STAPHYLOCOCCAL INFECTION IN THE MATERNITY HOSPITAL

JANET JELLARD

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

PREFACE

The work which is presented here is the result of two years' study of the epidemiology of Staph. aureus in two maternity hospitals. This was undertaken because there was an outbreak of severe infection at the end of 1953 in St. David's hospital (the older of the two). A small survey was made of the distribution of Staph. aureus at that time, but as the situation improved after the nursery and wards had been closed and cleaned, nothing further was done. By November 1954 sepsis had become a serious problem once more and a request was made for a detailed investigation. From this, two trends became clear;

(1) Staph. aureus was widely distributed in the environment and amongst the babies and staff.

(2) Most of the lesions were associated with the presence of a single strain of the organism, although it was not the most common one.

The problem of staphylococcal infection of the new-born has been approached in two ways according to these two observations, which might be described as quantitative and qualitative:

a) Why is this organism so common in maternity hospitals? How is it introduced and spread and how can its dissemination about the hospital be prevented?

b) Is there a difference in the behaviour of different strains as identified by phage typing, some being constantly more virulent than others? And if this is the case, can this difference be demonstrated in any in vitro tests?

This thesis is, therefore, divided into two sections, one on epidemiology and the other on the virulence of different phage types of Staph. aureus. Most of the work was carried out, not in the old hospital where the seriousness of the problem was first appreciated, but in a new one, the Cardiff Maternity Hospital, which was opened at the end of April, 1955. The opportunity of investigating the incidence of staphylococci among the staff and in the building before the hospital was opened for patients was a very valuable one.

The main work has been described in narrative form rather than written up as an experiment, for each investigation led to another one and chance observations often formed the basis of the next part of the study. Some laboratory experiments are described separately in Appendix I and II.

A list of the articles or books which I have read and referred to is given at the end of the thesis. It is arranged alphabetically according to the name of the author.

The word 'staphylococcus' occurs frequently in this work. I use it to mean Staph. aureus unless it is specifically stated otherwise.

I would like to thank a number of people who have helped me in this work: Dr. R. E. O. Williams for teaching me staphylococcal phage typing; Dr. J. Marks for his interest and encouragement; Dr. C. C. Spicer for advice on the statistical analysis of the results in Appendix I, and Dr. J. M. Dixon for putting down settling plates when I had to leave my investigations in May 1955. I am also grateful to Mr. J. Napper of the Pathology department at Cardiff Royal Infirmary for the photographs.

Lastly, I am very much indebted to Miss Dorothy Slade who has been responsible for typing this thesis and to my husband, Dr. C. H. Jellard, for help with reading and correcting the typescript.

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HISTORICAL INTRODUCTION

The history of staphylococcal infection of the newborn falls naturally into four periods. The first is the time before the discovery of bacteria, when extensive outbreaks of pemphigus neonatorum occurred, particularly in the large maternity hospitals on the Continent. Mortality was high and the striking clinical picture and obviously contagious nature of the disease led to the publication of a number of epidemiological studies. Once the bacterial cause of infections was recognised the second period was entered upon, with a search for the responsible organism. This continued until the 1920s and was carried on mainly in America because, during the first world war, the disease in its highly fatal epidemic form appeared in many maternity hospitals there. The third period began when the staphylococcal aetiology was finally determined and this organism began to be studied, both regarding its in vitro properties and its distribution among the population. The high carrier rate of potentially virulent staphylococci among normal people made the problem of preventing infection seem insoluble. The last period has seen a continuation of this work, perhaps with a slightly more hopeful outlook. The discovery of antimicrobial agents, advances in typing and classification, studies in air-hygiene and prevention of cross-infection all indicate ways in which the staphylococcal problem may be tackled. At the same time the appearance of antibiotic resistant organisms and the emergence

of what seem to be highly virulent strains have made the situation more serious than ever before.

As much of this later work was published since 1954 (when my own investigations began) I propose to discuss the history of this subject up to that date, and the more recent work after a description of my own studies.

1st Period: Before the Discovery of Bacteria

We now know that staphylococcal infection of the newborn may have various clinical forms of different severity, from small septic spots and mild sticky eyes to massive cellulitis, septicaemia or purulent infections of body cavities. There are few references in the earlier literature to this kind of clinical picture. The form which caused most of the mortality and morbidity in the nineteenth and early twentieth centuries was the affection of the skin known as pemphigus neonatorum. This was essentially a superficial skin condition, with the bulla as the diagnostic feature, which in severe cases developed into exfoliative dermatitis. This clinical syndrome, however, has other causes, which was responsible for much confusion in the past. It is difficult to tell whether the other manifestations of staphylococcal infection which we recognise nowadays also occurred; pemphigus neonatorum was the

one which was always described. It was also called infantile pemphigoid (Cole & Ruh, 1914), pyodermatits neonatorum (Reed, 1929), epidemic staphylococcal vesicular dermatitis (Falls, 1916) and bullous impetigo contagious of infants (Knowles & Munson, 1923).

Ochene, who wrote in 1773, is quoted by Cole & Ruh as providing the first description of this as an independent disease, but according to Rayer* (1835), Charles Lepois described a case of pemphigus in an infant in 1618. The textbook of Brocq (1902) - "La Practique Dermatologique" - is an excellent source of early references. In an historical summary the author says..."depuis longtemps on connait l'existance d'épidémies d'eruptions bulleuses. Thierry en 1736 en a relaté une chez les soldats francais qui occupaient Prague...main c'est surtout chez les enfants que survennent les semblement épidémies, d'où le nom qu'a été donné à cette affection de Pemphigus épidémique des nouveau-nés." He considers that the first incontestible description of an epidemic of pemphigus of the newborn was given by Rigby in 1835 in "Dr. Rigby's Midwifery Hospital Reports." A quotation from this paper gives a good idea of the behaviour of the disease at this time: "During the last year, a disease has prevailed, to a considerable extent, among the children born in hospital, which is otherwise of rare occurrence, viz. the

* quoted by Brocq.

pemphigus infantilis, appearing on the different parts of the body, particularly the face and neck, in minute vesicles, which gradually increase into bullae, and which burst, leaving a raw discharging surface: the ichorous fluid which is secreted inoculates the surrounding parts, and this keeps up the disease for some time. Generally speaking the children have recovered from it, but in two or three it has spread to a great extent, and produced such constitutional irritation as has proved fatal." After describing some of the cases he goes on: "This disease was not confined to the children only but some of the mothers had spots, Mrs. Martyn (the mother of a child who died) in particular, and in a few days after the post-mortem examination during which I pricked myself, several spots made their appearance on my face and chest, which were some days before they healed, and to the pain and irritation which they excited I can testify."

Brocq refers to a number of other papers which were published between this time and 1880, ending with an account of the work of E. Vidal, who, in 1875, studied fifty cases in the maternity department of the hospital of Saint-Louis in Paris. He continues: "Après ces observations precises, la nature contagieuse et épidémique de cette affection ne pouvait plus être mise en doute. Des recherches récentes sont venues mettre ces faits en complete lumière....." and

here follows a list of twenty-three papers written before 1880 and 1900. From their titles alone it may be seen that epidemics of pemphigus of the newborn were occurring often enough to be a problem in France, Germany, Scandinavia, Austria, Hungary, Russia, Hong Kong, America and Britain.

One paper of particular interest was published in England much earlier by Tilbury Fox, in 1864. He described a disease which he called Impetigo contagiosa or Porrigo, and which he said was different from both ordinary impetigo and pemphigus. It often followed on vaccination and usually occurred in children. It was characterized by umbilification, purulent contents, and by its highly contagious nature, which was proved by inoculation studies with material from a lesion. This may have been the kind of non-bullous infection which we see nowadays.

Although the contagious nature of the disease was by now in no doubt, there was a good deal of confusion about its aetiology. Devergie*, who made a classification of pemphigus in 1863, described pemphigus of the newborn as being always syphilitic. Brocq refers to "les discussions si passionnées qui ont lieu dans les sociétés savantes de 1835 à 1875 sur le pemphigus syphilitic ou non-syphilitic." An interesting paper belonging to this era is that of Kilham, published in America

* quoted by Brocq.

im 1889. He describes, as rarities, two small outbreaks of pemphigus neonatorum, and goes on to say: "I am unable to find any literature on the subject in American and very little in English works, though many sporadic cases have been reported, and also cases of syphilitic pemphigus. Very remarkable epidemics have occurred from time to time in the great Maternities on the Continent, the most notable being the one in Paris in 1867, reported by Hervieux, in which 150 cases were observed; the one in 1869 in Halle, of one hundred cases reported by Olshausen; and the one in Dresden in 1878-9, in which 170 cases were reported by Winkel. Olshausen, Koch and Dohrn report epidemics following in the wake of certain midwives, one woman enjoying the experience of 23 cases in her practice, while 200 infants delivered by other midwives in the same city escaped. All observers agree that the season of the year, sex and constitution of the child have no effect either on the development of the disease or on its usual benign course. Regarding the aetiology of the disease, the question still hangs in the balance. Henoch stated that he had seen only sporadic cases. Bohn regards it as only the desquamation which occurs physiologically in the first week, enormously increased by some external irritation, as, for instance, baths of too high a

temperature. Among those who have personally observed the disease in institutions, the weight of opinion is decidedly in favour of its being contagious. Numerous experiments have been tried in inoculating both the lower animals and man, but with unsatisfying results. In three cases, inoculation produced a bleb, but no crop of vesicles. In several cases, mothers have developed single blebs on the breast, and nurses on the fingers. The bacillus is said to have been discovered by Birch-Hirschfeld, and in 1881, by Gibbier... Against the common assumption that pemphigus in the newborn is always syphilitic may be urged that there are no other signs of syphilis and that recovery takes place without medication."

2nd Period: The search for a Bacterial Course of Pemphigus.

Kilham was not particularly interested in bacteriology, but among those who were, much work was in progress on the aetiologic agent of the disease. From the time that cocci were first seen in septic lesions (Koch 1879) and cultivated in broth (Pasteur, 1880) and on solid media (Rosenbach, 1884), attempts were made to discover the bacterial cause of the pemphigus lesion. Most workers considered this to be a coccus; some said staphylococcus, others streptococcus, and some thought that there was an organism which was specific to the disease. Demme, in 1886, is said to have been the

first to cultivate an organism, described as a non-chromogenic diplococcus, from a case of pemphigus neonatorum (Falls, 1917). Falls, who produced several papers on pemphigus neonatorum, wrote in 1917 that "the causative organism is a strain of Staphylococcus aureus, indistinguishable culturally and biologically from other strains of staphylococcus, but differing under certain circumstances morphologically and showing different pathogenic tendencies." He described how he produced a vesicle in his own skin by inoculating staphylococci which he had cultured from a lesion, and from which he recovered the organism. McCandlish, who wrote a good account of the disease and its history in 1925, stated that Matzenauer showed that Staph.aureus was the aetiologic agent, causing both impetigo contagiosa and pemphigus neonatorum. A paper by Cole and Ruh, which was published in America in 1914, summarizes the work done on the bacterial cause of the disease: "There has been much discussion concerning the aetiologic agent of the disease. Kaposi believed it to be simply an increased physiologic exfoliation taking place after birth and others of the older writers mentioned mycosis, while Vargas in his cases thought that toxins were to blame. The following authors found the staphylococcus in the skin lesions in a number of cases: Lewandowsky, Engmann, Call, Macguire, Schwartz and Kohler. Della Favera and Hofmann not only found the staphylococcus

in the vesicles, but also in the blood. Almquist in 1890 studied an epidemic in the Maternity Hospital in Gottenborg. Despite all attempts at disinfection and isolation, the epidemic lasted six months, and 134 of the 216 children were attacked and four died. Several of the mothers were also affected with lesions on the breast. He regularly isolated an organism much like Staph. aureus with which he inoculated himself and produced vesicles. He considered, however, on account of cultural characteristics, that it should be differentiated from the staphylococcus. Clegg and Wherry have isolated a diplococcus much like Almquist's organism, which they term Micrococcus pemphigi contagiosa. They state that it was found by them in all of the five cases investigated by them in the Manila Civil Hospital. Moreover, they state that practically every child in this hospital has pemphigus neonatorum. Manson has also found a diplococcus, while Holt, in one case, found the staphylococcus, streptococcus and B. lactis aerogenes at necropsy. Foerster asserts that there is no one causative organism, while Bloch in a carefully studied series of 15 cases from Baginsky's clinic, found on the eight coming to necropsy, pure cultures of streptococci in the blood and staphylococci in the skin lesions. He thought that in all of them there was a mixed infection and that the streptococci worked the deleterious influence. The

majority of writers, however, feel that the staphylococcus bears an aetiologic relation to the disease."

It was Sabouraud who was responsible for the confusion between streptococci and staphylococci. He used serum broth as an enrichment medium for the material he examined, and isolated streptococci from the majority of pemphigus lesions. He insisted that it was this organism which caused the disease, and that the staphylococcus was merely a secondary invader. The controversy lasted for nearly thirty years. In 1927, Haxthausen described how he was able to find the relative number of streptococci in lesions from various skin diseases by using a plate containing crystal violet. The numbers isolated from cases of pemphigus neonatorum were so small and so inconstant that he considered that this organism could not be the aetiological agent, and decided in favour of the staphylococcus.

Another contraversial point was the relationship to impetigo contagious and to exfoliative dermatitis (Ritter's disease). Cole and Ruh, in the paper already quoted, conclude that the former is a separate disease, caused by the streptococcus. They add, "There is one rare type of impetigo, however, from which we must admit that infantile pemphigus

can without question be contracted. We refer to impetigo Bockhart Staphylogenes, a disease characterized by isolated, discrete, thin, varnish-like crusts...." The authors also considered that Ritter's disease is simply a malignant type of pemphigus.

It may be guessed, from these American papers, that the disease in its highly infectious and virulent form had appeared on the other side of the Atlantic after the turn of the century. It is interesting to speculate on the reasons for this. It may have been because more confinements were taking place in Maternity Hospitals in the States, so that newborn babies were crowded together in nurseries. There was very little knowledge at that time of how organisms are spread in hospitals, and consequently cross-infection would be common. Numerous papers are available which give details of some of these catastrophic epidemics. Falls, for example, described an epidemic in Chicago in 1916 in which there were 100 cases and 4 deaths. Knowles and Munson in 1923 gave some mortality rates from recent epidemics:- 30% found by Biddle in Detroit in 1911, 25% of 27 by Schwartz in New York in 1908, 33% of 30 by Knowles in Philadelphia, 30% by Pusey in Chicago and half of the affected number of infants in a small outbreak in Philadelphia recorded by Hartzell. In the outbreak described in 1914 by Cole & Ruh there were 9 cases and 1 death.

Most of these workers were concerned with the termination of an outbreak once it was established, rather than how to prevent the infection from entering the hospital. In 1927, Falls wrote a paper discussing the origin of the disease, which he noticed occurred in well run up-to-date hospitals as well as in old-fashioned, inefficient ones, and attacked healthy babies as often as the sick. He considered that the infection might be introduced by anyone who had a staphylococcal lesion such as a boil, not only by those with pemphigus or impetigo. This was a new conception. It was discussed in 1931 by Swendson and Lee; they commented that if it were true, "the avenues of infection become appallingly numerous." There was no idea at this time of symptomless carriers; infection was seen to spread from child to child, presumably by the midwife, who, if she did not actually have a lesion, probably carried the contagion on her hands or clothes, (Cole & Ruh, 1914). Belding, writing in 1926 had advanced views which preceded the discovery of nasal carriers by ten years: "The principal source of human transmission is the nurse. In the nursery she is in constant intimate contact with the infants and serves as a means of mechanical transmission even if not a true carrier. The outbreak (described in this paper) on the second floor 12 days after the first followed closely the transfer of a nursery nurse from the first to the second floor. The possibility of true carriers among

the nurses or attendants must always be considered, but their detection is most difficult." Some of the preventive measures recommended by writers at this time are surprisingly modern. Swendson and Lee, for example, considered that a doctor should be appointed to be in charge of prevention in each hospital and that a three nursery system should be set up so that new-born babies should be separated from those already infected and those exposed to infection.

English midwifery at this time was not affected by these large-scale outbreaks, probably because most confinements still took place at home. Small outbreaks of pemphigus did occur, however, in domiciliary practice, which could often be traced to the wake of one particular midwife. For example, Smith, in 1910, described a 'malignant outbreak' which occurred in the practice of one midwife, in which three out of four babies died. Robertsom in 1914 refers to five outbreaks in midwifery practice in Birmingham in the past eight years, in one of which five out of thirteen babies died. In 1925 the disease was serious enough to deserve the publication of a pamphlet by the Central Midwives Board. Pemphigus neonatorum was described as a rare but highly contagious disease of the skin of the new-born, which occurred usually between the 4th and 14th day. The source of the infection was thought to be the midwife in most cases, and preventive measures included early reporting of cases and detection and isolation of the person responsible for the spread. In 1929 two papers appeared in the Lancet describing the type of staphy-

lococcal infection which was prevalent in Britain at that time. Collins and Campbell wrote:- "There does not appear to have been any serious epidemic in this country (of pemphigus neonatorum) for a long period, but during recent years there has been a very considerable increase in the number of cases arising in many areas throughout the country." They describe a series of fifty cases which occurred in domiciliary practice and which were definitely traced to a particular midwife. She had no septic lesion so it was concluded that symptomless carriage must be possible. Benians and Jones described two outbreaks of pemphigus neonatorum - again a rare but highly contagious disease - one of which was traced to a nurse with a boil. Staph. aureus was also found on normal babies, in the nose and on the umbilical cord. The authors conclude that the organism can be carried in what appears to be a harmless form.

