused microbiologists to seek alternative techniques for the recognition of these organisms in clinical specimens. Gorbach et al.(1974) were able to correlate the presence of B.fragilis in samples of pus with the production of isobutyric, butyric and succinic acids in the specimen and to provide a presumptive report within one hour. More recently these workers reported (Gorbach et al.1976) that the production of the three fatty acids was not specific for B.fragilis but that the presence of one or more of them was good evidence of an anaerobic infection. Similar results were obtained by Phillips et al. (1976b) who used a more straightforward extraction process, and the use of the uncoated stationary phase, Chromosorb 101, has been shown to be satisfactory without the need for ether extraction (Phillips - pers.comm.). Using this technique it is possible to demonstrate the presence of short chain fatty acids in 1 µl crude pus within 30 minutes. Rapid liquefaction of mucoid samples with pancreatin does not alter the GLC profile and the method of Phillips, modified in this way, is suitable for examination of pus from brain abscess. It was used in the present study and gave very good results.

Gas liquid chromatography of bacterial broth cultures has been widely used (Holdeman and Moor 1972, Sutter and Finegold 1972, Dowell and Hawkins 1974) and many anaerobic bacteria can be identified in this way. The procedure has

been extended into analyses of cellular fatty acids in other groups of organisms, and the extensive work of Bøvre and his colleagues (Janzen et al.1975) and Funderburk and Kester (1975) suggests that it could be used to detect the presence, within a bacterial culture, of amino acid dehydrogenases. These reports, together with the great improvements in the available stationary phases and technique (Hauser and Zabransky 1975) which are taking place, indicate the potential for the use of GLC in routine clinical microbiology. In the present investigation where, in spite of the careful technique used, the isolation rate for anaerobic bacteria was significantly lower than that reported by Heinemam and Braude (1963) and Ingham et al.(1975a and b), the application of GLC provided a useful control of the anaerobic procedure employed. Thus a GLC profile on pus, showing only short chain fatty acids, confirmed the absence of anaerobic organisms. The demonstration that pancreatin liquefaction of pus samples had no adverse effect on GLC profiles allowed the technique to be applied more widely than had been possible previously.
The results indicated that the longer chain fatty acids produced by anaerobic bacteria disappear rapidly after eradication of the organism. If this finding is confirmed then chromatography could provide a useful indicator to the efficacy of therapy in anaerobic infections.

Following the work of Heinemann and Braude (1963), who isolated anaerobic bacteria from $^{16}/18$ (88%) cases of brain abscess, there has been increasing interest and concern about the role of these organisms in intracranial sepsis. 'Anaerobic' streptococci were isolated from $^{12}/18$ (66%) of their cases, but in only two instances was an aerobic

streptococcus isolated, a finding at variance with the results of the present study. These workers did not speciate their isolates, nor did they incubate their cultures in 10% carbon dioxide, which may account for the discrepancy. Sixteen of thirty-seven streptococci in this series which failed to grow on primary aerobic culture at 37°C, grew in the presence of 10% carbon dioxide. Heinemann and Braude refer to some of their isolates of peptostreptococci as 'moderate anaerobes' which suggests that they were more aerotolerant than usual for this genus. Nevertheless, even if streptococci are discounted Heinemann and Braude isolated anaerobic bacteria from 60% of cases, an incidence which is significantly higher than that reported by others. These workers suggested that a comparable incidence of anaerobes would be found by other workers if sufficient attention were paid to anaerobic technique. The group of patients they studied was rather unusual: none had cyanotic heart disease, septicaemia or penetrating injury, and in 44% the abscess developed from otitic infection. In the present study 25% of cerebral abscesses followed otitic infection and the majority of the anaerobes (60%) occurred in this group, and there was a high incidence (54%) of mixed cultures. The microbial flora of temporal lobe abscess, which is usually otogenous, was thus similar to that reported by Heinemann and Braude. Workers in this country also have reported a higher overall incidence of bacteroides than the 22% that was found in this study (Ingham et al.1975b, Eykyn - pers.comm., Barrett - pers.comm.). The differences may be due either to

selection in the groups studied (Eykyn), or to the fact that the patients came from high density populations with a greater prevalance of chronic otitis media and, thus, of otitic brain abscess. This study has demonstrated a clear relation between the site of the abscess, the proximate predisposing cause (if discoverable),and the nature of the infecting organisms.

A wide range of micro-organisms have been incriminated in brain abscess, and it is difficult to determine to what extent the microbial flora of inflammatory lesions of the central nervous system has changed during the last 40 years. The majority of reports on brain abscess do not give details of the cultural methods employed and the many reports of bacteria seen in the Gram-stained film, but not cultured, indicate that the bacteriological procedures were probably inadequate to support the growth of fastidious microbes. Anaerobic culture for samples of intracranial pus only became a routine procedure in the late 1950's in one of the centres whose records were studied retrospectively in this thesis. The present work indicates that liquid anaerobic media, such as Brewer's thioglycollate broth, should be employed for fastidious organisms together with prolonged incubation for up to seven days.

It is apparent from the review of Samson and Clark (1973) and the studies of Liske and Weikers (1964) and Garfield (1969) that in the pre-antibiotic era brain abscess was caused predominantly by <u>Staph.aureus</u> and by aerobic and microaerophilic streptococci. The study by Garfield of 200 cases of supratentorial abscess, 100 between 1951-1957 and 100 between 1962-1967, provides valuable information on changes in the

distribution of the micro-organisms. He found that, while the numbers of streptococci remained constant in the two groups, the isolation rate of Staph.aureus during the period 1962-1967 was only 30% of that recorded during the earlier period, and that, while Staph.aureus accounted for 30% of all isolates during the first period, it represented only 11% of isolates during 1962-1967.

A comparable isolation rate for Staph.aureus (25%) was reported by Tutton (1953) for the period 1948-1952, but Samson and Clark (1973) found it in 26% of 42 cases studied in the period 1961-1971, in contrast to the findings of Garfield. These studies do not state the incidence of post-traumatic infection, nor whether an association between trauma and infection with Staph.aureus was apparent, as has been shown in the present investigation. The decline of serious staphylococcal disease, including septicaemia, is well attested, and fewer metastatic staphylococcal abscesses of the central nervous system might be expected. Only one case was encountered in this study.

