On the Bacteriology of Impetigo Contagiosa, with some Observations on Microbial Antagonism by Impetigo Staphylococci

by

George Ian Barrow

M.B., Ch.B., (Glasgow) Dip. Bact., (London)

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

Preface

Despite its familiarity, the precise nature of the causal organism of impetigo contagiosa has been disputed since the disease was first described by Tilbury Fox in 1864, and since its designation as a bacterial infection by Radcliffe Crocker in 1881. In the past it has been attributed variously to staphylococci, haemolytic streptococci, an unidentified virus, or to combinations of these agents. The most satisfactory explanation, though much contested, is that there are two kinds of impetigo, one caused by staphylococci and the other by streptococci; each kind may be distinguished clinically as well as bacteriologically, and each has prevailed at different times in different places. The work described in Part I of this thesis supports such a view. Staphylococci, largely of one particular bacteriophage 'type', were mostly isolated from sporadic cases of impetigo in Bradford during 1953-54. In contrast, an outbreak of impetigo at a school in Bradford in 1960 was different both clinically and bacteriologically, and was probably streptococcal in origin. An account of each of

these investigations has been published (Barrow, 1955, 1961).

During 1953-4, staphylococci of the same bacteriophage 'type' were also found in impetigo by other workers. They showed that these strains were able to prevent the growth of corynebacteria on solid media. This property, due to the production of a diffusible antibiotic substance, was possessed by very few other staphylococci. The experimental work described in Part II of this thesis concerns the nature and properties of this 'antibiotic'. Although not obtained in a pure state, it was found to be a heat-stable, slowly dialysable substance, probably protein or polypeptide in nature, which retained its activity for a considerable time under acid conditions. Also it was shown by 'direct' antagonism techniques that impetigo staphylococci were strongly active, not only against corynebacteria, but against all other strains of pathogenic staphylococci tested. The rôle of such microbial antagonism, and of other factors, in the initiation and establishment of impetigo infection was therefore considered.

This work, apart from the clinical aspects, was carried out in a routine bacteriological laboratory during several years. It is a pleasure to thank Dr. H.G.M. Smith, in whose laboratory it was done, and Dr. Allan Bigham, in whose department the

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Historical Review

Impetigo contagiosa

'Impetigo contagiosa' may be defined as an acute superficial infection of the skin, vesicular in onset and crusted in development, which heals completely without scar formation and leaves little or no immunity (O'Donovan, 1931). It was first described in 1864 by Tilbury Fox, who clearly recognised its contagious nature, and distinguished it from other infected or crusted diseases of the skin then referred to as 'impetigo'. Of some historical interest is his record (1869) that he 'had the pleasure of showing Dr. McCall Anderson* some cases a few weeks since, and he acquiesced in the special nature of the disease'.

Although Tilbury Fox and others searched for organisms in

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*Later Sir Thomas McCall Anderson, Professor of Clinical Medicine 1874-1900, and Regius Professor of the Practice of Medicine 1900-1908, in the University of Glasgow.

impetigo lesions, only occasional fungi were observed in the crusts. The presence of 'chains of cocci' in the vesicle fluid was first reported by Radcliffe Crocker (1881), who discounted the fungi as contaminants. In 1887 Bockhart concluded that impetigo was due to superficial staphylococcal infection, and this was supported by others (e.g. Dubreuilh, 1890; Wickham, 1892). However, Leroux (1894) cultivated both staphylococci and streptococci from numerous lesions. He considered that the streptococci were causative, because only they were recovered from impetigo lesions induced experimentally with fresh vesicle contents. This work was confirmed by Balzer & Griffon (1897), who obtained streptococci from many cases in pure culture.

Sabouraud (1900) greatly clarified the position by separating the impetigo of Tilbury Fox from the staphylococcal folliculitis which Bockhart (1887) and others had unfortunately regarded as impetigo. He overcame some of the disadvantages of liquid media, which he preferred for cultural purposes, by using enriched broth in Pasteur pipettes, the semi-anaerobic conditions in the capillary stem favouring the growth of streptococci. With this method, his extensive work clearly established the streptococcus as one of the causal organisms of impetigo contagiosa. Sabouraud stated, however, that impetigo was always caused by streptococci, and that

failure to isolate them was due to technical imperfections. He also insisted that staphylococci only caused lesions of the follicular type, and were therefore always secondary invaders in impetigo. These dogmatic assertions undoubtedly influenced subsequent work, and started a controversy which is only just being resolved.

Sabouraud also caused some confusion by asserting, correctly, though contrary to general opinion at the time, that pemphigus neonatorum was in reality bullous impetigo contagiosa of the newborn. Although his bacteriological evidence was unconvincing, he considered that this condition was streptococcal in origin, any staphylococci again representing contaminants. Pemphigus neonatorum, a misnamed highly infectious, bullous or exfoliative disease of infants, was first adequately described by Rigby in 1835. It was not until 1864 that Tilbury Fox again emphasised its contagious nature, and drew attention to its association with impetigo contagiosa. In 1886 Demme cultured 'non-chromogenic diplococci' from the lesions of pemphigus neonatorum, and Almquist (1891) isolated organisms indistinguishable from Staphylococcus aureus. Similar results were obtained by Matzenauer (1900) and Engman (1900), each of whom also maintained that pemphigus neonatorum was the infantile form of impetigo

contagiosa. This work has since been amply confirmed, both by culture and by experimental reproduction of the disease with staphylococci isolated from the lesions (e.g. Call, 1904; Cole & Ruh, 1914; Belding, 1926; Falls, 1927; Benian & Jones, 1929). Some workers, notably Clegg & Wherry (1906), Falls, (1917) and McCandlish (1925) described the presence of Gram-positive diplococci resembling gonococci in appearance, or short chains of cocci, in the lesions of pemphigus neonatorum, but obtained only staphylococci on culture. The possibility of a virus aetiology was considered by Benian & Jones (1929), but they were unable to substantiate it.

There are very few reports in favour of streptococci in pemphigus neonatorum. Apart from Sabouraud, only three possible cases were described by Whitfield (1903) and Adamson (1937) between them. Even Whitfield, observing the frequent association between pemphigus neonatorum and impetigo in adults, remarked how strange it was that one infection should be staphylococcal if the other was streptococcal in origin. Carter & Osborn (1936) isolated only staphylococci from pemphigus neonatorum, but they suggested that it was a 'subepithelial pyo-dermatitis', and therefore different from impetigo, in which effusion occurs between the stratum granulosum and stratum corneum. However, this suggestion

has not been generally accepted. The subject has been well reviewed by Poole & Whittle (1935) and Hart (1938), who consider that pemphigus neonatorum represents bullous impetigo in infants, and that Staphylococcus aureus has been fully established as the causal organism.

The literature on the bacteriology of impetigo from 1900 onwards is closely linked with that of pemphigus neonatorum, some considering both infections the same, others distinguishing between them. Many workers, using more selective methods for isolating streptococci, such as 'plating out' on solid media and the use of crystal violet for suppression of staphylococci, supported Sabouraud's findings in impetigo (e.g. Lewandowsky, 1909; Farley & Knowles, 1921; Haxthausen, 1927). Others were more doubtful and simply recorded the incidence of staphylococci and streptococci in pure and in mixed culture, some stating that it was impossible to say which organism was causative (e.g. Dubreuilh & Brandeis, 1910; Smith & Burky, 1924; Balmain, 1926).

On the other hand, some workers, particularly Engman (1901), Lewandowsky (1922) and Epstein (1940), maintained that there were two varieties of impetigo, one staphylococcal and the other streptococcal in origin, each of which could be distinguished clinically as well as bacteriologically, though perhaps not in

every individual case. Thus streptococcal impetigo was associated with thick, dirty-yellow crusts, often penetrating in character, and surrounded by inflamed margins. It frequently occurred in epidemics. In contrast, staphylococcal impetigo was primarily bullous in character, and was followed by thin, varnish-like crusts, usually without any inflammatory reaction, often showing some central healing. Numerous attempts, however, to reproduce impetigo experimentally with these organisms were only occasionally successful, and often gave conflicting results.

According to Epstein (1940) the clinical differentiation between these varieties had been made long before bacteriological investigations started. He considers that some priority should be accorded to Dunn (1863), who clearly described the streptococcal variety of impetigo, whereas Fox (1864) depicted not one but several clinical varieties, including both streptococcal and staphylococcal types. Indeed Fox quoted Dunn's description as a 'modification' of impetigo contagiosa.

Much of the controversy is understandable only in historical perspective. According to Tachau (1938), many outbreaks of staphylococcal impetigo occurred throughout the world before 1900, and these were often accompanied by epidemics of pemphigus neonatorum. From about 1890, outbreaks of staphylococcal impetigo

largely ceased in Europe, and by 1900 streptococcal impetigo had become predominant, staphylococcal impetigo occurring only sporadically or in small flare-ups. In moist, hot climates (Dohi & Dohi, 1912), however, and in infants, staphylococcal impetigo remained common.

This reasonable explanation, backed by clinical, bacteriological and some experimental evidence, elicited little support. The existence of staphylococcal impetigo was still contested, and Epstein (1940) cites an instance where Sabouraud's authority actually prevented its acceptance. Cruickshank (1941) for example obtained fewer streptococci than staphylococci from impetigo, but admitted that, rightly or wrongly, the staphylococci were simply regarded as contaminants. In contrast, Roxburgh (1941) stated that his clinical experience in London was mostly of staphylococcal impetigo. Further work among troops in wartime did much to alter general opinion that only streptococci were causative (Bigger & Hodgson, 1943, 1944; Sheehan & Fergusson, 1943). They obtained staphylococci from most impetigo lesions, and showed that the incidence of streptococci increased with the duration of infection, presumably due to secondary invasion. Indeed Bigger & Hodgson (1943) went as far as to state that streptococci were seldom if ever the cause of impetigo. They

also considered, but rejected, the possibility of a virus acting in association with staphylococci. There the matter rested, until Cruickshank (1953), in reviewing the epidemiology of some skin infections, suggested that the evidence supported the existence of the two varieties of impetigo, but that further work was required utilizing available methods of typing strains of staphylococci and streptococci.

Most of the work already mentioned was concerned primarily with identifying the bacterial species causing impetigo and pemphigus neonatorum. However type-specificity of the responsible organisms, whatever the species, was suggested as early as 1906 by Clegg & Wherry. They considered that the natural habitat, morphology and absence of invasiveness distinguished them from the ordinary pyogenic cocci. This was strongly supported on epidemiological grounds by Simpson (1941). By means of serological typing, May (1951) found that particular strains of staphylococci occurred in tropical bullous impetigo, and similar findings were obtained in cases of pemphigus neonatorum by Anderson (1943) and by Elliott, Gillespie & Holland (1951). As regards streptococci, Streptococcus pyogenes is usually involved, and Friedberg (1942) found some cultural differences between impetigo and other strains.

More recently it has been shown that, in this country, impetigo

staphylococci were mostly of one particular bacteriophage 'type' (Barrow, 1955; Parker, Tomlinson & Williams, 1955; Spittlehouse, 1955). This type was later also found in outbreaks of pemphigus neonatorum (Gillespie, Pope & Simpson, 1957). In addition, Parker, Tomlinson & Williams found that impetigo streptococci were largely of two serological 'types', and that impetigo staphylococci were also characterised by their ability to antagonise the growth of corynebacteria on solid media.

Microbial antagonism

When different species or types of organisms are present together under natural or experimental conditions, one may inhibit or retard growth of the others. Such antagonism among bacteria was probably first satisfactorily demonstrated by Pasteur & Joubert in 1877. They noticed that ordinary aerobic bacteria suppressed the growth of anthrax organisms in sterile urine. This observation subsequently aroused interest in the development of inhibitory properties in broth in which organisms had already grown. Media thus 'vaccinated' and so 'staled' frequently prevented the growth of other organisms, and occasionally failed to support further growth of the same organism. 'Direct' bacterial antagonism on

solid media was first described by Babès (1885), who sowed both active and passive organisms simultaneously on the same plate. In many instances Garré (1887) considered that inhibition was due, not to 'swamping' of one species by another, or to exhaustion of nutrients, or to adverse physico-chemical conditions in the medium, but to the production of specific diffusible substances by the antagonists. The term 'antibiosis' was used by Vuillemin (1889) to denote the elaboration of such substances by micro-organisms, and Waksman (1947) defined an 'antibiotic' as a 'chemical substance of microbial origin which possesses antibiotic powers'. More recently its scope has been extended to include anti-microbial substances not only from microbes, but from any living source, including animal and plant tissues. The earliest 'antibiotic' thus recognized was 'pyocyanase', obtained from Pseudomonas pyocyanea by Emmerich & Löw (1899). 'Antibiotic' agents are now known to range from simple organic compounds to complex proteins, which act by killing, lysing, or suppressing the growth of organisms.

Garré (1887) described several methods, which differ little from those used today, for detecting antagonistic organisms. They included 'deferred' antagonism methods on solid media, in which the growth of one organism was removed, and other organisms

were then inoculated across the site of the previous growth. He also observed that 'direct' microbial antagonism may be 'one-sided' or 'two-sided'. In the former, one organism repressed another which was not antagonistic to it, and in the latter both organisms repressed each other. By allowing one organism to grow first, as in 'deferred' antagonism, Garré showed that a 'one-sided' antagonism may become 'two-sided'. Further techniques for investigating the nature of microbial antagonism included the use of porcelain (Frankland & Ward, 1893), parchment (Lode, 1903) and collodion membranes (Frost, 1904) for the separation of growing cultures.

Thus at the beginning of this century the term 'antibiotic' and the idea of selective toxicity had been introduced, and many of the methods of searching for and investigating microbial antagonism had been elaborated. The number of antagonisms observed, between organisms of different species ('hetero-antagonism') and between different strains of the same species ('iso-antagonism'), steadily increased. Early reports gave little information about the nature of the substances responsible, and little was done to develop 'antibiotic' therapy. Indeed the suggestion of Cantani (1885) that microbial antagonism itself might be used therapeutically stimulated instead a vast amount

of work into so-called 'replacement therapy', the principle being to replace pathogens in the body with harmless organisms. Numerous observations, mostly clinical and many uncritical, were made on the protective effect of various antagonists against simultaneous or subsequent inoculation of animals with pathogens. In man, well-known examples were the use of coliform organisms and lactobacilli for various intestinal conditions (e.g. Metchnikoff, 1907; Nissle, 1916), and throat sprays of staphylococci in the treatment of diphtheria cases and carriers (e.g. Schiøtz, 1909; Dujardin - Beaumetz, 1934).

Microbial antagonism by staphylococci was first reported by Babès (1885) on solid media. Others found that media in which staphylococci had grown would not support the growth of anthrax organisms (Cornil & Babès, 1885), typhoid organisms (Freudenreich, 1888), gonococci (Grosz & Kraus, 1898), or some strains of staphylococci (Carpano, 1918). When studying morphological changes occurring in mixed broth cultures, Stoval, Scheid & Nicholls (1923) found that some staphylococci readily suppressed the growth of corynebacteria. Bogendörfer (1924) ascribed this to the production of thermolabile substances detectable in filtrates, but thought that they were common to many organisms. Inhibition of corynebacteria by staphylococci, both in liquid and on solid

media, was also mentioned by Adachi (1925) and Besta & Kuhn (1935).

In 1932 Dujardin - Beaumetz cultivated from a case of rhinitis a non-pathogenic staphylococcus ('Micrococcus antibioticus') active against most corynebacteria and Gram-positive organisms, but not staphylococci or streptococci, and against many Gram-negative organisms. The effect was seen only on solid media. Other staphylococci from many sources, including boils, osteomyelitis and mastitis of sheep, were examined, but were inactive. Duliscouët (1935) and Duliscouët & Ballet (1935) confirmed the inhibitory action of some staphylococci on corynebacteria, and in addition described a stimulating effect produced by others, although Steen (1949) found that this only occurred on media containing blood or serum. One of their strains, similar to that of Dujardin - Beaumetz, inhibited several strains of corynebacteria on solid media, and when mixed with diphtheria organisms, protected laboratory animals against otherwise fatal infection. Although unable to detect activity in liquid media, Duliscouët (1939, 1945) succeeded in extracting from agar cultures an active preparation, which he called 'staphyline', and which he thought was an enzyme.

Jennings & Sharp (1947) examined many strains of staphy-

lococci for antibiotic production, and found that about 10 per cent of random strains were active against corynebacteria. They obtained no evidence to suggest that more than one inhibitory agent was involved. One of their strains was later fully investigated by Gardner (1949). She found that the antibiotic formed was probably protein in nature and that aerobic conditions were essential for its production, but considered it unlikely to have any therapeutic value. A somewhat similar antibiotic substance, thought to be a polypeptide of low molecular weight, was obtained by Loeb, Moyer & Murray (1950) from a strain of Micrococcus epidermidis. Halbert, Swick & Sonn (1953) also obtained from various staphylococci different antibiotics, many of which were thought to be polypeptides. In contrast Magrassi & Spiga (1946) obtained from inhibitory staphylococci, after bubbling oxygen through broth cultures, active material which they subsequently identified as peroxide. Su (1948) also obtained a different antibiotic 'micrococcin' from a non-pathogenic staphylococcus isolated from sewage.

Using 'deferred' antagonism methods (Gratia, 1946) and suitable indicator strains, many more active organisms were detected. Numerous antagonisms were revealed, not only between staphylococci and corynebacteria (Jennings & Sharp, 1947), but also

between different strains of staphylococci (Frédéricq, 1946; Halbert, Swick & Sonn, 1953) and between different strains of corynebacteria (Thibaut, 1949; Terasse & Sohier, 1954). The high degree of biological specificity of these antibiotics has led recently to the development of limited epidemiological 'typing' systems for some organisms, including Corynebacterium diphtheriae (Thibaut & Frédéricq, 1956), Escherichia coli (Frédéricq, Betz - Bareau & Nicolle, 1956; Shannon, 1957) and Shigella sonnei (Abbott & Shannon, 1958). The individual 'types' so defined in general differ only in their ability to produce, or in their sensitivity to these substances, although Gratia (1932) and Frédéricq & Gratia (1949) found a close, but not complete, similarity between antibiotic production and bacteriophage lysogenicity.

Frédéricq (1946) demonstrated among staphylococci a situation similar to that of antibiotic 'colicine' production among enterobacteria (see Gratia, 1925; Gratia & Frédéricq, 1946; Heatley & Florey, 1946; Halbert, 1948a; Frédéricq 1948), and by analogy termed the active substances 'staphylococcines'. By means of differential inhibition, heat stability, cross-resistance and other tests Frédéricq showed that different inhibitors were produced by various strains, and that occasionally more than one

inhibitor was formed by the same strain. Inhibitory activity was apparently haphazard in distribution among staphylococci. It was not correlated with pathogenicity or other characteristics (Frédéricq & Betz, 1946), nor was it associated with a particular strain or 'type', until Parker, Tomlinson & Williams (1955) showed that most impetigo staphylococci were active against corynebacteria. Apart from the egg-yolk (Gillespie & Alder, 1952) and serum opacity (Tomlinson & Parker, 1956) reactions, Parker (1958) found no essential cultural differences between impetigo and other staphylococci. In subsequent work on the nature of the inhibitory substance Parker & Simmons (1959) were unable to obtain active bacteria - free preparations, but they showed that impetigo strains lost their inhibitory activity on storage in the laboratory, and that this loss was usually accompanied by changes in the range of bacteriophage susceptibility.

In much of the work already cited, interest was focussed mainly on the mechanism of antibiotic production or their possible applications. Little attention was devoted to the ecological significance of antibiotic production to the organisms themselves, particularly in relation to their natural micro-environment, where survival depends not only on suitable nutrients and metabolic conditions, but on successful competition with other organisms.

Experimental studies on microbial antagonism in mixed cultures are few, and are necessarily limited by the choice of organisms and available methods. A succession of dominant species occurring in broth cultures of faeces on continued incubation was reported by Topley & Fielden (1922). Régnier & Lambkin (1934) compared the growth of organisms in pure and in mixed cultures under identical conditions. With mixtures of staphylococci and coliform organisms they found that growth of the coliforms was unimpaired, but that growth of the staphylococci was hindered. The greater the number of coliforms, the greater was the effect. More recently, interaction between staphylococci, streptococci and corynebacteria in mixed broth cultures was studied by Hayes (1950) and Annear (1951). Complete inhibition of the corynebacterium was always found in the presence of the staphylococci. A more convenient approach to the quantitative study of interaction between organisms, using a chess-board procedure on solid media, was developed by Rosebury, Gale & Taylor (1954). This allowed observation of both inhibitory and stimulatory effects, and emphasized the importance of the initial concentrations and proportions of organisms in mixed bacterial populations in relation to the final outcome. The rôle of microbial antagonism in maintaining the normal bacterial

flora of the skin and mucous membranes of the eye was discussed by Evans, Smith, Johnson & Giblett (1950) and by Halbert, Swick & Sonn (1953). Its rôle in human infections with enterobacteria was considered by Frédéricq & Levine (1947). The relation of antagonistic coliform organisms to shigella infections was investigated by Halbert (1948, b, c), who found that the incidence, as well as the numbers, of antagonists were significantly greater in clinically ill patients than in others. There are, however, very few other studies on interaction between particular organisms isolated from particular infections. Because of their strong antagonistic action against corynebacteria, impetigo staphylococci were studied, together with streptococci cultivated from impetigo lesions, from this point of view, and the nature and significance of the antibiotic also further investigated.

Part I: The Bacteriology of Impetigo contagiosa

1. Introduction

In the past impetigo contagiosa has generally been attributed to haemolytic streptococci (e.g. Adamson, 1937; Cruickshank, 1941), although on occasions staphylococci were also isolated (Farley & Knowles, 1921; Balmain, 1926). The earlier literature has been reviewed by Percival (1929) and Forman (1938). Others maintained that each of these organisms may be causative (Tachau, 1938; Epstein, 1940). More recently staphylococci have again been incriminated (Bigger & Hodgson, 1943, 1944; Sheehan & Fergusson, 1943). A specific 'impetigococcus' has been postulated on epidemiological grounds (Simpson, 1941), and also the view that an unidentified agent - possibly a virus - may be concerned, either alone (Campbell, 1942) or in association with staphylococci (Bigger & Hodgson, 1943). Cruickshank (1953) has indicated the need for more detailed bacteriological studies.

The work described in Part I concerns first an investigation

during 1953-5 into the clinical, bacteriological and epidemiological features of impetigo contagiosa, referred to as 'impetigo', with particular reference to the phage-typing of staphylococci and serological typing of haemolytic streptococci (Barrow, 1955). Later an outbreak of streptococcal infection occurred in 1960 at a school for subnormal children (Barrow, 1961). A brief account of this is given because it provided an opportunity for further study of some aspects of impetigo.

Impetigo in 1953-54

In this investigation, mainly of sporadic impetigo among children, it was shown that staphylococci of one particular bacteriophage 'type' were largely involved.

2. Material and Methods

Clinical material

It was originally intended to examine only early cases of impetigo presenting with intact vesicles; this, however, was abandoned, as only one such case was seen after several weeks. Accordingly, all uncomplicated cases were investigated irrespective of their duration. As most cases were in the crusted phase when first seen, the term 'lesion' is used for all of the various

Table 1

Ages of Impetigo and control patients

Age group	SERIES			
	Impetigo	SI	ON	HN
2 and under	18	8	4	6
3-5	23	5	-	3
6-10	25	9	7	4
11-20	23	19	20	3
21-30	12	17	3	12
31-40	7	13	6	8
41-50	7	13	6	6
51-60	3	9	2	6
over 60	1	7	2	2
Total No. of cases	119	100	50	50

SI Staphylococcal infections other than impetigo

ON Outside 'normal' persons

HN Hospital 'normal' patients and staff

stages of impetigo, unless otherwise stated.

During the period June 1953 to July 1954 I examined 125 out-patients referred to me with impetigo at the Department of Dermatology, Bradford Royal Infirmary. Of these, 119 (69 male, 50 female) were considered to be true cases of impetigo. Their ages, together with those of three 'control' groups, are shown in Table 1.