By the mid-nineteen thirties the disease had spread to maternity hospitals in this country. Poole and Whittle described an outbreak in a Cambridge hospital in 1935 which was thought to be spread by a nurse. They say that "the epidemic form of pemphigus neonatorum has been rare until comparatively recently, but is now beginning to constitute a serious hospital problem." In the same year, Smith gave an account of an outbreak of staphylococcal pneumonia in a maternity hospital which killed four infants. This was preceded by several cases

of sepsis among babies and mothers, which included cellulitis and breast abscesses but no pemphigus. Apparently normal babies were swabbed and twelve out of fifteen were found to give very heavy growths of Staph. aureus. This is the first description which I have found of the type of outbreak which is so common to-day. Another outbreak of pneumonia was described by McGregor in Edinburgh in 1936. Eight out of ten cases were under a year old, and as there was no influenza in the city at the time, it was suggested that there was increased prevalence or virulence of the staphylococcus. Several other papers stress the increasing importance of staphylococcal infections of infancy in this country. Carter and Osborne introduced a paper in 1936 with, "the nomenclature is unscientific and misleading, the aetiology more than uncertain." The disease had been present in their area since 1926, and in 1933 there were 54 cases of pemphigus and many of folliculitis, with a mortality rate of 38%.

3rd Period: Epidemiological and Laboratory Studies of the Staphylococcus.

As these epidemics became more common and severe, it became a matter of urgency to determine the origin of the infection in order to discover better ways of preventing it. At the same time discoveries were being made in the course of laboratory studies

about the properties of the staphylococcus, and by epidemiological investigations into its distribution. These led to a new attitude to the disease, which was rather more pessimistic than before. Staph. aureus was found to be much more widely spread among the population than had previously been realised, and this common form of the organism appeared to be indistinguishable from that responsible for the most severe disease. To illustrate the despondence caused by these findings, a quotation may be made from a paper published by Brewer in 1937: "as the staphylococcus is almost universally epiphytic upon human hands and cannot be dislodged, anybody can spread it.... The cases (in the outbreak which he describes) were isolated, but as the only isolation likely to be of avail is isolation from human fingers - which is impossible - the isolation was as futile as it was expected to be."

The chief discovery which brought about this change in attitude was that of the high carrier rate in normal people. Hallman in America made the first study in 1937; and found a carrier rate for coagulase-positive staphylococci in 272 normal children of 58·4% and in 109 normal students of 36·7%. McFarlan made a similar investigation in Britain in 1938 and found that 34% of 101 students carried staphylococci which gave alpha lysis. Gillespie, Devenish and Cowan in the

following year made a more detailed study of staphylococcal carriers. They took nasal and skin swabs from 159 medical students for eight months and found the nasal and skin carriage rates to be 43·3% and 19·5% respectively.

At about the same time, much work was being published on the pathogenicity of staphylococci and how their virulence could best be determined. Now that these organisms had been shown to be widespread, it was important to find if they were all equally virulent. Although coagulase and haemolysins had been recognised since the beginning of the century, their relative importance in the pathogenesis of infection had never been made clear. Pigmentation of the colonies was often the only criterion used, or haemolysis on ordinary blood agar plates - which, being of horse blood, told nothing of alpha lysin. As early as 1928, Dudgeon and Hope Simpson compared virulence with the in vitro characteristics of different strains. In 1934, Chapman, Berens, Peters and Curcio published a comparison of coagulase and haemolysis as measures of pathogenicity. Other staphylococcal products and properties were discovered over the next 20 years, such as beta, gamma and delta lysins, hyaluronidase and fibrinolysin. It became generally recognised, however, following the work of Cruickshank in 1937, that coagulase was the final criterion of staphylococcal pathogenicity. It was this knowledge which gave the high carrier

rates of coagulase-positive staphylococci their significance. Each one of these coagulase-positive staphylococci had, for lack of a more specific test of pathogenicity, to be assumed to be fully virulent.

When hospital staff came to be swabbed - especially those working in theatre - the carrier rate was found to be even higher. Hart in 1937 described one of 60 - 80%. It was obviously impossible to exclude all these carriers and there seemed to be no way of finding out which strains were likely to be dangerous - so the situation was not an encouraging one. At this period, however, staphylococci were not as important in hospitals as streptococci, and prevention of cross-infection with the latter was still the more pressing problem.

4th Period: Antibiotics, phage typing and the study of Cross-Infection.

With the introduction of the sulphonamides and penicillin, it seemed as if both problems were about to be solved. But as early as 1945 penicillin-resistant staphylococci were being observed (Spink, Hall and Ferris) and by 1948 were as high as 38% in one hospital (Barber and Rozwadowska-Dowzenko, 1948).

Since then, the problem of hospital cross-infection with antibiotic-resistant staphylococci has become one of the most important of modern medicine, and the history of staphylococcal

infections of the new-born is closely linked to the larger one of cross-infection in general. The search for ways of preventing this may be said to have taken three directions: studies of air hygiene; of carriers and their importance in spreading infection; and the discovery of ways of identifying staphylococci so that their epidemiology could be investigated.

(1) Studies in Air Hygiene

Pioneer work on air hygiene was carried out by Wells and Wells (1936) in America. They showed that the length of time taken by droplets expelled from the nose and mouth to fall to the ground varies with their size, so that the fluid from the smaller ones evaporates and they remain suspended almost indefinitely. This means that these droplet nuclei would be carried much greater distances than had previously been thought possible. The damage caused by infection of wounds during the early years of the war led to studies on the prevention of this airborne spread, for example by the oiling of floors and blankets (Van den Ende and Spooner, 1941) and by ultra-violet light (introduced by Hart in 1937). Epidemiological investigations into the effectiveness of these procedures were made by Crosbie and Wright in 1941 with C. diphtheriae, Wright, Shone and Tucker (1941), Smellie and Wright in 1947 with measles, and Wright, Cruickshank and Gunn with streptococci (1944).

In 1941, a pamphlet was published by the Medical Research Council (War Memo. no. 6) outlining the procedures which should be followed in order to prevent hospital cross-infection. The studies of wound infection made during the war - Miles, Schwabacher and Spooner (1940) and Miles (1941) - were continued afterwards on the problem of industrial wounds, - Clayton-Cooper and Williams (1945), Williams and Miles (1945) and (1949), and burns - Bourdillon and Colebrook (1946). Lowbury in 1954 showed how infection of burns could be reduced from 38% to 17% by the use of special dressing-rooms in which the air was filtered and introduced under positive pressure. The study of aerial contamination was continued by Mackie (1942), Hare and Mackenzie (1946), Duguid and Wallace, (1948) Lidwell and Lowbury, (1950), Rubbo and Benjamin (1953) and Lowbury and Fox, (1953).

(2) Nasal Carriage of Staph. aureus.

Since symptomless carriers of C. diphtheriae and Strep. pyogenes were known to be important in the spread of disease, nasal carriage of Staph. aureus was made the subject of much study. In the first place, evidence had to be obtained that they did scatter their organisms and the mechanism by which this could take place also had to be investigated. Secondly, it was necessary to find out what was the best way of preventing spread from such carriers.

(a) Evidence that nasal carriers disseminate their organisms. Hare and Mackenzie investigated this subject in 1946. They found that while organisms from the throat were expelled into the air during any form of naso-pharyngeal activity, those in the nose were not. Nasal carriers were shown to contaminate the environment by spreading organisms onto their hands and clothes. Staphylococci are also to be found on the skin in 20% of people, as Gillespie, Devenish and Cowan showed in 1939, and usually these are of the same phage type as those in the nose, (Williams, 1946). Spread to the skin from the nose was also demonstrated where the aetiology of superficial skin sepsis was being investigated, by Miles, Williams and Clayton-Cooper (1944) in the case of industrial wounds, Hobbs, Carruthers and Gough (1947) with sycosis barbae and Moss, Squire and Topley (1948), Valentine and Hall-Smith (1952) and Tulloch (1954) with recurrent folliculitis. Duguid and Wallace (1948), in an important study of the spread of organisms from a subject carrying out various activities in a dust-proof cubicle, showed that large numbers of organisms were liberated by healthy people by ordinary movements, and especially by dressing and undressing. Nasal carriers of Staph. aureus spread these organisms from their clothes although they did not speak and wore masks. In 1956, Hare and Thomas made a detailed study of the spread of Staph. aureus from nasal carriers. They came to the conclusion that

the main route of transfer is by hands and handkerchiefs to the skin and clothes; from there, organisms are released into the air by friction and spread by air currents. They found that while patients with minor staphylococcal lesions had slightly more contamination of their clothes than nasal carriers without sepsis, non-nasal carriers with sepsis spread their organisms very much less. Some nasal carriers gave a much higher degree of air contamination than others and were probably more dangerous than clinically infected people. They also found a surprisingly large amount of spread during hand-washing, and that wet hands were more dangerous than dry ones.

(b) The prevention of spread from Nasal Carriers. Much work had been done on the use of nasal ointments containing anti-biotic substance for this purpose. Early experiments were made by Delafield, Straker and Topley (1941) on the value of antiseptic snuffs in the treatment of diphtheria carriers. Barber, Hayhoe and Whitehead, in 1949, used sulphonamide cream for staphylococci but found the effect to be only temporary. Gould and Allan (1954) treated 34 staff carriers with oxytetracycline cream. They found that this reduced the carriage rate a great deal, but that after three months it had returned to its original level. In 15 cases the phage type was the same as before. No resistant strains were found, and the infection rate in babies was said to

have fallen with the carriage rate in the staff. In 1955, Gould made a further study. 124 carriers were chosen, and a cream containing 1% antibiotic was applied to the nostrils every day for 14 days. 69% were negative 2 weeks later, 49% after 4 weeks and 20% after 20 weeks. Resistant strains were obtained from 24 carriers, but they were either a different type or untypable. In 1956, Rountree, Heseltine, Rheuben and Shearman carried out a similar trial, using an ointment containing Neomycin and Bacitracin. A week after this had been applied by 68 members of staff, 43·7% were negative, 34·3% carried their original strain and 22% had acquired a new one. Infection rates in babies were lowered while the trial was going on, and no resistant strains emerged.

Masks have not been found to be much use in preventing the spread of Staph. aureus from nasal carriers. They may have a value in reminding the wearer not to touch his nose, but often their use leads to more handling of the face than otherwise.

(3) Typing of Staphylococci.

The third approach to the problem of Staph. aureus cross-infection was to look for some way of classifying these organisms and if possible of identifying them so that their behaviour in an epidemic could be followed. If this became clear, a rational method of preventing the spread could be

worked out. The first important paper on this subject was Cowan's classification, published in 1938, which used antigenic differences for typing. This method was used in investigations of outbreaks described by Elliott, Gillespie and Holland in 1941, Hobbs in 1944 and Allison and Hobbs in 1947b. Meanwhile, phage typing had been introduced by Wilson and Atkinson in 1945, who gave an account of two outbreaks of pemphigus and showed how they were able to trace the carrier responsible in both cases. Similarly in 1947, Williams, Sims-Roberts and Cook were able to detect a nurse, a heavy carrier of type 3A which had caused an outbreak. This way of identifying strains of staphylococcus has been used in most of the subsequent investigations and has been one of the most important single discoveries in the history of staphyloccal infection. It was developed by Williams in this country, Rountree in Australia, and Laurell and Wallmark in Scandinavia and has now become a practical as well as a sensitive tool which is available to most routine laboratories in this country.

(4) Investigations into the Mechanism of Cross-Infection by Staph. aureus

Most of the earlier papers in this period stressed the importance of nasal carriers among the staff in the spread of staphylococci. Knott and Blaikley (1944) considered that fomites such as towels, were more often contaminated than dust,

and that spread was by the hands and clothes of nurses who carried the organism. (They found, however, that masking made no difference to the infection rate in babies). Allison and Hobbs, who made a large-scale study in Cardiff in 1947, also came to this conclusion. They said that "the main reservoir of infection was the nasal passages of the nursing staff, whence infection was spread to the infants, probably via the hands". Barber, Hayhoe and Whitehead, investigating an outbreak of sepsis in babies in St. Thomas's hospital in 1949, found that 75% of the staff carried staphylococci of which as many as 83% were resistant to penicillin. In an intensive study of the situation, lasting eight months, they found that a penicillin-resistant strain of phage-type 52A was spreading round the hospital and was responsible for most of the lesions in the babies. The nurses were, they thought, the main source of the infection. Four years later, a similar investigation was carried out (Barber, Wilson, Rippon & Williams, 1953) at a time when there was no severe sepsis. Over half the nurses were nasal carriers of penicillin-resistant Staph. aureus and again the predominant type was 52A. The nostrils of 75% of the babies were colonized and their strains resembled those of the nurses more often than their own mothers'. The actual mode of spread was not determined, though several possible routes of cross-infection were mentioned, nor was it possible to say why no sepsis occurred.

In 1949, Parker and Kennedy described an outbreak of true pemphigus associated with a staphylococcus of phage type 3A. Swabs were taken from all the babies every day for five and a half weeks and from mothers and staff. The epidemic strain was isolated once only from three members of staff and from none of the mothers. 38 out of 54 strains from infants belonged to type 3A during the epidemic, but three months later this type was not found. The significant point about this paper is the low carrier rate among the adults compared with that in healthy babies; for example, cases of pemphigus continued to occur over several weeks when no nasal carrier of type 3A could be found. The authors thought that while the ultimate source must presumably be the nasal passages of the adult, in a crowded nursery the infection may be propagated from 'generation to generation' of infants. Colbeck (1949) came to a similar conclusion: "the staff carrier seems to play a comparatively minor role after the introduction of the infection into the ward. The large reservoir of infection on the babies shows the futility of dealing with the staff alone.... The new-born infant's nose and throat is the important reservoir of infection, from which infection passes to the mother's breast. The initial stage of the epidemic is that of a respiratory infection, and every effort should be made to prevent airborne infection of

the babies." Miller in the following year, described 22 cases of pemphigus and conjunctivitis (of which one was fatal) from which phage-type 3A was isolated. He also considered that the nasal passages of the healthy infant were the main reservoir of infection and said that "it is more than probable that the air of the nurseries, mothers' wards and of the corridor became infected from these sources." (He did not suggest how this could take place). Forfar and his colleagues in 1953 described an outbreak of severe infection in which the antibiotic-resistance patterns of the strains isolated from the lesions and from the noses of the staff and cords of the babies were compared. They found that those from lesions and normal babies corresponded closely, while those of the staff tended to be different. "This suggests that cross-infection is more important than infection from carriers as far as lesions are concerned." They point out the dangers of spread from a lesion itself, for example, a heavily infected purulent conjunctivitis. Webb (1954) came to a similar conclusion after her investigations of an outbreak of breast abscess and infant sepsis in two hospitals in Winnipeg. She stated that it is not necessary for there to be a high proportion of carriers of an epidemic strain in a nursery for an outbreak to occur.

From these papers it may be seen that there was a tendency for opinion to change from the idea that nasal carriers among the staff are responsible both for the intro-

duction of the infecting organism and for its spread. Cross-infection in the nursery from baby to baby seemed to occur, though the methods by which this took place were not clear.

(5) The Possibility that Strains of Staph. aureus may vary in Virulence.

Another way in which opinion was developing was the idea that all staphylococci could not be of equal virulence. This had been assumed by many of the early workers, but the finding that coagulase and alpha lysin were produced in similar amounts by most staphylococci had caused this aspect to be neglected. Belding, in 1926, for example, said that "Since the resistance of the infants should prove the same, our observations and those of others indicate a marked difference in virulence in the strains of the infecting organism." Many workers also have commented on the fact that outbreaks do not occur only in overcrowded and inefficient hospitals: "We see," wrote Falls in 1927, "an institution under competant management function for months with no evidence of pemphigus. Suddenly, a typical lesion appears on one of the babies. From this an epidemic of typical cases can start." Williams, Sims-Roberts and Cook (1947) noticed the same thing: "The usual predisposing causes of the spread of infection, such as overcrowding and understaffing, were absent in this case." Colbeck (1949) and Webb (1954) also found that poor nursing was not inevitably present for an

outbreak to occur: "a nursing consultant reported that these hospitals had better physical facilities and practised more individual technique of infant care than others in the city."

Knott and Blaikley (1944) first tried to make use of this apparent difference in virulence. They devised a system in which an estimate was made of the degree of virulence of staphylococci isolated in a maternity department using coagulase, lysins, gelatin liquefaction and fermentation of sugars. Carriers whose strains came into the "pathogenic" group were excluded from the wards. As soon as these precautions were relaxed, lesions reappeared in the babies. However, these findings were questioned by Barber, Wilson, Rippon and Williams, (1953), who found that most of the staphylococci they isolated came into the "pathogenic" group, so that it would not have been practicable to have removed the carriers.

Much work has been done on the laboratory side in this question of virulence; this will be considered in a later section. So far it has not been found possible to predict this quality, and it may be that it is a question of enhancement by passage - Cass, (1940), Barber and Burston, (1955), Kourilsky and Mercier, (1940). On one aspect of the question there is much more evidence:- that a change in the character and severity of the

lesions caused by the staphylococcus has occurred in the last ten years - or since the emergence of antibiotic-resistant organisms. Whether this is cause or effect is a debatable point, but numerous papers give examples of this fact. In 1947, for example, Guthrie and Montgomery described an outbreak of staphylococcal pneumonia in children. 55 cases had occurred, two-thirds of them in children under six months of age. The babies collapsed and died within two days without any localizing signs, but Staph. aureus was recovered from 40 out of 54 lung punctures and 50% of blood cultures. An outbreak of staphylococcal infection occurred at the same time in a maternity hospital in which 90% of cases and 65% of healthy babies carried the organism. This may be compared with the report of Davis in 1920 that "there appears no clear evidence that staphylococci ever cause respiratory epidemics. As secondary invaders they may play a role, but in this regard they appear to be of decidedly less importance than streptococci or pneumonia." Colbeck described an extensive outbreak of severe staphylococcal infections from Canada in 1949 - mainly of maternal breast abscesses - as many as 15% of the mothers being affected at one time. The strain responsible was lysed by a simple phage filtrate which he called W, and this was isolated from

all the pustules in the babies which were examined and from ten cases of lung abscess which occurred. It was noticed that a number of boils and pustules appeared in the patients' families after they returned home, and also in the hospital staff. Felsen and his colleagues wrote a paper from America in 1951, in which they describe how in their experience, staphylococcal infection has changed in character since 1939, (or since the introduction of antibiotics). In 1941 there was an outbreak of diarrhoea and impetigo neonatorum in the Bronx hospital, and since that time staphylococcal infection has become sporadic, culminating in an outbreak of severe infection in 1950: - three cases of empyema, one of breast abscess, one of bacteriaemia, one abscess of buttock and one of osteomyelitis. Even the milder cases were of folliculitis rather than impetigo. A similar report came from Forfar et al, in Edinburgh in 1953, and Webb in Canada in 1954. The former presented six case histories of babies seriously ill with penicillin-resistant strains of staphylococcus, the disease taking the form of deep abscess and osteomyelitis. Webb described "an unusual incidence of staphylococcal infections of new-born infants and maternal breasts from 1947 to 1951, with its peak in 1948." The outbreak was only controlled when routine treatment of babies with chloramphenicol was started.