Intracranial suppuration due to Str.pneumoniae has been reported, in some cases arising as a complication of pneumococcal meningitis (Weider 1924, Benson et al.1960) but often not thus associated (McFarlan 1943, Garfield 1969, Shaw and Russell 1975). The single abscess due to Str.pneumoniae in this study followed meningitis. The recorded incidence of brain abscess caused by Str.pneumoniae is variable. McFarlan (1943), Garfield (1969) and Shaw and Russell (1975) isolated it from 10% of their patients, while Tutton (1953), Heinemann and Braude (1963), Beller et al.(1973) and Samson and Clark (1973), had isolation rates of less than 1%. Only on rare

occasions has this organism been isolated from cases of subdural empyema, except when this arose as a complication of pneumococcal meningitis.

The isolation rate of Enterobacteriaceae from intracranial abscess has changed since the introduction of antibiotics. Early studies (McFarlan 1943, Pennybacker 1948) reported few such infections, while later investigators (Tutton 1953, Samson and Clark 1963 and Shaw and Russell 1975) found that 25-33% of the bacteria isolated were Enterobacteriaceae. In Garfield's study (1969) the isolation rate for coliforms rose from 12% for the period 1951-1957 to 22% for the period 1962-1967. The incidence of subdural empyema due to these organisms is also rising, but not to the same extent as brain abscess. Pseudomonas species have been isolated from cases of non-traumatic intracranial sepsis only on rare occasions.

The introduction of antibiotics resulted in a reduction in the severity and duration of a number of infections and, in consequence, complicating brain abscesses are now rarely found. Amongst these conditions are acute and chronic pelvic sepsis (including puerperal sepsis), actinomycosis and tuberculosis. Krueger et al.(1954) and Turner (1954) reported that brain abscess was a consequence of nocardial infection in 30-50% of cases and Schneider and Rand (1949) considered that actinomycotic brain abscess, which occurs secondary to a focus elsewhere in the body, was a complication that invariably proved fatal. Members of the Actinomycetales, and _Mycobacterium tuberculosis_ are rarely found as causes of brain abscess nowadays.

Mycotic infections of the central nervous system are rare and, excluding infections due to yeasts, fewer than 100 cases have been reported. The majority have been due to species of Aspergillus (Visudhiphan et al.1973). Cerebral chromoblastomycosis has also been described (Symmers 1960, Summers 1968) and has been attributed to members of the genera Cladosporium, Hormondendrum, Phialophora or Fonsecaea. So far as is known, there has been no previous report of isolation of a fungus with the characteristics of the one described in this study. Although it failed to grow in any of the media used, it is probable that it was a type of water mould, many of which are known to be difficult to grow on artificial media (Dr. Stockdale - pers.comm.). The patient suffered no systemic illness and did not require antimicrobial treatment. This, coupled with the lack of abscess formation, seems to support the interpretation that the mould was contaminating a penetrating wound.

The prospective study reported here demonstrates a clear association between the site of an abscess in the central nervous system, and the causal micro-organisms. Abscesses in the frontal lobe commonly follow sinusitis, are streptococcal in origin (usually Str.milleri) and are usually caused by a single bacterial species (80%), except when following trauma. Those of the temporal lobe usually follow otitis media and contain a wide variety of micro-organisms that often occur as mixed infections (54%). The majority of the isolates of Proteus species and bacteroides group organisms occur in these patients. Non-traumatic subdural empyema is associated predominantly with Str.milleri (86%) which usually occurs in pure culture (71%). The commonest isolate in spinal abscesses

and in post-traumatic lesions at any site is Staph.aureus (83%) often in pure culture (58%). The demonstration of these clear differences in infecting microbe between abscesses in different sites may prove of value to neurosurgeons in deciding upon appropriate chemotherapy prior to a definite laboratory report.

There is considerable discussion and controversy on the antibiotic of choice for treatment of patients with brain abscess. Garfield (1969) found that the majority of organisms were sensitive to penicillin and, therefore, suggested it as the drug of choice. Bartlett (1974) felt that, in spite of the problems of toxicity, chloramphenicol should always be used until the causative organism(s) had been isolated and their sensitivities determined, after which therapy with penicillin might be considered. It was the opinion of Geddes et al.(1964) that lincomycin and clindamycin should not be used for intracranial lesions in view of their poor penetration into the central nervous system, a conclusion which is supported by Lerner (1969). In contrast, Khuri-Bulos et al. (1973) reported the successful treatment, with clindamycin, of a brain abscess due to B.fragilis, veillonella and bifidobacterium. The patient was, however, receiving ampicillin and probenicid at the same time and although some of the isolates were ampicillin resistant the possibility that ampicillin with probenicid contributed to the successful outcome cannot be ruled out. Mangi et al.(1973) concluded that the cephalosporins were unsuitable for the treatment of infections of the central nervous system and should be avoided. These workers claimed that the compounds are inactivated on crossing the blood brain barrier, resulting in poor levels of

active compound. They further suggested that the active agent may be metabolised to desacetylcephalosporin, a compound which has a quarter the activity of cephalosporin and to which some bacteria, eg. N.meningitidis, are far more resistant. Del Bene and Farrer (1972) suggested combined therapy with high dosage penicillin (20 mega units/day) and chloramphenicol (3-4 g daily) by the intravenous route, whilst accepting that some strains of Staph.aureus may not respond to this regimen. Combination of penicillin and chloramphenicol may be antagonistic, in vivo as well as in vitro (Mathies et al.1967).

The present study suggests that initial treatment with penicillin alone cannot be satisfactory since 53% patients were infected with bacteria which were resistant to it. From the results of routine sensitivity testing chloramphenicol would seem to be the drug of choice since 98% of the organisms isolated are sensitive to it, and it may achieve a ninefold concentration within the central nervous system (Kramer et al.1969). More work must be done on the penetration of this and other drugs into avascular brain tissue or abscess cavities before any recommendation on treatment can be made.

The value of local instillation of antibiotics should be assessed since, in the single case studied during the present investigation, chloramphenicol instilled into the abscess cavity rapidly diffused out. The less diffusible drugs should, perhaps, be considered for instillation rather than, as at present, those which diffuse most readily, and it might be necessary to modify the methods for invitro sensitivity testing of organisms isolated from brain abscess to take account of the high concentrations of drugs which can be directly instilled into the cavity.