As each patient attended, a full history was obtained, if necessary from a parent or guardian, and a complete clinical examination made. Single swabs were taken before treatment from the nose, throat and one recent lesion - the crust being lifted gently with sterile forceps and a dry swab carefully rubbed over the base. In many instances, direct films were examined after staining by Gram's method. In suitable patients, intact vesicles were cleansed with acetone and the vesicle fluid was aspirated into sterile capillary tubes; crusts were also obtained from some of these patients. Additional sets of swabs were taken from a few cases during and after treatment, and in others from the skin near lesions.

Control groups

When it was found that staphylococci of one phage 'type' were largely predominant in impetigo, swabs were taken from three

'control' groups in order to ascertain if this 'type' was common locally, or was special to impetigo.

Series SI: staphylococcal infections other than impetigo.

A total of 101 strains of staphylococci was obtained from 100 patients (55 male, 45 female), from boils (30), abscesses (36), osteomyelitis (7),septicaemia (1), and miscellaneous superficial skin infections (27). The superficial skin infections included various forms of eczema and dermatitis (4), sycosis barbae (2), ulcers (3), conjunctivitis (2), ecthyma (2) paronychia (2), and other infected lesions (8). This series was started in December, 1953, and completed in September, 1954.

Series ON: outside 'normals'

Staphylococci, representing thirty-two different phage-patterns, were cultured from nose (23) and throat (10) swabs of 50 persons (27 male, 23 female) who had had no direct contact with hospitals for some considerable time, but were referred to hospital with various non-infected conditions.

Series HN: hospital 'normals'.

Staphylococci, representing thirty-one different phage-patterns, were cultured from nose (29), and throat (4) swabs of 50 hospital patients and staff (22 male, 28 female). The patients were from general medical and surgical wards, and had been in

hospital for 2-3 weeks or more.

In addition to comparison of the control staphylococci with those of impetigo, series HN and ON also provided a measure for comparison with series SI, and with the nasal carriage rate in the impetigo cases. Streptococcal carriers were not studied because of the small number of streptococci isolated from the impetigo cases.

Bacteriological methods

All swabs were plated on 7 per cent layered horse-blood agar medium, usually on the same day, and always within 24 hours. Plates were examined after aerobic incubation overnight at 37°C. From impetigo lesions, staphylococci, or haemolytic streptococci, or both, were usually obtained in 'pure' culture; other organisms were uncommon. Single colonies of coagulase-positive staphylococci (slide technique - Cadness-Graves, Williams, Harper & Miles, 1943) were subcultured, and tested for penicillin sensitivity with discs containing 2.5 i.u. of penicillin. Coagulase-negative staphylococci were discarded. Haemolytic streptococci were grouped by a modified Lancefield's method (1933) and their penicillin sensitivity determined. Strains other than Group A were discarded.

Staphylococci were stored on nutrient agar slopes in bijou

Table 2

Phages used in the typing of Staphylococcus aureus, and the groups of staphylococci defined by them.

Group	Reaction with phages
I	29, 52, 52A, 79
II	3A, 3B, 3C, 55, (71) *
III	6, 7, 42D,** 42E, 47, 53, 54, 70, 73, 75, 77
Untypable	No strong reaction with any phage (< 50 plaques)
Not classifiable	Strong reaction with phages of more than one group.

* included in the 'basic' typing set during this work.

** now allocated to a separate 'Group IV'.

bottles at room temperature, and were phage-typed in batches at the Public Health Laboratory, Sheffield, by the method of Williams & Rippon (1952). Phages, comprising the 'basic set' of Williams, Rippon, & Dowsett (1953) with the omission of phage 44, and subsequent addition of phage 71 (Group II), were used at their routine test dilution (R.T.D.), and at 1000 times this concentration (Table 2).

Haemolytic streptococci were typed serologically at the Streptococcus Reference Laboratory, Colindale, by both the slide-agglutination (Griffith, 1934) and precipitin (Swift, Wilson & Lancefield, 1943) methods, as described in the Report (1954).

3. Clinical Features

All cases were characteristic of impetigo. Of the 119 cases studied, lesion swabs yielded neither staphylococci nor streptococci in 13 instances; eight of these patients had clinically resolving lesions. The remaining 106 cases were classified according to extent as mild (42), moderate (52) or severe (12), and according to duration as short (63), intermediate (26), or longstanding (17). The sites of infection were: face and/or neck, 94 cases; arms and/or legs, 31 cases; and body,

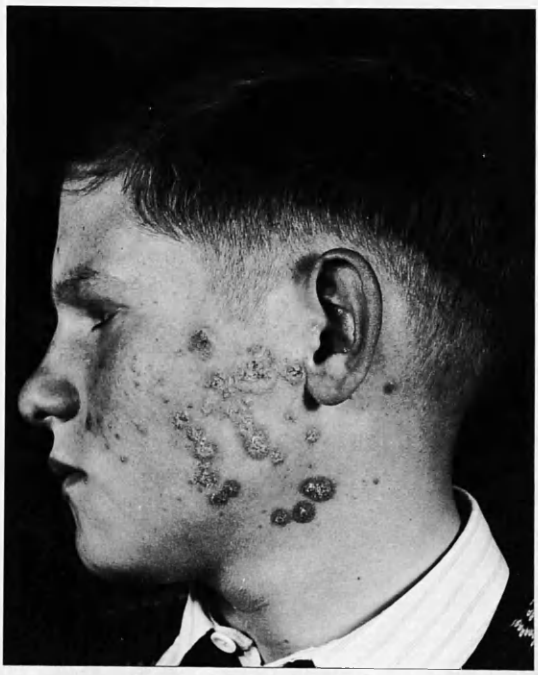
10 cases. The character of the skin was dry in 27 patients from all age-groups, and seborrhoeic in 17 youths or adults.

No clinical distinction was observed between lesions yielding a pure growth of staphylococci (Plate 1), and those yielding a pure growth of haemolytic streptococci. In these patients, lesions began as 'blebs' on normal skin, or as small 'red patches' on which 'blisters' quickly developed. Large or persistent bullae were not seen, nor were they described by patients. Blisters were readily broken by rubbing or scratching, and the ensuing serous exudate rapidly formed crusts. Intact, small vesicles were seen in only 7 patients. Recent lesions were small and covered with thin, flat, varnish-like crusts, frequently without any surrounding inflammatory margin. In older lesions, the crusts were larger, thicker, usually 'honey-coloured' and typically 'stuck-on' in appearance, often showing a tendency towards central healing. Removal of these crusts left a smooth, glistening 'raw' base which rarely bled, but exuded serum.

The presence of streptococci was suspected in some of the mixed infections, because of their severity or long-duration (Plate 2). In these patients, lesions were deeper and more penetrating. The crusts were difficult to remove, and left a

Plate 1

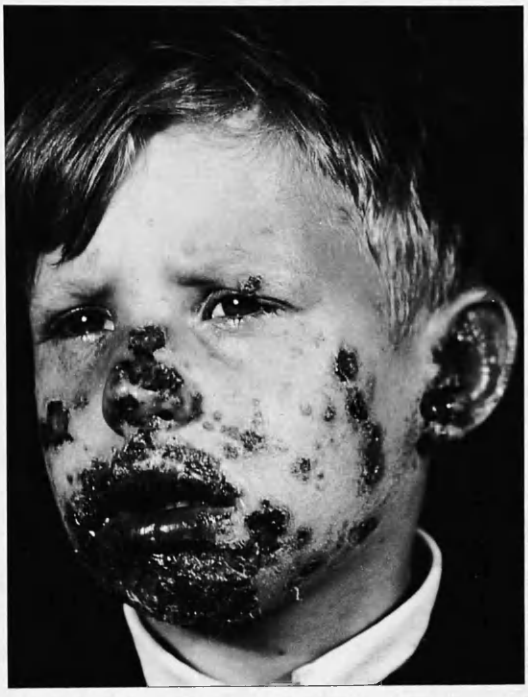
Staphylococcal impetigo



A typical mild case of impetigo of six days' duration, from which Staphylococcus aureus 'Type 71' was isolated in pure culture.

Plate 2

Mixed staphylococcal and Streptococcal impetigo



A moderately severe case of impetigo of fourteen days' duration, from which a mixed growth of Streptococcus pyogenes and Staphylococcus aureus was obtained.

bleeding base. They were dirty, yellow-green in colour, and were surrounded by marked inflammatory zones with, in a few instances, enlargement of the regional lymph nodes.

4. Bacteriology

Direct films from impetigo lesions were stained by Gram's method and examined microscopically in 71 cases. In films prepared from lesion swabs there was generally little to be seen, apart from scattered Gram-positive cocci, many of which were diplococcal in form. In smears of lesion scrapings, however, abundant polymorphs and large numbersof Gram-positive cocci, often in large clusters were always present. Most of these organisms were diplococcal in appearance, closely resembling gonococci in morphology, arrangement and in their intra-cellular position. In no instance was chain formation seen. Despite the examination of many films and smears from routine bacteriological specimens over several years, such a picture - Gram-positive intracellular diplococci - has not been found in any infection other than impetigo.

Only the two bacterial species Staphylococcus aureus (coagulase - positive staphylococci) and Streptococcus pyogenes

Table 3

Distribution of staphylococci and haemolytic streptococci from 106 cases of impetigo.

Source		Lesion	Nose	Throat
Staph. aureus only		86	48	14
Staph. aureus and haemolytic streptococci of Group	A	12	4	1
	C	-	-	1
	Not A,C, or G	2	2	4
Haemolytic Streptococci only of Group	A	6	3	6
	C	-	-	1
	Not A,C, or G	-	-	4
TOTAL		106	57	31

(Lancefield Group A) were considered. Of the 106 bacteriologically positive cases studied, Staph. aureus was obtained in pure culture from 86 (81%), and Str. pyogenes in pure culture from 6 (5.6%) lesions; both Staph. aureus and Str. pyogenes were isolated in 12 instances (11.4%), and Staph. aureus, together with streptococci not of Group A, C or G, were present in two cases. In all, therefore, Staph. aureus was cultured from 100 (94.4%) and Str. pyogenes from 18 (17%) impetigo lesions in the present cases (Table 3).

For convenience, the bacteriological findings are considered separately under (A) staphylococci, (B) haemolytic streptococci, and (C) mixed infections with Staph. aureus and Str. pyogenes. Virus studied (D) are reported last.

(A) Staphylococci

In general most phage-susceptible staphylococci fall into one or other of three broad groups, corresponding approximately to the three serological groups defined by Cowan (1939); those lysed by phages of more than one group are regarded as 'unclassifiable' and those not susceptible to any phage, even at 1000 x R.T.D., are termed 'untypable'. More recently, staphylococci lysed by phage

42D only at R.T.D. have been allocated to a separate fourth group (Rippon, 1956); these strains frequently occur in animals (Smith, 1948; Thatcher & Simon, 1956). The criteria involved in deciding whether or not strains showing small differences in phage-pattern are related have been reviewed by Anderson & Williams (1956). For this investigation, it was assumed that all staphylococci susceptible to phage 71 only, either at R.T.D. or at 1000 x R.T.D., were identical ('Type 71'), since such strains had not been encountered frequently before. The remaining staphylococci in Group II, lysed by one or more of the phages 3A, 3B, 3C, 55 and often also 71, have been classed together as 'other patterns'.

(i) Phage 'type' in impetigo lesions

Most of the first batches of impetigo staphylococci tested failed to show any lysis at R.T.D. with any of the phages in routine use, although they reacted with one or more of the phages 3A, 3B, 3C and 55 at 1000 x R.T.D. Such findings were unusual, and at this stage it was learnt that similar results had been encountered with impetigo staphylococci at Manchester by Parker, Tomlinson & Williams (1955). A few of their cultures were tested with less commonly used Group II phages, and all gave clear-cut strong reactions at R.T.D. with phage 71. Accordingly, all the

Table 4

Results of phage-typing Staphylococcus aureus strains from 100 cases of impetigo

Phage Group	Number of strains from		
	Lesion	Nose	Throat
I	3	3	2
II 'Type 71'	63	29	13
II Other patterns	26	18	4
III	3	1	1
Untypable	3	2	-
Unclassifiable	2	1	-
TOTAL	100	54	20

impetigo strains from Bradford were retested with phage 71, and this phage was included in all subsequent tests.

The results indicated that 97 of 100 staphylococci isolated from impetigo lesions were typable, and three were untypable (Table 4). Of the typable strains 89 were members of Group II, and two were not classifiable. As many as 63 of the Group II strains were the same, 'Type 71', being lysed only by this phage at R.T.D. The staphylococci comprising 'other patterns' in Group II varied slightly in their phage susceptibility; all but three were lysed by phage 71, amongst others, either at R.T.D. or at 1000 x R.T.D. They included 17 strains, closely related to 'Type 71', which were lysed by phage 71, and also by other Group II phages, at 1000 x R.T.D., but not by any phage at R.T.D. Such strains, regarded as 'weak 71' strains, were classified by Parker et al., (1955) as a subdivision of 'Type 71'. On this basis, 'Type 71' staphylococci accounted for 80 of the 100 strains cultivated from impetigo lesions. Six further strains, although not included within 'Type 71', were lysed at R.T.D. either by phages 55/71 or 3C/55/71; it is possible, for reasons given later by Parker & Simmons (1959) and cited in Part II of this thesis, that these staphylococci may have been degraded 'Type 71' strains, though this cannot now be proved. In addition, one unclassifiable lesion

culture of phage-pattern 29/79/3A/3B/3C/55/71/7/42E/54, was probably a mixture of two or more strains of staphylococci, one of which was 'weak 71'.

It is perhaps noteworthy that in 2 of the 3 cases yielding untypable strains from lesions, staphylococci of 'Type 71' in one instance and 'weak 71' in the other, were obtained from the nasal swabs. Staph. aureus was not isolated from the nose or throat of the third patient.

In seven patients fluid from unruptured vesicles was examined. Staphylococci of 'Type 71' were obtained in pure culture in four instances and 'weak 71' in one instance. The two remaining cases gave a mixed growth of 'weak 71' staphylococci and Str. pyogenes. Also in 9 of 15 cases, swabs from normal-looking skin near active lesions yielded staphylococci of the same type as in the lesions.

Staph. aureus of 'Type 71' was thus present in 80-90 per cent of the staphylococcal cases of impetigo. Furthermore, the control groups indicate that this 'type' is apparently uncommon in other situations.

(ii) Phage 'types' in control groups

Since one 'type' of Staph. aureus occurred so frequently in impetigo, search was made for its presence in other staphylococcal

Table 5

Phage-typing results of Staphylococcus aureus from the various lesions in staphylococcal infections other than impetigo

Phage Group	Superficial skin lesions	Boils	Abscesses	Osteomyelitis and Septicaemia	Total
I	3	7	10	3	23
II 'Type 71'	1	-	-	-	1
II Other patterns	8	8	10	5	31
III	10	4	5	-	19
Untypable	-	2	-	-	2
Unclassifiable	5	9	11	-	25
TOTAL	27	30	36	8	101

infections. Table 5 gives the source and phage groups of strains of Staph. aureus isolated from staphylococcal infections other than impetigo (series SI). There are considerably fewer strains of Group II, and considerably more of Groups I and III than in the impetigo series, although the proportion of unclassified staphylococci is perhaps higher than usual. They represented 43 different phage-patterns. In contrast to the cultures from impetigo, 'Type 71' was obtained in only one instance; this was from the cutaneous lesion of a suspected, but unconfirmed, case of anthrax. Other patterns in Group II included 9 'weak 71' strains, cultivated from hand infections (2), axillary boils (2), and from one case each of sycosis barbae, nasal pustule, breast abscess, peri-anal abscess, and osteomyelitis. No particular phage group was associated with any particular type of lesion.

The results of phage-typing nose and throat staphylococci from the hospital 'normal' (series HN) and outside 'normal' (series ON) control groups are summarized, and compared with those of series SI in Table 6. The distribution of staphylococci among the phage groups was similar in each of the 'normal' control series. In no case was Staph. aureus of 'Type 71' isolated, although one throat swab, and three nasal swabs in series ON yielded 'weak 71'

Table 6

Results of phage-typing Staphylococcus aureus strains from the control series

Phage Group		Control Series			Total of all Controls
		SI	ON	HN	
I		23	10	4	37
II	'Type 71'	1	-	-	1
	Other patterns	31	8	9	48
III		19	5	6	30
Untypable		2	3	7	12
Unclassifiable		25	6	5	36
TOTAL		101	32	31	164

SI Staphylococcal infections other than impetigo

ON Outside 'normal' nose and throat staphylococci

HN Hospital 'normal' nose and throat staphylococci

strains. Also the actual phage-patterns in each of the control series were similar, suggesting that these staphylococci were representative of the strains then prevalent locally.

In comparison with their frequency in impetigo lesions, it is striking that 'Type 71' was obtained only once out of a total of 164 control staphylococci. It seems, therefore, that in Bradford and district Staph. aureus of 'Type 71' is significantly associated with impetigo, and this association is not due to a local strain of unusual virulence, or to a high rate of nasal carriage amongst the population.

(iii) Relation of penicillin sensitivity to phage type

The penicillin sensitivity of 90 strains of Staph. aureus from impetigo lesions was determined, and 64 (71%) were found to be resistant. Some of these patients had used penicillin locally but this incidence was unexpectedly high for sporadic 'non-hospital' cases of infection. Penicillin resistance, although increasing (Barber & Rozwadowska-Dowzenko, 1948), had hitherto been confined almost entirely to hospital infections. Thus Forbes (1949) found that penicillin-resistance in staphylococcal infections inside hospitals was about 60 per cent compared with 12 per cent in those occurring outside. Ludlam (1953) showed that not only were the nasal carrier rates of infants born in one

Table 7

Relation of penicillin sensitivity to phage-typing results of *Staphylococcus aureus* from impetigo and control series

Phage Group	Impetigo lesions		Control Series						Total of all controls	
			SI		ON		HN			
	S	R	S	R	S	R	S	R	S	R
I	2	-	14	9	9	1	2	2	25	12
II 'Type 71'	11	44	1	-	-	-	-	-	1	-
II Other patterns	9	16	16	15	7	1	5	4	28	20
III	1	2	9	10	3	2	2	4	14	16
Untypable	2	1	1	1	3	-	3	4	7	5
Unclassifiable	1	1	14	11	6	-	2	3	22	14
TOTAL	26	64	55	46	28	4	14	17	97	67
%	29	71	54	46	87.5	12.5	45	55	59	41

(S = penicillin sensitive; R = penicillin resistant)

SI Staphylococcal infections other than impetigo

ON Outside 'normal' nose and throat staphylococci

HN Hospital 'normal' nose and throat staphylococci

hospital consistently higher than those born at home, but that almost all the strains were resistant to penicillin, and Roodyn (1954), in a survey from general practice, found that only 25 per cent of the strains tested were resistant. Other studies indicate that fewer penicillin-resistant staphylococci occur in phage Group II than in Groups I and III, which together include most of the hospital strains (Barber & Whitehead, 1949; Elwood, 1951; Williams et al., 1953; Rountree, 1953), although Parker & Lapage (1957) suggest that this may have been fortuitous.

It was, therefore, of some interest to see if there was any correlation between the unusually high incidence of both 'Type 71' and penicillin-resistant strains of Staph. aureus in this investigation (Table 7). Of 55 'Type 71' lesion strains, 44 (80%) were penicillin resistant. 'Other patterns' included 16 'weak 71' strains, 10 of which were resistant. Thus an inclusive total of 54 (76%) of these staphylococci were resistant to penicillin. In only two instances did staphylococci from the nose differ in sensitivity from those in the lesions. In both cases penicillin-sensitive Staph. aureus (type 55/71) was obtained from the lesions, and resistant strains (type 55) from the nose.

In the control groups, the proportions of Staph. aureus strains resistant to penicillin were much lower - 55 per cent in

series HN, 46 per cent in series SI, and only 12.5 per cent in series ON. In series SI, the single 'Type 71' strain, and 4 of the 9 'weak 71' strains were penicillin-sensitive, as were the four 'weak 71' staphylococci in series ON.

It seems, therefore, that a considerable proportion of 'Type 71' staphylococci are in fact resistant to penicillin, although fewer of the 'weak 71' strains are resistant.

(iv) Nasal carriage of staphylococci

Nose and throat swabs were taken from all impetigo cases, and from the 'normal' control groups. Since the throat swabs yielded relatively few staphylococci only those from the nasal swabs need be considered. Nasal carriage of Staph. aureus, whether occasional, intermittent or persistent, is common in healthy persons (Miles, Williams & Clayton-Cooper, 1944; Martin & Whitehead, 1949), the rate of carriage varying from about 85 per cent in infants, 57 per cent in children and 50 per cent in adults, to 65 per cent in hospital staff (Gould & McKillop, 1954). It is of considerable epidemiological importance in outbreaks of food-poisoning and other infective conditions (Wilson & Atkinson, 1945; Allison & Hobbs, 1947), and it has recently been implicated as the source of infection in some pyogenic skin diseases, including impetigo (Hobbs, Carruthers & Gough, 1947; Tulloch, 1954;

Table 8

Relation between duration of impetigo and incidence of Staphylococcus aureus in the nose.

Duration of impetigo	Nasal carriage of Staph. aureus of same type as in lesion		Total No. of cases
	No. of cases	% of cases	
Short (1-10 days)	22	38	58
Intermediate (11-20 days)	13	52	25
Long (21+ days)	11	64	17
TOTAL	46	46	100

Cruickshank, 1953).

Fifty-four of the 100 impetigo patients yielding staphylococci from lesions harboured Staph. aureus in the nose. In 8 of these 54 cases, the staphylococci cultivated from the nose were different in phage-pattern from those in the lesions. Thus based on single swabs only 46 per cent of the impetigo patients carried the same strains of Staph. aureus simultaneously in the nose and lesion. The rate of carriage of these strains in the nose increased progressively with the duration of infection (Table 8). When related to the extent of infection, the nasal carriage rates for the same 46 strains were: mild 41 per cent, moderate 52 per cent and severe 36 per cent. Considered separately, the incidence in 'Type 71' cases was similar, 28 (44%) of 63 patients carrying this strain in the nose. In general, the nasal carriage rate of staphylococci in the impetigo cases was similar to that of the 'normal' control groups - 46 per cent in series ON, and 58 per cent in series HN. There was no association with any particular age group. In six instances staphylococci were still present in the nose when the lesions were clinically resolving and bacteriologically negative, both during and after treatment.

The nasal carriage rate of Staph. aureus in the impetigo

Table 9

Distribution of Streptococcus pyogenes serotypes from impetigo patients related to the presence of Staphylococcus aureus and to the duration and severity of infection.

Streptococcus pyogenes					Staphylococcus aureus	Impetigo	
Serological type		present in					
Precipitin reaction	Agglutinin reaction	Lesion	Nose	Throat	Phage-pattern in lesions	Duration in days	Severity
1	1	+	+		-	5	++
** -	6	+			-	15	+++
28	28	+	+		-	7	++
F -	5/27/44	+			-	3	+
F -	3/13/B3264	+			-	4	+
F -	3/13/B3264	+			-	4	+
F -	3/13/B3264	+	+		52/52A/79*	2	+
-	8/25/10	+		+	29/52	17	++
1	1	+		+	71	13	++
-	8/IMP.19/10	+	+	+	71	28	++
-	5/11/27/44	+			71	14	++
-	4	+			71	4	+++
1	1	+	+	+	55	13	++
33	3/13/B3264	+			3C/55/71	28	+
28	28	+	+	+	3A/3B/3C/55/71*	14	++
F Not typable		+		+	79/3C/55/71*	21	+++
F Not typable		+			3C/55/71/53*	7	+
6	6	+	+		79/55/71/53*	7	+

F = family groups

* at 1000 x R.T.D.