In this way, information had gradually been accumulated on staphylococcal infections of the new-born and their mothers. It had been shown that the causal organism was Staph. aureus, and that infection had occurred in great waves, first on the Continent, then in America, and later in Britain, Australia, Canada and Scandinavia. Since staphylococci became resistant to many antibiotic substances, the epidemics they caused became increasingly serious, since the resistant organisms were favoured by the use of the antibiotics which had no effect on them. As to the method of spread, the concensus of opinion was that the organism was introduced by nasal carriers among the staff, and spread round the nursery from one baby to another, with its main reservoir in the nasal passages of the babies. Exactly how this spread took place was not clear, whether mainly by the air or by contact. Although there was no proof on this point, some workers were of the opinion that there were within the species Staph. aureus strains which appeared to be more virulent than others. A few workers had mentioned what seemed to them to be a change in the character of the lesions caused by staphylococci in recent years, from mild skin infections to deep abscesses and severe sepsis. This was, in general, the state of opinion when the work to be described in this thesis was begun in 1954.

MATERIALS AND METHODS.

The basic media used in the investigations described in this paper were the same throughout the work. They were those made in the Public Health Laboratory in Cardiff and used for the routine work of the laboratory.

(1) Nutrient Broth. This was a standard meat infusion made according to the method given by Mackie and McCartney (1950) "Handbook of Practical Bacteriology" 8th edition, page 144.

(2) Nutrient Agar. This was prepared from the broth and New Zealand powdered agar, as described by Mackie and McCartney (page 148).

(3) 8% salt broth was used as a selective medium for obtaining Staph. aureus from swabs taken from fomites. After being plated out, the swabs were immersed in the medium and incubated overnight.

(4) Sheep blood agar plates were used for the routine isolation of Staph. aureus. They were made in the same way as the horse blood plates used in the routine laboratory, but with 5% sheep cells. They were layered plates, the bottom layer being of nutrient agar. Defibrinated sheep blood was obtained each week from the Serum Research Institute at Carshalton. This medium was very satisfactory and proved most useful for the primary isolation of Staph. aureus from swabs as well as from settling plates. Colonies of Staph. aureus could be recognised

Plate I. A photograph of a Sheep Blood Agar plate on which two nasal swabs have been plated. Colonies of Staph. aureus may be recognized by their zones of lysis, and the presence of more than one strain in appreciated.

Plate II. A Sheep Blood Agar selling plate on which there are three colonies of Staph. aureus, one less haemolytic than the others.

after 18 hours' incubation by the zone of lysis round them, although the plates were not incubated in CO_2. After another 24 hours on the bench identification by pigment production could be used as an added check. Plate I shows the typical appearance of a sheep blood agar plate inoculated with two swabs from patients. Plate II is a photograph of a settling plate, on which colonies of Staph. aureus may be recognised by their zones of lysis.

Sheep blood agar plates were first used by Christie, North and Parkin (1946) who found that the production of lysis on them by the staphylococci they were testing for pathogenicity agreed closely with virulence for mice. Marks used them for primary isolation in his study of beat lesions in miners Atkins and Marks (1952), and in another paper published in that year (1952), suggested that they might give a more satisfactory index of pathogenicity than coagulase. He used sheep rather than rabbit blood as he found it less sensitive to non-specific lysins and because it could be obtained commercially. There have been some criticisms of this recommendation, for example by Lack and Walling (1954), but I found the plates ideal for routine work. They are simple to make and interpret, more so, in my opinion, than the other special diagnostic media for Staph. aureus which are in use.

The following tests were used in the epidemiological investigations:

(1) The Coagulase Test. The slide coagulase test of Cadness-Graves, Williams, Harper and Miles (1943) was used on every strain before it was recorded as being Staph. aureus. I intended to use the tube test for the identification of any organisms which had the appearance of Staph. aureus on sheep blood agar plates but did not give clumping in the slide test, but in fact no such strains were encountered.

(2) Antibiotic Sensitivity Tests. These were carried out on a number of representative strains chosen from those phage typed each week. Six-inch plates were used, poured with nutrient agar. A ditch was cut in the agar and filled with 10 ml. agar containing the antibiotic against which the staphylococci were to be tested.

Final concentrations were as follows:

penicillin	-	10 units/ml
tetracycline	-	50 µgm/ml
streptomycin	-	50 " "
chloramphenicol	-	50 " "
erythromycin	-	50 " "

(3) Phage Typing. This was carried out according to the method used at the Staphylococcal Reference Laboratory at Colindale and described by Anderson and Williams (1956). A set of 20 phages, which were supplied from Colindale, were used at first, and later phages 80 and 81 were added. Pools of

phages were not used. The phages were tested each week on their propagating strains of staphylococci (also supplied by the Reference Laboratory) and the routine test dilution adjusted according to the amount of lysis obtained.

EPIDEMIOLOGICAL STUDIES.

Introduction.

The first part of this work is an investigation into the epidemiology of Staph. aureus in a maternity department. This was not planned from the outset but gradually took shape according to events in the hospital. The main idea in my mind, however, was that a long-term study of the behaviour of different strains of staphylococcus, followed by means of phage typing, might be more informative than several single swabbings of all the staff and babies. I was interested to discover something about the following points:

a) How soon babies became colonized by Staph. aureus and which were the first places from which it could be isolated.

b) Whether nasal carriers among the staff or the babies themselves were more important as distributors of staphylococci.

c) Whether spread by the air or by contact played the larger part in staphylococcal cross-infection.

d) Which were likely to be the best methods of preventing staphylococcal cross-infection.

Something was learned about these problems in the course of the following investigations in which phage typing was employed to study the behaviour of different strains of staphylococci in the hospital. These are described in part I. But there was still a great deal to be found out about the mechanism of cross-infection in a maternity hospital and possible ways of preventing it:- points c) and d). Therefore three investigations were

planned which studied the effect on infection rates of various changes im technique. Descriptions of these make up part II of the epidemiological studies.

PART I.

EPIDEMIOLOGY of Different Phage Types.

I. St. David's Hospital.

The maternity unit of St. David's Hospital in Cardiff is housed in an old building which used to be part of the Municipal Hospital. Staphylococcal sepsis had occurred in this hospital for a number of years (it was called St. John's Lodge at that time), and it was the hospital investigated by Allison and Hobbs in 1947. There had been a sharp outbreak of infection among the babies in the winter of 1953-4 which had been controlled by closing the affected nursery and spring-cleaning it and the wards one by one. The strain of staphylococcus which had been isolated from the lesions had been phage-typed at the Staphylococcus Reference Laboratory at Colindale, and shown to be lysed only by phages 52 and 52A at low dilutions. It was resistant to penicillin and oxytetracycline. The clinical picture had been one of deep abscesses and cellulitis in the severe cases and septic spots and conjunctivitis in the mild ones.

In November, 1954, it was reported once more that cases of staphylococcal sepsis were occurring, this time mainly in the premature unit, and a thorough investigation into the whole situation was requested. This was a large undertaking, for the staff numbered 127, and there were eight post-natal wards, a nursery for normal babies and a premature unit which served a large area. However, nasal swabs were taken from 119 members

of staff and a routine for regular swabbing of the babies and sampling of the air in the nursery prepared. This was designed to give a long-term survey of the epidemiology of the hospital rather than the 'cross-section' which would be obtained if all the babies in the hospital were swabbed at one time. Swabs were taken from each baby, wherever possible, on the day of its birth and every day subsequently until Staph. aureus was isolated. Various sites on the babies were also swabbed, their bed linen and clothes and several other places in the nursery. Sheep blood agar plates were put down regularly in the nursery and elsewhere. This regime was continued, with short intervals, for six months.

A great many facts were collected in this investigation, but few conclusions could be drawn from them which had not already been demonstrated by other workers. The dominant impression was of the ubiquity of Staph. aureus. 62% of the staff were nasal carriers on the first examination, and on repeated swabbing this figure rose to 77·3%. Settling plates put down in the wards, corridors and nursery grew many colonies of the organism. It was isolated from clean linen, from the front of nurses' gowns (4 out of 4 swabs were positive)

TABLE 1.

To Show the Early and Widespread Occurrence of Infection with Staph. aureus in Babies less than 27 hours old.

Initial	M	F	W	T	H	W	K	H	K	C	A	B	J	L	K	Y	M	K	M
Age in hours	20	26	27	24	25	$2\frac{1}{2}$	$1\frac{1}{2}$	25	23	22	5	15	15	14	13	$14\frac{1}{2}$	1	5	5
Nose	(+)	+	+	±	+	-	-	++	±	-	-	-	±	++	+	+	-	+	±
Eye						-			(+)	-		-				+	-	-	
Face	(+)																		
Head		++			+	±		+											
Mouth	-	++	+		-												±		±
Hands	+		++			-	+	+								+++	-	++	
Chest	-	++		-	+	±		+++			-								
Axilla	-			-										+++	+	+++			
Back									+	-									
Cord	+	+++	++	++	+++	-	+	+++	+	-	±	-	+++	+++	+	+++	±	+++	++
Groin														+++	+				
Top sheet		++			+	-	+	+			±		++						
Bottom "		+			+	-		+			±	-	++						
Blanket		±					+												
Pillow		+					(+)												

(+) indicates that the swab was only positive after enrichment in 8% salt broth

from the cuffs of doctors' white coats (5 out of 12) and from many sites of the babies and their bed linen. Table 1 shows the results of swabs taken from 19 young babies and their cots, all less than 27 hours old. It may be seen that the umbilical cord was the site most often and most heavily infected. The predominant strain of staphylococcus was not 52/52A, the one which had been isolated from the lesions both in the earlier and the present outbreak, although that was present, but a penicillin-sensitive strain of group III - 6/7/47/54. This was isolated most often from babies and the hospital environment. The nurses, on the other hand, commonly carried another strain, of phage type 52A/79. The distribution of phage types among staff and babies is recorded in the following table:

TABLE 2.

The percentage distribution of three phage types among staff and babies.

	Total no. of staphylococci isolated	52/52A	6/7/47/54	52A/79	other types & not typable
staff	92	15•2	13•0	26•1	45•7
babies	358	16•2	55•6	5•3	22•9

These findings suggest that the babies were not infected entirely by the staff.

Because these strains were so common, it was difficult to make any worthwhile investigations into their epidemiology. Several points are, however, worth recording:

I. Evidence that cross-infection was taking place could be gathered from the following incidents in which the infecting strain was of an unusual phage-type, so that it could be identified easily.

a) A baby was admitted to the premature unit from a hospital in the Rhondda, and a staphylococcus of phage-type 71 was isolated from its cord on March 29th, the day after admission. This type had not been seen in the hospital before. This baby died on April 4th. On April 7th, type 71 was grown from the cord of a new-born baby in a different cubicle and on April 8th from two other day-old infants. The staff of the unit were all swabbed, and no carriers of type 71 were found.

b) A baby born on November 23rd gave a growth of staphylococcus phage-type 7/54/77 from its cord on the day of birth. This type was also carried by the mother. It was isolated from the cords of four other babies subsequently on November 25th, 28th and December 3rd and 13th.

c) On March 1st., a medical student came to work in the nursery for two weeks. He was a heavy nasal carrier of phage type 30; a rich growth was obtained from his nose on March 8th and from the sleeve of his white coat on March 11th. On March 2nd this strain was isolated from a baby's nose and subsequently from other infants on the 4th, 11th, 14th and 15th - eight babies in

all. It was also isolated from settling plates on the 11th, 13th, 14th and 15th. March 15th was this student's last day of work in the nursery and his strain was not isolated after his departure.

d) A doctor's nasal swab grew a staphylococcus of type 55/71 on December 21st. On January 1st. a similar but not identical strain, 3c/55/71 was isolated from the nose of a nurse. On January 12th, the doctor sent in a swab from a boil on his neck, which was recorded as phage-type 3B/71. On 15th January one baby was found to be carrying this strain and on the 23rd it was isolated from the bullae of an infant with pemphigus. On January 31st, a swab from another nurse gave a growth of strain 55/71.

II. A small experiment was carried out on the reputed bactericidal power of vernix caseosa; it is often believed to have this quality and is for that reason left on the babies' skins.

A piece of vernix was obtained from a case delivered by caesarian section, with sterile precautions. It was placed in the bottom of a universal container. 0·02 ml of a 1 in 10,000 dilution of a 3-hour culture of Staph. aureus was

dropped onto this and incubated overnight. Similar drops were allowed to fall onto a blood agar plate. Next day 1 ml. of $\frac{1}{4}$ strength Ringer's solution was added and the bottle shaken for 2 mins. Dilutions were made from this at 1 in 10, 100 and 1000.

A second experiment was carried out in the same way using a 1 in 10,000 dilution of a 24 hour culture.

Results: Expt. 1 - inoculum: 3950 cols./ml.
colony count after incubation in vernix:
uncountable at 1 in 1000 dilution.

Expt. 2 - inoculum: 9,000 orgs./ml
colony count after incubation:
500,000 colonies/ml.

Allison and Hobbs made a study of vernix obtained from ten infants in 1947. One sample was sterile; Staph. aureus was isolated from two. Five were tested for lysozyme and all were found to contain some.

III. The results of the main work, the daily swabbing of new-born babies and sampling of the air are worth recording. In order to make conditions as standard as possible, only swabs from infants aged 12 to 24 hours were considered. A record was kept of the number of babies in the nursery each day and of the average number of Staph. aureus colonies isolated from four blood agar plates put down for four hours each day.

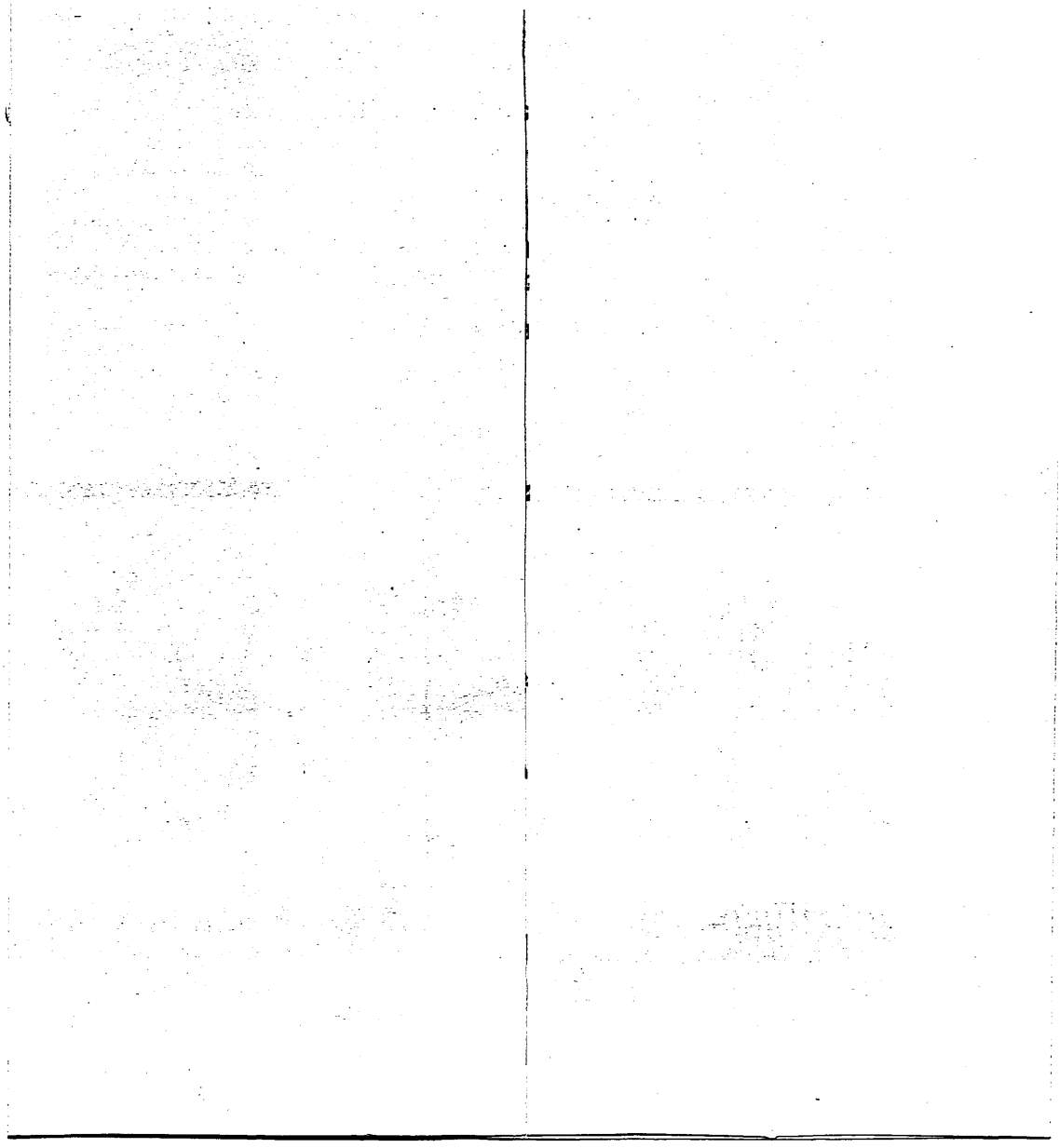
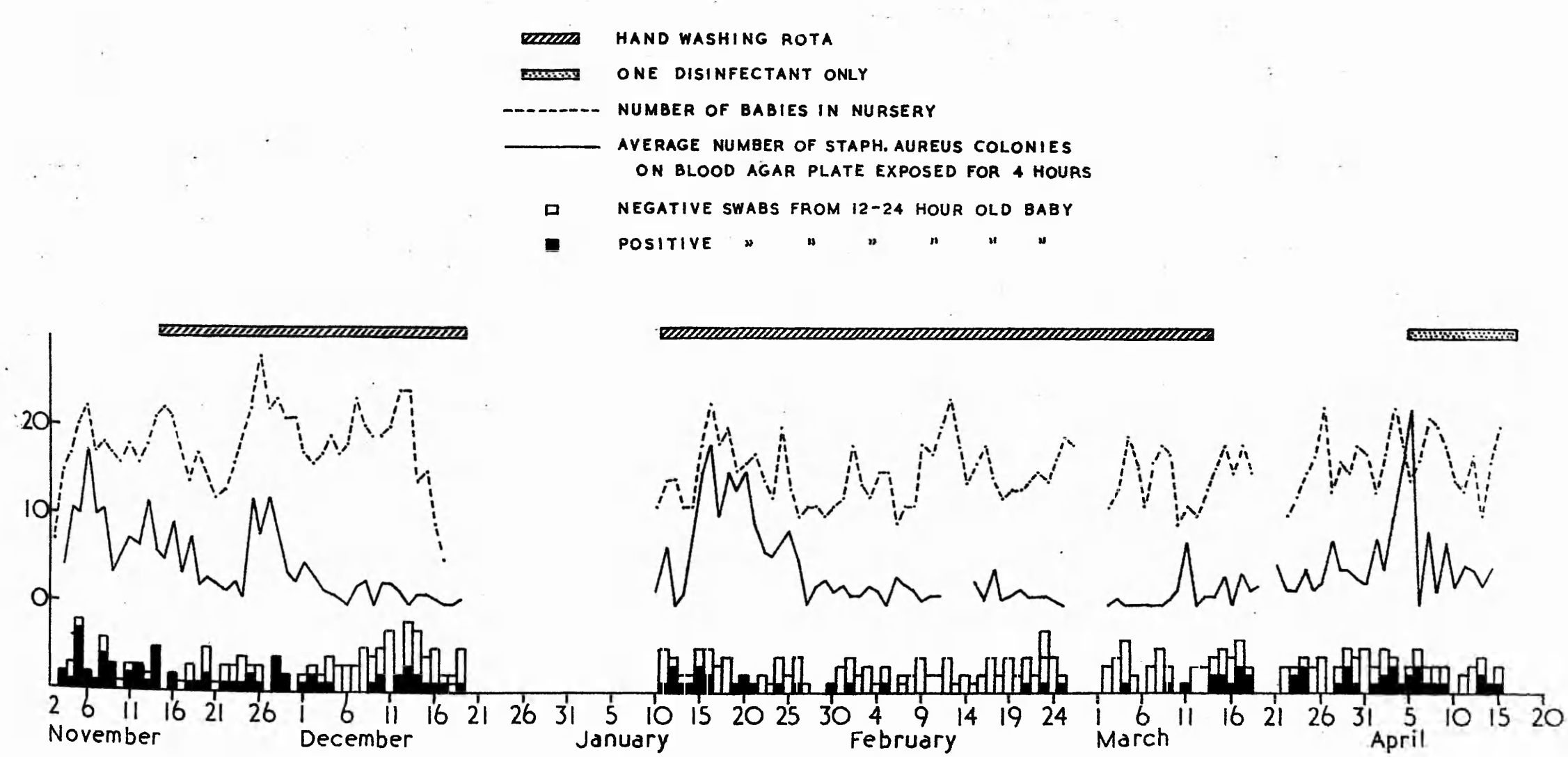