The activity of antimicrobial drugs within a purulent milieu should be studied more closely in view of the extraordinarily rapid inactivation of penicillin and cephalosporin shown by pus from some patients in vitro (and demonstrated in this thesis). The metabolism of drugs, for example cephalosporin (Mangi et al.1973), their non-specific inactivation by pus, or their specific inactivation by bacterial β-lactamase and similar microbial enzymes produced by the infecting organisms (Del Bene and Farrer 1973), are all factors which could affect the successful treatment of intracranial sepsis. The use of probenicid (Benemid) to increase the serum and tissue concentrations of the penicillins and cephalosporins (except cephaloridine) in cases of intracranial sepsis does not appear to be widespread, and there are no published studies to show whether the combination of penicillin and probenicid produces a significant increase of active penicillin in intracranial pus. Although it is outside the scope of this thesis, it should be recorded that dexamethazone therapy is widely used in the management of brain abscess to reduce brain oedema. The effects of dexamethazone therapy on the penetration of antibiotics and the relationship between brain oedema and effective antibiotic concentrations are unknown.

The present study has shown that antimicrobial drugs can be assayed satisfactorily in pus, with or without pancreatin liquefaction. The level of active drug shows considerable variation between one patient and another on comparable dosage regimens. The number of patients studied was too small to determine whether the size of the lesion or the presence of a capsule affected the concentration of antibiotic

in the pus.

The finding that $^{4}/22$ samples of pus were able to neutralise the activity of the penicillins was of considerable interest and could have important implications for clinical treatment. Were it to occur in vivo it might account in part for hitherto inexplicable failures of penicillin treatment. Penicillin is inactivated through being bound to protein, and is destroyed by acid. The protein content of the pus samples that inactivated penicillin did not differ substantially from that of other specimens, and pH for all the samples was within the same range. Moreover, the detectable activity of acid-stable ampicillin was also diminished by specimens active against penicillin. The possibility that the effect was due to the presence of B-lactamase producing organisms cannot be discounted, for bacteroides or Esch.coli were isolated from three of four specimens that inactivated penicillin rapidly, and B-lactamase production has been described both for the bacteroides group (Anderson and Sykes 1972) and for Esch.coli (Percival et al.1963). Unless the enzyme accumulated within the loculated pus it is unlikely to have produced so substantial an effect, as enzyme production in these organisms is low. Bacterial B-lactamase may not always be extracellular (Sykes and Matthew 1976) and penicillin may be inactivated within the bacterial cell. The failure to detect the enzyme in supernatant pus, even after repeated freezing and thawing that should have disrupted the bacteria, suggests that inactivation was not caused by a bacterial enzyme of this type.

Penicillin inactivation may be a property of the host. Work quoted by Hamilton-Miller (1967) shows that animal tissues, especially those of the mouse, are able to hydrolyse

a number of penicillin esters by means of ester-specific β-lactamase enzymes, but similar activity has not been shown in human tissues. Attempts to demonstrate activity against benzylpenicillin in tissue extracts and serum from healthy men and women have been unsuccessful (Richardson et al.1945), although O'Callaghan et al.(1972) suggested the possibility that animal and human tissues may contain β-lactamase-like enzymes, quoting the work of Shaer and Siminova (1970) who found such an enzyme in mouse kidney. Unlike the present findings, the enzymes were demonstrable in the fluid portion of tissue homogenates. So far as is known there has not been a previous report which describes the inactivation of penicillin by pus, other than that due to bacterial β-lactamase. Since both cephaloridine and benzylpenicillin were inactivated, the active principle is unlikely to be an esterase, and the results indicate that it is not likely to be β-lactamase. If the property is enzymic in nature, an amidase may be involved. The report of Barnes and Waterworth (1977) supports the view that the ability to inactivate the penicillins is a property of the cellular material in the pus and is not due to a bacterial enzyme. These workers described a case showing that the effect occurs *in vivo* and has clinical significance. Were this phenomenon to occur in patients with brain abscess or meningitis it might account for the erratic levels of penicillin obtained in blood and pus and for failure of treatment where penicillin is used as the sole agent against a fully sensitive organism.

Heinemann and Braude (1963) suggested that there was a

consistent pattern of bacterial identity and antibiotic sensitivity in brain abscesses, and that in many instances a presumptive aetiological diagnosis could have been made days or weeks earlier. They investigated the possibility of initiating antibiotic treatment without surgical intervention at what they reasoned to be a reversible stage of the infection, presumably the stage of active bacterial encephalitis (Heinemann et al.1971). In their study six patients were included on the basis of EEG and radiological investigation, and all showed focal slow waves in the EEG. In four patients this was the only sign and the subsequent brain scan, sono-encephalogram or carotid angiogram were normal. The other two patients had a brain scan with sodium pertechnetate which showed an increased uptake. The choice of antibiotic was made with reference to the sensitivity pattern of the bacteria isolated from the presumptive source of the intracranial lesion. Five patients had otitic infection and one a post-operative wound infection. Four patients were treated with penicillin or cephalothin, and tetracycline, one received penicillin and methicillin and the other penicillin alone. All were reported to have recovered although one required neurosurgery one month later for an established abscess. The possibility of reversing an intracranial lesion by the use of antibiotics alone before it becomes established as an abscess, has considerable attraction. However, as discussed below, one of the major problems in successful treatment of infections of the brain parenchyma is that of early diagnosis. Experience in the present study was similar to that reported by Black et al. (1973a) and to the experimental results of

Wood and Smith (Wood and Smith 1956, Smith and Wood 1956a,1956b) all of whom found that once a cavity had formed surgical intervention was essential. It is not possible from the results of the present investigation to comment on the assertion by Heinemann (1974) that even when a small abscess has formed, a successful outcome can be achieved by antibiotic treatment alone. Heinemann also suggests that there is no substantial role for antibiotics in the treatment of the residual local infection following aspiration of a brain abscess. This is contrary to the findings of this study and to those of Black et al.(1973a). In this study, where antibiotic treatment was adequate and appropriate, fewer aspirations of the cavity seemed to be needed. In two cases where apparently inadequate dosage regimens were employed the patients failed to progress; when the dosage was increased, improvement was rapid. The only patient treated conservatively failed to respond.

The high mortality rate which is still associated with suppurative lesions of the central nervous system is a matter of considerable concern to neurosurgeons and should be viewed with equal regard by microbiologists. Generally, although there are exceptions, there has been a fall in the mortality rate since the introduction of antibiotics, but it has not been so great as was anticipated or hoped (Grant 1941). The mortality rate in unselected groups of patients remains in excess of 40%. This figure does not compare with the 13% mortality reported by Tutton (1953) or the 6% mortality reported by Pennybacker (1961), which an editorial

in the British Medical Journal (1969) considered "represented the high points of achievement in the field." It went on to suggest that the true mortality rate in cases of brain abscess may be higher than that reported by neurosurgeons since patients with brain abscess may die before reaching a neurosurgical unit.