** Brother had impetigo of 7 days duration - yielded pure Staph. aureus Type 71.

patients (46%) was, therefore, surprisingly low, especially as active, infective lesions were present on the face or neck in 89 per cent of these cases. Moreover, although 44 per cent of 'Type 71' staphylococci is a considerable frequency of nasal carriage for one 'type' of organism, nevertheless, if the nose were the source of infection in impetigo, a greater measure of carriage would have been expected.

(B) Haemolytic streptococci

Six of 18 strains of Str. pyogenes obtained from impetigo lesions were isolated in pure culture, and 12 in mixed culture with Staph. aureus. Of these 18 strains, 8 reacted in both precipitin and slide-agglutination tests, 8 only in the slide-agglutination test, and two were not typable. The type-distribution of these strains and their relation to the duration and severity of infection, as well as the presence of staphylococci, are summarised in Table 9. The penicillin sensitivity of 13 of the streptococci was determined, and all were sensitive.

In the precipitin test, which depends on the 'M' protein antigens, cross-reactions between types are uncommon, but many

strains fail to react. The slide-agglutination test is less precise than the precipitin test, because of the frequency of cross-reactions, and patterns of agglutination are usually obtained. Consequently, it is often only possible to assign strains to a group of types with 'T' antigens in common (Report, 1954). Thus four of the 16 typable strains were each agglutinated by sera for the types 3 and 13 and the provisional type B 3264; three of these strains were obtained from members of one family, in two cases in pure culture. In this family, infection of all the members occurred more or less simultaneously. Table 9 shows that staphylococci of 'Type 71' were not associated with Str. pyogenes of agglutination - pattern 3/13/B3264.

(C) Mixed infections with Staphylococcus aureus and Streptococcus pyogenes

Both Staph. aureus and Str. pyogenes were obtained in mixed culture from 12 patients. Although no clinical distinction was observed, 5 of the 6 'pure' streptococcal series were early cases of mild or moderate severity, with negative throat swabs. The remaining case was severe and of longer duration, with a history of sore throat; his brother, seen at the same time, yielded a

pure growth of 'Type 71' staphylococci.

In general, the 12 cases with mixed infections were of greater severity and of longer duration than those of the 'pure' streptococcal series. Str. pyogenes of the same type as those in the lesions was isolated from the nose and/or throat of eight of these cases, some of whom gave an antecedent history of sore throat. In several instances the streptococci were probably secondary invaders, as their incidence increased with the age of the lesions, but the numbers are too small to be significant. Also, some of the staphylococci shown in Table 9, lysed mainly by Group II phages at 1000 x R.T.D., may have been degraded 'Type 71' strains (Parker & Simmons, 1959). Conversely, in the mixed series, infection of the patient yielding Str. pyogenes of agglutination pattern 3/13/B3264 and Staph. aureus of phage-pattern 52/52A/79 was probably primarily streptococcal, since this was an early case and a member of the same family as that in the 'pure' streptococcal series.

(D) Virus studies

The possibility of a virus being associated with impetigo was mentioned by Campbell (1942) and Bigger & Hodgson (1943).

Before this investigation into impetigo started, the virus of Herpes simplex was isolated, unexpectedly, from the fluid obtained from unruptured vesicles in two typical cases of impetigo. Since this virus is widely distributed, its association with impetigo, either causal or as a secondary invader, seemed possible. Accordingly, it was decided to look for this virus in a small number of cases. Material, including vesicle fluid, lesion crusts, and saliva, from 10 cases was examined by inoculation onto the chorio-allantoic membrane of embryonated hens' eggs at the Virus Reference Laboratory, Colindale, and from a further 10 cases at the Department of Bacteriology, University of Leeds. In no instance was this or any other virus obtained.

It is concluded that the virus of Herpes simplex is not related to impetigo, and that its rôle in the two cases cited was coincidental.

5. Epidemiology

The present impetigo series, apart from 13 family groups, comprised sporadic cases from different districts and all social classes. Although sought, there was no evidence of association with streptococcal infections, in particular with tonsillitis or

scarlet fever, nor was there any outstanding seasonal or sex distribution. Neither did other staphylococcal or streptococcal infections develop in any patient with impetigo. Those affected were mostly children (Table 1) of whom 16 had previously suffered from impetigo. There was, however, no correlation between the age-groups of impetigo patients and the isolation of 'Type 71' staphylococci. In 13 instances spread of impetigo occurred within families, accounting for 29 patients. In 11 of these family groups, staphylococci gave rise to impetigo in various members of the house-hold over differing periods of time - from a few days to several weeks - suggesting that the immediate infectivity of the organisms concerned was probably low. 'Type 71' Staph. aureus was concerned alone in seven of these families, embracing 14 patients. In one family 'weak 71' strains were involved. Members of other three families yielded the following staphylococci: (1) 2 cases, 71 and untypable; (2) 3 cases, 55, 55/71 and 55/71; (3) 2 cases, 71 and 55/71.* Again, it is possible that some of these strains represented degraded 'Type 71' staphylococci. In yet another family, Staph. aureus of 'Type 71' was obtained from one member, and Str. pyogenes of agglutination-

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* at 1000 x R.T.D.

Table 10

Relation between duration of impetigo and phage-patterns of staphylococci isolated from lesions.

Staphylococci from impetigo lesions	Duration of Impetigo			No. of Cases
	Short (1-10 days)	Intermediate (11-20 days)	Long (21+ days)	
'Type 71'	40 (69%)	15 (60%)	8 (46%)	63
Other Group II patterns	11 (18%)	7 (28%)	8 (47%)	26
All other phage Groups	7 (12%)	3 (12%)	1 (5%)	11
TOTAL	58	25	17	100

type 6 from the other. The 'pure' streptococcal series included the only family in which all the members were affected at about the same time.

Undue bias in favour of 'Type 71' staphylococci in impetigo was reduced by considering each family group as one impetigo 'incident'. This showed that 55 of 88 impetigo 'incidents' (62%) yielded 'Type 71' organisms from lesions, compared with 63 per cent for all cases before adjustment. When related to the duration of infection, the incidence of 'Type 71' staphylococci in impetigo decreased, and conversely, the incidence of 'other patterns' in Group II increased with the age of the lesions (Table 10).

Detailed histories were taken from each patient in an attempt to correlate the mode of spread with known impetigo contact, or with other possible sources of infection within the vicinity. It was soon evident, however, that the majority of cases remembered contact, in one way or another, with various septic conditions, and their significance is therefore doubtful.

On the other hand, a definite history of previous impetigo contact, whether at home or at school, might prove of value if related to 'Type 71' staphylococci. Of 39 patients who were known to have been in contact with impetigo, 31 yielded 'Type 71'

and 5 'weak 71' staphylococci from lesions. Staphylococci of 'Type 71' were isolated from 32, and 'weak 71' from 12, of the 67 patients without such a history. Thus over 90 per cent of those cases with known impetigo contact yielded Staph. aureus of 'Type 71', compared with 65 per cent in those without definite contact - suggesting that the mode of spread is by the transfer of organisms from case to case.

6. Treatment

Many patients, when first seen, had tried a variety of local applications without effect, including 24 known to have used penicillin. After investigation each patient was instructed to apply ointment (aureomycin in 87 cases; neomycin in 32) to all lesions thrice daily after cleaning and decrusting, and to attend for review one week later. Eight extensive cases were treated in hospital.

The response to treatment was rapid, most patients stating that fresh lesions soon ceased to appear, and that healing had usually occurred within 3-5 days. In five cases there was little or no response to systemic aureomycin therapy, but rapid response to subsequent local aureomycin ointment. At review, all except

seven cases were clear, apart from residual erythema and scaling indicating the site of former lesions. Complications of impetigo, or of treatment, were not seen. No case, so far as known, recurred within a few months of treatment. Similar results with aureomycin and neomycin locally were reported by Solomons (1951) and Church (1954).

Impetigo in 1960

In an outbreak of infections, mostly of sore throat and impetigo, in a day-school for educationally sub-normal children, a wide and unexpected variety of haemolytic streptococci, differing either in group or in type, was isolated (Barrow, 1961). It was shown also that some children were carrying two or more different streptococcal strains simultaneously in the throat, thus making precise epidemiological assessment difficult. An account of this outbreak is given because, despite limited material, the clinical, bacteriological and epidemiological features of impetigo were different from those found in 1953-5, and because the results illustrate some of the difficulties which may be encountered in bacteriological studies of skin infections.

7. Material and Methods

A total of 364 throat swabs and 21 swabs or crusts from impetigo lesions was taken from the children and school staff during the course of the outbreak and submitted to the laboratory.

All swabs were plated on 7 per cent layered horse-blood agar medium, with and without the addition of 1 in 500,000 gentian violet. Plates were examined after aerobic incubation for 24 and 48 hours. From each positive swab, one colony showing characteristic β haemolysis was sub-cultured for purity and provisionally identified as Streptococcus pyogenes by sensitivity to bacitracin discs (Maxted, 1953). Each strain was sent to the Streptococcus Reference Laboratory, Colindale, for type-identification and confirmation of its group. Strains of Str. pyogenes were typed serologically by the slide-agglutination and precipitin methods, as described in Report (1954).

Strains of Staphylococcus aureus cultivated from impetigo lesions were phage-typed at the Public Health Laboratory, Leeds, as described by Anderson & Williams (1956). In addition, they were examined for (1) penicillin sensitivity, (2) egg-yolk opacity reaction of Gillespie & Alder (1952), (3) serum opacity reaction of Tomlinson & Parker (1956) and (4) ability to inhibit the growth

of corynebacteria on solid media, as described by Parker (1958).

8. The outbreak

The school consisted of 120 children, aged 5-16 years, and 14 members of the staff, who attended daily from various districts in or near Bradford. All the children were mentally retarded, and many also had physical defects. Most of them had a low standard of personal hygiene, usually reflecting their home background. The outbreak started in mid-September, 1960, shortly after the school term began, and within 3-4 weeks had affected most of the children and staff. Altogether, 119 of the children and staff were absent at various times and for different periods.

Throat infections. About 100 of the children or staff suffered from throat infections of varying degrees of severity. The onset was usually sudden, often occurring at school, with headache, malaise and pyrexia, followed by 'sore throat' and painful swelling of the cervical lymph nodes. Transient scarlatiniform rashes were frequent. In several children not complaining of sore throat, inspection revealed acute inflammation, and it is probable that everyone at the school was infected in this way at some stage. The tonsils, when present, were also affected. Recovery normally

ensued within 7-10 days, but was occasionally delayed by complications, such as otitis or laryngo-tracheo-bronchitis.

Impetigo. The lesions of impetigo occurred on the limbs, face or body of 41 children and one member of the staff during the course of the outbreak. In these cases, the initial symptoms and pyrexia were generally of short duration, and throat infections milder. Early lesions consisted of small, thin-walled vesicles. Thick crusts and inflamed margins rapidly developed. Removal of the crusts was difficult and painful, and left a bleeding, sodden base. Many of these lesions slowly enlarged, progressing into deep, indurated painful ulcers typical of ecthyma, and covered by dirty green crusts. They frequently gave rise to regional lymphadenitis, and when healed left faint marks which faded slowly.

Other infections. Other clinical manifestations of streptococcal infection occurring during the outbreak included cases of lymphangitis (6), cellulitis or erysipelas (3), and scarlet fever (2).

Control of outbreak. Advice was sought by the Headmistress because of apparent reinfection in some children. After preliminary confirmation of the nature of the outbreak, the extent of streptococcal involvement was roughly assessed from throat swabs taken from all the children and staff. A few swabs were also obtained from

some impetigo lesions. Closure of the school was avoided, mainly because of the difficulty of ensuring adequate treatment for elimination of streptococci at home. With the consent of the family doctors concerned, children harbouring streptococci in the throat were given treatment at school. A long-acting sulphonamide preparation was used, and Neomycin ointment was applied liberally to all skin lesions. The response to these measures was rapid. Treatment was continued at home by the family doctors in children too ill to attend school. Throat swabs were taken from absentees on their return to school.

Repeat throat swabs taken from all the children six days later showed a fall in the number of carriers, most of the treated children giving negative results. Fresh carriers were treated as described, after which no further cases of throat infection or of impetigo occurred. The half-term holiday of one week, due in a few days' time, allowed further time for the clearance of streptococcal carriers. Throat swabs taken again from the whole school immediately after the holiday revealed only a few carriers, who were kept at home until clear.

Table 11

Group distribution of haemolytic streptococci isolated from throat swabs.

Lancefield Group	No. of Cultures	No. of Patients
A	48	37
C	9	6
G	4	2
Not A, C or G	8	6
TOTAL	69	*

* Three of 48 children yielded streptococci of different groups on different occasions.

9. Bacteriological results

A few preliminary throat swabs yielded a heavy growth of haemolytic streptococci. Even though the school was not a closed community, it was thought that the outbreak was probably due to one, or possibly two particular types of Str. pyogenes. To confirm this view, five cultures were sent for type identification. The results, however, indicated that each culture was different in type. Because of this unexpected finding, most subsequent strains of streptococci isolated from the outbreak were grouped and typed.

Throat swabs. Altogether 77 strains of haemolytic streptococci were isolated from throat swabs from fifty-six of the children. In eight instances the strains were not grouped or typed. The remaining 69 cultures from forty-eight children were grouped serologically, and their distribution is shown in Table 11. Of these 69 cultures, 48 were strains of Str. pyogenes isolated from thirty-seven children.

The single colony subcultures of each of the 48 strains of Str. pyogenes were also typed, and the results are shown in Table 12 (facing page 51). Only twelve strains were fully identified by the presence of M antigens in the precipitin test. The re-

Table 12

Type distribution of strains of Group A streptococci isolated from throat swabs.

Serological Type	No. of Cultures	No. of Patients	Antigens Recognized
1	2	2	T
2	1	1	T
3	7	6	M(2), R(5), T(7)
4	13	10	T
6	2	1	M,T
11	4	3	M(1), T(4)
12	5	3	T
15	1	1	M
18	6	6	M(6), T(3)
19	1	1	T
22	1	1	T
28	1	1	T
4/28	1	1	R, T
1/B3264	1	1	T
8/IMP.19	1	1	T
Not typable	1	1	-
TOTAL	48	*	M(12),R(6), T(43)

* Three of 37 children yielded different types on different occasions.

mainder were less precisely identified by slide-agglutination, although most strains reacted with T antisera for single types only, and cross reactions were uncommon. The presence of at least 14 different types was found among the children, of which Type 4 was the commonest, followed by Types 3 and 18. Some strains represented single isolations from individual children, and their significance is therefore uncertain. It is unlikely that all the strains isolated were actually causing infection, though some additional evidence was obtained indicating the pathogenicity of Group C streptococci. During the course of the school outbreak, throat swabs from five children living in a local authority 'home' and suffering from throat infections, yielded Group C streptococci only. Subsequently it was learnt that one of these children attended the school under investigation, and that Group C streptococci had already been isolated from her.

Impetigo. Material comprising 21 lesion swabs or crusts, was obtained from fifteen children with impetigo. Some of this material was not entirely satisfactory, either because of treatment or because the crusts were not removed before swabs were taken. In 16 instances material from eleven patients yielded haemolytic streptococci or staphylococci, either alone or together in mixed culture. The distribution and type - identity of these

Table 13

Distribution and type identity of strains of streptococci and staphylococci isolated from the lesions and throat of impetigo cases.

Patient No.	Streptococci from lesions. Group	Type	Staph. aureus from lesions. Phage pattern	Streptococci in throat
1.	A	12	-	Strain died
2. (a)	A	5/12/27/44/28+	-	Type 28
(b)	A	28	-	
(c)	A	28+	-	
3.	A	28	3B/55+(S)	Not A, C, or G
4.	A	3	52/52A/79/80/7/54(R)	Type 3
5. (a)	A	28	52A/79(S)	Group C
(b)	C	-	52A/79(S)	Type 3
6. (a)	C	-	-	-
(b)	C	-	52/52A/79/80/7/54(R)	
7. (a)	C	-	52/52A/79/80/7/54(R)	Type 19
(b)	C	-	52/52A/79/80/7/54(R)	Group C
8.	C	-	-	-
9.	-	-	29/77/83+*(R)	Type 3
10.	-	-	52A+*(R)	-
11.	-	-	7/54/81(S)	-

* At 1000 x R.T.D. (S) = penicillin sensitive

(R) = penicillin resistant

(a), (b), (c) = Swabs taken at the same time from different lesions

organisms is given in Table 13. Altogether Str. pyogenes was cultivated from 7 lesions from five patients; only T antigens were detected in these strains. Six lesion swabs from four patients yielded streptococci of Group C, and 10 lesion swabs from eight patients yielded Staph. aureus. Neither streptococci nor staphylococci were obtained from the remainder.

On two occasions different strains of streptococci were cultivated from swabs, taken at the same time, from different lesions from each of two patients (Table 13). In one case, the streptococcal colony identified as 5/12/27/44/28+ was itself probably a mixture of two strains, 28 and 5/12/27/44. The strains of Staph. aureus isolated varied considerably in phage pattern, and were not related to 'Type 71' staphylococci previously found in sporadic impetigo, nor did they possess any of the other characteristics subsequently associated with 'Type 71' strains (Parker, 1958). All gave positive egg-yolk and negative serum opacity reactions, and none inhibited the growth of corynebacteria on solid media. For these reasons they were regarded as secondary invaders in streptococcal impetigo.

Carriage of different streptococci. In eight instances throat or lesion swabs, taken on two or more separate occasions, yielded strains of streptococci different either in group or in type.

Table 14

Identity of several single colonies of streptococci from individual throat swab cultures

Swab Culture No.	Type or Group identity of single colonies of Streptococci (a)	(b)	(c)	(d)	(e)	(f)	(g)	Previous Isolations from Throat
1	4	3	4	4	4	-	-	4
2	1	4	4	-	-	-	-	-
3	6	6	6	6	6	3	3	6
4	8/IMP. 19	8/IMP. 19	8/IMP. 19	4	1	1/B3264	-	-
5	12	12	12	12	12	4	4	12 Not A, C, or G
6	G	G	G	Not A,C, or G	Not A,C, or G	-	-	-
7	3	3	3	3	3	-	-	C
8	18	18	18	18	-	-	-	1
9	C	C	C	-	-	-	-	C
10	11	11	11	11	11	-	-	-
11	18	18	18	18	18	-	-	-

Because of this, and the wide variety of groups and types already identified in the outbreak, the possibility that some children were carrying two or more strains simultaneously in the throat was investigated. Throat swabs were streaked out on blood agar medium and several single colonies of haemolytic streptococci were picked at random from each positive culture. In this way 54 sub-cultures of haemolytic streptococci were obtained for grouping and typing from eleven children. The results showed that 6 of the 11 children were in fact harbouring at least two different strains in the throat when the swabs were taken (Table 14). In one instance, examination of six separate colonies from the primary culture revealed four different 'T' strains of Str. pyogenes: three colonies of agglutination - pattern 8/IMP.19, one colony of pattern 1/B3264, and one colony each of agglutination - Type 1 and Type 4. In the remaining cases, all the colonies derived from individual swabs were identical, although two more children yielded streptococci different from those previously isolated. If several colonies from the impetigo cultures had been examined in this way, it is probable that the simultaneous presence of different streptococcal strains would also have been found.

Part II: Microbial antagonism by Impetigo staphylococci

10. Introduction

It has long been known that some strains of staphylococci are capable of inhibiting the growth of other organisms, particularly corynebacteria, by the production of diffusible 'antibiotic' substances. The earlier literature has been well reviewed by Florey, Chain, Heatley, Jennings, Sanders, Abraham & Florey (1949). Such strains were not thought to be associated with any particular kind of infection, until Parker, Tomlinson & Williams (1955) demonstrated that most 'Type 71' staphylococci isolated mainly from impetigo lesions, and very few others, were able to inhibit the growth of Corynebacterium diphtheriae on solid media, with the formation of sharply defined zones of inhibition. A number of other strains of staphylococci with inhibitory properties formed wider zones of inhibition with 'hazy' edges. The few strains, other than 'Type 71', forming sharply defined zones were found to have other '71-like' characters, and were also associated with

superficial vesicular infections of the skin, including impetigo (Parker, 1958). In subsequent work, the nature of this inhibitory activity was studied by Parker & Simmons (1959), although they were greatly hampered by failure to obtain active bacteria-free preparations. Since 'Type 71' organisms are so restricted in their invasive power, the possible rôle of inhibitory activity in natural infection and its importance in the epidemiology of impetigo was considered, and the nature of the antibiotic further investigated.

The work described in Part II therefore concerns observations made during 1958-61 on the nature, properties and significance of the inhibitory agent produced by 'Type 71' staphylococci.

11. Material and Methods

Organisms

Staphylococcus aureus. A collection of 100 strains of coagulase-positive staphylococci, isolated from routine material submitted to the laboratory during 1959-60, was phage-typed at the Public Health Laboratory, Leeds. They were derived from infections such as boils, abscesses, and various skin lesions, as well as

from ear, nose and throat swabs, and were representative of all the phage groups. In addition, two strains of Staph. aureus antagonistic to corynebacteria were obtained from the National Collection of Type Cultures (NCTC): No. 6507 - strain 'Inhibitor' isolated from an empyema, and deposited by A. Fleming in 1943; and No. 8004 - strain E755, used by Gardner (1949).

Each strain was examined for (1) sensitivity to penicillin by means of Evans Sentest discs containing 2.5 i.u. of penicillin (2) ability to inhibit the growth of corynebacteria on solid media (3) production of opacity in horse-serum agar medium, as described by Parker (1958), and (4) egg-yolk opacity reaction, as described by Alder, Gillespie and Herdan (1953) with Oxoid egg-yolk broth. They were also examined for inhibitory activity against each other, and against other organisms. They were kept at room temperature in the dark on Lemco nutrient agar slopes in bijoux bottles, and subcultured infrequently.

Coagulase - negative staphylococci. Twenty-four strains of coagulase - negative staphylococci were isolated from routine bacteriological specimens and from normal skin. One strain, Staphylococcus saprophyticus (NCTC No. 7291), originally cultivated from healthy skin, was also used. Each strain was examined

for inhibitory activity against, and susceptibility to other organisms.

Corynebacteria. A suitable 'indicator' strain, highly susceptible to inhibition by 'Type 71' staphylococci, was selected from a collection of corynebacteria obtained from routine specimens. This strain, an unnamed diphtheroid, referred to as 'Bradford' (BFD), was isolated from an ear swab, and was a short, broad rod forming flat, whitish, opaque colonies with lobate edges and a dull granular surface. It fermented glucose and sucrose only, and showed some enhancement of growth around the zone of inhibition. It was thus similar in almost all respects to the original 'Bradford' strain sent to, and used by Parker & Simmons (1959). Later, a subculture of their nitrate-negative, avirulent strain of Corynebacterium diphtheriae mitis (No. 51 in the collection of the Public Health Laboratory, Manchester) was also obtained, and used in parallel with the 'Bradford' diphtheroid. This 'Manchester' organism (MC) showed typical mitis morphology and cultural characters. Both 'indicator' organisms were subcultured weekly on blood-agar medium and kept on the bench. Stock cultures were also kept at 4°C.

Haemolytic streptococci. Twenty representative strains of streptococci, including different sero-types of Group A and

members of other Lancefield groups, originally isolated from the school outbreak described in Part I, were used. In addition 18 'T' strains, and 20 'M' strains of Str. pyogenes, each representing most of Griffith's types, as well as 12 different strains from impetigo lesions, were obtained in the lyophilized state from the Streptococcus Reference Laboratory, Colindale. They were maintained by frequent subculture on blood-agar medium, and were examined for inhibition of, and susceptibility to the action of staphylococci.

Other organisms. These were used in various inhibition tests, and comprised miscellaneous strains of pneumococci, viridans streptococci, various enterobacteria, and aerobic and anaerobic spore-bearing organisms isolated in the laboratory. One strain of Corynebacterium acnes (NCTC No. 737) was also used.

Media

All media, except for specified references and commercial products, were prepared as described by Cruickshank (1960). Incubation was aerobic at 37°C overnight, unless otherwise stated.