Diagram 1.

A Comparison of the Aerial Contamination in a Nursery, the number of Babies present, and the Infection Rate in Babies.


HAND WASHING ROTA
ONE DISINFECTANT ONLY
NUMBER OF BABIES IN NURSERY
AVERAGE NUMBER OF STAPH. AUREUS COLONIES ON BLOOD AGAR PLATE EXPOSED FOR 4 HOURS
NEGATIVE SWABS FROM 12-24 HOUR OLD BABY
POSITIVE » » » » » »
20
10
0
2 6 11 16 21 26 1 6 11 16 21 26 31 5 10 15 20 25 30 4 9 14 19 24 1 6 11 16 21 26 31 5 10 15 20
November
December
January
February
March
April

These figures are plotted in diagram 1. It may be seen that there is at times a similarity between the curves for the number of babies and the number of colonies on the settling plates. There is another factor, however. While this investigation was going on, Dr. Jacobs, the paediatrician at St. David's, was carrying out a clinical investigation on the effect of the use of various hand creams on the incidence of clinical infection in babies. Whatever the effect of the different substances, which were used in a three-day rota, the general result was to make the nurses highly conscious of their hands. When the times at which the hand-washing rota was in force was added to the diagram, an apparent correlation is seen between this and the infection rate in the babies. An exception to this is at the beginning of March, which is the time when the heavy carrier mentioned in the previous section was present in the nursery.

On the whole, the investigations in this hospital were a disappointment. I did, however, receive a number of impressions which I was anxious to verify:-

(1) that cross-infection was common; but whether it took place by airborne spread or by contact needed to be investigated further.

(2) that the babies themselves were rich sources of staphylococci.

(3) that not all strains of staphylococcus behave in the same way, for example strain 6/7/47/54 which was common in normal babies, strain 52/52A which appeared to be associated with many

of the lesions and strain 52A/79 which was isolated more often from the staff than from the babies or the environment.

(4) that while nasal carriers could introduce infection, their part in its spread round the hospital needed to be investigated.

At this point, in the spring of 1955, I was informed that a large new maternity hospital was about to be opened in Cardiff, and was asked if I would carry out research on staphylococci there. This provided the ideal opportunity for attempting to confirm the impressions gained in the old hospital, using the experience acquired there. The work that follows is a description of the subsequent investigations.

II. Cardiff Maternity Hospital.

The new hospital is situated close to Cardiff Royal Infirmary. It had a staff of about 60 at the time that it was opened, of whom ten were pupil midwives who came from hospitals all over the country. A number of student nurses, in training at the Royal Infirmary, also worked there for periods of about three weeks at a time. They lived in the Infirmary Nurses' Home, while the pupil midwives and midwives had rooms on the 4th and 5th floor of the Maternity Hospital.

In order to assess the importance of nasal carriers in the introduction of Staph. aureus into the hospital, a special point was made of taking nasal swabs from all the staff before they started work, or on the first day that they came into the hospital. After that, all the nursing staff were swabbed every two months, at least, during the time of the investigations, which lasted from April 1955 to January 1956, that is, four times altogether.

The ground floor of the hospital consists of offices, laboratories, clinics and a lecture theatre, and the wards occupy the first three floors. When the hospital was opened in April, 1955, only the first floor (floor A) was used; floor B was opened on August 22nd, and floor C on September 6th. Each floor contains seven or eight single rooms and five small wards with about five beds in each. There is a labour ward placed centrally on each floor and a nursery opening on the other side of the corridor. This is divided into five small rooms opening off a central vestibule. The nursery on floor C is used for premature babies and has more cubicles than the other two. All the equipment is well-designed and up-to-date. The nurseries have windows on three sides and the partitions are of glass. Cleaning is carried out by vacuum-cleaner (although there was a delay of several months

in obtaining one for floor B) and damp cloths are used for dusting. Blankets are washed but not otherwise treated after every patient has left. The only criticisms which occurred to me were:

1) the cubicle doors were heavy and on a strong spring so that they had to be opened from the inside by grasping the handle and from the outside by pushing on the door plate;

2) there was no wash-hand-basin in the nurseries except in the duty room. Instead, each cubicle was fitted with a baby's bath in which the tap, worked by handles on the outside, was situated in a recess (so as not to injure the baby). Unfortunately, it was impossible to wash the hands under this tap and the whole bath had to be filled for this purpose - which was slow and cumbersome;

3) Linen hand towels were used, which got very wet, were dried on radiators and often fell to the floor. Later a machine for issuing paper towels was fitted in each nursery, but the paper was remarkably non-absorbent and no-one liked using them.

A certain routine was followed every day during the investigation, first on floor A and then on floor B when it opened. Three sheep blood agar plates were put down on the floor of the nursery every day in two of the cubicles and the vestibules. They were put there

at nine o'clock in the morning and removed at one p.m. This time was chosen because conditions were standard as far as possible. The babies had been bathed, dressed and their napkins changed, and taken to their mothers in the wards. The floors were cleaned in the course of the morning, but otherwise the nurseries were quiet. Any particles which fell on the plates would be those left in the air from the morning's activity or raised by the cleaning. Large particles, splashes and scraps from blankets would not be present (I had found that these tend to give misleading results). At 1 p.m. the babies which had been born four days before were visited in the wards and swabs were taken from the umbilical cords and in some cases from the nose and elsewhere. At this time the babies had not been disturbed since their ten o'clock feed. Swabs were also taken, on occasions, from different sites, such as door handles, nurses' hands and hand towels. On floor C, samples of dust from the vacuum cleaner were cultured for a time as an alternative to the use of settling plates. Nasal swabs were also taken from the mothers for the first few weeks, until it became clear that, except in one instance, they contributed very little to the staphylococcal population of the hospital.

The results of this work are contained in diagram 2.*

* This diagram is in a pocket at the end of the volume.

In it, each swab from a 4-day-old baby which yielded a staphylococcus is represented by a square. A colour has been used to represent the commoner phage types; non-hospital types are represented by various forms of shading. The page is divided into three horizontal sections, each row representing a floor of the hospital. The lower part of each row shows the phage types of a sample of staphylococci isolated from settling plates or from fomites in the nursery; the upper part, the swabs from the babies.

At the beginning of this programme, I was called away from this work because of illness at home, so none of the babies were swabbed between April 22nd and June 6th. I was fortunate that Dr. Michael Dixon was good enough to put down the settling plates for me over that period, and to pick a number of staphylococcal colonies from each, which I was able to phage type later.

RESULTS

181 members of staff were swabbed during this investigation and Staph. aureus was isolated from 113 of them (62•5%). 72 of these, mainly cleaners and student nurses, worked in the hospital for a short time so that they were only swabbed once and the carriage rate from them was consequently lower - 45•8%. The rate for the 109 people who were swabbed two or more times was 73•3%. 36 (33%) were of the "permanent" carrier type - Miles, Williams & Clayton-Cooper (1944); the same strain was isolated from them on two or more occasions at an interval of at least a month. 44 (40•4%) were "intermittant" carriers; sometimes no staphylococci would be isolated from them, while at other times various strains, usually those present in the hospital. 20 carried two or more strains at the same or different times. 29 (26•6%) never carried Staph. aureus at all, however highly contaminated the environment. This phenomenon of persistant non-carriage has been described by many workers and is discussed by Hutchison, Green and Grimson (1957) in a recent paper on nasal carriage in nurses. It would be very interesting to know the reason for this state of resistance to colonization, whether it might be the nature of the nasal secretion or the presence of organisms inimical to staphylococci. Some people were found to carry the same strain for

long periods; eight who were working in the hospital all the time that investigations were going on did so for periods of seven, eight, ten, eleven, fourteen, fifteen and sixteen months.

One of the facts which emerged from this investigation was that the spread of Staph. aureus occurred in two ways. One was a long-term wave of infection which lasted several months and involved many babies. Strains which become endemic in this way have come to be known as "Hospital strains". It is their presence which so often makes investigations into the epidemiology of Staph. aureus in hospitals inconclusive, for they are so widespread that it is difficult to determine their origin or method of spread. The other pattern of infection which became obvious was the comparatively short epidemic which could often be shown to be associated with the presence of a carrier. When this person left the unit, the strain was no longer seen. These types of outbreak correspond to the first two of the three kinds described by Williams in 1956:- "In one type of outbreak only one or two carriers of the epidemic type can be found, and sometimes it is possible to demonstrate reasonably clearly that one of these is in fact responsible for the spread of infection. These epidemics are terminated by the exclusion of the carrier; they are not, however, particularly common. In the second type the epidemic type of staphy-

lococcus is far more widespread - colonizing the noses of many of the staff, and being widely distributed in fomites (e.g. Blowers, Mason, Wallace and Walton, 1955). Such epidemics cannot be terminated by the exclusion of a few carriers; they may be controllable by extensive revision of aseptic technique, with, perhaps, the addition of local chemotherapy aimed at eliminating the staphylococcus from the noses of the carriers." (The third type, which is considered to be due to a general lowering of standards was not seen in this hospital). Williams was, of course, writing about outbreaks of clinical infection, but the results of this investigation show that staphylococci behave in the same way when they are not causing lesions.

For clarity I shall call the two kinds of epidemic Williams' type I (limited and associated with the presence of a single carrier) and type II (widespread and with numerous carriers among staff and babies).

The epidemiology of the strains causing these two types of epidemic is illustrated in the diagram.

80. This strain was isolated from the dust on the first day that the hospital opened. It was known to be carried by one student nurse at that time, but as it was also endemic in Cardiff Royal Infirmary it could very easily be carried on

hands or clothes the short distance between the two hospitals. On July 3rd., a student nurse from the Infirmary who was a scanty carrier of this strain entered the unit, and on August 1st. a pupil midwife, also from the Infirmary, who proved to be a permanent carrier. On August 10th, the organism was isolated from a settling plate, and on the 16th from the cord of a 4-day-old baby. From that time until the 20th. of October, it appeared on this floor in two other babies, both of whom developed lesions. The mother of one of them developed a breast abscess. It was isolated from four swabs taken from door handles or towels in the nursery and 24 times from settling plates. At the end of August, two more student nurses who carried this strain entered the unit from the Infirmary; one to work on floor A, where this strain was already endemic, the other on floor B and floor C which was just about to be opened. On September 10th, the pupil midwife who was a heavy permanent carrier went to work on floor C. On the 15th, the organism was isolated from the cord of a four-day-old baby on this floor. This was then followed by a sharp outbreak. 22 babies were infected between this date and the end of October, of whom all but two developed some sort of sepsis. Two mothers had breast abscesses and a staff midwife went off sick with a dental abscess and was found to be carrying this strain. The

organism was isolated from the dust on 24 days over this period. By this time, it was recognised that this strain had abnormally high virulence and steps were taken to exclude the people who were carrying it. The three student nurses had either left the unit or were no longer carriers. The permanent carriers were given an ointment containing Neomycin and Gramicin ("Graneodin") to rub into their nostrils; while the Staff Midwife did not lose the strain but left at the end of October. A system of obtaining swabs from all new staff before they entered the unit was organised - if necessary by arrangement with the nurse's own doctor at home. Seven carriers of phage type 80 were found in this way, one with a boil on her neck. Six lost the strain after treatment with the nasal ointment; the seventh who did not was removed from duty in the nurseries. Latterly all staphylococci were screened for tetracycline resistance, which picked out strains of this phage type more quickly than phage typing could give results. This strain appeared once in the unit after these procedures had been adopted; on November 10th. it was grown from the cord swab of a baby on floor A. This child had been given a replacement transfusion, which involved an amount of handling by people who also worked in the main hospital. Two days later another baby developed extensive spots from which

this strain was isolated, and a third at the end of November was also infected. It was isolated from two settling plates at about this time, but during the rest of the investigations it was not found.

52/52A/6/7/54/73/81. This strain was also isolated from settling plates on the first day. The only carrier at that time was a staff midwife who proved to be a permanent carrier. At once the air became heavily contaminated and during the course of the investigation this strain has been isolated from 64 babies and from the environment on 139 days. It was acquired by 12 other members of staff. The nurse who was suspected of introducing it left the unit at the end of October, but by that time two sisters, working on floors B and C, and a Staff Midwife on night duty had acquired it. Towards the end of the investigation it became rare on floor A but persisted on floor B.

52A/79. This strain has also been present from the start. The only known carrier at that time was one of the sisters who also had a non-typable strain in her nose and who lost 52A/79 after five weeks. By this time a student nurse carrier was present; nine other members of staff have been found to be carrying it and it has been isolated regularly from all floors

over the whole period. It has appeared sporadically, rather than in outbreaks; altogether it has been isolated 46 times from babies, 77 times from the environment.

42D/77. Present on floor A from the first day, this strain was widespread until the middle of September. It appears to have been introduced by one of the sisters who worked in the building before the unit opened. She went to work in the Ante-Natal Clinic in May and had no further contact with the nurseries. This strain was very common in the first few months and was isolated from 43 babies and from the environment on 90 occasions. Four other members of staff also acquired it.

7/54. This strain first appeared in a baby on June 21st on floor A. It had a very characteristic appearance, being a bright lemon yellow colour, and had not been seen before. A medical student, who was swabbed on June 10th, and who had just come to work in the unit was found to be carrying the same strain. He left at the end of July but his strain persisted on floor A until the end of the investigation. Only one other member of staff acquired it, a pupil midwife at the end of January. Another medical student and a cleaner were found to be carrying a strain of similar phage type at the beginning of December, but it had not the typical

yellow colour of the original strain and may have had a different origin. It was isolated from 3 babies and 8 samples of air on floor B at this time. The original yellow strain was obtained from 41 babies and 100 times from the environment. All the isolations, except 7 from babies and one from a settling plate were made after the original carrier had left.

6/47. This is a common strain outside this hospital and is endemic in St. David's. It was not seen, apart from two isolations from the air on September 14th and 21st, until November 13th., when it appeared in a baby on floor B. This was followed by a shower of isolations from settling plates, and 3 more infants were found to be infected in the next day or two. On the 16th., it was isolated from a baby on Floor A; no others were found to be infected on this floor, but a student nurse had acquired the strain by November 17th. It continued to be obtained from the environment there until January 12th., on 10 separate days. It persisted longer on Floor B, where 8 babies were colonized and was isolated 24 times from the environment . The only known carrier at the beginning of November was a pupil midwife, a permanent carrier, who worked on floor C until December 10th. when she was transferred to floor B.

52A. This strain gives rather a confused epidemiological picture. It is only faintly lysed by the phage and may sometimes have been classed as untypable. There have been three permanent carriers of it present in the unit from the beginning, the night superintendent, a staff midwife and a pupil midwife. It has been isolated at intervals throughout the investigation, but has not caused any noticeable epidemic. It became more common towards the end, possibly because the pupil midwife re-entered the unit after having been ill. Altogether it was isolated from 38 babies and from settling plates on 63 occasions.

These seven strains are the ones which I considered to be the "hospital" strains - or those which caused the second type of epidemic described by Williams. They are represented by different colours in the diagram. The first type of epidemic appeared to be associated with some staphylococci which will now be described, illustrating an association between carrier and outbreak. These strains are represented in the diagram by various patterns such as cross-hatching.