A number of factors are believed to contribute to the high mortality rate. The major of these relate to the problems associated with early diagnosis. Beller et al. (1973) and Samson and Clark (1973) reported that a significant percentage of their patients died before treatment could be initiated and were only diagnosed at postmortem. In their review of the factors affecting mortality, Karandanis and Shulman (1975) observed that the mortality rate was associated with the level of consciousness of the patient on admission, reporting a mortality rate of 88% in the seven patients in their study who had been admitted in coma. Garfield (1969) found a comparable mortality (80%) in a similar group of patients, as did Morgan et al. (1973), Samson and Clark (1973) and Liske and Weikers (1974). Not only the initial diagnosis but subsequent localisation of the abscess once the diagnosis has been reached poses problems (Garfield 1969). He reported that when the decision to place a temporal or frontal burrhole for aspiration of the abscess was made purely on the site of ENT sepsis, the results showed a degree of accuracy unrivalled by any other method of localisation. Recent developments in neuroradiology offer hope of greater accuracy in the diagnosis and localisation of the brain abscess.

In this study of 46 patients with intracranial or intraspinal abscess, the overall mortality was 24% and the operative mortality was 20%. In the 35 patients with brain abscess the mortality was 20% and 14% respectively. The figures are significantly lower than the majority of those published and are comparable with the 13% operative mortality reported by Tutton (1953). It may be considered that inclusion of the two patients with cyanotic heart disease biases the mortality rate. One patient died while undergoing corrective cardiac surgery six months after his abscess had been satisfactorily treated, and the other, having shown every sign of complete recovery from the infection, died following cardiac arrest three weeks after operation.

The apparently higher mortality in men (8 deaths - 29%) compared with women (3 deaths - 17%) is explained by the finding of this study that non-traumatic subdural empyema occurred only in men and carried a 37% ($^{3}/8$) mortality. The mortality rate among patients with intracranial abscess was 21% ($^{4}/19$) for men and 19% ($^{3}/16$) for women. The eleventh death occurred in one of the three cases of spinal abscess.

Every effort was made to study patients who had not been selected in any way, the only restriction being the limitation imposed by the transport service. It is possible that the low mortality rate reflects a more favourable prognosis in those who are operated upon during week days, for few specimens were despatched at night, or on Sundays, from centres using the City Link service. Since there was no difference in mortality between the centres served by City Link and those served by mini-cab, where there was a 24-hour service, seven days a week, this would not seem to be the case.

Other than this patients from all the centres constituted an unselected group, and no single factor was found to predominate in the group that died, although the incidence of mixed infections among those who died (56%) was higher than for the population as a whole (30%). Str.milleri or related organisms were isolated from $^{10}/11$ (91%) patients dying from abscess of non-traumatic origin.

The space occupying effect of a brain abscess may result in raised intracranial pressure, that sometimes causes brain stem compression (Kiser and Kendig 1963, Wright and Ballantine 1967). Samson and Clark (1973) suggested that the space occupying effect could be reduced more effectively by surgical excision than by aspiration. Other workers (Morgan et al.1973, Shaw and Russell 1975) were less convinced that total excision was the treatment of choice. In the present study $^{10}/35$ patients had encapsulated abscesses and in eight cases they were excised (Figures 6-4 and 6-5). Three abscesses which lacked a discernible capsule were also excised. Excision of the abscess did not influence the mortality in this study. The nature and location of the abscesses is clearly relevant to the mortality. The prognosis is poor for patients with multiple lesions and also for those in whom the abscess ruptures. Liske and Weikers (1964) and Garfield (1969) report mortality rates of 70-100% in such patients.

The persistently high mortality associated with brain abscess could be ascribed to changes in the natural history and presentation of the disease, yet Garfield (1969) considering this possibility, found that in comparing two groups of cases collected during two seven year period between 1951 and 1967, there was no difference in patients' ages, the sites of the

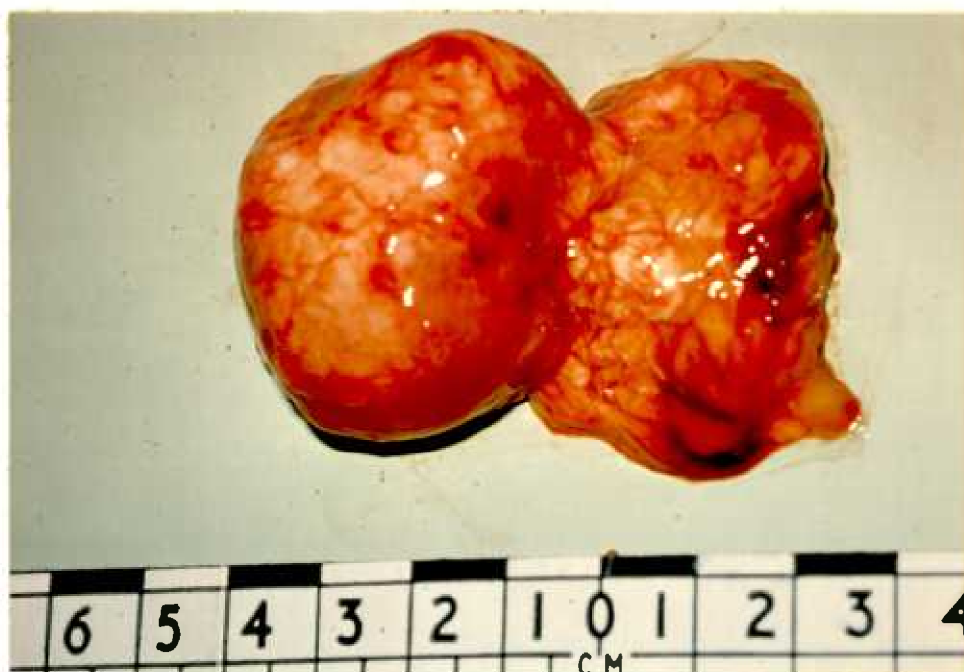

Fig.6-4 Intact bilocular, encapsulated abscess removed from a man aged 33 years from which was isolated Str.milleri and Fusobacterium species.