With solid media, satisfactory antibiotic activity occurred on the blood-agar medium in routine laboratory use, and it was

therefore generally employed. The nutrient base was Lab Lemco infusion broth at pH 7.5, with the addition of 0.002 per cent cystine, and 1.6 per cent New Zealand agar. After autoclaving, 7 per cent Evans defibrinated horse-blood was added, and the medium poured into Petri dishes. When required, other additions were made as well as, or instead of blood.

With liquid media, preliminary work indicated the importance of suitable broth for antibiotic production. Horse flesh trypsin-digest broth was usually found satisfactory for this purpose. As considerable batch to batch variation occurred, a large quantity was prepared, tested and kept for subsequent use.

The effect of variations, such as composition of media, temperature and period of incubation, inoculum size, metabolism and other factors is considered later.

Methods used in the study of Inhibition on solid media

(A) 'Direct' antagonism

In these methods, the active or test organism is placed on a solid medium shortly after seeding the surface evenly with a sensitive 'indicator' organism. The term 'direct' is used instead of 'simultaneous' antagonism (Gratia, 1946) because it

avoids the suggestion that each organism is active against the other.

(i) 'Spot' test. The technique described by Parker (1958) was used at first. Blood-agar medium was sown with the BFD or MC organism, either by spreading with a loop as evenly as possible over the surface, or by flooding the plate with a broth suspension, faintly turbid to the naked eye, and drying. Small loopfuls of overnight broth cultures of staphylococci were then deposited on the surface of the medium. After incubation, the corynebacteria had grown as confluent lawns, with zones of inhibition round some of the staphylococcal 'spots'.

(ii) Stab-inoculation method. Instead of placing loopfuls on the surface, staphylococci were later stab-inoculated with a straight wire throughout the depth of the medium. This technique was simple, rapid, and gave consistent results with wider inhibition zones. It was also eminently suitable for examining single colonies.

In accordance with Parker & Simmons (1959), inhibition of corynebacteria was considered positive (DI+) when the zones were 1.0 mm. or more in width; zones with sharply defined edges were designated DI+S and those with 'hazy' edges DI+H.

(iii) Overlapping drop technique. This method was based on the

cross-titration procedure of Rosebury, Gale & Taylor (1954), in which pairs of overlapping drops of dilutions of two different broth cultures were placed on solid media. It was used to demonstrate direct antagonism and competitive inhibition among staphylococci. Drops were deposited with a standard loop; the second drop was added as soon as the first had dried, and overlapped the first by approximately one-third of its area. After incubation, plates were examined for inhibition within the area of overlap.

(iv) Cellophan method. After seeding blood-agar media with indicator organisms, autoclaved sheets of commercial cellophan were placed on the surface, allowed to dry, and then inoculated with spots or streaks of staphylococci. After incubation, the cellophan was removed, and any inhibition recorded.

(B) 'Deferred' antagonism.

In these methods, active or test organisms were grown on solid media for 1-3 days before the passive 'indicator' strains were inoculated (Gratia, 1946). They allowed growth of both test and indicator organisms under different conditions, as well as detection of inhibitory substances sometimes not revealed by 'direct' antagonism.

(i) Slide inocula. Test strains were grown as central spots or

as streaks across plates. After incubation, passive organisms were inoculated at right angles to the primary growth, using the edges of sterile glass microscope slides seeded from culture-soaked filter papers in Petri dishes. This method allowed inoculation close to the primary culture without touching it.

(ii) 'Colicine' method. At first the method described by Abbott and Shannon (1958) for the colicine typing of Shigella sonnei was applied to staphylococci. Thick primary streaks were grown across blood-agar media for 1-3 days. The resulting growth was scraped off with the short edges of clean glass slides, after which the remaining organisms were killed by exposure for 30 minutes to chloroform (1-2 ml.) placed in the lids of the inverted plates. The medium was then exposed, face downwards, to the air for not less than two hours; otherwise growth of the Manchester organism was impaired. Loopfuls of broth cultures of passive strains were then stroked across. After incubation, any inhibition was recorded. Normally one primary culture and eight or nine cross-streaks could be placed conveniently on one plate.

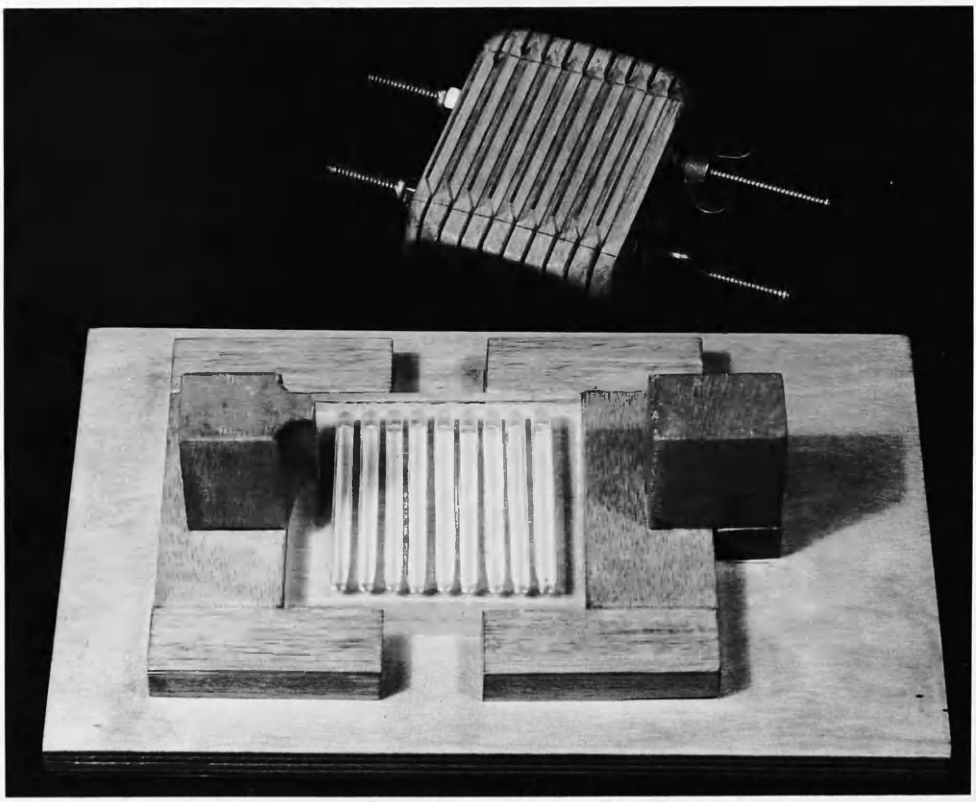
For large numbers of organisms this method was tedious and required much time and media. The following procedure, using slide inocula, was therefore devised. A supply of sterile glass microscope slides (some shortened by 1-2 cm.), circular filter

papers (Whatman No. 1 were suitable), and Petri dishes with flat bases was prepared. Two different primary streaks were grown across one blood-agar plate, and each culture scraped off as already described. Cross-inocula were made thus: a sterile filter paper, placed in the base of a sterile Petri dish, was flooded with 2-3 ml. of broth culture by means of a sterile Pasteur pipette. The long edge of a sterile glass slide, held horizontally between thumb and fingers, was pressed firmly on the soaked filter paper, and then placed across the medium using gentle pressure. Other plates were similarly quickly inoculated with the same slide and culture. The lid of the same Petri dish, with a fresh filter paper and slide, was used for the next culture, and so on. With large numbers of organisms, this technique saved much time, media and bench space. It gave fairly standard, even inocula across the primary growth without 'trailing', and also allowed upto 20 passive, as well as two primary strains, to be placed on one plate. A simple multiple slide device (Plate 3) for the simultaneous and replicate inoculation of several different cross-streaks has since been developed for this and other purposes (Barrow & Ellis, 1962).

(iii) Cellophan methods. Autoclaved sheets of commercial cellophan were placed on the surface of solid media and allowed

Plate 3

The multiple-slide inoculation apparatus



The apparatus is assembled ready for use. Sterile glass microscope slides are gripped between the wooden sections of the slide holder. A Perspex trough unit, with absorbent paper strips placed in the troughs, is in position in the frame on the base of the apparatus. The troughs are partially filled with different broth cultures. The slides are charged from the troughs by lowering the rods of the holder between the two wooden guide blocks. The face of the slide holder bears against the front guide block to give precise location of the slides in the troughs. The construction and use of this apparatus is fully described by Barrow & Ellis (1962).

to dry. Spots or streaks of staphylococci were placed on the cellophan, and the plates incubated. The cellophan was then removed, the surface of the medium inoculated with an indicator organism, and the plates re-incubated.

Alternatively, cellophan (Visking) tubing was cut into sections, autoclaved, and the dry, sterile 'cylinders' placed in solid media before setting. Active organisms were inoculated inside the tubing, and passive organisms outside - either at the same time as, or after incubation of, the active strains. Several organisms could be examined for diffusible inhibitory agents in this way on one plate.

Methods used in the study of Inhibition in liquid media

In principle, 'Type 71' broth cultures were assayed for inhibitory activity against corynebacteria or staphylococci. The methods used to obtain bacteria-free preparations are described later. In general, organisms were killed by heat or acid treatment and then separated by centrifugation. Alternatively cultures were grown inside cellophan (Visking) tubing immersed in broth. Two assay procedures were used.

(i) Agar-cup method. Blood-agar media of standard depth and

even surface were seeded with broth suspensions of indicator corynebacteria, as in the 'spot' test. When dry, cups 8.0 mm. in diameter were punched out with a sterile cork-borer. It was found unnecessary to seal the floor of the cups. Two-fold dilutions of the assay material were made in quarter-strength Ringer's solution by means of a 0.5 ml. automatic syringe. Each cup was filled appropriately by a Pasteur pipette. The plates were left undisturbed on the bench for about 30 minutes to allow some diffusion to occur, and so prevent overspill on moving. After incubation, the width of any inhibition zones (i.e. the distance from the edge of cup to the edge of growth) was measured and recorded. This method was sufficiently accurate for comparative purposes.

(ii) Dilution method. Dilutions, ranging from 10^{-1} to 10^{-6}, were made from a broth culture of a staphylococcal strain known to be inhibited on solid media. Equal quantities (approximately 1.0 ml.) of normal broth and of the preparation to be assayed were distributed in two sets of sterile tubes. Standard volumes (0.02 ml.) of each culture dilution were then placed in one tube of each set. Readings were made after incubation for 24 and 48 hours. The end-point was taken as the largest inoculum showing no visible growth in the assay material, but visible growth in the

Table 15

Phage groups and some properties of 100 strains of Staphylococcus aureus isolated from routine specimens

Phage Group	Number of strains				Total No. of Strains
	Penicillin resistant	Egg-yolk positive	Serum opacity positive	DI+	
I	27	38	1	0	41
'Type 71'	6	0	6	6	7
II Other Patterns	4	7	1*	1*	8
III	12	15	3	0	19
Not typable	11	10	2	0	13
Not classifiable	8	12	0	0	12
TOTAL	68	82	13	7	100

*This strain yielded inhibitory colonies of 'Type 71' and non-inhibitory colonies of phage-pattern 55/71.

corresponding control tube.

EXPERIMENTAL

12. Antibiotic activity on solid media.

Inhibition occurred on different media, but blood-agar was generally used because of better growth of the Manchester organism, and because the zones of inhibition were more easily seen. The blood also provided a source of catalase, thus excluding the possibility of inhibition due to peroxide formation.

(A) Inhibition of corynebacteria

(i) Distribution of inhibitory activity among Staphylococci.

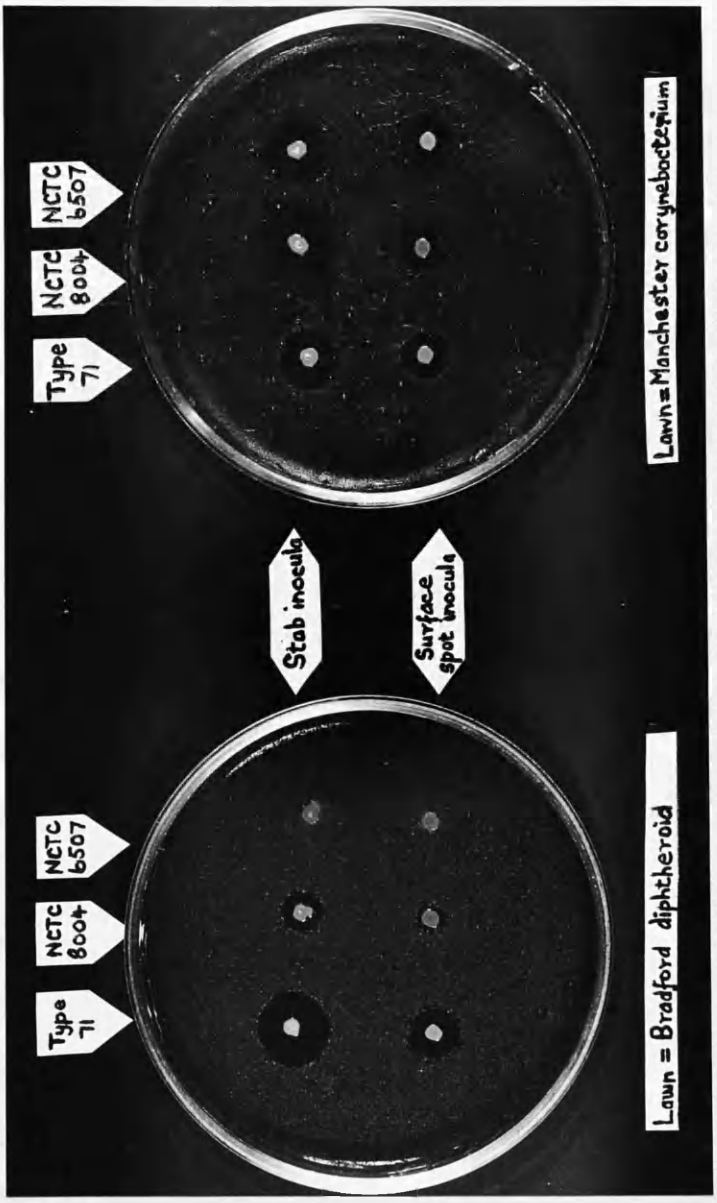
The inhibitory activity of 100 strains of Staph. aureus obtained from routine bacteriological specimens was first ascertained. Each strain was tested against both BFD and MC indicator corynebacteria, using the direct antagonism 'spot' test. The results, together with the phage groups and some properties of these staphylococci are shown in Table 15. Seven strains were active (DI+), giving sharply defined zones of inhibition (DI+S)

with both corynebacteria (Plate 4). They represented six 'Type 71' strains derived from two cases of impetigo, one case of conjunctivitis, and from superficial skin infections in three infants, and one strain of phage pattern 55/71, isolated from a patient with conjunctivitis. These inhibitory strains were all penicillin - resistant, produced opacity in horse-serum agar medium, and gave negative egg-yolk opacity reactions. Of the remaining 93 staphylococcal strains, only one susceptible to phage 71 was identified. This strain, isolated from a urethral discharge, was atypical. It gave additional minor reactions with other phages, did not inhibit corynebacteria, was penicillin-sensitive, and gave negative egg-yolk and serum opacity reactions.

As no inhibitory strains other than 'Type 71' were found, two NCTC strains of Staph. aureus active against corynebacteria were obtained and used for comparison in some later experiments. Strain No. 8004 (E755), not susceptible to any of the typing phages in routine use, gave narrow, poorly defined zones of inhibition with the BFD diphtheroid, and wide zones with hazy, ill-defined margins (DI+H) with the MC diphtheria organism (Plate 4). Strain No. 6507 ('Inhibitor'), weakly susceptible to phage 29, also gave wide, hazy zones with MC, but had little or no inhibitory effect on the BFD organism.

Plate 4

Direct inhibition of corynebacteria by staphylococci



Results given by three active strains of Staphylococcus aureus tested for direct antagonism against the Bradford and Manchester indicator organisms. With the Manchester organism, 'Type 71' strains gave sharply defined inhibition zones (DI+S), whereas the two NCTC strains gave zones with hazy, poorly defined margins (DI+H). Stab inocula produced wider inhibition zones than surface spot inocula. x 2/3. Reflected light.

(ii) Loss of inhibitory activity.

Parker & Simmons (1959) found that DI+S 'Type 71' and '71 - like' strains regularly lost their inhibitory activity after storage in the laboratory for 3-12 months. When tested at intervals, the zones of inhibition became progressively smaller, although no change was observed in other cultural characters.

Accordingly, the seven inhibitory strains of Staph. aureus were retested repeatedly for activity against both BFD and MC organisms, and these findings were confirmed. With the 'spot' test, 4 of the 7 strains had lost their activity after six months, and the others gave much narrower inhibition zones. Three of the four strains showing loss of inhibitory power in the 'spot' test were, however, still active by the 'colicine' method. When dilutions of these cultures were seeded on plates, together with the corynebacteria, so as to produce separate staphylococcal colonies on the indicator lawns, a number of inhibitory colonies was observed among the many inactive colonies. On subculture these active colonies were indistinguishable from freshly isolated cultures, and behaved in exactly the same way on storage, showing gradual loss of inhibitory ability. Active variants were never obtained from non-inhibitory colonies. Neither of the NCTC strains showed loss of activity during this time.

Although loss of inhibitory power was not associated with any apparent alteration in cultural properties, Parker & Simmons found that it was accompanied by increased susceptibility to other Group II phages. This finding was again confirmed with the present strains. Those lysed at first only by phage 71 at R.T.D. became susceptible also to phage 55, and sometimes to phages 3C and 3B as well. It was shown that the active culture originally of phage pattern 55/71 yielded on dilution a mixture of inhibitory colonies of 'Type 71' and non-inhibitory colonies of 55/71. Thus all seven DI+S staphylococci identified in this study were in fact 'Type 71' strains.

It was observed that loss of activity by direct antagonism always preceded loss by deferred antagonism techniques. Similarly, ability to inhibit growth of the MC organism was always lost before ability to inhibit the BFD organism.

(B) Inhibition of staphylococci

(i) Staphylococcus aureus.

(a) Deferred antagonism. At first, active (DI+) 'Type 71' strains were tested for inhibitory power against a few non-inhibitory (DI-) staphylococci by the colicine method. It was

Table 36

Inhibitory activity between representative strains of *Staphylococcus aureus* in deferred antagonism tests

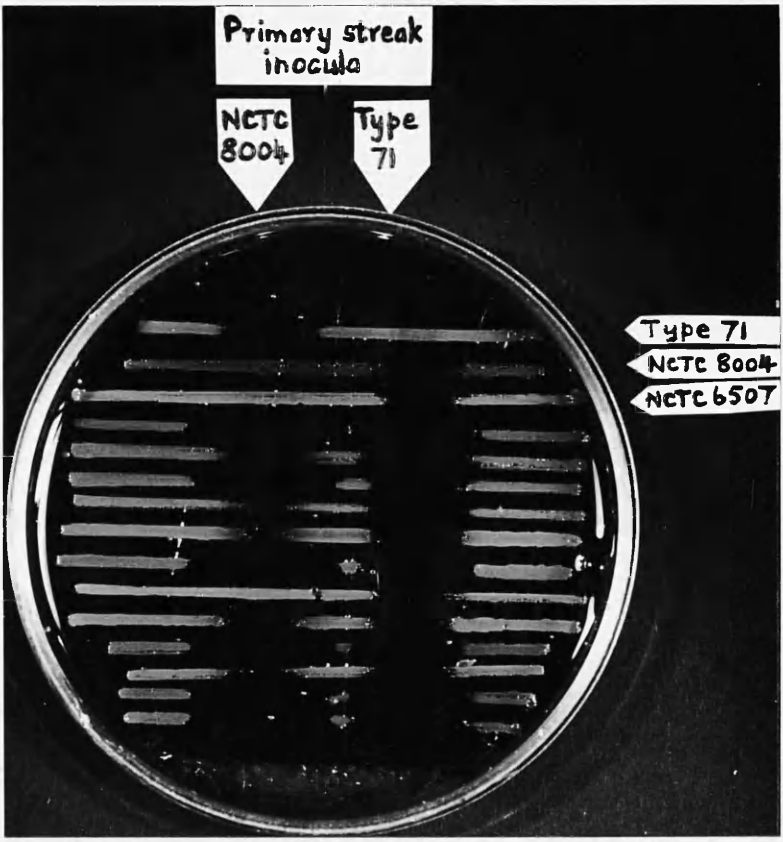
Phage Group	Primary Streak Strain No.	Passive cross-streaks Strain No. 1	2	3	4	5	6	7	8	9	10	11	12
I	1	-	-	-	-	-	-	-	-	-	-	-	-
	2 (NCTC 6507)	++	-	+	++	++	+++	+	-	+	-	++	-
'Type 71'	3	+++	+++	-	-	+++	+++	+++	+++	+++	+++	+++	+++
II	4	+++	++	-	-	+++	+++	+++	+++	+++	++	+++	+++
Other	5	-	-	-	-	-	-	-	-	-	-	-	-
Patterns	6	-	-	-	-	-	-	-	-	-	-	-	-
III	7	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
Not typable	9	-	-	-	-	-	-	-	-	-	-	-	-
	10 (NCTC 8004)	++	-	±	+	++	++	++	+	-	-	++	+
Not classifiable	11	-	-	-	-	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-	-	-	-	-

+++ = Complete inhibition extending beyond area of overlap
++ = Complete inhibition over area of overlap
+ = Complete inhibition within area of overlap
± = Partial inhibition
- = No inhibition

found that the DI+S 'Type 71' strains were also active against the DI- staphylococci. Next, these DI+S strains, and the two DI+H NCTC strains, were tested similarly against all the DI- staphylococci isolated from routine specimens, and then vice versa. In addition, 50 of the DI- strains were subsequently tested against each other. The combined results gave a chess board indicating mutual inhibitory activity. This showed that all DI+S 'Type 71' strains inhibited the growth of every DI- strain examined, and conversely that no DI- strain had any inhibitory activity against either DI+S or DI+H staphylococci. Each of the two DI+H NCTC strains inhibited most, but not all DI- staphylococci; resistance was not, however, associated with any particular phage pattern. The inhibition zones produced by DI+ staphylococci were distinct (Plate 5), although resistant colonies sometimes occurred within them. Among the DI- staphylococci only weak, doubtful activity against occasional strains was observed. The results of a typical experiment with representative DI+ and DI- staphylococci are given in Table 16. When used as test organisms, active 'Type 71' strains did not inhibit other DI+S strains, nor did the DI+H NCTC strains inhibit each other. Both DI+H strains, however, inhibited all the DI+S 'Type 71' strains, and similarly every 'Type 71' strain inhibited both DI+H NCTC strains.

Plate 5

Deferred 'colicine' inhibition by staphylococci



Inhibition of different strains of Staphylococcus aureus by two active strains, using the deferred antagonism colicine method. x 4/5. Reflected light.

Loss of inhibitory power of 'Type 71' strains against corynebacteria was found to coincide with loss of activity against staphylococci. Indeed DI- variants derived directly from DI+S 'Type 71' strains were completely inhibited by the active parent cultures.