79/7/42E. This was carried by one person only, a pupil midwife, who was present when the unit first opened. It appeared on settling plates within 11 days and a small

outbreak of aerial contamination followed - 9 isolations over 29 days, (babies were not being swabbed at this time). This nurse then went on night duty and her strain did not re-appear until the beginning of August, when she returned to floor A. Four babies were infected on August 3rd, 6th and 9th, and settling plates were colonized on 8 occasions between August 5th and 25th. On September 4th. this nurse was transferred to floor C. Three babies were infected there before she left the hospital at the end of October.

6/7/47/53/54/75. This strain was first isolated from the skin and clothing of a pupil midwife on August 8th. Another type was grown from her nose, which persisted. The skin strain was not isolated from her or from anyone else until it was found to be carried by a student nurse at the end of November. Whatever its source, it caused a sharp outbreak of environmental contamination on floor A, although no sepsis resulted. Between August 18th and September 23rd., 13 babies were infected and 22 samples from the environment. Three babies also acquired this strain on floor B, it was found 4 times on settling plates and once on floor C. It was last

seen ten days after the carrier left the unit.

79/3A/3B/7/42E/54/70. This was the strain which was carried in the nose of the same nurse. She first worked on floor A where it was isolated from 3 babies and from settling plates or door-handles on 6 occasions. Next, she went to work on night duty on all floors and her strain was found to be carried by a baby on floor B and ~~five times~~ once on settling plates. In January a cleaner acquired this strain.

47/53/75/77. This strain was carried by a pupil midwife who joined the unit at the beginning of August; she continued to carry it until the end of October. She first went to work on floor B and the same strain was isolated from a door-handle on September 9th, and from 2 babies and a settling plate on the 12th. On being transferred to floor C she infected 5 babies and two settling plates. Two babies on floor A yielded this strain on September 28th, and a settling plate on October 1st. No more was seen of it after the carrier became negative. This strain is one of the six described by Williams (1959) as being responsible for a number of hospital outbreaks. It was resistant to streptomycin as well as to penicillin. It is interesting that although there was ample opportunity, no epidemic of infection occurred in Cardiff.

42E/77. The carrier of this strain was also a pupil midwife who joined the unit at the beginning of August, working mainly on floor B. It was isolated from the environment there 18 times and from 3 babies. It also appeared on floor A - on two settling plates - where she worked for a short time, and similarly was isolated from 3 babies on floor C.

55. This strain appeared suddenly on floor A at the beginning of October; it was grown from the environment five times and from 2 babies. A medical student who started work on October 1st, was found to be carrying it.

71. This strain appeared sporadically on floors B and C during the last three months of the investigation. A similar but not identical one was isolated from a student nurse who worked on those floors and from the orderly on floor C. It was grown altogether from 3 babies and from the environment 11 times.

7/47/54/77. This strain, which was resistant to streptomycin as well as penicillin, was carried by a member of the paediatric staff who was present in the hospital throughout the investigation. It was not isolated from any babies but was obtained from the environment on 8 widely separate

occasions and on all three floors.

3A. Two varieties of this phage type were isolated in the hospital. One, which produced no alpha lysin and was sensitive to penicillin, was carried by a cleaner who was present from the beginning of June onwards. She may have been responsible for an epidemic of environmental contamination between June 24th and July 23rd. on that floor, in which this strain was grown from settling plates on 7 occasions. A staff midwife who came to work in nursery A at the beginning of December carried a similar strain, but she appeared to be responsible for no dissemination except for one isolation from a settling plate on December 14th. A pupil midwife who entered the unit at the beginning of November carried a lytic, penicillin-resistant strain of type 3A, but she had lost this strain when reswabbed on December 21st., and January 25th. She worked on floor B from November 1st to December 10th and after this she went on night duty. Altogether, 8 babies were infected with this strain between November 14th and December 2nd, and it was isolated from the environment on 16 occasions over this period.

3C. This strain was carried by a student nurse who worked on floor C before it was opened, and later there and on floor B. It was isolated from vacuum cleaner dust from

floor C on September 10th., 13th and 28th, and from a baby on September 11th. Later it appeared on floor A, on January 20th, from a baby and 21st and 22nd from settling plates, but the origin of this infection was not known.

39/71. Staphylococci of this phage type were isolated from settling plates on floor A on May 15th, 18th, 20th and 26th. As no swabbing was being done at this time, the origin of this little outbreak was not discovered.

75/77. Another strain, besides the one mentioned before, is of interest because it did not cause an epidemic. This was one of phage type 75/77, resistant to penicillin, streptomycin and the tetracyclines. The student nurse from whom this staphylococcus was isolated on October 1st and 21st, worked on floor A from the beginning of October until November 11th. This strain was not isolated at all from settling plates and only once from a baby, on December 2nd. It is surprising that no further cases occurred, for this also is one of the six strains which Williams found to be commonly associated with outbreaks of sepsis.
All the staphylococci described here, except those of type 3A, already discussed and some of 6/47, were resistant to penicillin.

These are the principal strains which appeared to be spreading round the hospital and were associated with the presence of a nasal carrier among the staff. In nearly every case there is evidence that the carrier was positive for that strain before she or he started work. A number of other strains were isolated which have not been included in this list; some were not typable, or were not easy to define as phage types; others were only seen once or twice or not enough to be called epidemic. One small outbreak on floor A may be mentioned, as it is an unusual example of spread from a mother. She was found to be carrying strain 5/53/54/75/77 on admission on June 17th. It was not isolated from her own baby but was grown from a settling plate on June 26th, and from another baby on June 27th. If was found on 4 more settling plates between then and July 11th.

Another incident that is of interest is a clear-cut case of spread from a baby.

This infant was admitted from the district on August 15th and found to be carrying strain 53/75/77 on its nose and cord on that day. It appeared on a settling plate on the 18th., was isolated from another baby on the 22nd, and from door handles on that day and the 25th. There were 7 more isolations from settling plates between then and September 25th. The baby left the unit on September 14th., carrying one of the epidemic strains as well as its own.

One of the facts which I was anxious to determine from this investigation was the part played by nasal carriers in the spread of staphylococci. The most interesting carriers from this point of view were those who were permanent carriers of strains which were new to the hospital. Where they had been shown to be carrying these strains before they started work, it could be assumed that any of these staphylococci which appeared among the babies or in the environment must have come from them originally. Table 2 shows these carriers, classified according to their type of work.

From this table it may be seen that nasal carriers do spread their strains to the babies and the environment. All 23 of these permanent carriers of non-hospital strains were responsible for some spread, though the amount varied greatly. Presumably carriers of hospital strains would be responsible for a similar dissemination, but this would be much more difficult to demonstrate because of the ubiquity of these strains already. It might be expected that people who work most closely with babies would spread their organisms to them most often. The number of non-hospital strains are too small to prove this here, but there did appear to be

less spread from the doctors, medical students, sisters and cleaners than from the pupil midwives who carry out most of the routine nursery care. Student nurses help them in this, but in the present investigation remarkably few in this group carried easily recognisable strains. This was probably because they had worked before in Cardiff Royal Infirmary, while pupil midwives came from hospitals all over the country.

TABLE 3.

The Spread of Staph. aureus from Nasal Carriers of Non-hospital Strains.

Type of work	Phage type	Type of outbreak	No. isolated from babies	No. isolated from environment
Sisters	42D/70	-	2	2
	29/52	-	3	6
	42D/77	II	43	89
Staff midwives	3A	-	-	1
	52/52A/6/7/53/81	II	64	132
Pupil midwives	79/7/42E	I	7	11
	42E/77	I	7	18
	80	II	25	56
	52/77	-	3	3
	6/7/47/53/54/75	I	4	27
	79/3A/3B/42E/70	I	4	13
	47/53/75/77	I	9	7
	6/47	II	10	39
	3A	I	8	16
Student nurses	75/77	-	1	-
Doctors	7/47/54/77	I	-	8
Medical Students	55	I	-	5
	7/54	II	41	100
Domestic staff	71	I	2	11
	52	-	-	4
	7/54	I	3	7
	3A	I	-	7

PART II

Investigations into the Mechanism and Prevention of Cross-Infection.

I. Dust Suppression by Vacuum Cleaner.

Introduction

This is a study of the use of a cylinder model vacuum cleaner and its effect on airborne bacterial counts and nasal infection rates in infants. This type of machine was investigated by Rogers in 1951; he found that, provided the bag was not brand new, it would hold back staphylococci and other organisms. Experiments showed that both in rooms where the floor was oiled and where it was not, the use of a vacuum cleaner greatly reduced the airborne bacterial count.

Dry sweeping was carried out in Cardiff Maternity Hospital during May and June until a cylinder-type cleaner was acquired at the beginning of July. It was not until September that two others were bought for the other floors, so that during August and September floors A and B had to share one machine. This was an ideal arrangement for a controlled trial in which infection rates and dust counts might be compared on the two floors while the cleaner was used on one and not the other. Like Rogers, I found that there was no cross-infection between floors. My attention was drawn to the part played by a vacuum

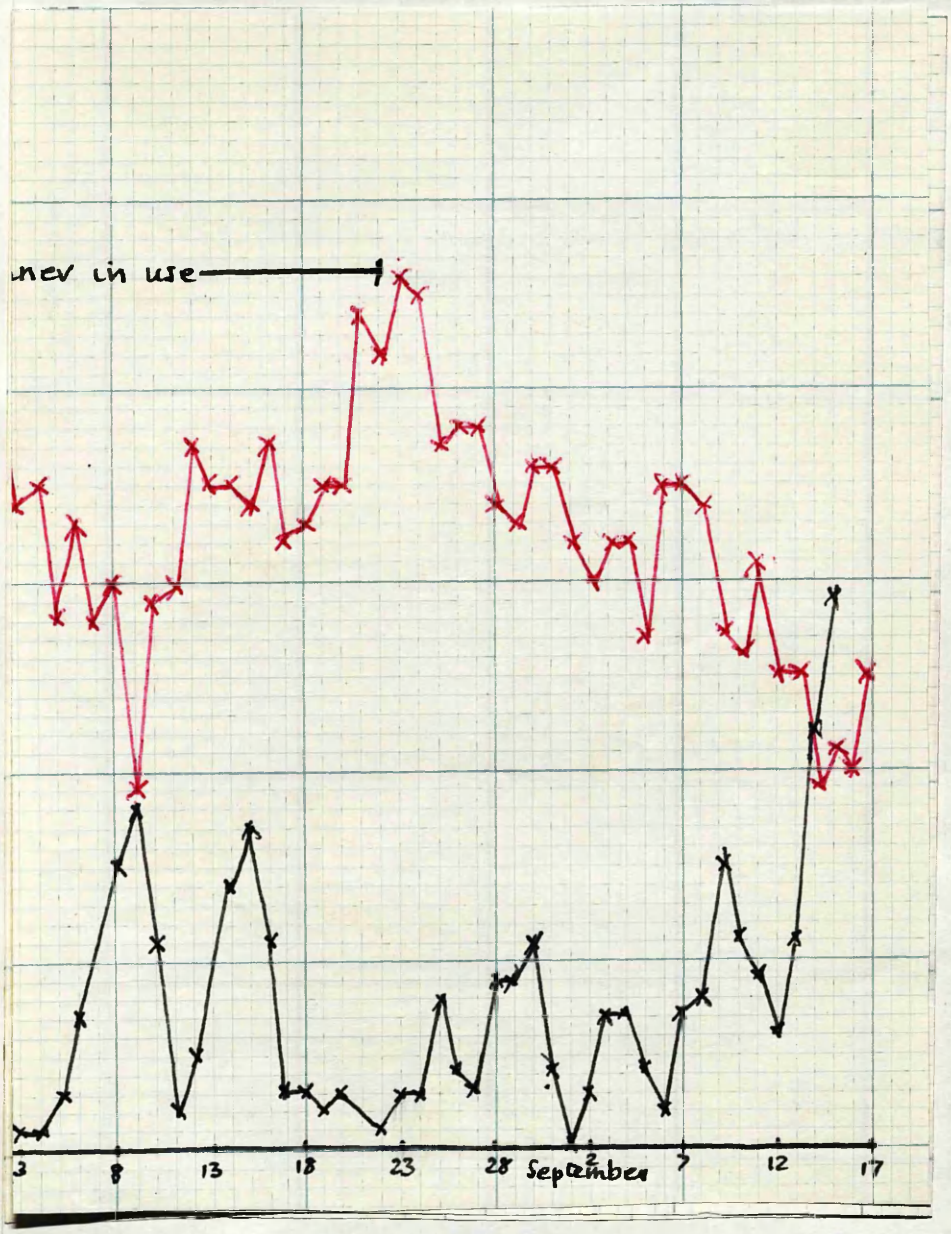


Diagram 3.

A Comparison of the Number of Staph. aureus Colonies isolated from Settling plates and the Maximum daily Temperature Readings.

cleaner because I noticed that the total amount of bacterial growth and the number of Staph. aureus colonies were lower in July than before. In May and June, they had varied greatly and had sometimes been very high. I first thought that this fall might be associated with the unusually hot weather which was occurring at that time. Indeed, a graph (Diagram 3) of the maximum daily temperature and the total number of staphylococcal colonies shows a remarkable inverse correlation between the two. Then I discovered that the vacuum cleaner was brought into use at the beginning of July and that a very energetic spring clean of the whole hospital was carried out at that time in preparation for the official opening of the hospital by the Duchess of Kent, on July 12th. Which of these two factor contributed most to the low counts in July and August it is difficult to say. Increased ventilation from open windows, or the bactericidal effect of sunlight (as shown by Garrod in 1944) may have been responsible. The nursery windows were whitewashed on August 28th. as the rooms were becoming overheated, but the temperature began to fall after that so this effect cannot be proved.

The controlled trial of the effect of the cleaner, first on floor B and then on floor A, took place from August 23rd. to October 29th. As counts were made on both floors at the same time allowance was made for the effects of the heat wave.

The investigation was in three parts: a) to compare the total numbers of airborne bacteria on the two floors, b) to compare the numbers of Staph. aureus colonies isolated from the air, and c) to see if there was any effect on infection rates in babies as shown by nose and cord swabs.

Methods.

In this experiment the babies were swabbed while they were still in the nursery, being between 12 and 24 hours old. Settling plates were put down as described in the previous section. For the first 25 days the cleaner was used on floor B and not on floor A: After an interval of four days it was transferred to floor A and the experiment repeated. The total bacterial growth on three plates was recorded by an arbitrary system of numbers, 1 representing less than 20 colonies, 2 between 20 and 50, 3 more than 50, and 4 a very heavy growth. The number of Staph. aureus colonies on the three plates were counted; these figures are shown graphically in diagram 4a; the infection rates in babies are illustrated in diagram 4b.

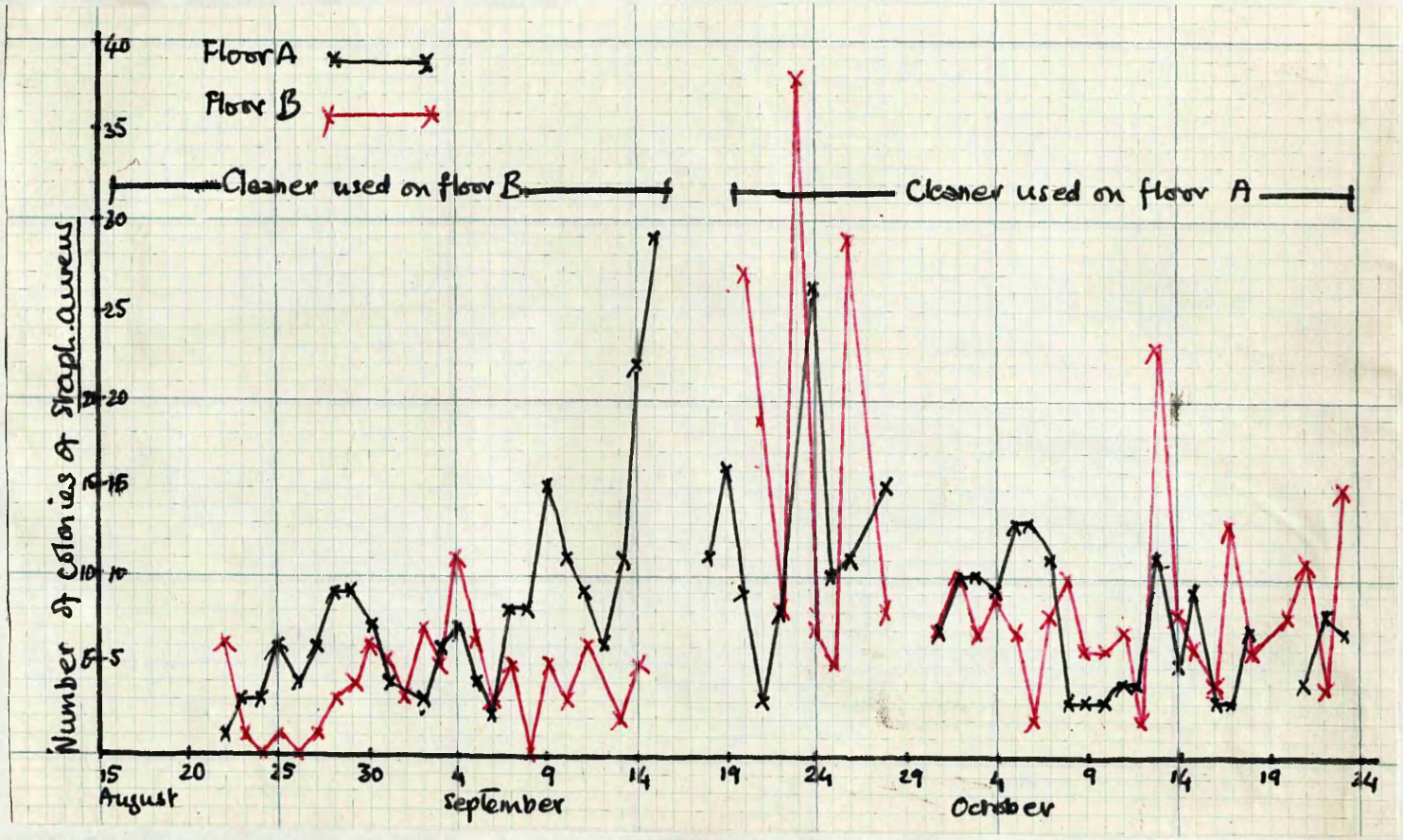


Diagram 4a. The Average Number of Staph. aureus colonies isolated each day on floors with and without a vacuum cleaner.