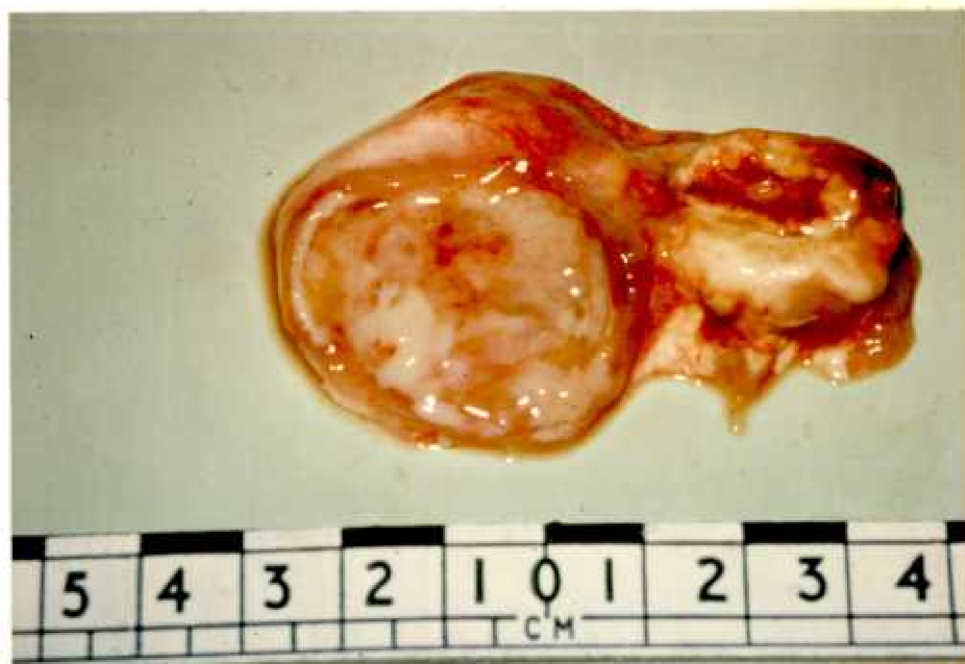

Fig. 6-5 Cut surface of the abscess shown in Fig.6-4 showing a dense fibrotic capsule and gelatinous pus.

abscesses, the aetiology, the level of consciousness on admission or the length of neurological history. He concluded, therefore, that there had been no significant change in the natural history of intracranial suppurative lesions during this time.

The prophesy made by Grant in 1941 that, for uncomplicated abscesses, diagnosed early and adequately treated, the mortality should be 20%, has been achieved by some, (Tutton 1953, Karandanis and Shulman 1975). The results of the majority of published studies, however, fall far short of this goal and do not bear out Grant's second prediction that the overall mortality from brain abscess, whether diagnosed or not, should be 40%. It is clear that many of the problems associated with early diagnosis and adequate treatment have still to be resolved (Garfield 1969).

There is considerable confusion and disagreement regarding changes in the incidence of brain abscess. Pennybacker (1951) predicted that following the introduction of antibiotics the incidence of chronic otorhinolaryngological infection would drop, with resulting decrease in the complications of chronic infection, including brain abscess. Kiser and Kendig (1963) reported a declining incidence of brain abscess; Garfield (1969), Morgan et al.(1973) and Shaw and Russell (1975) all found no change in incidence over periods of 20 years or more, while Beller et al.(1973) and Samson and Clark (1973) reported that the incidence was rising. A notable feature of the report by Shaw and Russell on 47 cases of cerebellar abscess was that while the incidence did not change, the mortality increased from 25% in the period 1950-59 to 55% for the period 1970-73.

The retrospective study carried out at two London neurosurgical units and reported in this thesis illustrates the difficulties of comparison of one study with another. The data from Centre 1 indicates no change in the incidence of intracranial or intraspinal abscess over a period of 23 years, while that from Centre 2, which is only 12 miles from Centre 1, shows a 60% decrease in incidence over the same period. Nothing is known about changes in the catchment area, referral system, or other factors which could account for the differences between the two units.

The real incidence of abscess of the central nervous system cannot be determined unless those patients who are not referred to neurosurgical units are also known. All patients who are known to have died with inflammatory disease of the central nervous system are included in the Registrar General's returns. However, for purposes of analysis, records relating to patients who survive are available only for those treated within neurosurgical units. As a result it is not possible to determine the true incidence of intracranial abscess within the United Kingdom.

Tutton (1953) referred to changes in the socio-economic status of the population and equated this with decreasing incidence of brain abscess, and an editorial in the British Medical Journal (1975) suggested that changes in the pattern of otological disease during the last thirty years (Reading and Schurr 1956) have been reflected in a general decrease in the incidence of otogenic brain abscess (Wright and Grimaldi 1973). This opinion is supported by the findings of Shaw and Russell (1975) who noted a persistently high incidence

of chronic ear disease in West Scotland and a constant incidence of intracranial abscess as a result.

The wide variation in the mortality, incidence and bacteriological findings in the published reports of intracranial suppuration is disturbing until they are examined more closely. A few reports have been confined to cerebellar abscesses (Pennybacker 1948, Shaw and Russell 1975); and subdural empyema (Bhandari and Sarkari 1970, Coonrod and Dans 1972, Anagnostopoulos and Gortvai 1973) is usually considered separately from other suppurative lesions of the CNS. The majority of studies, however, have included abscesses from any part of the brain substance and the distribution of their sites varies considerably from study to study (for example, see Tutton 1953, Garfield 1969, Samson and Clark 1973). The reported predisposing causes of intracranial abscess also vary. A number of studies refer specifically to lesions of otogenic origin (Pennybacker 1950 and 1961, Dawes 1961, Wright and Grimaldi 1973, Baitykov 1975), but there are few studies dealing exclusively with abscesses of metastatic (Gregory et al.1967), sinusitic (Pennybacker 1950) or cryptogenic origin. The group of 18 patients studied by Heinemann and Braude (1963) was unusual since 44% of patients had otogenic abscesses and none had cyanotic heart disease, septicaemia or antecedent injury. Pennybacker (1950) and Tutton (1953) reported that 40-50% of brain abscesses were of otogenic origin. However, the majority of later reports have recorded a lower percentage of abscesses arising from otitic disease. The cases studied by Beller et al. (1973) reporting from Israel, were unusual in that 43% of their 89 patients were under 15 years of age. Before

meaningful comparison can be drawn between one study and another, more data on patient populations, the general incidence of chronic disease, the predisposing causes, and the distribution of infected sites in relation to predisposing causes must be made available, since these factors affect the incidence, the ease of diagnosis, the infecting organisms and the prognosis of the condition.