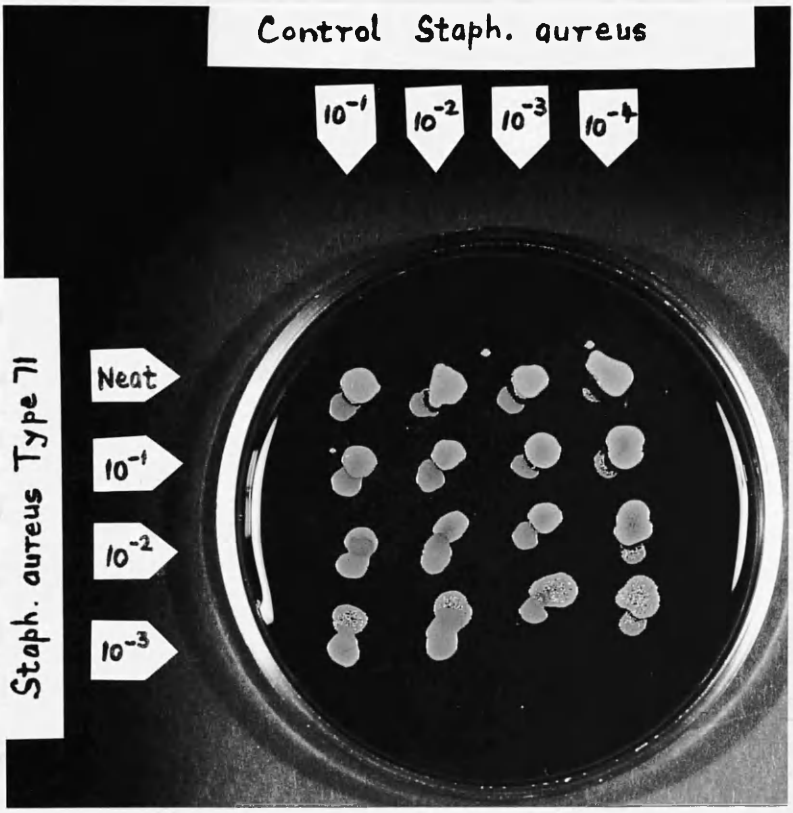
(b) Direct antagonism.

The finding that DI+S 'Type 71' strains could inhibit the growth of all other staphylococci in deferred antagonism tests suggested that this ability might be of some advantage to them under natural conditions. It was therefore considered important to determine whether 'Type 71' strains could suppress the growth of, or at least compete with, other staphylococci in direct antagonism experiments.

With the 'spot' test, inhibition was not generally observed when loopfuls of active 'Type 71' broth cultures were deposited on lawns of DI- staphylococci. The effect of varying the ratio of organisms in the spot inocula and in the lawns was therefore investigated by means of the overlapping drop procedure of Rosebury, Gale & Taylor (1954). It was found that undiluted DI+ broth cultures readily inhibited diluted broth cultures of DI- strains, but not vice versa. The results of a typical experiment with an active 'Type 71' strain are shown in Plate 6. When

Plate 6

Cross titration of staphylococci



Direct inhibition of a control (DI-) strain of Staphylococcus aureus by stronger concentrations of a 'Type 71' strain. Loopfuls of each dilution of the control strain were first deposited in rows on the surface of the medium, and allowed to dry. They were then partly overlapped by loopfuls of 'Type 71' dilutions, as indicated, forming a complete chessboard. x 4/5. Reflected light.

examined in this way, a few representative staphylococci, including the two NCTC strains, gave results corresponding entirely with those obtained in deferred antagonism tests.

Although direct antagonism between staphylococci was thus demonstrated, this method was not satisfactory for examining numerous strains, and a more suitable technique was therefore sought.

(c) Stab - inoculation method.

It was observed that active 'Type 71' strains stab - inoculated with a straight wire throughout the depth of solid media inhibited the corynebacteria to a greater extent than larger surface 'spot' inocula (Plate 4). When tried with lawns of staphylococci prepared from broth cultures, good inhibition of DI- by DI+ strains was also observed, particularly when the straight wire was heavily charged with growth from solid media. This technique was therefore investigated, and was found to provide a simple, rapid method by which larger inocula of one organism could easily be tested for 'direct' antagonism against lower concentrations of other organisms.

It was thought that the smaller zones of inhibition given by surface inocula of active staphylococci were probably due to lowering of the local antibiotic concentration by downward

diffusion, and that antibiotic production throughout stab inocula largely obviated this loss by giving a higher surface gradient. This idea was supported by experiments in which antibiotic discs were placed on the surface, in the middle, and at the bottom of solid media, the surface of which had been seeded with a sensitive organism. After incubation it was observed that the surface discs gave narrower inhibition zones than the other discs. The importance of such loss of antibiotic by diffusion was also emphasized by deferred antagonism experiments with media containing different concentrations of agar. It was observed that inhibition was weaker and zones smaller in media containing one per cent or less agar compared with media containing higher concentrations of agar. Further improvement in the stab - inoculation method was achieved by limiting diffusion of the inhibitory agents in media: the available water content was reduced by increasing the agar concentration to two per cent, the customary saline-agar bases were omitted, and the depth of media was made slightly less than normal.

With this technique all the strains of Staph. aureus were re-examined for direct antagonism against each other. The results were almost entirely the same as in the deferred antagonism tests. All DI+ staphylococci, including the NCTC cultures, inhibited most of the DI- strains, including non-inhibitory variants derived from

active 'Type 71' cultures. Conversely no DI- strain showed any inhibitory activity whatever against any other staphylococci. Again, both DI+H NCTC strains were susceptible to the action of DI+S 'Type 71' staphylococci, and these in turn were inhibited though to a lesser extent, by the NCTC strains (Plate 7). Neither NCTC organism inhibited the other, nor were any of the DI+S 'Type 71' strains active against each other. In general the DI+S 'Type 71' strains produced wider zones of inhibition of staphylococci than the DI+H strains.

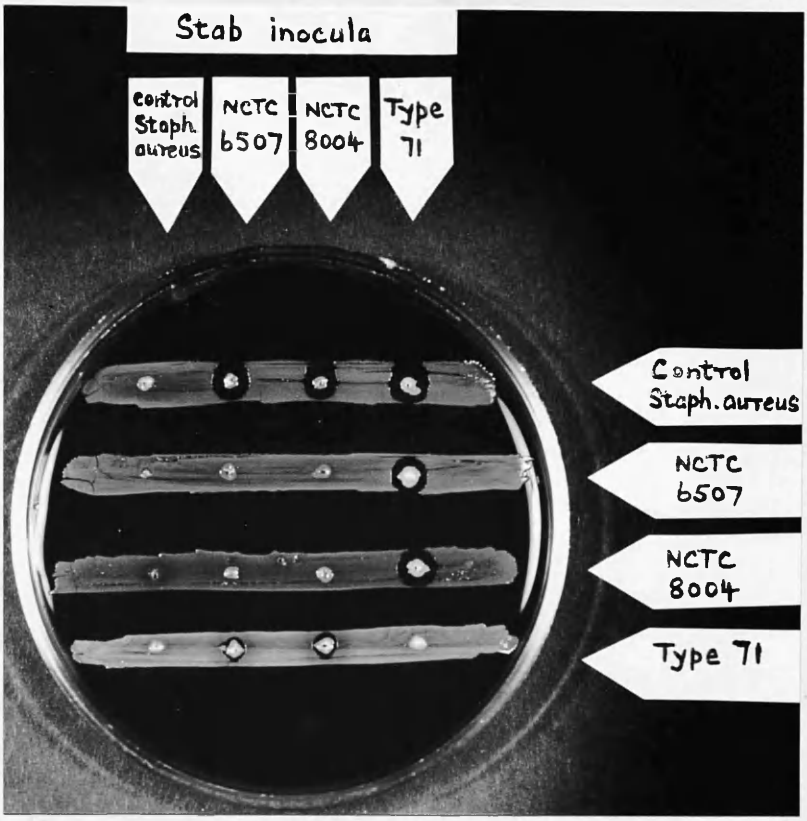
By this method, 'Type 71' strains were thus shown even more clearly to be strongly and directly antagonistic to other strains of Staph. aureus.

(ii) Staphylococcus albus.

The remarkable inhibitory powers exhibited by 'Type 71' staphylococci against other strains of Staph. aureus suggested that similar activity against other organisms might be one of the factors concerned in the initiation of skin infections. Interaction between DI+ 'Type 71' strains and 25 coagulase - negative staphylococci was therefore studied by the stab - inoculation technique. Sixteen of the Staph. albus strains were isolated from urines (6), urethral or vaginal discharges (4), and from boils or other septic skin lesions (6). The remaining nine

Plate 7

Inhibition by staphylococcal stab inocula



Inhibition results given by one DI- control and three active strains of Staphylococcus aureus tested against each other by the direct antagonism stab-inoculation method. The parallel streaks represent growth from single loopfuls of overnight broth cultures. The stab inocula were from growth on solid media. x 4/5. Reflected light.

strains were derived from normal 'healthy' skin, one of which was Staphylococcus saprophyticus NCTC No. 7291.

It was found that 14 of the Staph. albus strains, including four from normal skin, were inhibited by DI+ 'Type 71' stab inocula. On the other hand, only one of ten random DI- Staph. aureus strains was active, inhibiting three of the Staph. albus strains. Conversely, two coagulase - negative strains, both isolated from normal skin, were active against all the DI+ 'Type 71' strains, as well as against several DI- staphylococci. A few DI- Staph. aureus strains were also weakly susceptible to some, though not necessarily to the same, Staph. albus strains.

When tested against the indicator corynebacteria, ten of the Staph. albus strains inhibited the BFD diphtheroid, two giving sharply defined zones and eight hazy zones. Only three Staph. albus strains inhibited the MC organism, all producing sharply defined zones; they included the two Staph. albus strains active against 'Type 71' staphylococci. There was thus no close association, compared with Staph. aureus, between ability to inhibit corynebacteria and ability to inhibit other strains of Staph. aureus.

Loss of power to inhibit corynebacteria in 'Type 71' strains again coincided with loss of activity against coagulase-negative

staphylococci, and in one instance at least, resulted in susceptibility to other Staph. albus strains.

(C) Inhibition of streptococci

The strains of streptococci and staphylococci isolated from the school outbreak of infection described in Part I were first tested against each other for mutual inhibitory activity by both direct and deferred antagonism techniques, but none was found. Next, interaction between 'Type 71' staphylococci and different types of haemolytic streptococci was studied to try to assess their relative importance in impetigo. Twenty representative streptococci, including strains other than Group A, kept from the school outbreak, were examined for inhibition by 'Type 71' stab inocula. Streptococci of Group G were susceptible, but only doubtful activity was observed against occasional strains of Str. pyogenes. As M antigens, necessary for complete type-identification of Str. pyogenes, were not found in most of these strains, other cultures with known M and/or T antigens, obtained from the Streptococcus Reference Laboratory, were investigated. These included 20 stock 'M' strains and 18 'T' strains of Str. pyogenes, together representing 26 different serotypes. In addition 12

Table 17

Inhibition of serotypes of *Streptococcus pyogenes* on blood agar medium by stab inocula of 'Type 71' staphylococci.

Streptococcus pyogenes				
	'T' strains		'M' strains	
Sero-type	Antigens present	Direct inhibition	Antigens present	Direct inhibition
1	T	-	M + T	+
2	T	-	M + T	±
3		Not tested	M + T	+
4	T	-		Not tested
5	T	-	M + T	±
6	T	-	M	±
9	T	-	M + T	+
11	T	-	M + T	-
12	T	-	M + T	+
13	T	-		Not tested
14		Not tested	M + T	+
15	T	-	M	±
17		Not tested	M + T	±
18		Not tested	M + T	±
19	T	-	M	±
22	T	-	M + T	±
23	T	+++	M + T	+++
24		Not tested	M	±
25	T	-		Not tested
26		Not tested	M	+
27	T	-		Not tested
28	T	-	T + R	-
29		Not tested	M	+
30		Not tested	M	±
44	T	++		Not tested
B3264	T	-		Not tested

+++, ++, +, ± = Degrees of inhibition
- = No inhibition

different strains originally isolated from impetigo lesions and giving characteristic agglutination patterns, such as 3/13/B3264, 8/IMP.19 and 5/11/27/44, were obtained.

None of the 12 impetigo strains was inhibited by stab inocula of 'Type 71' staphylococci on blood agar medium. The results obtained with the type strains of Str. pyogenes are given in Table 17. Only the M and T cultures of Type 23 were clearly and consistently inhibited by 'Type 71' stab inocula. Most 'T' strains were unaffected, but weak activity, giving rise to hazy zones of minute colonies, was observed against several 'M' strains. Examination of selected single colonies from streptococcal cultures revealed variation in susceptibility to 'Type 71' staphylococci, but inhibition could not be related to colonial morphology or to the presence of particular antigens. Many of the results shown in Table 17 were obtained in this way. DI- staphylococci used as controls had little or no effect on any of the streptococci. Blood-agar media at different pH values, incubated under different conditions, failed to give more definite results. Inhibition of every streptococcal strain, however, was produced on nutrient agar medium without blood by many staphylococci, including DI- cultures, and was therefore not comparable with 'Type 71' activity.

Loss of power by 'Type 71' strains to inhibit corynebacteria

resulted in loss of activity against Str. pyogenes Type 23, but weak activity against other strains was not affected. None of the streptococcal strains was active in deferred antagonism tests against 'Type 71' staphylococci.

(D) Inhibition of other organisms

(i) Gram-negative species. Active staphylococci were tested by the stab inoculation method against several different strains of Salmonella, Shigella, Proteus, Pseudomonas, Haemophilus, Neisseria and coliform organisms. In no instance was inhibition observed.

(ii) Gram-positive species. Several organisms, including strains of pneumococci, enterococci and viridans streptococci, as well as different species of Corynebacterium and Bacillus, were inhibited by 'Type 71' staphylococci and by both NCTC strains. Under anaerobic conditions, different species of Clostridium were also inhibited by 'Type 71' stab inocula, but not by the NCTC strains. One strain of Corynebacterium acnes (NCTC No. 737) was susceptible to the action of 'Type 71' staphylococci, but not to the DI+H NCTC strains. Conversely, the C. acnes culture was not active in deferred antagonism tests against these staphylococci.

13. Antibiotic activity in liquid media

(A) Production of antibiotic

(i) Antibiotic material.

Active bacteria-free preparations were essential for further study of the inhibitory substance produced by 'Type 71' staphylococci. Preliminary attempts to detect activity against corynebacteria in broth cultures were equivocal, partly because of low titres and partly because of difficulty in obtaining suitable material. Seitz or sintered-glass filtrates were negative, and despite repeated centrifugation, contamination of the supernatant with the original organism frequently occurred, giving false positive results. Other methods were therefore sought, three of which were used.

(a) Cellophan-sac cultures. A piece of washed Visking tubing was tied at each end to inlet and outlet tubes in the stopper of a conical filtration flask. The loop of tubing so formed was partly submerged in 100 ml. of nutrient broth placed in the flask, which was autoclaved. The cellophan loop was then filled with sterile broth through the inlet tube, and inoculated with 'Type 71' organisms. On incubation, growth occurred inside the tubing.

The broth outside remained sterile, and presumably contained the antibiotic substance as judged by inhibitory activity against corynebacteria. Controls, obtained similarly after growth of DI- staphylococci, were inactive.

(b) Heat treatment. It was found that suitable broth cultures could withstand a considerable amount of heat without much loss of inhibitory activity. Usually cultures were placed in boiling water for only a few minutes, sufficient to kill all the organisms, and then rapidly cooled by immersion in cold water. After centrifugation, the supernatant was removed, and, if necessary, neutralised. Again, this crude material was active against corynebacteria.

(c) Acid treatment. Antibiotic activity was found to be retained in solution after precipitation of organisms and proteins in broth cultures by the addition of an equal volume of 10 per cent trichlor-acetic acid (TCA). Other concentrations and different amounts of TCA were not so satisfactory. After centrifugation the supernatant was carefully neutralized with 5N NaOH using phenol red as an indicator. It contained antibiotic activity, whereas DI- staphylococcal cultures treated in the same way were inactive. Although diluted two-fold, this material gave inhibition titres against corynebacteria equal to those of undiluted heated pre-

Table 18

Antibiotic production by 'Type 71' staphylococci in different liquid media

Medium	Inhibition of corynebacteria in cup assays of 'Type 71' broth cultures	
	Neat supernatant	Maximum titre obtained
Fresh trypsin - digest broth*	+++	1/64
Old trypsin - digest broth*	+	1/8
Fresh meat - infusion broth*	+	1/4
Old meat - infusion broth*	±	1/2
Lab lemco infusion broth*	±	1/2
Robertson's cooked meat medium*	-	-
Todd-Hewitt broth*	±	1/2
Saline broth*	-	-
Casein hydrolysate medium*	-	-
Lactalbumin hydrolysate medium*	-	-
Oxoid nutrient broth	-	-
Oxoid nutrient broth No. 2	+	1/4
Oxoid Hartley's digest broth	+	1/4
Oxoid brain-heart infusion broth	++	1/16
Labacta nutrient broth	-	-

*Prepared in the laboratory

+++, ++, +, ± = Degrees of inhibition

\- = No inhibition

parations, and it was therefore mostly used.

(ii) Importance of medium.

Cup assays of crude solutions against corynebacteria indicated that broths varied considerably in their ability to support antibiotic production. Different broths, including meat infusions and digests, Lemco broth, casein and Lactalbumin hydrolysate media, and several commercial products were therefore compared. The results (Table 18) showed that broth freshly prepared by tryptic digestion of meat usually gave the greatest antibiotic yields, many of the others showing no inhibitory activity at all. Variation was, however, found between different batches of digest broth, and deterioration also occurred during prolonged storage.

Antibiotic activity was not enhanced in media prepared by either replacement or addition of Lab lemco, yeast extract, haemoglobin, different kinds of peptone, and other substances, to different broths. With digest broth, however, antibiotic yields were appreciably increased by the addition of fermentable carbohydrates such as glucose or mannitol. An initial pH of about 7.8 was also beneficial; staphylococcal growth produced a final pH of 6.0 - 6.8 in this medium. The highest crude antibiotic titre against corynebacteria, 1 in 128, was obtained after over-

night incubation of 'Type 71' organisms in digest broth containing one per cent mannitol.

(iii) Conditions for antibiotic production.

Digest broth cultures grown under different metabolic conditions were compared for antibiotic activity. In general, aerobic incubation was best; reduced O_2 or increased CO_2 tension made little difference, but antibiotic production was poor under anaerobic conditions. Aerobic growth followed by anaerobic incubation, and vice versa, were not satisfactory for antibiotic production. Re-inoculation of the supernatant from 'Type 71' digest broth cultures failed to increase activity. The volume, depth and aeration of the medium were also considered, but shallow and deep cultures, with and without intermittent shaking, all gave similar results. Screw-capped containers filled with digest broth were therefore generally used for antibiotic production by 'Type 71' staphylococci.

Samples from cultures grown under different conditions were assayed for inhibitory activity each day for seven days. Under aerobic conditions, maximum activity was reached within 24 hours, after which the titre slowly fell. Similar cultures, grown at different temperatures, were also assayed for activity. Incubation at 37 °C was clearly best, and was directly related to the

Table 19

Inhibition of DI- staphylococcal growth in bacteria-free 'Type 71' antibiotic material, using the dilution assay technique.

Material dispensed in 1.0 ml. amounts	*Staph. aureus* inoculated	Period of incubation	Growth from 0.02 ml. inocula of broth culture dilutions 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Normal broth	'Type 71'	24 hrs	++++	++++	++++	++++	++++	++++
		48 hrs	++++	++++	++++	++++	++++	++++
	DI -	24 hrs	++++	++++	++++	++++	++++	++++
		48 hrs	++++	++++	++++	++++	++++	++++
'Type 71' culture supernatant	'Type 71'	24 hrs	++++	++++	+++	+++	++	+
		48 hrs	++++	++++	++++	++++	++++	+++
	DI -	24 hrs	++	-	-	-	-	-
		48 hrs	++++	+++	++	+	±	-
DI - culture supernatant	'Type 71'	24 hrs	++++	++++	++++	++++	+++	++
		48 hrs	++++	++++	++++	++++	++++	++++
	DI -	24 hrs	++++	++++	++++	++++	+++	++
		48 hrs	++++	++++	++++	++++	++++	++++

++++, +++, ++, +, ± = arbitrary visual assessment of growth

\- = no visible growth

amount of growth.

(B) Antibiotic activity in mixed cultures

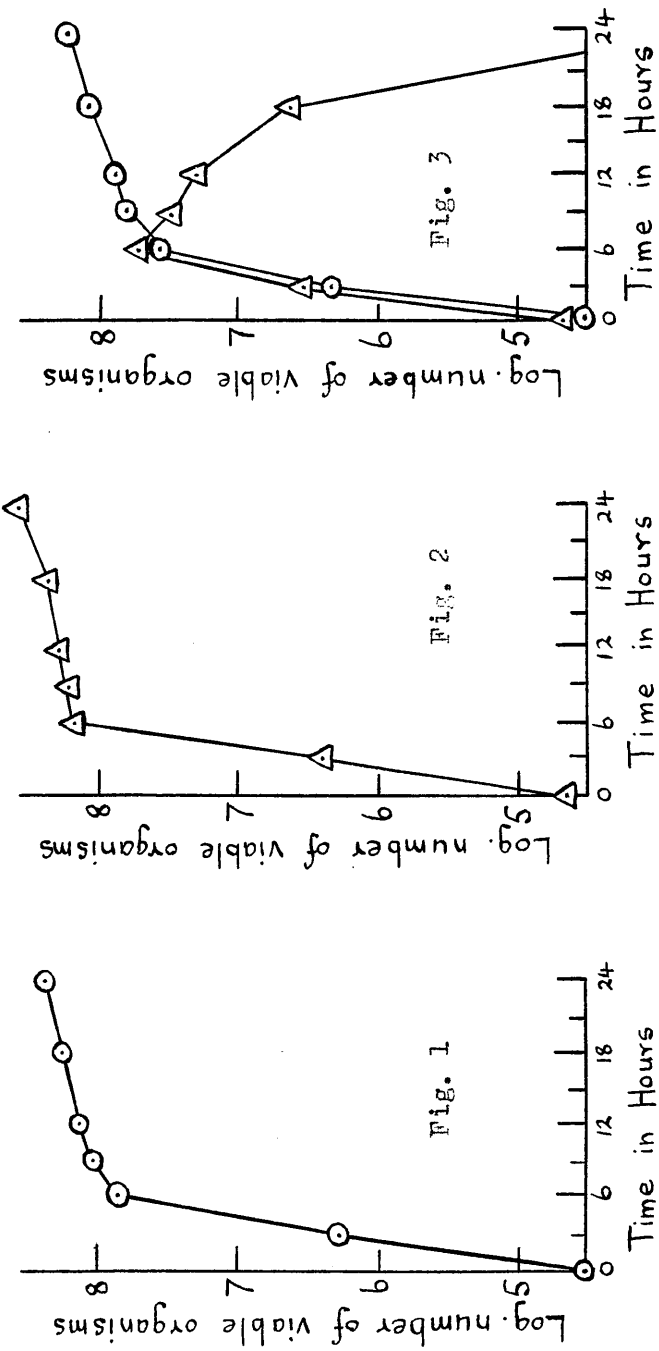
Although antibiotic activity against corynebacteria was readily detected in cup assays of 'Type 71' digest broth preparations, none was found against DI- staphylococci. The dilution assay method was therefore used to try to demonstrate such activity. In crude antibiotic broths, growth of the corynebacteria was completely suppressed, no matter how large the inocula, but difficulties were experienced with staphylococci because of (1) inhibition of some 'Type 71' control inocula in the antibiotic material, and (2) the frequent failure of small staphylococcal inocula to grow in normal broth. Some satisfactory results were, however, obtained (Table 19) indicating partial inhibition of DI- staphylococci after overnight culture, but growth usually occurred on further incubation.

Because of these difficulties with dilution assays, mixed cultures were examined for inhibitory activity in broth against DI- staphylococci. For this purpose, a DI- strain of Staph. aureus was chosen with characteristic colonies, readily distinguishable from those of 'Type 71' staphylococci. Colony

counts of each organism, cultured alone as well as together in digest broths, were made by the method of Miles & Misra (1938) on nutrient agar medium. An average of two samples was taken for estimation of viable counts. Samples were withdrawn at 3 hourly intervals, disturbing the cultures as little as possible. Duplicate counts, determined by inhibition of corynebacteria seeded on blood agar medium, were substantially similar.