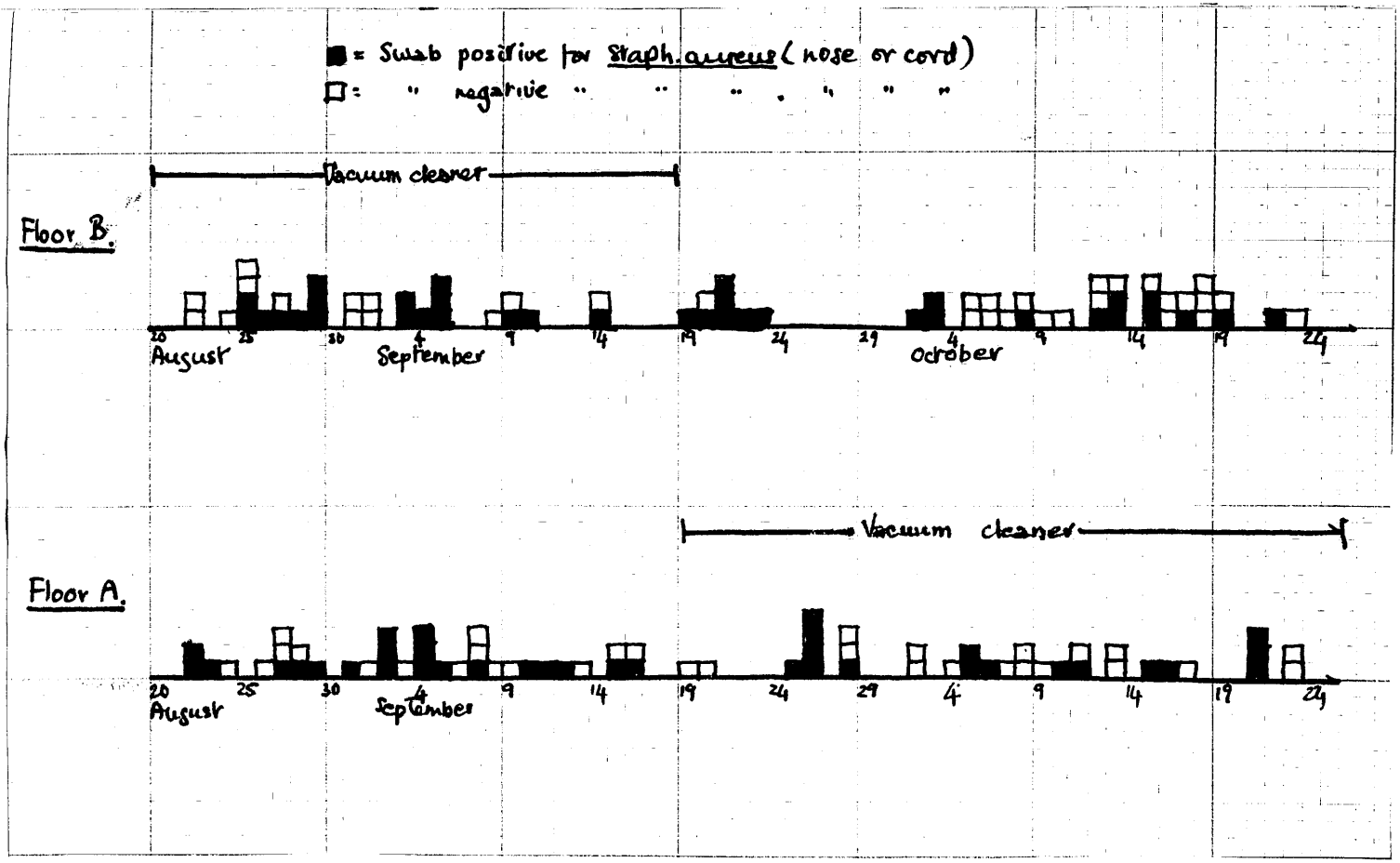


Diagram 4b. Infection rates on the two floors with and without a vacuum cleaner.

TABLE 4a,b and c.

Results:

a) To show the total bacterial growth on three settling plates in four hours

Test floor					Control floor				
	Amount of bacterial growth								
	1	2	3	4		1	2	3	4
Floor					Floor				
A	26	44	5	-	A	1	49	22	2

b) To show the average number of Staph. aureus colonies isolated on three settling plates.

Test floor		Control floor		No. of days	Difference	Standard Error
B	4·68	A	7·64	25	2·96	1·42
A	8·32	B	10·58	31	2·26	1·72

c) To show the effect of vacuum cleaning on infection rates in babies

Nasal swabs:

Test floor		Control floor	
B. 7 positive out of 24	29·1%	A. 11 positive out of 36	30·6%
A 4 " " " 27	14·8%	B. 6 " " " 32	18·7%

Cord swabs:

B 16 " " " 27	59·3%	A 16 " " " 36	44·5%
A 15 " " " 33	45·5%	B 17 " " " 40	42·5%

It may be seen from these tables that where the vacuum cleaner was in use, fewer organisms were isolated on settling plates and fewer colonies of Staph. aureus (though the difference in the second part of experiment b) was not significant. There was no difference in the infection rates of the babies, either of nose or cord.

Discussion:

The findings in this investigation raise a problem which is still under discussion - the importance of dust in hospital cross-infection. The earliest methods of dust suppression were the oiling of floors and blankets. In 1944, Wright, Cruickshank and Gunn demonstrated a reduction of haemolytic streptococci of 97% and of total bacteria of 91% in the air of a measles ward by these means. When floors alone were oiled there was no difference in amount of infection, but when blankets were treated too, the streptococcal cross-infection rate and incidence of middle-ear complications fell. In a later study, however, Begg, Smellie and Wright (1947) found that while these proeedures were successful in suppressing dust-borne bacteria, there was no alteration in the amount of cross-infection with haemolytic streptococci. Marsh and Rodway, working in a maternity hospital in 1954, made a study of the problem as it affected nasal carriage of Staph. aureus in infants. They found that disinfection of blankets, mattresses and pillows (but not the

oiling of floors alone) reduced the bacterial contamination of ward dust but not the number of infants carrying Staph. aureus. Clarke, Dalgleish, Parry and Gillespie in the same year published an account of a similar investigation in two surgical wards. They found that oiling the floor, screens and bedclothes in one of the wards was followed by a reduction in the numbers of bacteria in the air of the treated ward, but the nasal and wound cross-infection rates were unaffected. The part played by contamination of blankets in cross-infection is still a contraversial question. When they are disinfected, either by quaternary ammonium compounds or by formaldehyde, airborne bacteria are greatly reduced. (Blowers and Wallace, 1955, and Schwabacher, Salsbury and Fincham, 1958) but although it appears that the infection rate is also decreased, this has not been proved to happen as a direct result. Presumably blankets can spread infection by contact as well as by scattering organisms into the air.

The conclusions which may be drawn from the present study are similar to those of Wright et al, Marsh and Rodway, and Clarke et al. - that a decrease in the numbers of Staph. aureus in the air is not necessarily followed by a fall in the cross-infection rate. Airborne infection did not appear to be the most important route of cross-infection in this hospital.

II. Treatment of Nurses' Hands with Chlorhexidine Cream.

Introduction.

From the previous investigation it appears that a reduction in the total amount of airborne staphylococci does not diminish the airborne infection rate in babies. This indicates that transfer of organisms by another route may be more important. In order to study this, a way of cutting down cross-infection by hand contact was required. In 1955, Murray and Calman published a paper on the control of cross-infection by means of an antiseptic hand-cream. The substance tested, Chlorhexidine, was found in laboratory experiments to be "capable of greatly reducing the bacterial count of the skin. Use of the cream in the wards led to a marked decrease in the number of clinical cases of staphylococcal infection; during the period of trial no groups of infection were seen in any ward or nursery." No clinical trial was carried out. I decided, therefore, to see how the use of this hand cream by the staff in Cardiff Maternity Hospital would affect the number of staphylococcal colonies on settling plates and the nasal carrier rates in babies, as in the previous experiment.

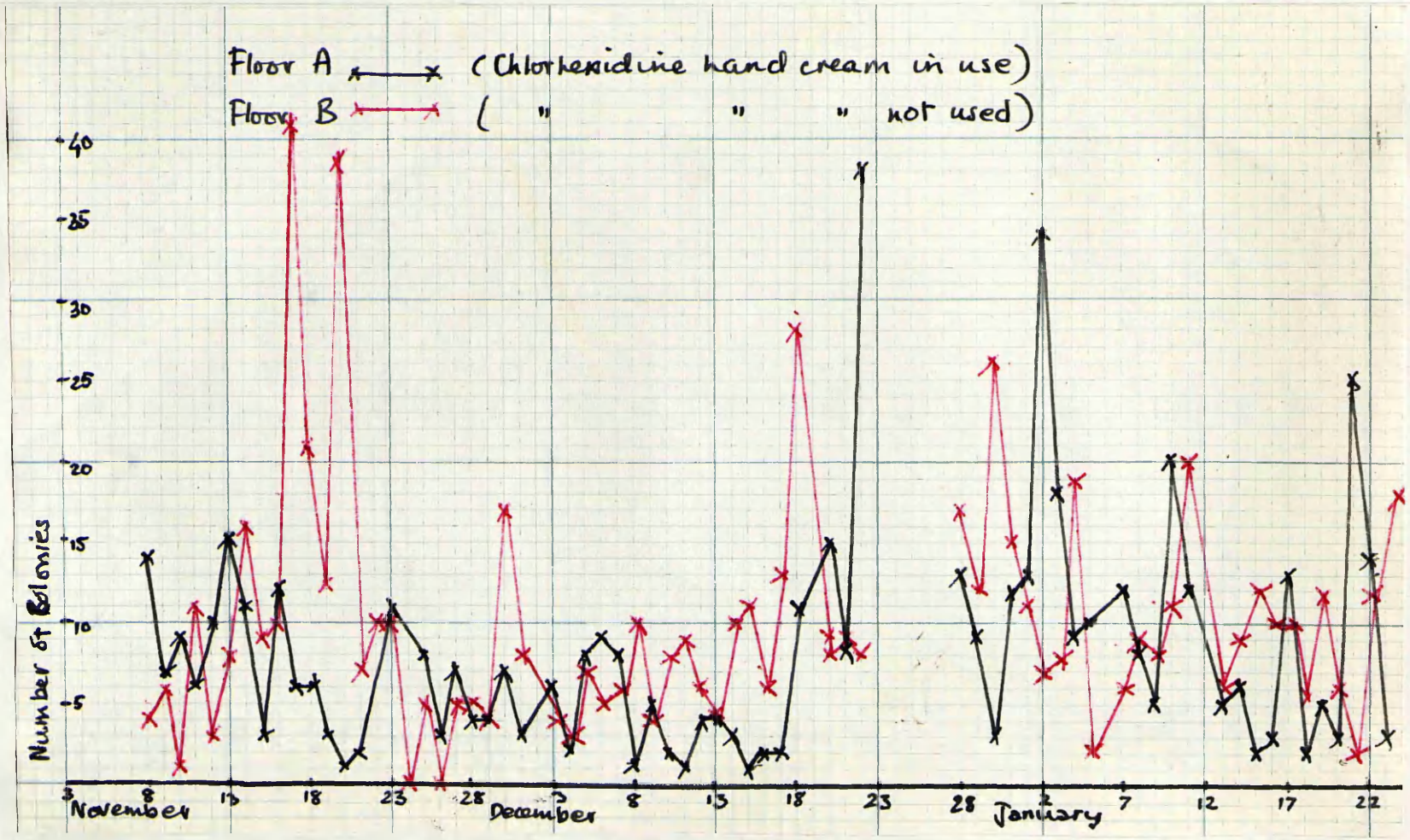


Diagram 5a. The Average no. of Staph. aureus colonies isolated each day on floor A where Chlorhexidine was used and floor B where it was not.

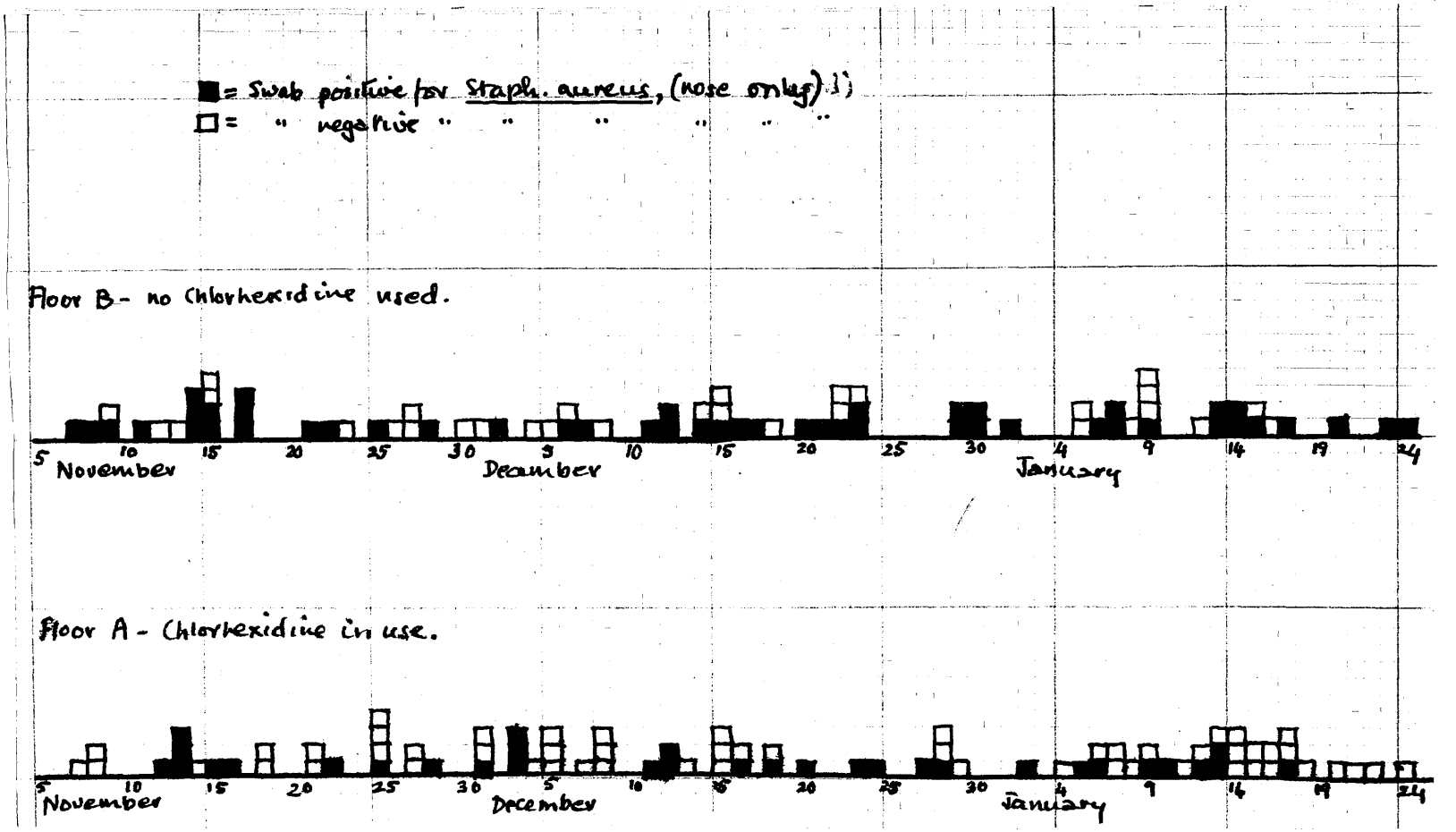


Diagram 5b. Infection rates in babies on floor A, where Chlorhexidine was used, and floor B, which acted as a control.

Methods.

Tubes of the cream were put beside each wash-hand basin and all the staff on floor A instructed to rub the cream into their hands every time they washed them. Floor B was used as a control; the investigation was carried out for 67 days, from November 7th until January 24th. Unfortunately I was not able to carry out the second part of this experiment, in which the cream should have been used on floor B and not on floor A. It must be considered incomplete for this reason and no definite conclusions drawn from it.

Results.

TABLES 5a and b.

a) To show the average number of Staph. aureus colonies isolated on three settling plates in four hours.

Test floor	Control floor	Difference	Standard Error
A 7·99	B 10·28	2·29	1·25

b) To show the effect on infection rates in babies of the use of Chlohexidine hand cream by the staff. (nasal swabs only).

Test floor - A - 32 positive out of 87 - 36·8%
Control floor - B - 49 " " " 80 - 61·2%

Difference 24·4 Standard Error 7·5

These results are illustrated in diagrams 5a and 5b.

These findings show that there was a significant difference between the number of new-born babies carrying Staph. aureus in their nostrils on the test and control floor. It may be seen from diagram 5b that the number of positive swabs became fewer towards the end of the period; this may be the result of a campaign to make sure that the nurses really did use the cream in the wards and theatre as well as in the nursery. Several notices were put up to this effect. The difference between the number of Staph. aureus colonies isolated was not large enough to be significant. It is possible that other factors, which would have been allowed for if the test had been repeated with the test and control floors reversed, were at work to give this result. If babies' nostrils were infected only from the air, one would have expected the difference in the airborne staphylococci to have been as great as that in the nasal carrier rate. Is it possible that the babies' noses were infected from a source other than the air of the nursery - their own hands, blankets or clothes? This point is not clear from the limited data available.

Discussion.

My attention was drawn to the dangers of inadequate disinfection of hands when dealing with Staph. aureus by an incident which occurred in the course of my laboratory work.