The higher incidence of brain abscess among men reported by the majority of early workers has been less discernible in recent years. In the retrospective study of 257 patients over 23 years (1951-1973) reported in this thesis the male/ female ratio was 2.6:1, while in the prospective study (1973-1976) the ratio was 1.2:1. Tutton (1953) believed that improvement of social conditions led to decline in the incidence of brain abscess. The levelling of the sex distribution evident from this work may reflect better working conditions for males in the industrial sector and the smaller percentage of the work force associated with heavy industry and mining, occupations which predispose to chronic infection. Chronically discharging ears are no longer socially acceptable among the labouring classes, and medical treatment is freely available. The majority of the patients in both studies were from the south-east of England, which may account for the differences in sex distribution and infecting organisms reported here and those reported by Ingham et al. (1975a and b), all of whose patients came from the industrial north-east. With the exception of a single case of post-operative infection, the seven cases of subdural empyema reported in this thesis occurred in men. These findings are different from those of Hitchcock and Andreadis (1964), Bhandari and Sarkari (1970)

and Anagnostopoulos and Gortvai (1973) all of whom reported that while the incidence of subdural empyema was lower than that of brain abscess the male/female ratio in the two conditions was essentially the same.

All commentators agree that the collection of lumbar CSF is a dangerous procedure in patients with brain abscess, if the intracranial pressure is raised (Duffy 1969), and it is perhaps surprising that in spite of the general awareness of the potential danger, $^{12}/43$ patients in this study had a lumbar puncture at some stage. In many instances, this was performed at the referring hospital. The majority of specimens were found to have normal levels of protein and sugar. In $^{4}/12$ the polymorphonuclear leucocytes in the CSF were within normal limits. With the exception of one patient with an extradural spinal abscess and another with a subdural abscess, all the CSF cultures were negative. These findings are in agreement with those of Karandanis and Shulman (1975) who observed that the finding of an alphahaemolytic streptococcus, other than Str.pneumoniae, or a mixed bacterial population in cultures of spinal fluid,should alert the clinician and the microbiologist to the possibility of a ruptured brain abscess, since both findings are very rare in cases of uncomplicated meningitis.

There was only one patient in this study whose abscess is known to have ruptured into the ventricle. Cerebrospinal fluid was at normal pressure and there was no deterioration in the patient's condition following collection. The abscess was thought to have ruptured after instillation of penicillin following primary aspiration of pus. The patient subsequently died (Case 10, Appendix A, page 243).

In view of the few bacterial species associated with meningitis, the low incidence of brain abscess in patients with chronic otorhinologic infection and the difficulties which have been experienced in establishing satisfactory experimental models in laboratory animals, it would seem that mammalian brain tissue is well protected against bacterial invasion. Previous attempts to produce experimental brain abscess have been largely unsuccessful unless sizeable inocula have been used together with local trauma or irritant material, eg. agar, injected with bacteria (Falconer et al.1943). Even when isolates from clinical cases of brain abscess were used, they failed to produce lesions experimentally.

Waggoner (1974) suggested that cerebral infarction was necessary before an infecting organism could establish itself in the brain. It may be that prior injection into the carotid artery of material which results in the production of microemboli would render the brain more susceptible to experimental abscess formation.

The experimental study reported here was undertaken to establish whether meningitis and brain abscess could be produced by streptococci in the absence of any foreign material, and to study the natural history of these diseases. Str.milleri was used as the postulated pathogen implicated in brain abscess, Str.agalactiae because of its established role as a cause of neonatal meningitis and Str.mutans as a control, since its isolation from cases of intracranial suppuration had not at that time been reported. Contrary to expectation, the histological study showed that cavitating abscesses are produced most frequently in mice by Str.mutans and less so by

Str.milleri. Although intracerebral injection of Str.agalactiae resulted in brain abscess the reaction was predominantly meningitis even if the injection was given intravenously or intrahepatically, and in this the experimental findings mimicked the clinical situation. Small inocula did not provoke suppuration and if sublethal doses were administered the animals recovered without ill effect. Even following direct injection of large numbers of viable Str.milleri into the brain, the formation of brain abscesses was less marked than in the case of Str.mutans. Str.mutans is not often associated with cerebral infection in man but provokes marked suppuration in the mouse suggesting that species specificity may be a factor in the pathogenesis of brain abscess in mammals. The size of the inoculum necessary to produce an abscess caused early death and macroscopic lesions were not seen at necropsy. Lesions in man may be small and Gregory et al.(1967) reported that in $^{7}/29$ cases of metastatic abscess the lesion had only been visible microscopically.

Of some interest is the demonstration that the degree of inflammatory response varied with the site of inoculation of the organism. Thus, following injection into the junction of the white and grey matter there was a profound inflammatory reaction, while if the inoculation site was deep in the brain substance, or in the ventricles, the inflammatory reaction was minimal. If this occurs in man it could account in part for the poor prognosis associated with abscess originating in the white matter (Gregory et al.1967).

The study of Falconer et al.(1943) suggests that the rabbit is a more satisfactory experimental animal for the investigation of inflammatory disease of the central nervous

system in man. Abscesses in rabbits are histologically very similar to those found in man and the animals do not develop meningitis following intracranial injection with microbes causing brain abscess in man. Further, in two rabbits, aspiration of a cerebral abscess resulted in complete cure. The rabbit thus seems to survive experimental infection for a sufficiently long time to enable it to be used as a model for regimens of therapy.

Several of the streptococci show considerable specificity for single mammalian hosts or a few closely related species, and their biotypes are fairly strictly adapted to certain animal hosts. Notable among these is Str.agalactiae, the pathogenicity of which is reported to be low for small laboratory animals (Wilson and Miles 1975 p 739). Serious disease in man is unusual except in the newborn. This may reflect immunological immaturity, either humoral or cellular since both occur in the neonate, and it is of interest that the experiments reported here indicate that the streptococcus is virulent enough in young mice to kill them within 48 hours of intravenous, intraperitoneal or intracerebral injection. The target organ, as in the human neonate, is the brain and purulent meningitis is a constant feature of experimental infection. The group B streptococcus,in nature,and in the experimental situation lacks the versatility of adverse effect shown by streptococci of Lancefield's group A, or even Str.pneumoniae, preferentially selecting the meningeal membranes as the site of multiplication. This predilection may be associated with a particular biotype but there is little information on the point and it was not investigated in this thesis. The type

and quantity of capsular polysaccharide determines the virulence of Str.pneumoniae and similar factors may be operative in Str.agalactiae. Certainly, it is more virulent for mice than either Str.milleri or Str.mutans, which may be injected in low doses without apparent ill effect. The pathogenic mechanisms of these two species have not been studied, since their taxonomical position has only recently been resolved. However, observations made on Str.pyogenes may apply to strains of Str.milleri possessing the Lancefield group A antigen, although, as this thesis and the work of Parker and Ball (1976) and others have indicated, the pyogenic members of Str.milleri are not confined to those possessing the group A polysaccharide. The group F polysaccharide is undoubtedly important in the genesis of deep infections of the brain of man. All isolates of Str.milleri from patients with brain abscess studied in the present series possessed it, and the group F strain of Str.milleri studied experimentally caused suppuration of the brains of young mice when injected intracerebrally. The group F streptococcus has been associated with meningitis and can induce meningitis experimentally although it is not so marked as that caused by Str.agalactiae. It would be interesting to study pathogenic effects of strains of Str.milleri not possessing the group F antigen in experimental animals. All strains of Str.milleri isolated from brain abscess possessed, in addition, the type O III Ottens and Winkler antigen, suggesting that it is this serotype that is specifically adapted to infect the deep tissues of the central nervous system in man. The distribution of Str.milleri

in man has not been studied systematically, and there is little information on the ecology of its serotypes.