The results obtained with mixed broth cultures, starting with approximately equal inocula, are shown as growth curves in Figs. 1, 2 and 3. Each organism multiplied normally during the log phase of growth, after which the number of surviving DI- staphylococci declined rapidly compared with the control culture. In contrast, growth of 'Type 71' organisms was similar in both the mixed and control cultures. In further experiments with different proportions of organisms in the inoculum, it was found that growth of DI- staphylococci was almost completely suppressed when they were outnumbered initially by 'Type 71' organisms in a ratio of more than 10: 1. Conversely when DI- organisms were similarly in excess, their growth was unimpaired by 'Type 71' staphylococci. Inhibitory activity by 'Type 71' organisms was thus demonstrated in mixed broth cultures, and was similar to that obtained with the overlapping drop technique on solid media.

Growth curves of staphylococcal broth cultures



Inocula = 0.02 ml. of 1:10 dilutions of overnight broth cultures of each organism

Fig. 1. Growth of Staph. aureus 'Type 71' in digest broth

Fig. 2. Growth of DI - Staph. aureus in digest broth

Fig. 3. Growth of DI - and 'Type 71' staphylococci together in digest broth

14. Nature and Properties of the antibiotic

The experiments already described indicated that the inhibitory substance produced by 'Type 71' staphylococci was water-soluble, diffusible, and to some extent resistant to the action of heat. It was investigated further as follows.

(i) Concentration of antibiotic.

Extraction, or filtration, of the antibiotic substance from crude broth was unsuccessful. Addition of alkali, HCl, glacial acetic acid and concentrated TCA in the cold resulted in complete loss of activity. Separation was not obtained by shaking with methyl alcohol, ethyl alcohol, acetone, chloroform, ether, xylol, or pyridine. Absorption with activated charcoal removed all activity from solution, but attempts at subsequent elution failed. Doubtful activity was found in the precipitate given by addition of an equal volume of saturated $(NH_4)_2SO_4$ solution. Considerable activity was present in the neutralized supernatant, obtained after precipitation of organisms and proteins in broth cultures with an equal volume of 10 per cent TCA. No activity was detected in the residue from this culture supernatant after evaporation to dryness. The antibiotic was concentrated, however, by evaporating the neutralized TCA supernatant in Visking tubing in a current of

Table 20

Titration of 'Type 71' antibiotic broth material in agar cups against corynebacteria, showing relation between dilution and zone size.

Dilution of antibiotic	Width of inhibition zone in mm.	Dilution of antibiotic	Width of inhibition zone in mm.
Neat	10.0	1/64	2.0
1/2	9.0	1/128	1.0
1/4	8.5	1/256	0.5
1/8	6.0	1/512	0
1/16	4.5	1/1024	0
1/32	4.0	DI- control Neat	0

warm air to about one-eighth of its volume. This gave a copious, inactive deposit, which was removed by centrifugation. After dialysis, the resulting solution was found on testing to contain about four times the activity of the original culture preparation. The results of a typical titration of this material, showing the relation between antibiotic dilution and zone size, is given in Table 20. In cup assays, weak inhibition of DI- staphylococci was also observed. This concentrated antibiotic material was used in further experiments.

(ii) Stability of antibiotic.

In an early experiment, 'Type 71' plates were prepared for cross-streaking by the colicine method, and then placed in an alloy anaerobic container. This was partly immersed in boiling water for 3 hours; the maximum air temperature reached inside the container was 83°C. After removal, the plates were cooled and inoculated with sensitive organisms. Inhibition occurred on incubation and was similar to that produced on unheated control plates, indicating some degree of heat stability.

Later, concentrated antibiotic preparations, adjusted to pH 3.0, 7.0 and 8.5, were assayed against corynebacteria after heating at (a) 60°C for one hour (b) 100°C for 15 minutes, and (c) 120°C for 15 minutes in the autoclave. Under neutral and

acid conditions, activity remained substantially the same after heating at 60°C. Slight loss occurred at 100°C, and activity was completely destroyed by autoclaving. When alkaline, activity was completely destroyed by any form of heating, and rapid loss occurred even without heating. Care was therefore necessary when neutralizing antibiotic preparations. There was gradual loss of activity when kept at 4°C, or for long periods at -40°C, if the solution was alkaline. Under acid conditions, the antibiotic remained stable at these temperatures.

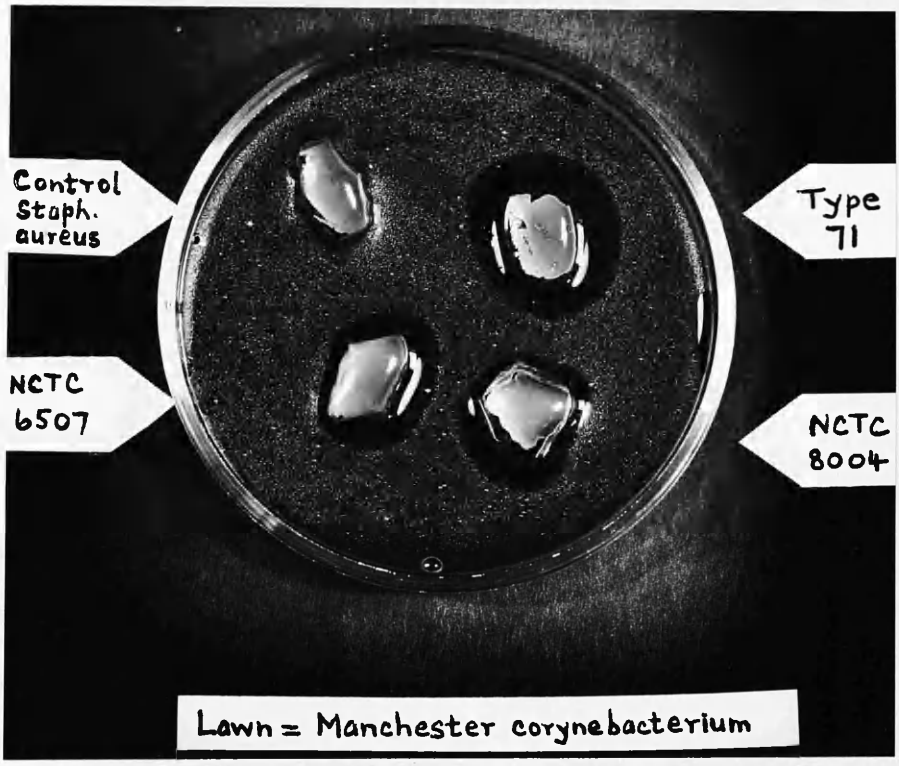
(iii) Dialysis of antibiotic.

Crude antibiotic material obtained from heated cultures was placed in washed, sterile cellophan (Visking) tubing, sealed by tying each end, and immersed in an equal volume of sterile water or normal saline. These were kept at 4°C, and the material inside and outside the cellophan bags assayed against corynebacteria each day for seven days. No evidence of dialysis was found. However, slow passage of the antibiotic through cellophan occurred when unheated, concentrated TCA material was similarly tested.

In contrast, inhibition of corynebacteria was produced by growing cultures inoculated on solid media as shown in Plate 8, indicating that the antibiotic readily passed through the cellophan tubing 'cylinders' placed in the medium before setting. This

Plate 8

Diffusion of antibiotics through cellophan



Direct inhibition of the Manchester corynebacterium by three active strains of Staphylococcus aureus: diffusion of the antibiotics through cellophan tubing 'cylinders' inserted in the blood agar medium before setting. Inhibition was not produced by the DI- control culture.
x 4/5. Reflected light.

was also demonstrated in direct and deferred antagonism experiments with sheets of commercial cellophan placed on the surface of solid media.

(iv) Susceptibility to enzyme digestion.

Equal amounts of sterile enzyme preparations (one per cent w/v in physiological saline) were added to concentrated antibiotic material in tubes, and the pH adjusted to 3.0 for pepsin (BDH 1: 2500), and 8.0 for trypsin (Difco 1: 250). The tubes were incubated at 37°C in a water bath for one hour. They were then neutralized and assayed in agar cups for activity against corynebacteria. Controls, similarly tested, included the same antibiotic material (a) with the addition of saline instead of enzymes (b) with the addition of heat-denatured enzymes, and (c) without pH adjustment. The results showed that antibiotic activity was completely destroyed in one hour by trypsin, but was only slightly reduced by pepsin.

(v) Mode and specificity of antibiotic action.

The inhibitory substances produced by active staphylococci were lethal to corynebacteria, but did not cause lysis of the organisms. Zones of inhibition were unaffected by prolonged incubation, and corynebacteria could not be cultivated from them until the outer edge of growth was approached, nor were they

recovered from antibiotic broth preparations. With staphylococci, the inhibitory effect was probably bacteriostatic since in dilution tests, growth occurred after further incubation in tubes showing initial inhibition. Subculture from zones of inhibition usually yielded staphylococcal growth, and some reduction in zone size occurred on prolonged incubation, presumably because of lowering of the local antibiotic concentration by diffusion.

Active broth preparations were not obtained with the DI+H NCTC organisms, and their range of activity was different from that of 'Type 71' strains, suggesting that the inhibitory substances concerned were different. The seven DI+S 'Type 71' strains all behaved in the same way and showed the same anti-bacterial spectra. With each, complete loss of activity against one organism always coincided with similar loss against other organisms, suggesting that one and the same substance was responsible for 'Type 71' activity.

Experiments with colonies of staphylococci selected for their resistance to these inhibitory substances indicated considerable specificity in antibiotic action. Variants of DI- staphylococci resistant to the action of one 'Type 71' strain were equally resistant to all 'Type 71' strains, but they remained sensitive to the DI+H NCTC organisms. Similarly variants resistant to the

action of DI+H NCTC strains were not resistant to 'Type 71' staphylococci. From 'Type 71' organisms which had lost their inhibitory power, it was possible to isolate variants that, unlike the parent culture, were resistant to the 'Type 71' antibiotic, but which showed no inhibitory activity against corynebacteria. These variants nevertheless remained susceptible to the DI+H NCTC strains. Further confirmation of the specific nature of antibiotic action was obtained from absorption experiments. The inhibitory activity of concentrated 'Type 71' antibiotic solutions was greatly reduced by absorption with sensitive staphylococci, but not by absorption with 'Type 71' organisms or with resistant DI- staphylococcal variants.

Antibiotic activity by 'Type 71' staphylococci was not prevented by standard commercial staphylococcal antitoxin, or by an immune serum prepared against these organisms.

(vi) Effect of some factors on antibiotic activity.

A few experiments were made to ascertain whether antibiotic production occurred, and if so, whether it was effective under some of the conditions which might be encountered in the skin. The factors considered were (a) E_h and pH (b) temperature (c) salt concentration (d) presence of unsaturated fatty acids, and (e) presence of blood, plasma or serum.

Antibacterial activity by 'Type 71' staphylococci was observed on solid media, including pH gradient plates (Sacks, 1956), under both aerobic and anaerobic conditions at 37°C over a range of pH from 5.0 to 9.0. It also occurred and was almost as effective when incubated at 30°C and 25°C under similar conditions of E_h and pH, even in the presence of five per cent NaCl. In contrast, the DI+H NCTC strains were not active when grown anaerobically, but otherwise produced inhibition under aerobic conditions. Antibiotic activity by these staphylococci was detected by deferred antagonism techniques after aerobic growth for as little as 5 hours.

Oleic acid (BDH) was used to represent the unsaturated fatty acids present in sebum. Although not miscible with water, it was incorporated as fine globules in suspension by adding it to hot, molten agar media, mixing constantly until viscous, and then pouring rapidly into plates. Some staphylococcal strains grew with difficulty on 0.5 or 1.0 per cent oleic acid media but 'Type 71' strains, the DI+H NCTC organisms, and the BFD diphtheroid, grew well. Direct inhibition of corynebacteria or DI- staphylococci was not observed however, on these plates.

Inhibitory activity by 'Type 71' staphylococci on solid media was not affected by the presence of serum from several different

animal species, or by human blood or pooled human plasma or serum. Finally, no significant reduction in antibiotic broth activity occurred in the presence of 50 per cent pooled human serum, with or without preliminary incubation for two hours at 37°C before diluting for assay against corynebacteria.

Discussion

Impetigo contagiosa remains one of the common bacterial infections of children, and occasionally of adults, although its severity and incidence have diminished considerably since it was first described by Tilbury Fox in 1864. It was then apparently highly contagious, characterized by fever and general malaise, and liable to complications now recognized as streptococcal. Despite antibiotic therapy, a recent increase in its frequency has, however, been observed (Sneddon, 1953). Seasonal variations in incidence, as well as correlation with some streptococcal infections have also been described (Newman, 1935; Cruickshank, 1941). Although sought in the 1953-4 investigation, no association with other diseases was found, nor was there any significant seasonal or sex distribution.

Impetigo has been classified bacteriologically into staphylococcal and streptococcal types (Engman, 1901; Lewandowsky, 1922; Epstein, 1940), although clinical differentiation in individual cases was not always possible. In the former, cases are frequently

sporadic, and the lesions primarily bullous in character, followed by thin crusts, frequently healing in the centre, with clean bases. The latter is apt to become 'epidemic', and is said to be associated with thick 'stuck-on honey-coloured' scabs with inflamed margins and 'sodden' bases (Lancet, 1943).

In Part I, the cases seen during 1953-4 were mostly of sporadic impetigo among children from different districts and from different social classes, and it is significant that nearly all yielded staphylococci. Early lesions were vesicular, subsequently developing yellow crusts of moderate thickness, often showing signs of central healing, with little inflammatory reaction and clean bases. The finding of numerous Gram-positive diplococci morphologically resembling gonococci in stained smears from impetigo lesions confirmed earlier observations (e.g. Clegg & Wherry, 1906; Simpson, 1941). Altogether Staph. aureus was isolated from 94 per cent of the cases, and Str. pyogenes similarly from 17 per cent of cases. The use of selective media, or of moistened or serum impregnated swabs (Rubbo & Benjamin, 1951), might have yielded some additional streptococci, but it is doubtful if they would have affected the present results appreciably. Although Staph. aureus was isolated alone in 86 instances, and Str. pyogenes alone in six instances, no clinical distinction was

observed between these cases. Indeed, the isolation of haemolytic streptococci in pure culture from early lesions was unexpected. Their presence was, however, suspected in some of the mixed infections, either because of greater severity or longer duration. The 'pure' streptococcal series were all early cases of mild or moderate severity, and included the only 'epidemic' incident in which one entire family was affected almost simultaneously. It is notable that Parker & Williams (1961) now consider streptococcal impetigo to be a milder infection than staphylococcal impetigo.

Substantially similar bacteriological results were obtained from adult cases by Sheehan & Fergusson (1943), Bigger & Hodgson (1943, 1944) and by Davies, Dixon & Stuart-Harris (1945), though the proportions of streptococci isolated were greater. Sheehan & Fergusson observed that staphylococci were present in early impetigo lesions, whereas streptococci occurred only after the initial blister had become crusted. Bigger & Hodgson (1943) cultivated staphylococci from nearly all their cases, and showed that the incidence of streptococci isolated increased progressively with the age of the lesions, presumably due to secondary invasion, as was found in burns by Colebrook and his colleagues (1944 a,b). Bigger & Hodgson concluded therefore that Staph.

aureus was either the causative organism or was of importance in the aetiology of impetigo, and that streptococci were seldom, if ever, the cause. They did not, however, attempt to differentiate their cases clinically, and Stuart-Harris (1948), whilst agreeing that most of their cases probably were staphylococcal in origin, has pointed out that the higher incidence of streptococci in older lesions could have been due to the elimination of more rapidly healing staphylococcal cases. Davies, Dixon & Stuart-Harris, studying the bacterial flora of some skin infections, found little evidence of a constant association of particular organisms with particular conditions, except in impetigo and ecthyma. They obtained more staphylococci and streptococci from impetigo cases than would be expected from 'normal' skin carriage, and also observed that vesicular lesions yielded only staphylococci, whereas thickly crusted lesions usually yielded streptococci as well as staphylococci. Streptococci were always found in ecthyma, but the remainder of their cases, mostly of infected seborrhoea, yielded either staphylococci, or both streptococci and staphylococci.

The mechanism of infection is uncertain, but it is generally agreed that some degree of trauma is essential for its initiation. Stuart-Harris (1948) has emphasized that staphylococci and strepto-

cocci are seldom 'primary' causal agents, as for instance the tubercle bacillus is in relation to tuberculosis. Some crack, abrasion, or other defect, such as obstructive closure of pore orifices as suggested by O'Brien (1950), are probably necessary for lodgement and multiplication of pathogens, with consequent characteristic tissue reaction. The fact that almost any breach in the continuity of the epidermis becomes a site of bacterial proliferation makes it difficult to distinguish lesions initiated by pathogens from those infected by secondary invaders. Nevertheless, there is a fundamental difference between 'primary' conditions such as impetigo, pemphigus neonatorum and erysipelas, which exhibit epidemic or contagious features, and those in which conditions such as eczema or contact dermatitis become 'secondarily' infected.

In the past repeated attempts to reproduce impetigo experimentally in laboratory animals have failed, and despite its superficial nature and readily accessible situation, only limited success has been obtained in man. Bigger & Hodgson (1943) for instance tried several different methods without success, whereas Sheehan & Fergusson (1943) managed, with difficulty, to induce lesions in a few adults, but only with material containing impetigo staphylococci, and only after vigorously scraping the skin. They

pointed out however that, although impracticable, children would be more suitable than adults for such experiments. Probably environmental or host factors, such as alterations in the normal bactericidal power of the skin, are also involved.

The fact that at least 80 per cent of the staphylococci isolated from impetigo patients in 1953-4 were of the same or closely related phage 'Type 71' suggests that their rôle was directly connected with infection, and that they were not merely 'opportunists'. Indeed their incidence was found to be greater in early than in older lesions. Their infrequent occurrence in the control groups indicated that they were not a local strain of unusual virulence. The same bacteriophage type has been found widely distributed in cases of impetigo (Parker et al; 1955; Spittlehouse, 1955) and of pemphigus neonatorum (Parker, 1958; Howells & Jones, 1961) in different parts of England. The association of this type with impetigo is also supported indirectly by the small proportion of staphylococci of phage-group II obtained by Tulloch (1954) from skin infections other than impetigo. The presence of 'Type 71' staphylococci in impetigo has since been confirmed in Denmark by Schmidt, Eriksen & Rosendal (1957), and in Holland by Van Toorn (1961), although in each country most of the strains were sensitive to penicillin. A

close association of 'Type 71' strains with impetigo was not, however, observed in a survey of staphylococcal infections of the skin in general practice in Australia (Johnson, Rountree, Smith, Stanley & Anderson, 1960). Further information on the geographical distribution and pathogenicity of this 'type' is therefore needed.

In the past, organisms cultivated from fluid withdrawn from early, unbroken impetigo vesicles have usually been regarded as causative. During 1953-4, fluid from intact vesicles was obtained in only seven cases, but each yielded staphylococci, either of 'Type 71' or 'weak 71', thus further supporting their aetiological rôle. From two of these cases, brothers seen at the same time, Str. pyogenes was isolated as well as 'weak 71' staphylococci. In both instances the streptococci were not typable, and a similar strain was obtained from the throat of the longer-standing case. This patient also gave an antecedent history of sore throat, suggesting that the streptococcal strains may not have been concerned directly with the lesions. Since impetigo vesicles are formed primarily in the epidermis, not by haematogenous dissemination, but by the growth and multiplication of organisms at sites of supposed minor injury, there is no reason why other organisms, not necessarily pathogens, should not co-exist

ab initio. Failure to recognise this possibility may account for some of the conflicting earlier results.

In an effort to trace the source of the infecting organisms in impetigo, Bigger & Hodgson (1944) swabbed the nose, throat and skin near lesions in a small number of patients. From their results they surmised that, in some cases at least, the staphylococci came from the nose, and streptococci from the throat. Cruickshank (1953) also considered that the nose was probably the source of infection in staphylococcal impetigo. Nasal carriage rates of 68-80 per cent were recorded by Valentine & Hall-Smith (1952) in cases of furunculosis, and as high as 91 per cent in sycosis barbae, the staphylococci in the nose and lesion being similar in phage pattern in almost every instance. Similar rates of nasal carriage were obtained by Roodyn (1954) and Gould & Cruickshank (1957) in cases of staphylococcal infection in general practice, though in 17 per cent of Roodyn's cases the strains in the nose were different from those in the lesions. On the other hand, nasal carriage in various infective conditions was found in only 40 per cent of cases by Davies et al., (1945).

In the present cases, only 46 per cent carried the same strains of Staph. aureus in the nose and lesion at the time of swabbing. This figure was much the same as found in the 'normal'

control groups, and was considered low, since active lesions were present on the face or neck in 89 per cent of these patients, most of whom were seen in the first or second week of disease. If the nose were the source of infection in impetigo, it would presumably remain infected while the skin lesions were active, and a much higher incidence of nasal carriage would be expected. Further, if this were the case, some relapses might be expected, but despite the fact that no effort was made to suppress staphylococci in the nose, no case recurred within a few months of attendance. Also, it was shown that the longer the duration of impetigo, the higher was the nasal carriage rate of staphylococci. Moreover Str. pyogenes was not obtained from the throat of any patient in the 'pure' streptococcal series. It is doubtful, therefore, if the nose or throat are the source of the causal organisms of impetigo though they may harbour some of the secondary invaders. It seems more likely, especially in children, that organisms are transferred by direct contact, and that the nose becomes secondarily infected from lesions. In some instances, primary contamination of the nose may occur, with subsequent infection of the skin, or transmission to other persons. No doubt occasional carriers occurred amongst these cases, even though nasal carriage of 'Type 71' strains was not detected in the control

groups. A high incidence of nasal carriage of 'Type 71' staphylococci among the population was also considered, but excluded by Parker et al. They obtained 92 strains of Staph. aureus from nasal swabs from 100 children in each of two schools, but only three strains belonged to 'Type 71', and these were from a school in which impetigo had occurred.

As regards streptococci, surveys of the relative prevalence of various streptococcal serotypes in England and Wales, undertaken by the Public Health Laboratory Service, have shown a close association of Str. pyogenes of agglutination pattern 3/13/B3264 with impetigo and ear infections (Reports, 1954, 1957). It was not suggested, however, that these organisms were necessarily causing infection. Similarly Parker et al., (1955) observed that strains of Str. pyogenes isolated from impetigo were mostly confined to two agglutination patterns, 3/13/B3264 (12) and 5/11/12/27/44, neither of which were common in other infections at that time. These strains rarely possessed M antigens, and were usually only identifiable by slide agglutination with T antisera. Parker et al., also cited earlier unpublished work in which other impetigo streptococci were agglutinated by sera for the types 8, 25 or IMP. 19. However, although they obtained a mixed growth of Str. pyogenes and Staph. aureus from 65 of 190

impetigo swabs, they did not indicate to what extent the two streptococcal 'types' overlapped with 'Type 71' strains.

In the 1953-4 investigation Str. pyogenes was isolated from a very small number of patients, but of the 16 typable strains obtained, four belonged to the 'type' 3/13/B3264. Three of these strains were from the entire family already mentioned, and none was associated with 'Type 71' staphylococci. In addition, two strains of both the 'types' 5/11/27/44 and 8/IMP. 19/10 were isolated, one of each occurring together with 'Type 71' staphylococci. These numbers are too small to be significant.

The presence of Str. pyogenes and the rapid response to sulphonamide therapy confirmed the bacterial nature of the school outbreak of infection in 1960. The clinical features and epidemic spread, both of throat infection and of impetigo, suggested that one, or possibly two streptococcal types were concerned. Even though the school was not a closed community, the finding of such a wide variety of serological groups and types of streptococci within so short a time was unexpected. In addition the isolation of two or more strains of streptococci from several children, both on different occasions and from single throat swabs, made epidemiological assessment difficult. It did,

however, indicate that the spread of different strains was extensive; the type of children and special circumstances of the school probably contributed largely to this.