I contaminated my hands accidentally with this organism and at once scrubbed them thoroughly with soap and water. It occurred to me to take a swab from them afterwards; the result was quite a heavy growth of Staph. aureus. I repeated this several times and found that even after washing them for two minutes, my hands still yielded a few organisms. This seemed to indicate a dangerous state of affairs. Most people who work in hospitals assume that a thorough scrubbing with soap and water will disinfect their hands, and so, relying on this, they may handle a susceptible patient immediately after touching contaminated material. Even the most conscientious nurses rarely have time to wash their hands for more than a minute, unless they are actually working in theatre. To make sure about this, I paid a visit to the nurseries several times while the morning routine of changing and cleaning the babies was going on. The hands of 11 nurses were swabbed before and after they were washed in the usual way. Six were positive for Staph. aureus on both occasions, one acquired the organism and one lost it. The staphylococci isolated were of the phage type endemic at the time, and not the nurses' own nasal strains. Swabs were taken from door-handles of cubicles and hand-towels in the nurseries over a period of several months; 49% of 147 door-handles were positive for Staph. aureus and 47% of 53 towels. The average time which a nurse was able to spare for hand-washing between the handling of each baby was 15 seconds.

The ineffectiveness of soap against Staph. aureus has been known for a long time. Walker in 1924 investigated the germicidal properties of chemically pure soaps, using broth cultures of organisms which he inoculated into different concentrations of pure soaps such as sodium linoleate and laurate. He found that pneumococci were killed by a N/12,240 solution of linoleate, while S.typhi resisted 2000 times this concentration. Most of the strains of staphylococci tested were not killed by any of the concentrations used, even in 15 minutes. Price (1938) found that, while hands were never completely sterilized by washing with soap and water, two minutes was the average time taken for the removal of "transient" organisms. Colebrook and Maxted (1933) reported that staphylococci could still be recovered from the hands after they had been washed for as long as five minutes. In order to limit this degree of hand contamination, various skin disinfectants may be used, but many are not suitable for prolonged use. Chlorhexidine which was first described by Davies in 1954, was found to be the best general antiseptic for the purposes of midwifery by Murray and Calman in 1956. It was used by Lowbury in an investigation into methods of preventing cross-infection of burns with penicillin-resistant staphylococci in 1955. He found that it exerted a definite prophylactic effect and that no resistant organisms were

found. Since then, Chlorhexidine has been used widely in hospitals where a regime designed to cut down cross-infection has been instituted. I have not seen an account of a complete clinical trial of its effectiveness as a hand cream in reducing cross-infection.

An alternative way of dealing with the problem is that gloves should be used more often, as Colebrook suggested in 1955 . These could be disinfected on the hands during the nursery routine, much stronger concentrations being used than the skin could tolerate, provided the disinfectant was thoroughly rinsed off each time. The disadvantage of gloves is that nurses do not like using them for handling babies and find that their hands become uncomfortable if they are covered for long periods. The use of gloves might be limited to certain procedures, such as the handling of patients who were known to be infected, or heavily contaminated areas such as umbilical cords. It is most important that the staff realise that their hands probably are contaminated after they have touched any baby of more than a day old and that soap and water do not disinfect them.

III. Elimination of Reservoirs of Infection: the Umbilical Cord*

Introduction.

During my work at St. David's Hospital, I took swabs from various places on the babies and found that the umbilical cord was infected more often than any other site. There was a very heavy growth there and Staph. aureus was usually in pure culture. Therefore, I considered that this site was the most convenient one for routine swabbing in investigations into the incidence of Staph. aureus as Forfar and his colleagues had done in 1953. In June, 1955, I was asked to investigate the case of a baby who had died in a small maternity hospital from streptococcal septicaemia following liver abscesses. Nose and throat swabs were taken from 17 members of staff and 25 mothers, and cord swabs from the 14 babies present in the nursery. As might be expected, 15 of the staff carried Staph. aureus, 10 of the mothers and all the babies. The distribution of haemolytic streptococci was interesting. The organism was isolated from none of the staff or mothers, except for the mother of the dead baby whose nose yielded a heavy growth of Griffith type 12 (the type grown from the post-mortem culture). This type was also isolated from the umbilical swab of every baby. It is not clear how this strain was introduced into the nursery, though possibly the mother who carried it was responsible. It appears to have spread round

* the investigations were published as a separate paper in Brit. med.J. (1957) i, 925.

the nursery without the means of an adult nasal carrier by cross-infection from one baby's cord to another.

At about this time, a paper was published by Lowbury (1955) on cross-infection of wounds with antibiotic-resistant organisms. The problem was approached in three ways:

a) elimination of reservoirs of infection

b) blocking routes of transfer and

c) increasing the resistance of the patient.

Lowbury says that "elimination of the primary reservoirs of infection and of the targets for infection are obviously of the first importance." In the Birmingham Burns Unit where he was working, the burns themselves acted both as reservoirs and targets. In the same way, in a surgical ward, discharging wounds and large infected areas are the dangerous places where organisms are present in large numbers, a source of infection for the whole ward. At first sight there appear to be no reservoirs of that kind in a nursery for healthy babies. But the umbilical stump has been compared to an open wound - by Coventry and Isbister in 1951. They write "here an open wound, communicating with the liver and large blood vessels has been exposed to a virulent infection and local resistance could not be expected to be as high as in the naso-pharynx."

They took nose and cord swabs from every baby in the nursery and the results, graded according to age, were as follows:

TABLE 6.

Age (days)	No. of swabs	Nose	Cord
0 - 2	31	8	6
3 - 4	30	17	27
4 - 6	36	18	21
7 -14	58	32	33

In another paper in the same year, Isbister also found a higher rate of infection of the cord than of the nose - 49•7% of 54 babies as compared with 42•9%. She thought that cross-infection from baby to baby on nurses' hands was the most likely method of spread. In 1953 she published a paper about the umbilicus of the infant, from which the following is relevant: "The healing umbilicus is a potential site of entry for infecting organisms that must never be overlooked. With institutional midwifery and the greater risk of cross-infection, particularly by staphylococci, supervision of the umbilicus becomes even more important.... the severity of the local reaction is no indication of the severity of the lesion.. in a maternity hospital with a high standard of nursery care and asepsis, over 50% of infants were carrying Staph. aureus by the time the cord separated...The orthodox procedure (of religation and dressing the cord) means undesirable handling

of the open wound." Forfar, Balf, Elias-Jones and Edmunds in 1953 swabbed 120 babies at the time of separation of the cord (about the eighth day) and found an infection-rate of 60%. Two years later (Edmunds, Elias-Jones, Forfar and Balf, 1955) they recorded a lower rate - 40% of 604 infants of the same age. My own findings for the incidence of Staph. aureus on the cord were:- 88% of 84 four-day-old babies at St. David's Hospital and 85% of 59 at Cardiff Maternity Hospital. I also compared the times at which nose and cord became infected, to see which occurred first; the results are shown in Table 7.

Table 7.

The Number of Times that the First Positive Swab was Obtained from Nose or Cord.

Hospital	Nasal swab pos. first	Cord swab pos. first	Both swabs positive	Total No. of swabs
St. David's	7	34	19	60
Cardiff Maternity	6	37	24	67

It may be seen that the cord was almost always infected before or at the same time as the nose, while it was rare for the nose alone to be positive.

It was also thought to be of some importance to make a rough comparison of the amount of growth obtained from nasal and cord swabs taken in the first four days of life. Cord swabs proved to give a heavy growth of Staph. aureus more often than nasal ones, and the organism was frequently in pure culture. The results are shown in Table 8.

TABLE 8.

A rough comparison of the Amount of Growth obtained from Nasal and Cord Swabs taken on the First Four Days of Life.

Age (in days)	No. of babies swabbed	Growth from Nose			Growth from Cord		
		Heavy.	Mod.	Light	Heavy.	Mod.	Light
0	12	0	1	11	1	2	9
1	22	6	1	15	13	6	3
2	17	3	-	14	13	-	4
3-4	5	1	-	4	5	-	-

It was also found that 8 out of 22 babies with a positive swab on the first day of life had become negative on the second. Only 5 out of 37 cord swabs showed a similar loss of the organism, and two of these were overgrown with Proteus spp.. This suggests that the isolation of Staph. aureus from a nasal swab may not necessarily represent the multiplication of organisms there but merely that they have been filtered off from the air.

These findings show that the umbilical stump does resemble

an open wound in being both a site where any organism in the environment may multiply freely and a source of contamination of the air and the attendants' hands. It is likely to provide a better medium for multiplication than most wounds, however, for it is cut off from its blood supply and consists of gangrenous tissue. Unlike the wounds and burns described by Lowbury, which are dressed in a special room supplied by filtered air under positive pressure and are covered by protective dressings and local applications, the cord stump is usually left uncovered and is handled many times every day. The treatment which the cord of a new-born baby received in Cardiff Maternity Hospital was as follows: Immediately after delivery the cord of a new-born baby was clamped by the accoucheur, ligated and cut. The baby was put in a cot and any treatment or cleaning-up considered necessary was carried out with ungloved hands. The cord was inspected frequently to make sure that it was not bleeding; it was sometimes handled or squeezed during this inspection. About six hours after birth it was re-ligated and cut short to about an inch; surgical spirit was then applied and the cut end sealed with collodion. If the cord was thick with an abnormal amount of Wharton's jelly, this was squeezed out before the second ligature was applied. Each morning the spirit treatment was repeated. It was difficult for the

napkin to be changed without the cord touching the nurse's hands, and since soap and water was the only method used for disinfecting these it is easy to see how organisms could be spread from cord to cord by the nurses.

As the uncovered cord is in direct contact with the cot blankets and the baby's gown it must also be a source for the contamination of the air. Every time the baby is picked up, many organisms must be scattered from its clothes.

In order to find out if the cord is as important a reservoir of infection in a nursery as these findings suggest, an investigation was planned on the same lines as the previous ones, to determine the effect of reducing the growth of Staph. aureus on the cord. Triple Dye* was used for this purpose because the dyes it contains kill staphylococci, it is harmless to the tissues in effective concentration and staphylococci do not become resistant to it. It is also useful in a trial of this sort because it is easy to see if it has been applied or not. It appears to be quickly fixed to the tissues, for it does not stain the clothes or become smeared over the baby's skin as much as one might expect.

* Brilliant green 10 gr. Proflavine hemisulphate 5 gr.
Crystal violet 10 gr. Water 10 fluid ozs.

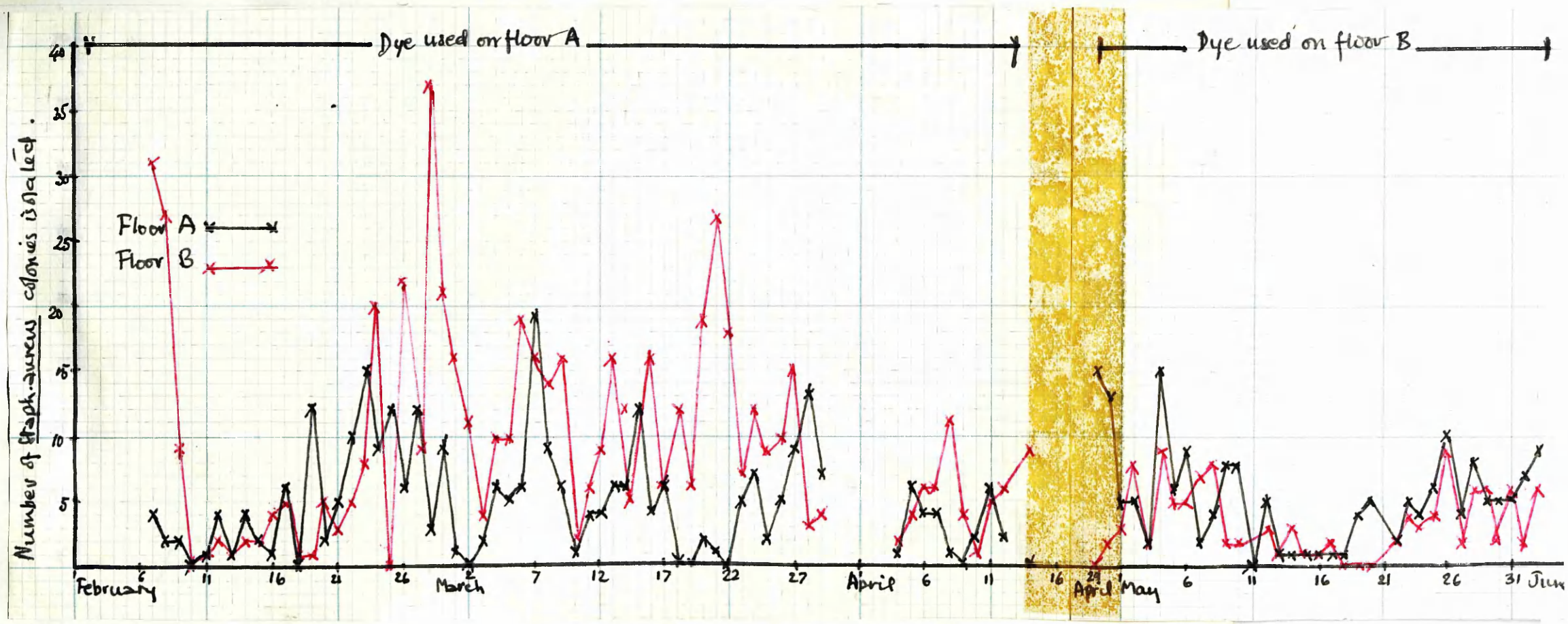


Diagram 6a. The Effect of Using Triple Dye on Umbilical cords: the average numbers of Staph. aureus colonies isolated on TSV and control floors.

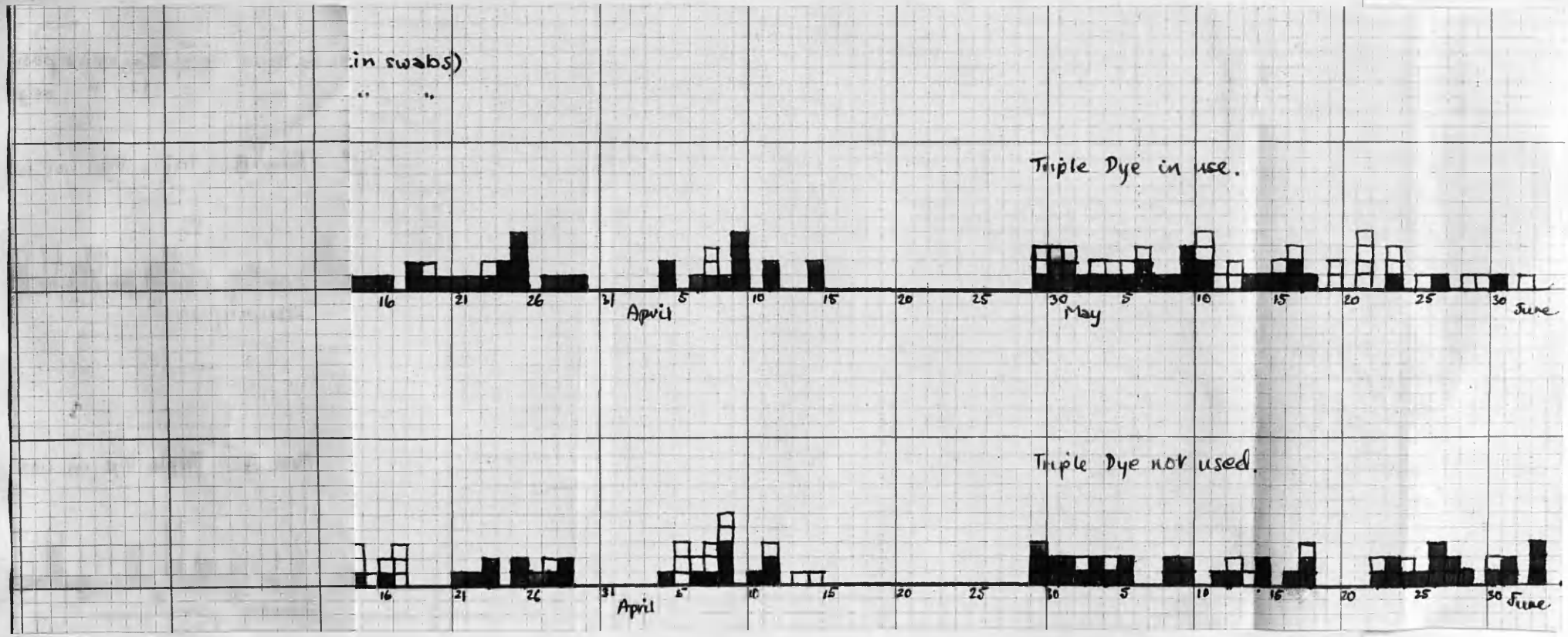


Diagram 6 b. The effect of using Triple Dye on Umbilical Cords: a comparison of Infection Rates on Test and Control Floors.

Methods.

Swabs were taken from each baby when it was four days old, at the same time each day. One swab was taken from both nostrils and one moistened in broth, from a small area of skin (about 2 in. in diameter) at the lower end of the sternum. Settling plates were put down as before. These procedures were carried out first on floor A, with floor B acting as a control, and then after an interval of a fortnight, the dye was used on floor B and not on floor A.

The results of these investigations are shown in Tables 9.10, and are illustrated in figures 6a and b.

TABLE 9a. and b.

(a). To Show the Effect on Infection Rates in Babies of the Treatment of Umbilical Cords with Triple Dye.

Swabs.	Treatment of Cord	No. of Babies	No. of Swabs Positive	Difference	Standard Error
Nasal	Dye (floor A)	91	46 - 50.5%	24.2%	6.95
	No dye " B	91	68 - 74.7%		
Skin	Dye (floor A)	91	32 - 35.2%	25.2%	7.61
	No dye " B	91	55 - 60.4%		

(b) The Average Number of Staph. aureus colonies isolated on three settling plates.

Treatment of Cord.	No. of days.	Average Count:	Difference:	Standard Error.
Dye (floor A)	62	4.77	4.93	1.14
No Dye " B	68	9.70		

TABLE 10a and b.

(a) To Show the Effect on Infection Rates in Babies of the Treatment of Umbilical Cords with Triple Dye.

Swabs	Treatment of Cord	No. of Babies	No. of swabs Positive	Difference	Standard Error.
Nasal	Dye (floor B)	54	19 - 35·2%	36·2%	9·16
	No Dye " A	49	35 - 71·4%		
Skin	Dye (floor B)	54	17 - 31·5%	39·9%	9·03
	No Dye " A	49	35 - 71·4%		

(b) The Average Number of Staph. aureus colonies isolated on three Settling Plates.