Although small blood vessels are involved in the disease caused experimentally by Str.milleri there is little evidence that it disseminates widely in the naturally infected, or the experimental host, and it does not show the invasive properties of Str.pyogenes or Str.agalactiae. The tissue reaction to Str.mutans and Str.milleri in the brain though localised, is extensive, with suppuration a marked feature, suggesting that 'spreading' enzymes are less likely to be part of their pathogenic armamentarium than enzymes such as the diphosphopyridine nucleotidase reported to be associated with some group A streptococci. All these points remain to be investigated and elucidated.

SUMMARY AND CONCLUSIONS

The investigations described in this thesis were prompted by observations made in 1973 on three patients with cerebral abscess. Streptococci were isolated from all three and isolates from two were identified as *Streptococcus milleri*, an organism of the viridans group first described by Guthof (1956). The coincidence of two brain abscesses yielding streptococci of unusual type suggested that *Str. milleri* might have a predilection for the central nervous system of man as a cause of brain abscess. As a result of these findings a study was undertaken to investigate the role of streptococci in intracranial infections.

The effects of *Str.agalactiae*, an organism with proven pathogenicity for the central nervous system, especially in human neonates, of *Str.milleri*, the organism under investigation and of *Str.mutans*, an organism which had not at that time been incriminated in intracranial infection, on the central nervous system of white Swiss mice were compared using different routes of inoculation. *Str.mutans* was as virulent as *Str.milleri* following intracranial inoculation and more so by the intravenous route. *Str.agalactiae* was highly virulent irrespective of the route. Histological investigations showed that *Str.agalactiae* spread rapidly throughout the meninges within fifteen minutes of injection. The bacteria multiplied freely and within two hours there was a well established meningitis. Streptococci could be seen in the Virchow-Robin spaces, and septic infarcts were visible. Within eighteen hours there was a severe purulent meningitis, with formation of submeningeal abscesses. Abscesses formed also in relation to the needle track, and

at sites that were apparently anatomically remote from both the meninges and the needle track. The most striking lesion was the meningitis.

Str.mutans showed the most marked deep abscess formation, usually within eighteen hours and always related to the needle track. Streptococci were abundant within the main abscess, and those forming adjacent to it, and although meningeal lesions were found there were no lesions elsewhere in the brain substance.

The spread of *Str.milleri* through the meninges was much slower than *Str.agalactiae* although it did occur and was apparent in 18-24 hours. The initial meningeal reaction was due to mononuclear cells which were later replaced by polymorphonuclear leucocytes. Pus formed either deep to the meninges or at the inoculation site but in both cases there were relatively few streptococci to be seen and the infection showed no signs of spreading to remote parts of the brain. The cellular defences of the mouse appeared to be more effective at combating infection with *Str.milleri* than with *Str.mutans* or *Str.agalactiae*, since the lesions were more circumscribed at all stages of the disease, and contained fewer bacteria.

Procedures for the assay of antimicrobial drugs in pus were investigated. The results presented show that antibiotics can be assayed accurately from pus samples and that the use of pancreatin to liquefy mucoid samples does not affect the assay result. Four of twenty-two samples of pus, two of which came from cases of brain abscess, inactivated up to 90% of added penicillin within one hour *in vitro*. Ampicillin and cephaloridine were also inactivated but

there was no effect on streptomycin and fusidic acid. The effect was not related to the protein content of the pus, nor to it pH. Microbes that may produce β-lactamase in small quantities were isolated from three of the four specimens but the enzyme was not detected in the pus by physical methods nor by microbiological inactivation assay. The inactivating effect was shown to be a property of the solid portion of the pus, and was absent from the filtrate. The possibility that this effect is an intrinsic property of the host and its important implication for clinical practice are discussed.

The role of streptococci in intracranial infection of man was investigated prospectively and retrospectively. A prospective study was set up with the co-operation of a number of neurosurgical units to investigate the problems of 'sterile pus'; to establish the causative organisms using modern taxonomic groupings; and to determine whether parenteral antibiotics penetrate abscess cavities and if so whether this has any effect on the ultimate outcome of the disease.

Specimens from 50 patients were examined, 35 cases of brain abscess, eight of sub or extradural empyema, three of spinal abscess and three of post craniotomy infection. The fiftieth case was of a man who sustained a penetrating wound of the orbit which was apparently contaminated with a water mould.

The studies showed that pus from intracranial lesions is not bacteriologically sterile provided that cultures are incubated for long enough and antimicrobial drugs present in the sample were neutralised or diluted. There were 69 isolates from the patients studied. Streptococci were

isolated most frequently, occurring in 36 (72%) patients and samples from 20 patients (40%) contained Str.milleri. Staph.aureus was isolated from 24% patients and was commonly associated with lesions of traumatic origin and with spinal abscesses. Organisms of the bacteroides group were isolated from 20% patients and Enterobacteriaceae from 16%; they were commonly associated with abscesses of otitic origin. Mixed bacterial populations were isolated from 14 patients (29%). The majority ($^{12}/14$) occurred in patients with brain abscess (34%), and were associated with temporal lobe abscess of otitic origin (54%).

Streptococci were isolated from $^{27}/35$ (79%) patients with brain abscess and from $^{8}/8$ (100%) patients with sub or extradural empyema. The isolation rate for Str.milleri in these two groups was 50% and 75% respectively. All of the isolates of Str.milleri reacted with antisera to Lancefield group F and Ottens and Winkler type O III. The significance of these findings is discussed.

Abscess in the temporal lobe was associated with otitis. A variety of organisms were responsible and these lesions were characterised by a high incidence of anaerobic bacteria (70% cases) and of mixed bacterial populations (54% cases). In contrast abscesses of the frontal lobe which were associated with sinusitis were characterised by a low incidence of anaerobic bacteria (27%). Mixed bacterial populations were less frequent (36% cases) and the commonest single isolate was Str.milleri (63% cases).