It is possible that this outbreak consisted of separate incidents caused by different strains. It is unlikely however, that all the strains isolated were causing infection, although some additional evidence was obtained indicating the pathogenicity of Group C streptococci in throat infections. As regards impetigo, the clinical development and epidemiological features were different from those previously described. None of the staphylococci isolated were related to 'Type 71' strains, nor did they show any other characteristics of strains associated with superficial infections (Parker, 1958). For these reasons, the cases were probably streptococcal in origin, the staphylococci representing secondary invaders. The particular strains of streptococci causing impetigo were, however, uncertain because of the number of different types isolated. None of them possessed 'M' antigens, although patterns of agglutination were unusual. Some of the streptococcal 'types' previously associated with impetigo were at least present in the school, and Group C streptococci may also have been concerned. As these cases were not seen in the early stages of infection, the possibility that some of the streptococci

were actually secondary invaders cannot be entirely excluded. It is probable that many more swabs would have yielded different strains of streptococci if several colonies from each culture had been type identified. This outbreak thus illustrates some of the difficulties and sources of error encountered in bacteriological studies of skin infections. It also emphasized the importance of examining more than one colony from primary cultures, particularly in epidemiological studies in 'open' or semi-closed communities.

From these, and other investigations, there is good clinical, bacteriological and epidemiological evidence in favour of the existence of at least two kinds of impetigo, caused by staphylococci and streptococci respectively; and in each kind, particular strains of staphylococci and streptococci appear to be largely concerned. This explanation has the merit of resolving many previous anomalies, such as the relation between impetigo contagiosa and pemphigus neonatorum; differences in the reported age incidences; variations in geographical and seasonal incidence; the possible relationship to other streptococcal infections; and differences in the effect of certain earlier forms of local treatment - staphylococcal impetigo responded best to gentian violet, and streptococcal impetigo to mercurial applications. According

to Epstein (1940), failure to correlate clinical features with bacteriological findings was usually due either to mistakes in diagnosis, faulty techniques, or to secondary invasion of staphylococcal lesions by streptococci. Epstein states that he has never seen streptococcal impetigo change clinically to the staphylococcal type, but that he has observed the reverse. Indeed Sulzberger (1940) has even proposed a third kind of impetigo caused by the combined action of staphylococci and streptococci, but this has not been generally accepted. It is suggested that sporadic impetigo is largely due to Staph. aureus, whereas increases in prevalence or severity are probably associated with a greater proportion of the streptococcal form. Further combined clinical, bacteriological and epidemiological inquiries are needed to confirm this view.

As early as 1906, Clegg & Wherry stated that the natural habitat and absence of invasiveness distinguished impetigo organisms from the ordinary pyogenic cocci. However repeated attempts failed to reveal any cultural differences between them (Epstein, 1934, 1935). More recently, Simpson (1941) has again indicated that impetigo is a distinct infection which breeds true; that contacts of impetigo do not develop other pyogenic lesions, and conversely, that contact with boils and other septic conditions

does not give rise to impetigo. He postulated on these grounds that, whatever its precise nature, the causal organism of impetigo contagiosa was 'special to that disease'; he suggested for it the title 'impetigococcus'. Staphylococci of 'Type 71' at least, and possibly also some streptococci, appear to fulfil these criteria.

It was of considerable interest that so many impetigo staphylococci were similar in 'type'. Although a general correlation had been observed between staphylococci producing enterotoxin and phage-group III (Allison, 1949), and those causing superinfection in influenzal pneumonia with phage-group I (Williams et al., 1953), the strains concerned varied in their actual phage patterns. Apart from strains of 'Type 71', and possibly of phage 'Type 80', the association of a particular bacteriophage 'type' of Staph. aureus with a particular kind of lesion has not previously been observed. 'Type 80' strains were identified first in Australia (Ibister, Durie, Rountree & Freeman, 1954; Rountree & Freeman, 1955), and subsequently in this country (Duthie, 1957) as the cause of epidemic infection in hospitals. They were responsible for skin infections in infants and abscesses in older patients, and frequently invaded deeper tissues.

Impetigo staphylococci are characterized not only by susceptibility to phage 71 alone, but to a considerable extent by

their resistance to penicillin, absence of invasive power, and by their ability to survive and multiply in the superficial layers of the skin, where they provoke only a mild pyogenic response. They possess all the usual attributes of virulent staphylococci. They produce pigment, coagulase and fibrinolysin, liquefy gelatin, ferment mannitol, and produce at least five antigen-antigody lines when tested by the method of Elek and Levy (1950). According to Parker (1958), they form both α and δ toxins, as well as abundant hyaluronidase. They are also able to inhibit the growth of corynebacteria (Parker et al., 1955) and to produce opacity in horse serum agar media (Tomlinson & Parker, 1956). This kind of evidence, supporting the existence of certain strains with particular properties within the species is not, however, entirely conclusive, unless such strains can be shown to occur in association with the same infection throughout the world. Elek (1959) has emphasized that bacteriophage susceptibility is only one arbitrary biological character, and that impetigo-causing factors, if such exist, may be quite independent. Thus a given phage 'type' should not be regarded as necessarily synonymous with an epidemic strain.

Parker (1958) examined 1389 staphylococcal strains from a variety of human sources. He found that ability to inhibit the growth of corynebacteria on solid media, with the formation of

sharply defined zones, was limited almost entirely to 'Type 71' organisms. These were all penicillin-resistant, gave negative egg-yolk opacity reactions, and produced opacity on horse serum agar media. An inverse relationship was observed between the serum opacity and egg-yolk opacity reactions, and Parker concluded that, whatever its mechanism, the serum opacity test provided a valuable indication of 'superficiality' among Staph. aureus. Recently Gooder (1961) has also described a serum opacity reaction produced in broth by certain serotypes of Str. pyogenes, including those found in superficial skin infections. A number of other staphylococcal strains active against corynebacteria gave inhibition zones with hazy edges, but had little else in common. Most of the few remaining strains forming sharp zones possessed other '71 - like' properties, and were also associated with superficial infections. Although examples of '71 - like' strains occurred in all the phage groups, the majority belonged to Group II, or were untypable, suggesting that their differences in phage susceptibility from 'Type 71' strains were quantitative rather than qualitative. Later Parker & Simmons (1959) found that old 'Type 71' cultures lost their inhibitory activity, and that this loss was accompanied by increased phage susceptibility.

Similar findings, on a smaller scale, were obtained in

Part II of this thesis. Only seven strains active against corynebacteria were found among 100 cultures of Staph. aureus isolated from routine bacteriological specimens in 1959-60. They produced sharply defined inhibition zones, possessed all the other characteristics of 'Type 71' strains, and were derived from superficial infections. They lost their inhibitory activity on storage, and this loss coincided with increased susceptibility to Group II phages. In contrast, two NCTC organisms, one untypable and the other belonging to Group I, formed hazy zones against corynebacteria, but showed no apparent changes after storage.

As regards the distribution of inhibitory activity among staphylococci, Parker et al., (1955) suggested that other workers (e.g. Dujardin - Beaumetz, 1932; Jennings & Sharp, 1947) may have failed to observe a correlation with particular infections because the strains concerned probably produced hazy zones of inhibition. This is supported by the findings of Váczi & Mihályfi (1954), who stated that nearly all their inhibitory strains, derived mostly from throat swabs, were penicillin sensitive. They were, therefore, probably not 'Type 71' strains, especially since Parker & Lapage (1957) have given reasons for supposing that 'Type 71' strains were naturally resistant, because

of their ability to produce penicillinase, even before the introduction of penicillin.

In further work on the nature of 'Type 71' inhibitory activity, Parker & Simmons (1959) were unable to demonstrate direct antagonism against other staphylococci, although they observed some activity in deferred antagonism experiments. They concluded that inhibitory activity between different staphylococci was weak and probably of little significance. However, it has been shown in this study that, with suitable methods, 'Type 71' strains were strongly and directly antagonistic to nearly all other Staph. aureus strains tested, and that DI+H organisms were similarly active against many, though not all staphylococci. The stab inoculation technique proved very useful for demonstrating such activity. It allowed inhibition which would otherwise not have been observed, and directed attention to the importance of limiting diffusion in order to maintain high local antibiotic concentrations. This is particularly important when examining organisms with similar growth rates, since a critical antibiotic concentration may be attained before growth of the sensitive population reaches a 'resistant' level, as was found by Cooper, Linton & Sehgal (1958).

Inhibition by 'Type 71' staphylococci was clearly not due

to exhaustion of nutrients or to pH changes in media, nor to peroxide or enzyme production, nor to bacteriophage action. The fact that all 'Type 71' strains behaved in the same way, and that loss of power against one organism coincided with loss against other organisms, suggested that one particular substance was responsible for their activity. This antibiotic substance was water-soluble and diffusible, but Parker & Simmons failed to obtain satisfactory bacteria-free preparations and were therefore unable to investigate its nature further.

In the present study, preliminary work indicated that different broths varied considerably in their ability to support antibiotic production. It was found that broth freshly prepared by tryptic digestion of meat usually gave the greatest antibiotic yields, as measured by agar cup assays against corynebacteria. Extraction of this antibiotic substance from broth was not, however, achieved. It was retained in solution after precipitation of organisms and proteins in broth cultures with tri-chlor-acetic acid, and some of its properties were determined after concentrating this material by evaporation. It was relatively heat and acid stable, inactivated by trypsin but not by pepsin, adsorbed by charcoal, readily destroyed under alkaline conditions, and was slowly dialysable. In contrast, rapid passage of the

antibiotic through cellophan was observed with actively growing cultures, suggesting that some changes occurred during the manipulation of broth preparations, particularly after heat treatment. These properties suggest that it is one of the class of substances considered by Waksman (1947) to represent polypeptides, proteins, organic bases, or adsorption compounds on protein molecules, most of which have not been obtained in a pure state. It is different from the antibiotic substance formed by Staph. aureus NCTC No.8004, investigated by Gardner (1949) and thought to be a protein. This organism was used for comparison in the present work, but active bacteria-free preparations were not obtained with the methods described for 'Type 71' staphylococci. A group of nine similar antibiotic substances, many considered to be polypeptide in structure, were extracted in a crude state by Halbert, Swick and Sonn (1954) from a series of 22 active micrococci isolated from eye lesions. Several of these agents differed only in their heat stability or in their specificity of action, and were thus analogous to the colicins produced by enterobacteria (Fredericq, 1957). Further identification of the 'Type 71' antibiotic depends on chemical extraction and purification, and is beyond the scope of this work.

Numerous bacterial species are now known to form active

metabolites of this kind, and it is hard to believe that they have no ecological significance to the organisms. Their importance in clinical infections is however, almost impossible to assess since so many other factors are concerned. The experiments with mixed staphylococcal broth cultures suggest that 'Type 71' organisms, if in excess, could prevent invasion of impetigo lesions by other staphylococci or by corynebacteria. Indeed, despite its superficial nature, there is no evidence that mixtures of different organisms or strains occur in impetigo as frequently as they are found in eczema or in burns. Experimentally 'Type 71' organisms were able to inhibit the growth of some skin saprophytes, such as Staph. albus and C. acnes; similarly two Staph. albus strains isolated from adult skin were active against 'Type 71' staphylococci. Interaction between infecting organisms and skin residents, as well as their properties and numbers, may therefore be concerned in the initiation of clinical lesions. Although such a mechanism has been postulated for many kinds of infection, actual proof of its occurrence in vivo is still lacking. Nasal carriage of staphylococci was also investigated from this point of view by Rountree & Barbour (1951), but no evidence was obtained to suggest that inhibitory activity influenced the ability of one strain to supplant another in the nose.

It is possible that the original pathogen may be replaced, or that treatment may allow other organisms to be isolated, but again there is no evidence that this actually happens in impetigo. If there are two kinds of impetigo caused by staphylococci and streptococci respectively, then the association of specific staphylococci with non-specific streptococci, and vice versa, might be expected in mixed infections. The 'Type 71' results obtained in Part I would support this hypothesis to some extent, but the numbers of 'specific' streptococcal 'types' isolated are too small to draw any conclusions.

Alternatively, secondary invasion by some streptococcal 'types' might show an apparent association with impetigo because they were not, unlike other strains, suppressed by the action of inhibitory staphylococci, or because they were better able to survive in the skin. It was found that very few of the streptococcal strains tested were directly inhibited by 'Type 71' stab inocula. There was, however, some evidence to suggest that streptococci yielding satisfactory M antigen extracts were more susceptible than those not giving them. Strains of Staph. aureus isolated from human secretions and active against mucoid colonies of Str. pyogenes were described by Murray & Loeb (1950). Few of the corresponding non-mucoid variants were inhibited, nor were members of Lancefield's

Groups B, C or G. As most of the streptococcal serotypes used in the present study were laboratory strains, it is possible that freshly isolated streptococci forming M antigens might be more susceptible.

The antibacterial mechanisms affecting the fate of organisms lodging on the skin were reviewed by Burtenshaw (1948). Desiccation, acidity, desquamation, and washing played some part in removing organisms, but the fatty acids present in sebum were considered more important for Gram-positive cocci, although their effect will presumably be less before puberty. Burtenshaw found that strains of Str. pyogenes were rapidly killed by unsaturated fatty acids, such as oleic acid, whereas staphylococci were less susceptible. These organisms were not type-identified, and it is doubtful if impetigo strains were included. Similar studies by Ricketts, Squire & Topley (1951) and Joiris (1957) showed that different staphylococcal strains varied considerably in their sensitivity to fatty acids, but again impetigo strains were probably not examined. In the present study, inhibitory activity occurred under some of the conditions found in the skin, except in the presence of oleic acid, although 'Type 71' staphylococci appeared to grow well on this medium. Microbial antagonism may, however, still occur in the micro-environment of the skin, especially

since serum proteins neutralize the bactericidal power of these fatty acids. Also, limited work by Evans, Smith, Johnson & Giblett (1950) has suggested that in children the resident population of the skin is quite different from that of adults, in whom anaerobes were found to greatly outnumber aerobes, the organisms mostly living in the sebaceous glands or ducts. Further studies are therefore needed on the normal flora of the skin, especially in children, as well as on the ability of impetigo staphylococci and streptococci to survive and multiply under the conditions existing in the skin.

Summary and Conclusions

Part I

1. The literature on the bacteriology of impetigo contagiosa has been reviewed.
2. An investigation during 1953-4 into the clinical, bacteriological and epidemiological features of impetigo, with special reference to the type identification of staphylococci and haemolytic streptococci, is described.
3. The cases studied were mostly of sporadic impetigo among children. Clinically the lesions showed little inflammation; they were covered by fairly thick yellow crusts, removal of which left clean bases exuding serum.
4. Numerous polymorpho-nuclear leucocytes and Gram-positive cocci, closely resembling gonococci in morphology, arrangement and intra-cellular position, were observed in stained smears prepared directly from impetigo lesions. Only scanty Gram-positive cocci were seen in stained films from impetigo

swabs.

5. Of 106 impetigo cases examined, Staphylococcus aureus was isolated alone from 86 lesions (81%), Streptococcus pyogenes alone from six lesions (5.6%), and a mixed growth of Staph. aureus and haemolytic streptococci from 14 lesions (13.2%).

6. Of the 100 strains of Staph. aureus isolated from impetigo lesions, 63 were identical in bacteriophage susceptibility ('Type 71'), and at least another 17 strains were closely related ('weak 71').

7. Only one representative of 'Type 71' and nine of 'weak 71' were obtained from 164 strains of Staph. aureus isolated from 200 persons in three control groups.

8. Of 90 strains of Staph. aureus from impetigo lesions, 64 (71%) were resistant to penicillin. Of these penicillin-resistant strains, 54 (84%) were 'Type 71' or close variants.

9. Staphylococci of 'Type 71' were isolated more frequently from early cases of impetigo than from cases of longer duration.

10. Nasal swabs yielded staphylococci of the same type as in the lesions in only 46 of 100 cases; the rate of carriage of these strains in the nose increased with the duration of impetigo. It is doubtful, therefore, if nasal carriage is as important in the epidemiology of impetigo as in other

staphylococcal infections.

11. Str. pyogenes was probably causative in at least 6 of the 18 patients yielding this organism from lesions; it was presumed to be a secondary invader in the remainder.

12. The virus of Herpes simplex is not associated with impetigo.

13. The response to local treatment with Aureomycin or Neomycin ointment was rapid.

14. An account of an outbreak of infection, mainly of sore throat and impetigo, in 1960 at a day school for subnormal children, is given. The clinical, bacteriological and epidemiological features of impetigo were different to those observed during 1953-4.

15. A wide and unexpected variety of serotypes of Str. pyogenes, as well as haemolytic streptococci of other Groups, was isolated from throat and impetigo swabs during this outbreak.

16. Several children yielded streptococci, differing either in group or in type, on different occasions. Other children were shown to be carrying two or more streptococcal strains simultaneously in the throat, thus making precise epidemiological assessment of the outbreak impossible.

17. Seven strains of Str. pyogenes, six of Group C streptococci, and ten of Staph. aureus were cultivated from impetigo

material. None of the staphylococci isolated possessed any of the characteristics of 'Type 71' strains; they were regarded, therefore, as secondary invaders in streptococcal impetigo.

18. It was not possible to specify the actual streptococci causing impetigo because of the number of different strains obtained, and because of the possibility that more than one strain may have been present simultaneously in lesions.

19. In epidemiological studies in open or 'semi-closed' communities it is important to type-identify more than one colony from primary cultures.

20. It is concluded that there are at least two kinds of impetigo contagiosa, one caused by staphylococci and the other caused by haemolytic streptococci.

21. There is one particular strain of Staph. aureus, at present 'Type 71', associated with this form of impetigo in this country.

22. There may also be particular strains involved in streptococcal impetigo.

23. It is suggested that sporadic impetigo is largely staphylococcal in origin, whereas the streptococcal form is epidemic in character and may be associated with increases in

the prevalence or severity of impetigo.

Part II

24. The literature on microbial antagonism by staphylococci has been reviewed briefly.

25. The distribution and nature of inhibitory activity among staphylococci was studied by direct and deferred antagonism techniques using susceptible corynebacteria as indicator organisms.

26. Sterile glass microscope slides were useful for rapid cross-inoculation of organisms in deferred antagonism tests on solid media.

27. Stab inoculation of test strains in solid media provided a simple, rapid method of examining organisms for direct antagonism against each other. It gave wider zones of inhibition than similar surface inocula by reducing antibiotic loss from downward diffusion.

28. Of 100 strains of Staphylococcus aureus isolated from routine bacteriological specimens, seven were active, producing sharply defined zones of inhibition. These all belonged to bacteriophage 'Type 71', were all penicillin resistant,

produced opacity in horse serum agar medium, and were derived from superficial infections. They lost their inhibitory activity on storage, and this loss was accompanied by increased susceptibility to Group II phages.

29. Inhibitory power by 'Type 71' staphylococci was due to the production of a diffusible antibiotic substance. On solid media, all strains of Staph. aureus other than 'Type 71', and some strains of streptococci, coagulase - negative staphylococci, and many other Gram-positive species were susceptible, but Gram-negative organisms were not inhibited. Loss of activity against corynebacteria coincided with similar loss against other organisms. All seven 'Type 71' strains behaved in the same way.

30. Inhibitory activity by 'Type 71' organisms against other strains of Staph. aureus was also demonstrated in mixed broth cultures. The growth of all but overwhelming numbers of other staphylococci was suppressed.

31. Antibiotic production in liquid media, as measured by agar-cup assays against corynebacteria, was poor. Broth freshly prepared by tryptic digestion of meat was usually satisfactory, but variation in ability to support antibiotic production was observed between different batches, and deterioration

also occurred during prolonged storage. An initial pH of 7.8 and the addition of a fermentable carbohydrate were also beneficial. The yield was greatest after aerobic incubation overnight at 37°C.

32. The antibiotic substance was not obtained in a pure state, but was concentrated by evaporation of crude solutions. These were prepared either by heating, or by adding 10 per cent tri-chlor-acetic acid to broth cultures and centrifuging to remove the organisms. Alternatively bacteria-free material from cellophan-sac cultures was used.

33. The antibiotic was relatively heat resistant, stable under acid conditions but quickly destroyed when alkaline, was slowly dialysable through cellophan, and was inactivated by trypsin but not by pepsin. These properties suggest that it may be polypeptide in nature.

34. On solid media, inhibitory activity by 'Type 71' staphylococci was observed after incubation at 25°C and 30°C, as well as at 37°C, over a wide range of pH under aerobic or anaerobic conditions. It also occurred under these conditions in the presence of high salt concentrations and pooled human serum, but not in the presence of oleic acid.

35. In contrast, two NCTC cultures of Staph. aureus, one weakly

susceptible to phage 29 and the other untypable, both formed wide, hazy zones of inhibition against Corynebacterium diphtheriae. They were not active under anaerobic conditions, and were not susceptible to each other.

36. The antibiotics produced by active staphylococci were specific in their action. Variants selected for their resistance to 'Type 71' organisms remained susceptible to the action of the NCTC strains, and similarly vice versa. From 'Type 71' colonies which had lost their inhibitory power and were therefore sensitive to the active parent culture, it was possible to isolate resistant variants which remained inactive, and which were still susceptible to the NCTC organisms.

37. Using laboratory strains of Str. pyogenes, little experimental evidence was obtained to support the hypothesis that 'Type 71' staphylococci suppressed the growth of some streptococcal serotypes, thus giving an apparent association of others with impetigo. Streptococci producing satisfactory M antigens were slightly more susceptible than those with only T antigens.

38. It was not possible to relate microbial antagonism to the initiation of infection, but it is suggested that, once

established, 'Type 71' activity probably prevents secondary invasion of impetigo lesions by many different organisms.

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APPENDIX A

Details of impetigo patients and the strains of *Staphylococcus aureus* isolated from them in 1953-4

	Case No.	Age in years	Lesion	Nose	Throat	Extent	Duration in days
	1	9	71*(R)	-	-	++	21
	2	1 4/12	71 (R)	-	-	++	15
	3	35	71 (R)	-	-	+	21-28
	4	2	71 (R)	71 (R)	-	++	13
	5	16	71 (R)	3B/3C*(R)	-	+	8
F	6	4	71 (R)	-	-	+	14
F	7	28	71 (R)	-	-	+	7
	8	23	71 (R)	3B/3C*(R)	-	+	10
	9	8	3C/55/71*(S)	-	-	+	28
	10	13	3C/55/71*(R)	-	-	+++	17
	13	1 3/12	52/52A/79*(S)	52/52A/79*(S)	-	+	2
	15	4	79/55/71*(R)	55/71*(R)	55/71*(R)	++	14
	16	8/12	79/55/71*(S)	Not typable(S)	-	++	7
F	17	15	71 (S)	7/54/73 (S)	-	+++	10
F	18	27	71 (S)	-	-	+	3
F	19	1 9/12	71 (R)	-	71 (R)	++	10
F	20	26	Not typable(R)	3B/3C/55/71*(R)	-	+	11+
	21	47	71	71	71	++	10
	22	6	71	71	-	+	6
	23	48	71	-	-	++	10
	24	55	3C/55/71*	3C/55/71*	-	+	10
	25	12	71	55/71*	-	+	14
	26	16	29	29	-	+	5
	27	11/12	71	71	-	++	21+
	28	6	71	71	-	++	5
F	29	13	71	71	-	++	7
F	30	12	71 (R)	71 (R)	71 (R)	+	7
	31	14	71	-	-	+	5
	32	40	71 (R)	-	-	+	4
	33	19	71 (R)	-	-	+++	21
	34	3	79/55 (R)	79/55 (R)	-	++	22
	35	5	71 (R)	-	-	+	10
	36	21	71 (R)	-	52A/79/55(S)	++	14

* At 1000 x R.T.D. F = family groups

(S) = Penicillin sensitive; (R) = penicillin resistant

'Type 71' and 'weak 71' strains are shown in red.