Antibiotic sensitivity tests on the 69 isolates showed that 28 of them from 26 patients (53%) were resistant to penicillin. In no case was the organism isolated more resistant to the drug being used for treatment than sensitive

members of the same species or group. On the basis of sensitivity testing chloramphenicol would appear to be the most appropriate drug for the initial treatment of brain abscess. In six cases patients were receiving antibiotics which were inappropriate for treatment as judged by in vitro sensitivity testing. Two patients who failed to respond to low doses of an appropriate antibiotic showed clinical improvement when the dosage of antibiotic was increased.

During the period of the study 11/49 patients died (22%). The overall mortality of patients with intracranial or intraspinal abscess was 23% and the treated mortality was 19%. Brief clinical details on the patients who died are presented and the criteria used to determine mortality rates are discussed.

Encapsulated abscesses were demonstrated in 10/35 (29%) and eight of these together with three unencapsulated abscesses were excised. There was no correlation between mortality and excision, encapsulation or multiplicity of the abscess in this study.

In addition to the 66 samples of pus, 38 blood cultures, 160 swabs, three cerebrospinal fluids and an antral washout were examined. Bacteriological examination of these specimens was restricted, in the main, to the presence of Str.milleri and of those organisms isolated from the pus sample. In 5/11 patients with lesions due to Staph.aureus organisms of the same phage type were also present in other specimens and 5/7 patients who had temporal lobe abscesses which contained Proteus sp. organisms of the same species were isolated from the ear swabs. In the majority of the remaining patients the micro-organisms present in the pus sample were not isolated from other specimens.

Forty-nine samples were examined by gas liquid chromatography (GLC), and a procedure for liquefying mucoid samples is described. Good correlation was found between cultural results for anaerobic bacteria and the presence of long chain fatty acids in the GLC profile of pus samples. GLC was also found to be of value in monitoring the elimination of anaerobic bacteria from the pus during treatment.

One hundred and twenty-five antibiotic assays were performed on samples of intracranial pus, serum or cerebrospinal fluid. No correlation between the dose of antimicrobial drug administered and the concentration of active agent present in pus was established. Assays on pus and serum from a number of patients who were apparently receiving chemotherapy were negative. In the majority of cases where the serum level of penicillin was in excess of 1 mg/l there was also detectable drug in the pus sample. The number of patients treated with drugs other than penicillin was small. Chloramphenicol, fusidic acid and lincomycin showed good penetration into abscess cavities. The penetration of ampicillin, cloxacillin and cephaloridine was erratic and that of gentamicin was poor. No attempt, on a quantitative basis, was made to distinguish between antibiotic which had diffused into the cavity and that present as a result of haemorrhage. However, many of the highest levels were obtained from samples of blood stained pus. It was not possible to draw any general conclusions on the adequacy of treatment in terms of the level of active agent at the site of infection.

Countercurrent immunoelectrophoresis of sera from patients infected with Str.milleri showed that specific antibody had been produced. Using one of these sera it was

possible to detect the presence of antigen to Str.milleri in samples of pus within 1½ hours of receipt.

A retrospective study of two London neurosurgical units showed that during the period 1951-1973 there had been 257 cases of intracranial or intraspinal abscess. During the period 1951-1955 the mortality rate was 39%. Over the next 20 years it fell steadily and for the period 1961-1965 was 10%. During the period 1966-1970 there was an unexplained five-fold increase in mortality at both centres with an overall figure of 48%. Since 1970 the mortality has again been dropping. Differences in the incidence and mortality from intracranial and intraspinal sepsis between the two centres and in the bacteriological findings are reported and discussed.

The Registrar General's report for the years 1963-1973 shows that there are approximately 600 deaths per year from inflammatory disease of the central nervous system and that 12% of these are the result of intracranial or intraspinal suppuration. The mortality during the period 1968-1973 was 25% lower than for the preceding five year period although the male to female ratio remained the same at 2:1. These findings are discussed.

As a result of the findings reported in this thesis, it is proposed to examine the pathogenicity of Str.milleri in rabbits with a view to developing an experimental system in which the long term effects of cerebral abscess can be studied. Further studies on the apparent affinity of Str. milleri for the central nervous system of man are also planned. Further investigations on the penicillin inactivating properties of purulent exudates will also be undertaken. Finally the factors affecting the penetration of chemotherapeutic

drugs into cerebral abscesses have still to be elaborated and with the co-operation of a number of neurosurgeons it is proposed to study these factors in detail.

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ACKNOWLEDGEMENTS

I wish to express my gratitude and appreciation to Professor Rosalinde Hurley, M.D., my supervisor, for her advice and encouragement during this course of study.

I am grateful to Mr.Peter Gortvai, F.R.C.S. for his help and advice on organising the prospective study and to the neurosurgeons and morbid anatomists from Atkinson Morley's Hospital, London; Frenchay Hospital, Bristol; Oldchurch Hospital, Romford; St.Bartholomew's Hospital, London; Wessex Neurosurgical Centre, Southampton and Walton Hospital, Liverpool, for sending me specimens, case summaries and/or postmortem reports on their patients and for allowing me access to patients' case notes. In addition, my thanks are due to Professor J.A.N.Corsellis, Neuropathologist, Maudsley Hospital, London for providing the photographs shown as Figures 5-2 and 5-3.

I am grateful to Dr. John Pryse-Davies, M.D., Consultant Histopathologist at Queen Charlotte's Hospital for Women for his advice, for allowing me to work in his department and for providing the photographs shown as Figures 6-1, 6-2 and 6-3.

I should like to thank Professor Ian Phillips, M.D. for allowing me to use the gas liquid chromatography equipment in the Department of Clinical Microbiology, St.Thomas' Hospital, London.

I should like to thank Dr.M.T.Parker for advice and for allowing me access to his records (Table 6-1).

My thanks are also due to Mr. Peter Marriott and Mr. Keith Napier for their help and advice on the histological aspects of this study, to Mr. John Arthur for photographic advice and for taking the macrophotographs and to Mr. David Jarvis for reading the manuscripts and for his helpful comments.

Finally, I should like to thank my wife for so patiently typing the manuscript and Mrs.Paulette Mullings for secretarial assistance.

PREPARATION OF THESIS

This thesis is a record of my own work carried out between December 1973 and March 1977.

All the investigations and the preparation of this thesis were carried out by me with the exception of the macrophotography and the typing which have been acknowledged.

My research was carried out in the Department of Medical Microbiology, Queen Charlotte's Hospital for Women, London W6 where I held the position of Principal Microbiologist.