APPENDIX A (Continued)

	Case No.	Age in years	Lesion	Nose	Throat	Extent	Duration in days
	37	16	71 (R)	-	-	+	10
	38	6	71 (R)	71 (R)	-	+	14
	39	14	29/79/3A/3B/3C/55/71/7/42E/54*(S)	52A/79/55(S)	29/79/55/71/6/7/42E/53/54*(R)	+	4
	40	6	71 (S)	71 (S)	-	+	13
	42	6	55 (S)	-	-	++	13
	43	76	71 (S)	71 (S)	-	++	10
F	44	14	71 (R)	-	-	+	7
F	45	3	71 (R)	-	-	+	21
F	46	6	71 (R)	71 (R)	71 (R)	+	12
F	47	4	71 (R)	71 (R)	71 (R)	+	5
	48	26	71 (R)	71 (R)	-	++	7
	49	4	3C/55/71*(R)	-	-	+++	3
	50	2	71 (R)	-	-	++	7
	51	51	Not typable(S)	-	-	+	7
	52	20	71 (R)	-	-	++	7
	53	20	71 (S)	71 (S)	-	++	14
	54	3	71 (R)	-	-	++	5
	55	9	3C/55/71*(R)	3C/55/71*(R)	-	++	21
	56	4	29/52 (S)	-	29/52(S)	++	17
	57	21	29/55/71*(S)	-	-	+	28+
	58	51	53 (S)	-	-	++	10
	60	24	71 (R)	-	-	++	14
	61	39	71 (R)	-	-	+	10
	62	8	71 (S)	71 (S)	-	++	28+
	63	36	3A/3B/3C/55/71*(R)	3A/3B/3C/55/71*(R)	-	++	14+
	64	5	71 (R)	-	71 (R)	++	6
	65	22	3C/55/71*(R)	3C/55/71*(R)	-	+	21
	66	21	6/42E/53/75/77*(R)	-	-	++	7
	67	16	53 (R)	-	-	++	11
	68	9	71 (R)	71 (R)	71 (R)	++	14
	69	2/12	71 (R)	71 (R)	-	++	4
	70	47	3C/55/71*(R)	-	-	++	7
	71	38	3C/55/71*(S)	-	-	++	7
	72	11/12	71 (R)	71 (R)	71 (R)	+++	7
	73	48	71 (R)	-	-	+++	14
	74	7	71 (R)	-	-	++	7
	75	16	71 (R)	-	-	+	7

* At 1000 x R.T.D. F = family groups

(S) = Penicillin sensitive; (R) = penicillin resistant

'Type 71' and 'Weak 71' strains are shown in red

APPENDIX A (Continued)

	Case No.	Age in years	Lesion	Nose	Throat	Extent	Duration in days
F	76	4	55 (R)	55 (R)	-	+	14
F	77	4	55/71 (S)	55 (R)	-	+	3
F	78	2	55/71 (S)	55 (R)	-	+	1
	79	20	55/71 (R)	-	-	+++	4
	80	4	71 (R)	-	-	++	14
	81	19	55 (R)	55 (R)	-	++	14
	82	41	55/71 (R)	55/71 (R)	-	++	28
	84	8	55/71 (R)	55/71 (R)	55/71 (R)	++	21
	85	13	3C/55/71 (R)	3C/55/71 (R)	-	+	21
	86	5	79/55/71/53* (S)	-	-	+	10
	87	17	71 (R)	71 (R)	71 (R)	++	14
	88	46	71 (R)	71 (R)	-	++	21
	89	2	3A/3C/55/71* (S)	3A/3C/55/71* (S)	52A/79*(S)	++	10
	90	2	Not typable(S)	71 (S)	6/7/42E/54 /75 (R)	+++	10
F	91	16	71 (R)	71 (R)	-	++	7
F	92	22	71 (R)	71 (R)	-	+	28+
	93	10	71 (R)	-	-	+	5
	94	4	71 (S)	-	-	+	4
	95	1	71 (S)	Not typable(S)	-	++	7
	96	3	71 (S)	-	-	++	4
	97	11/12	71 (R)	71 (R)	71 (R)	++	7
F	98	5	79/3C/55/71* (R)	79/3C/55/71* (R)	-	+++	21+
F	99	6	3C/55/71/53* (R)	-	-	+	7
	100	11	71 (R)	71 (R)	-	++	11
F	101	5	71 (S)	71 (S)	71 (S)	+++	7
F	102	4	71 (S)	71 (S)	71 (S)	+++	4
	103	8	71 (R)	71 (R)	-	++	5
	104	8	71 (R)	71 (R)	71 (R)	+	6
F	105	20	55/71*(R)	-	-	+	11+
F	106	2	71 (R)	-	-	++	6

* At 1000 x R.T.D. F = family groups

(S) = Penicillin sensitive; (R) = penicillin resistant

'Type 71' and 'weak 71' strains shown in red.

APPENDIX B

Details of patients with infections other than impetigo (Series SI) and the strains of Staphylococcus aureus isolated from them in 1953-54.

Case No.	Age in years	Phage-pattern	Penicillin sensitivity	Source
1	7	52A/79	S	Paronychia
2	71	47	S	Ecthyma
3	54	52A/79	S	Osteomyelitis
4	28	7	S	Perinephric abscess
5	52	3C/55/71*	S	Sycosis barbae
6	16	3C/55/71	R	Fatal septicaemia
7	4	7	R	Axillary abscess
8	40	52A/79	S	Carbuncle
9	17	3C/55/71	S	Brodie's abscess
10	17 3/12	3C/71	R	Osteomyelitis
11	51	79/42D/6/7/42E/53/54/70/73/75*	S	Paronychia
12	24	79/55+	R	Breast abscess
13	54	75/55*	R	Axillary abscess
14	37	79/55*	R	Eczema
15	41	52A/79	S	Ischiorectal abscess
16	19	79/55/71*	R	Peri-anal abscess
17	10	52A/79	S	Atopic eczema
18	59	79/55/71/6/42E/47/53/54/73/77	S	Whitlow
19	7	79	R	Osteomyelitis
20	42	52A/79	R	Boil
21	28	79/55/71*	R	Hand infection
22	32	52A/79	S	Boil
23	39	79/55	R	Whitlow
24	6	29/52	S	Cervical abscess
25	40	79/55*	S	Boil
26	6	55	S	Inguinal abscess
27	4/12	42D/3C/55/71	S	Axillary abscess
28	30	42D/55/71	S	Peri-anal abscess
29	62	52A/79/55	S	Boil
30	31	29/71/47/53*	S	Breast abscess
31	28	55	R	Breast abscess
32	9/12	55	R	Axillary abscess
33	44	55/7	R	Contact dermatitis

* At 1000 x R.T.D. 'Type 71' and 'weak 71' strains are shown in red.

S = penicillin sensitive; R = Penicillin resistant.

APPENDIX B (Continued)

Case No.	Age in years	Phage-pattern	Penicillin sensitivity	Source
34	30	55	S	Boil
35	6	55	S	Boil
36	11	42D/3C/55/71	S	Boil
37	12	42D/3C/55/71	S	Boil
38	35	3C/55/71	S	Boil
39	12	79	S	Boil
40	18	52/52A*	S	Boil
41	60	55	R	Boil
42	75	55	S	Whitlow
43	14	52A	S	Osteomyelitis
44	72	55	R	Incision abscess
45	25	29/79/53/42E/75/77	R	Empyaema
46	40	79/55	R	Axillary abscess
47	1	79/55	S	Cervical abscess
48	32	55	R	Breast abscess
49	2	55	S	Papular urticaria
50	10	3A/3B/3C/71*	S	Osteomyelitis
51	49	79/55	R	Boil
52	39	79/55/71/53*	S	Boil
53	49	79	R	Breast abscess
54	73	55	S	Boil
55	27	79	R	Axillary abscess
56	47	79/55/71*	R	Hand infection
57	5/12	79*	R	Axillary abscess
58	27	79/55/71*	S	Boil
59	54	6/7/54/75	R	Varicose ulcer
60	19	55/71/53	S	Boil
61	11/12	79/3A/3C/55/71/54/70+	R	Osteomyelitis
62	43	79	S	Seborrhoeic eczema
63	40	79/55/71/53*	R	Breast abscess
64	18	3B/3C/55/71	S	Cervical abscess
65	5	3C/55/71	S	Boil
66	28	79/6/42E/53/54/70/75	S	Ulcer of hand
67	14	79/3B/3C/71	S	Boil
68(A)	20	3C/55	S	Boil
68(B)	20	6/7/42E/53/54/75	S	Nasal pustule
69	45	79	S	Boil
70	32	79/55/71*	R	Boil
71	64	79/71/53	R	Ischio-rectal abscess

* At 1000 x R.T.D. 'Type 71' and 'weak 71' strains are shown in red.

S = penicillin sensitive; R = pencillin resistant.

APPENDIX B (Continued)

Case No.	Age in years	Phage-pattern	Penicillin sensitivity	Source
72	18	47	R	Conjunctivitis
73	24	79/3B/7/54*	S	Ecthyma
74	44	79/55/47*	R	Boil
75	37	79/55*	R	Breast abscess
76	30	52A/79	R	Knee abscess
77	28	42E	R	Boil
78	16	75*	S	Sycosis barbae
79	44	52A/6/7/47/54/73	S	Boil
80	30	71	S	Pustule ?anthrax
81	36	52A/79	R	Varicose ulcer
82	14	3C/55	R	Cervical abscess
83	4	52A/79	R	Inguinal abscess
84	14	3B/3C/55	S	Osteomyelitis
85	6	52	S	Inguinal abscess
86	12	7/47/53/75/77*	S	Infected haematoma
87	7/12	Not typable	R	Boil
88	2	7/47/54/70/73/75/77	R	Boil
89	58	7/47	R	Inguinal abscess
90	50	7/47/53/54/75/77	R	Incision abscess
91	22	53	R	Conjunctivitis
92	18	52A/7/42E/53*	R	Axillary abscess
93	3/12	52A*	S	Cervical abscess
94	10	3C/71	S	Folliculitis of leg
95	35	79/42D*	R	Breast abscess
96	44	3B/55/71	S	Incision abscess
97	44	Not typable	S	Boil
98	53	52A/79/42D*	R	Incision abscess
99	62	42E/53	R	Incision abscess
100	25	7/70	S	Boil

* At 1000 x R.T.D. 'Type 71' and 'weak 71' strains are shown in red.

S = penicillin sensitive: R = penicillin resistant.

APPENDIX C

Details of 'outside' normal persons (Series ON) and the strains of Staphylococcus aureus isolated from them in 1953-54

No.	Age in years	Nose	Penicillin sensitivity	Throat	Penicillin sensitivity	Nature of disease
1	56	-		-		Warts
2	18	-		-		Mollusca contagiosa
3	35	-		-		Warts
4	23	7/54	S	-		Sprained ankle
5	45	55/71	S	-		Normal
6	58	-		6/7/42E/75	S	Enlarged prostate
7	36	29/79/3C/ 55/71*	S	-		Normal
8	41	79*	S	-		Normal
9	11	-		7/54	R	Sprained ankle
10	48	-		-		Normal
11	38	79/55/73*	S	-		Normal
12	42	29/52	S	-		Acne rosacea
13	40	29/52/52A /79/53/7	S	-		Dermatitis
14	36	-		-		Eczema
15	43	29/52/55	S	-		Normal
16	41	-		-		Normal
17	67	79/55	S	-		Seborrhoeic eczema
18	23	52A/79/55	S	-		Pityriasis rosea
19	12	-		-		Alopecia areata
20	34	-		-		Mole
21	7	-		-		Sprained wrist
22	63	55	S	-		Palmar Hyperkeratosis
23	16	55	R	Not typable	S	Warts
24	16	-		29/79*	S	Warts
25	28	52/70	S	42D	S	Contact Dermatitis
26	13	79/55/71*	S	53	R	Sprained ankle
27	12	29*	S	-		Injury
28	13	-		-		Normal
29	17	-		-		Normal

* At 1000 x R.T.D.

S = penicillin sensitive; R = penicillin resistant

'Weak 71' strains are shown in red

APPENDIX C (Continued)

No.	Age in years	Nose	Penicillin sensitivity	Throat	Penicillin sensitivity	Nature of disease
30	10	-		-		Normal
31	12	-		-		Normal
32	12	Not typable	S	-		Normal
33	14	-		29/79*		Warts
34	17	29/3C/ 55/71*	S	55/71*	S	Warts
35	13	52A/79/ 55/71*	S	52A/79*	S	Warts
36	13	79/71*	S	-		Alopecia areata
37	14	-		-		Warts
38	17	79	R	79	R	Warts
39	12	52/52A/ 79/7/47	S	-		Warts
40	14	-		-		Warts
41	4/12	-		-		Haemangioma
42	6/12	-		-		Naevus
43	8/12	-		-		Naevus
44	10	-		-		Warts
45	7/12	-		-		Naevus
46	9	-		-		Warts
47	6	-		-		Warts
48	8	79/3B/3C/ 55/71	S	-		Warts
49	11	Not typable	S	-		Warts
50	6	-		-		Warts

* At 1000 x R.T.D.

S = penicillin sensitive; R = penicillin resistant

'Weak 71' strains are shown in red.

APPENDIX D

Details of 'hospital' normal patients and staff (Series HN) and the strains of Staphylococcus aureus isolated from them in 1953-54

No.	Age in years	Nose	Penicillin sensitivity	Throat	Penicillin sensitivity	Nature of Disease
1	27	-		-		Staff
2	20	-		-		Staff
3	40	-		-		Staff
4	24	-		-		Staff
5	18	52A/79/55	R	-		Staff
6	30	-		-		Staff
7	22	-		-		Staff
8	26	Not typable	R	-		Staff
9	23	52A/79	R	-		Psoriasis
10	74	-		-		Hemiplegia
11	50	-		-		Duodenal ulcer
12	52	-		-		Varicose ulcer
13	79	-		-		Prostatic Carcinoma
14	45	Not typable	R	-		Eczema
15	38	52/79/55/71*	R	-		Varicose ulcer
16	33	6/7/42E/47/-54/73/75/77	S	-		Psoriasis
17	56	Not typable	R	-		Eczema
18	49	79/55*	S	-		Eczema
19	45	52A/79	R	-		Seborrhoeic eczema
20	35	Not typable	S	-		Hyperthyroidism
21	37	55/7	R	-		Eczema
22	54	Not typable	S	-		Cholecystitis
23	50	-		-		Contact dermatitis
24	21	-		-		Eczema
25	40	52A/55	R	-		Intermittent claudication
26	45	-		-		Varicose veins
27	39	Not typable	S	Not typable	S	Reticulosis
28	54	-		-		Lymphadenopathy
29	55	-		-		Rectal neoplasm
30	26	55	S	-		Seborrhoeic eczema
31	56	29*	S	-		Erythrodermia
32	29	55/7	S	-		Pruritis ani

* At 1000 x R.T.D.

S = penicillin sensitive; R = penicillin resistant

'Weak 71' strains are shown in red.

APPENDIX D (Continued)

No.	Age in years	Nose	Penicillin sensitivity	Throat	Penicillin sensitivity	Nature of Disease
33	25	29/79/55	S	-		Appendicitis
34	27	-		-		Atopic eczema
35	40	3B/3C/55	S	-		Varicose ulcer
36	13/12	Not typable	R	-		Infantile eczema
37	2	29*	S	-		Infantile eczema
38	12	55	R	-		Chest investigation
39	9	53*	S	-		Atopic eczema
40	7	-		-		Chest investigation
41	1	3B/3C/55	S	-		Loss of weight
42	8	55	R	-		Seborrhoeic eczema
43	12	-		-		Aseptic meningitis
44	2	-		-		Aseptic meningitis
45	5	3B/3C/55/71	R	-		Splenomegaly
46	7	42E/75/77	R	42E/75/77	R	Anaemia
47	1	-		75/77	R	Haemangioma
48	10/12	42E/75/77	R	-		Infantile eczema
49	5	7/42E/54/73	R	-		Enuresis
50	1	-		55	S	Infantile eczema

* At 1000 x R.T.D.

S = penicillin sensitive; R = penicillin resistant

'Weak 71' strains are shown in red

APPENDIX E

Details of impetigo patients and the strains of *Streptococcus pyogenes* isolated from them in 1953-4

Case No.	Age in years	Lesion A*	Lesion P**	Nose A*	Nose P**	Throat A*	Throat P**	Extent	Duration in days
4	2	1	1	-	-	-	-	++	13
9	8	33	3/13/ B3264	-	-	-	-	+	28
F 11	4	-	3/13/ B3264	-	-	-	-	++	4
F 12	3	-	3/13/ B3264	-	-	Group C		++	4
F 13	1 3/12	-	3/13/ B3264	-	3/13/ B3264	-	-	+	2
F 14	15	-	5/27/ 44	-	-	-	-	+	3
41	3	-	6	-	-	-	-	+++	15
42	6	1	1	1	1	1	1	++	13
56	4	-	8/25/ 10	-	-	-	8/25/ 10	++	17
59	9	28	4/28	28	4/28	-	-	++	7
62	8	-	8/IMP. 19/10	-	8/IMP. 19/10	-	8/IMP. 19/10	++	28+
63	36	28	28	28	28	28	28	++	14+
68	9	-	5/11/ 27/44	-	-	-	-	++	14
83	34	1	1	1	1	-	-	++	5
86	5	6	6	6	6	-	-	+	7
F 98	5	Not typable		-	-	Not typable		+++	21+
F 99	6	Not typable		-	-	-	-	+	7
102	4	-	4	-	-	-	-	+++	4

F = family groups

* A = Slide agglutination reaction

** P = Precipitin reaction

APPENDIX F

Origin, phage-pattern and some properties of 100 strains of Staphylococcus aureus isolated from routine specimens during 1959-60

Strain No.	Phage Pattern	Penicillin sensitivity	Serum opacity reaction	Egg-yolk opacity reaction	Inhibition of corynebacteria	Origin
1	42E	R	-	+	-	Conjunctivitis
2	3C/55+	S	-	+	-	Boil
3	80*	R	-	+	-	Lung P.M.
4	Not typable	R	-	+	-	Throat swab
5	52/52A/79/80/7/42E	R	-	+	-	Lung P.M.
6	52A/80*	S	-	+	-	Abscess
7	Not typable	R	+	-	-	Skin infection
8	52A/7/42E+	R	-	+	-	Boil
9	29/77*	R	-	+	-	Ear swab
10	52A/79+	S	-	+	-	Ear swab
11	52A/79+	R	-	+	-	Breast abscess
12	52A/79*	R	-	+	-	Abscess
13	Not typable	R	-	+	-	Pustule
14	52A/80/7+*	S	-	+	-	Nose swab
15	52/52A/79/80/7/42E	S	-	+	-	Nose swab
16	52A/80/7/54+	S	-	+	-	Nose swab
17	52A/80/7+*	S	-	+	-	Nose swab
18	79/7	R	-	-	-	Hand infection
19	Not typable	R	-	+	-	Boil
20	52A/79+	R	-	+	-	Throat swab
21	42E	R	-	+	-	Abscess
22	52A/80/7/42E/54+	S	-	+	-	Pustule
23	7	R	+	-	-	Leg infection
24	29/80+*	R	-	+	-	Skin infection
25	52/52A/79/80/55/71/53/77+	S	-	+	-	Nose swab
26	52/52A/79/80*	S	-	+	-	Conjunctivitis
27	3C/55/71	S	-	+	-	Abscess
28	7/47/53/54/75+	R	-	+	-	Sinus of femur
29	52	S	-	+	-	Ear swab
30	3C/55/71	S	-	+	-	Boil

* At 1000 x R.T.D. S = penicillin sensitive; R = penicillin resistant

'Type 71' strains are shown in red

APPENDIX F (Continued)

Strain No.	Phage Pattern	Penicillin sensitivity	Serum opacity reaction	Egg-yolk opacity reaction	Inhibition of corynebacteria	Origin
31	42E	S	-	+	-	Hand infection
32	42E	S	-	-	-	Skin infection
33	71	R	+	-	+	Impetigo
34	29/52/80+	R	-	+	-	Conjunctivitis
35	Not typable	R	+	-	-	Ear swab
36	Not typable	S	-	+	-	Throat swab
37	42E	R	-	+	-	Throat swab
38	80/42E+	R	-	+	-	Throat swab
39	29+	S	-	+	-	Boil
40	42E	R	+	-	-	Throat swab
41	79/42E/53/77*	R	-	+	-	Abscess
42	42E	S	-	+	-	Pustule
43	52A/79+	R	-	+	-	Nose swab
44	3A	R	-	+	-	Wound infection
45	52A*W	R	+	-	-	Ear swab
46	53/83	S	-	+	-	Nose swab
47	52A	R	-	+	-	Boil
48	29/52/80+	R	-	+	-	Nose swab
49	29/52/80+	R	-	+	-	Throat swab
50	Not typable	R	-	+	-	Nose swab
51	29/47/53/75/77+*	R	-	+	-	Throat swab
52	Not typable	S	-	-	-	Nose swab
53	29/52/52A/79/80+	S	-	+	-	Nose swab
54	80/47/54/75+	S	-	+	-	Faeces
55	29/52*	R	-	+	-	Conjunctivitis
56	Not typable	R	-	+	-	Ear swab
57	79/53/77+*	R	+	-	-	Conjunctivitis
58	52/52A/80/7/42E/81+	R	-	+	-	Throat swab
59	52A	R	-	+	-	Conjunctivitis

* At 1000 x R.T.D. S = Penicillin sensitive; R = Penicillin resistant

'Type 71' strains are shown in red

APPENDIX F (Continued)

Strain No.	Phage Pattern	Penicillin sensitivity	Serum opacity reaction	Egg-yolk opacity reaction	Inhibition of Corynebacteria	Origin
59	52A	R	-	+	-	Conjunctivitis
60	29	S	-	+	-	Faeces
61	77+	R	-	+	-	Eye swab
62	52/79/80*	R	-	+	-	Faeces
63	79/80*	R	-	+	-	Food
64	29/52/80+*	R	-	+	-	Skin lesion
65	80*	R	-	+	-	Nose swab
66	52/52A/80/7/42E/81+	R	-	+	-	Boil
67	Not typable	R	-	+	-	Throat swab
68	29/52/80+	R	-	+	-	Eye swab
69	52A+	R	-	+	-	Finger infection
70	3C/55/71	R	-	+	-	Boil
71	55/71	R	-	+	-	Skin infection
72	71	R	+	-	+	Impetigo
73	55/71	R	+	-	+	Conjunctivitis
74	79	S	-	+	-	Boil
75	29+	R	-	-	-	Nose swab
76	Not typable	R	-	+	-	Boil
77	Not typable	R	-	+	-	Umbilical swab
78	52/80/53/54*	S	-	+	-	Throat swab
79	71	R	+	-	+	Conjunctivitis
80	79/80+	R	-	+	-	Nose swab
81	81*	S	-	+	-	Hand infection
82	29*	S	-	+	-	Skin infection
83	52/80/7+	R	-	+	-	Wound infection
84	71+	S	-	-	-	Urethral discharge
85	Not typable	R	-	+	-	Eye swab
86	29/52/80+	R	-	+	-	Umbilical swab
87	42E	R	-	+	-	Throat swab
88	7/53/54/75/77/83w	R	-	+	-	Umbilical swab

* At 1000 x R.T.D. S = Penicillin sensitive; R = Penicillin resistant

'Type 71' strains are shown in red

APPENDIX F (Continued)

Strain No.	Phage Pattern	Penicillin sensitivity	Serum opacity reaction	Egg-yolk opacity reaction	Inhibition of corynebacteria	Origin
89	80/81	R	-	+	-	Nose swab
90	71	R	+	-	+	Pemphigus neonatorum
91	71+	R	+	-	+	Pemphigus neonatorum
92	52/52A/79/80/81/7/54+*	R	-	+	-	Throat swab
93	55/71	S	-	+	-	Skin infection
94	71	R	+	-	+	Pemphigus neonatorum
95	7/47/75+	S	-	+	-	Eye infection
96	83*	S	-	+	-	Wound infection
97	29/52+	R	-	+	-	Pustule
98	83+*	R	-	+	-	Wound infection
99	42E	S	-	+	-	Pustule
100	29/52+	S	-	+	-	Skin infection

* At 1000 x R.T.D. S = Penicillin sensitive; R = Penicillin resistant

'Type 71' strains are shown in red