Treatment of Cord	No. of Days	Average Count	Difference	Standard Error
Dye (floor B)	34	3·41	1·82	0·81
No Dye " A	34	5·23		

When the cords of all babies on floor A were treated with the dye, both the nasal and the skin infection rates were reduced by a significant amount when compared with those on the control floor. This difference might have been due to variations in nursing technique or to the presence of heavy nasal carriers on one floor and not on the other. However, this seems unlikely because when the dye was introduced on floor B and discontinued on floor A after only a fortnight's interval, both infection rates fell on floor B and rose significantly higher on floor A. The number of colonies on settling plates showed a similar distribution. Thus it appears that treatment of all babies' cords on one floor was

associated with a lower infection rate of nose and skin and with reduced contamination of the air.

Over this period a second type of investigation was carried out on floor C. The cord of every second baby born in this unit was painted with the dye, but treated and un-treated infants were kept in the same nursery and handled by the same staff, so that apart from the dye on the cord all conditions were the same for both groups. Swabs were taken from each treated baby when it was four days old and from an un-treated one of the same age, which acted as a control. The techniques used were the same as before, but settling plates were not put down. This floor also contained the premature unit, so special precautions were used here which were not employed on the other floors, such as the wearing of masks and the application of chlorhexidine cream after washing hands. Premature babies were not included in the investigation. The results are shown in Table 11.

TABLE 11.

The Effect on Infection Rates in Babies of the Treatment of the Cords of Alternate Infants Only.

Swabs.	Treatment of Cord	No. of Babies.	No. of Swabs Pos:	Difference	Standard Error.
Nasal	Dye	50	20(40·0%)	-	-
	No dye	49	19(38·8%)	-	-
Skin	Dye	50	10(20·0%)	33·1%	9·1
	No dye	49	26(53·1%)		

This investigation was carried out in order to eliminate the effect on the infection rate of nursing techniques and nasal carriers. The results, however, show an unexpected pattern. While the skin swabs of treated babies gave a significantly lower infection rate than those of the control group, the nasal infection rates were the same. The babies on this floor all breathed the same air, so this finding suggests that, though organisms may spread from the cord on to the skin, nasal infection comes mainly from the air. The nasal infection rate in both groups was lower than usual; it is possible that the treatment of the cords of half the babies in this unit had decreased the level of airborne contamination which was reflected in the number of organisms inhaled by the babies. The additional precautions mentioned before may also have contributed to this reduction.

Discussion.

The results of these investigations suggest that the umbilical stump is an important reservoir from which staphylococci may be spread round a nursery for new-born babies. After this work was completed, several papers were published which confirm this view. In September 1957, two papers appeared on the spread of beta haemolytic streptococci from this site. Kwantes and James described two outbreaks of streptococcal infection in which a large number of infants

were found to be carrying haemolytic streptococci on the umbilicus without showing signs of clinical illness. Boissard and Eton showed how an outbreak of puerperal pyrexia in mothers had its origin in the infected umbilicus of infants which acted as a reservoir for the organism so that the epidemic continued when there were no carriers among the staff. Like the incident which I have already described, these epidemics show the importance of swabbing the umbilicus when infection occurs in a maternity hospital. Hutchison and Bowman (1957) in a study of staphylococcal epidemiology in a maternity hospital, took 878 routine nasal and cord swabs from 439 babies, and found that on the third day, 77·2% were infected at each site. They concluded that the infants, which acted as a culture medium for the growth of Staph. aureus, were the main source of infection for each other and for contamination of hospital dust. Of the many sites on a baby which are known to yield a growth of staphylococci, none are so easy to treat as the umbilical cord, for there local applications are effective in suppressing growth whereas nose, eyes or faeces could not be disinfected except by the use of antibiotics, and this is undesirable as a routine. In 1958, Cook, Parish and Shooter at St. Bartholomew's Hospital made a study of various techniques which might prevent cross-infection in a Maternity unit, in an attempt to find out how this occurred. They compared the effect on infants' nasal carriage rate of the use of Chlorhexidine hand cream by nurses,

of individual gowns for the care of each baby, and of the application of Triple Dye to the infants' cords. In the hand cream trial, 9·7% of 31 babies were negative for Staph. aureus on the 12th day; with individual gowns, 24% of 29; and with Triple Dye, 31% of 55.

The chief criticism of the use of Triple Dye on cords is that separation of the cord is delayed, often until after the 10th day, when most mothers and babies leave hospital. This does occur and would do so if any bactericidal substance were applied to the cord. There is said to be a danger of infection of haemorrhage from the umbilicus if the cord remains attached, so the baby should remain under nursing care. This causes administrative difficulties; either the baby has to stay in hospital or a nurse has to visit the baby at home until separation has taken place. This may be inconvenient for the authorities but is not altogether a bad thing for the mother,. In America it is usual for mother and baby to leave hospital earlier than in this country, often at about the fourth day after delivery. I have not heard that there is a higher rate of complications connected with the umbilicus as a result of this practice. Other objections to the use of the dye have been to the colour itself, which is rather startling for the mother and is difficult to remove. Any substance which would

limit the growth of staphylococci could be substituted, Chlorhexidine in spirit, for example, or one of the antibiotics such as Neomycin or Gramicidin which are suitable for local application. Surgical spirit alone does not kill staphylococci; I took many swabs from the cord after spirit had been applied and found no decrease in the usual heavy culture obtained.

These criticisms do not outweight the value of any procedure which helps of limit cross-infection among newborn babies, for it is a very real threat to life and health in maternity hospitals nowadays.

Discussion.

The results of these investigations may be considered under four headings. They are different from those put forward in the introduction to this section, because as the work proceded, new ideas of the subject developed and new problems arose.

(1) The relative Importance of Staff-to-baby and Baby-to-baby Spread.

It is impossible to be dogmatic about which is the more important of these two methods of spread. It is, as it were, a matter of seed and soil; the nurses supply the seed, the babies the soil. In the Cardiff Maternity Hospital, when the staff were active in preparing the unit before it was opened, staphylococci were not isolated at all from 6 settling plates put down in the unoccupied nursery three days before it was opened. As soon as babies were admitted, staphylococci appeared, on an average of 5 colones per plate, and this figure soon rose to high levels. On floor C, where nurses worked for six weeks before deliveries began to be carried out there, using it for sewing and general preparations, samples of dust from the vacuum cleaner were examined for staphylococci on 34 occasions. Colonies of Staph. aureus were isolated seven times; they were of the same phage types as those carried by the staff who worked there. Again, as soon as babies were

present, staphylococci were isolated every day. This shows that babies must be present before nasal carriers can spread their organisms in very large numbers; conversely, there would be no cross-infection in a nursery if all carriers of Staph. aureus could be excluded. Some workers describe the effect of removing nasal carriers of certain strains from the nursery and found that these strains were no longer found among the babies. For example, Baldwin, Rheins, Sylvester and Shaffer (1957) removed 11 carriers of erythromycin-resistant strains from the nursery (10 carried the same strain, type Va 4/54). Subsequently only two infants were colonized by this strain and none of the nursing staff became transient carriers. I found, as several workers have done since (Clarke, McGeogh and Sippe, 1956, and Gillespie and Alder, 1957), that it was possible to terminate an outbreak due to a particular phage type by treating or removing from duty the people who carried it. Rountree, Heseltine, Rheuben and Shearman (1956) state that, where a virulent strain was concerned, it was essential for it to be eliminated from the staff before infection could be controlled.

The importance of baby-to-baby spread is, of course, due to the fact that the babies themselves act as the chief reservoir of infection in the nursery. It has been shown how staphylococci flourish on their skin and mucous membrane, in their

faeces and umbilical stump. Cross-infection in a nursery is different from that in other wards because a) so many reservoirs are present and b) it is so difficult to prevent spread from them. Wounds can be dressed in a specially ventilated room; patients coughing up or excreting large numbers of staphylococci can be isolated; but babies with staphylococci on their skins, cords and nappies still have to be bathed, dressed and their nappies changed in the same room as a dozen or more others. A point which was not recognised, or at least was not made clear until recently, is the difference between 'reservoirs' where organisms grow in large numbers and the places where they fall and may remain viable but do not multiply. Failure to appreciate this difference has, in my opinion, led to an undue amount of stress being laid on the isolatioh of staphylococci from dust, air, and fomites such as blankets, curtains and other materials. These are responsible for a certain amount of infection, no doubt, and their presence cannot be ignored because it is an indication that there is an abundant reservoir in the ward. But their importance as a source of new infection is relatively small compared with that of a reservoir site on which many millions of staphylococci are proliferating. The most important point is to realise how dangerous these reservoirs are as a source of organisms, which may be transmitted both by the air and by contact.

(2) The Relative Importance of Airborne and Contact Spread.

This used to be the subject of much discussion, but I think that again it is impossible to say that one route is all-important and the other negligible. It has been found, however, in several studies, that hospital cross-infection does not cease when measures are taken against airborne spread alone, such as the oiling of floors and blankets, the use of ultra-violet-light and, as in the present investigation, of a vacuum cleaner.

(3) Methods of Preventing Cross-Infection.

These may be considered under three headings:

(a) Prevention of the introduction of virulent staphylococci: How to tell these will be discussed in the section on virulence. It is impossible to remove all carriers of Staph. aureus from their work, but those who carry strains which are resistant to more than one antibiotic, or are known to be causing sepsis which is unusually severe, should be treated with a nasal ointment containing a suitable antibiotic. The substances used should be those which are active locally but are not used parenterally. In this way, even if resistance is acquired it will not interfere with treatment. Rountree et al (1956) used one containing neomycin and bacitracin, while Gillespie and Alder (1957) and Duthie (1957) used as I did, neomycin and gramicidin. Later Gillespie changed to neomycin and bacitracin

which he found to be more effective (1957). Everyone who has reported on this method of preventing the entry of virulent organisms has found it valuable, although a small proportion of carriers are said to be resistant to this form of treatment.

(b) Elimination of reservoirs: the treatment of umbilical cords with an antiseptic comes under this heading, but other methods are equally important. In the United States, infants are bathed with Hexachlorophene solutions and this has been considered to be useful, (Shaffer, Baldwin, Rheins and Sylvester, 1956). The isolation of babies infected with the epidemic strain is obviously sensible if not too many are involved. It is of great importance that any member of staff who has sepsis must not go on duty until Staph. aureus can no longer be isolated from the lesions.

(c) Blocking of routes of spread: In my opinion, the most important single method of spreading staphylococci in a nursery is by the hands. This problem has been discussed already. Many of the papers published recently on cross-infection in nurseries mention that the staff used either Chlorhexidene or Hexachlorophene on their hands - Shaffer, Baldwin, Rheims and Sylvester (1956), Munro and Markham (1958), Cook, Parrish and Shooter (1958). The last paper also draws attention to the value of using individual gowns for the care of each baby.

Several workers have mentioned the isolation of staphylococci from nurses' gowns, and I am sure that this technique should become generally adopted. As Hare stated in 1957: "No baby should ever be handled by the bare hands of the nurse or touch any part of her clothing."

(4) The Pattern of Spread by Staphylococci.

Two kinds of staphylococcal epidemic are described in this section:

a) An extensive one lasting for several months and involving numerous babies and a number of members of staff; (Williams' type II).

b) A series of short incidents associated with the presence of an individual carrier, (Williams' type I).

A similar pattern has recently been described by several workers. Shooter et al (1958) in a study in a surgical unit, called the first type staphylococcal "broadcasts". Hutchison and Bowman (1957) described them as follows: "waves of one or more major strains waxing and waning over a period of weeks or months with undercurrents of minor types occurring sporadically or at low levels, the whole system in a state of flux and liable to sudden variation without warning." Baldwin et al. (1957) show how the epidemiology of staphylococci in the hospital where they were working could be used to illustrate Williams' classification of staphylococcal epidemics. They considered that when Hexachlorophene baths were used, the widespread dissemination

of staphylococci round the unit by baby-to-baby spread was prevented. Williams' first type of epidemic remained - the direct infection of babies by a nasal carrier. They uncovered nurse-to-baby spread by this means, as I was able to demonstrate it in the present investigation by following the spread of non-hospital strains from new members of staff.

In this way the two types of outbreak appear to correspond roughly with the two types of spread, nurse-to-baby and baby-to-baby. The former occurs when there is a carrier who is an efficient distributor of staphylococci, but poor opportunities for baby-to-baby spread, or a strain which is not well-qualified as an epidemic one. The latter is an example of baby-to-baby spread which may occur in the absence of a nasal carrier and means either good opportunities for transmission or a highly communicable strain, or both.

STUDIES OF THE VIRULENCE OF STRAINS WITHIN THE SPECIES STAPHYLOCOCCUS AUREUS

Introduction.

It is a commonplace nowadays to remark on the unusual virulence of some strains of staphylococcus, notably phage type 80. The first published account of an outbreak for which it was responsible was given in 1954 by Isbister, Durie, Rountree and Freeman. This was before phage 80 had been isolated, so the staphylococcus at this time was described as being lysed weakly by phage 52A. Infections with this strain had been identified first in July 1953. It belongs to phage group I and Rountree, in her study of the distribution of phage types in Australia, published in 1953 did not find a preponderence of infections of babies or nurses within this group, so it is unlikely that this strain was widespread then. The phage 80, which was found to lyse this strain specifically, was isolated in November 1953. In 1955, Rountree and Freeman showed how this new phage lysed numerous other staphylococci from outbreaks of neonatal sepsis which had been received from all over Australia. They found also that this strain was becoming more commonly isolated from adult patients admitted to hospital with severe staphylococcal lesions. It was responsible in addition for outbreaks of furunculosis among the hospital staff. The types of lesions were unusually severe in these, with much oedema and toxaemia. During 1954, strains from outbreaks of neonatal

sepsis were received from 24 hospitals throughout Australia, and 19 of them proved to be phage type 80. Again, the skin lesions were described as being more severe than usual, and 8 out of 10 strains from empyemata belonged to this phage type.

In 1956, Bynce, Elder and Comtois gave an account of the strains isolated in a general hospital in Ottowa between 1953 and 1954. They found that one lysed by phage 81 was responsible for 50% of boils, carbuncles and abscesses; this has now been shown to be the same phage type as the Australian strain. Since then, outbreaks due to similar strains have been reported from Australia - McCartney and Yates (1956), Clarke, McGeosh & Sippe (1956): from the United States - Schaffer, Sylvester, Baldwin and Rheins (1957), Wysham and Kirby (1957), Cooper and Keller (1958) and Blair and Carr (1958): and from Britain - Duthie (1957), Gillespie and Alder (1957) and Barber and Dutton (1958). Several of these papers, those by Blair and Carr, Shaffer et al. and Cooper and Keller, describe the world-wide incidence of this phage type and summarise the numerous outbreaks it has caused. Blair and Carr compare the phage types encountered before 1954 with those received for typing between 1954 and 1957. The most striking difference was the prevalence of type 80 as a cause of severe infection in all parts of the United States. It was reported as the predominant strain in

most of the hospital outbreaks which were investigated. In one hospital it was first isolated in 1955; in 1956 it made up 5·3% of the staphylococci isolated, and in 1957, 13·7% during the first six months and 38·5% in the second.

INVESTIGATION IN ST. DAVID'S HOSPITAL

At the time when my own investigations began in the autumn of 1954, this phage type was unknown in Britain. St. David's Hospital had experienced an outbreak of severe infection in the winter of 1953 - 54 and strains from this had been typed at the Staphylococcal Reference Laboratory at Colindale and reported as being weakly lysed by phages 52 and 52A at a thousand times the routine test dilution only. When I started to type the strains which I was isolating in 1954, it became apparent that this strain was present in a much greater proportion of babies with sepsis than of normal ones, although there was no outbreak at the time. It was not the commonest strain; this was one of phage type 6/47, which although almost ubiquitous, appeared to do very little harm to the babies which carried it. In the course of the routine weekly swabbing, which was described in an earlier section, 193 cultures of Staph. aureus were isolated in nurseries from babies within a few days of birth. The subsequent history of these babies was determined and a record kept of whether they developed sepsis or not during their stay

in hospital; 61 of them (31·6%) were found to do so. The strains from these babies were typed and the results analysed in two ways:-

a) The number of staphylococci in phage types 52/52A and 6/47 and other strains were recorded according to whether they came from clinically infected or normal babies, (Table 12)

b) The percentage in each phage type which developed sepsis was worked out. From this it was possible to estimate the chance a baby would have of developing sepsis according to the phage type it carried, (Table 13).

TABLE 12.

The Distribution of Phage Types among 193 babies with and without Sepsis.

	Total	52/52A	6/47	other strains
Babies without sepsis	132	13 (9·9%)	76 (57·7%)	43 (32·4%)
Babies with sepsis	61	20 (32·8%)	31 (50·8%)	10 (16·4%)
" " "	60	28 (46·6%)	25 (41·7%)	7 (11·7%)

The third row of figures represents the typing results of 60 strains sent by the Hospital Laboratory for phage typing, and obtained separately from babies with lesions.

TABLE 13.

The Percentage of Strains in Each Phage Type grouped according to the Subsequent History of Infection in the Babies Carrying them.

Phage type	Total	No. later developing lesions	Percentage
52/52A	33	20	61•6
6/47	107	31	24•0
other types	53	10	28•0

Strains from any cases of unusual staphylococcal disease encountered in other hospitals in the region were usually sent to be phage typed. If these are considered along with those from cases at St. David's, the total number of severe infections from which type 52/52A was isolated between November 1953 and April 1955 was:- 4 deaths - 2 from empyema, 1 from peritonitis and one from an abscess; 1 empyema from which the baby recovered; 2 cases of pneumonia of the new-born (52/52A was assumed to be the cause of these, as it was isolated from the nose and throat swabs); and 6 of severe abscesses and cellulitis. The following accounts are given of infection among adults to show the type of disease which was occurring:-

(1) A patient was admitted to one of the outlying hospitals because of an infected injury to the hand. Subsequently the doctor attending him, one of the nurses and two patients developed boils or other form of sepsis. Because of the severe nature of the infection and clear-cut epidemic form, staphylococci from all five cases were sent to me for phage

typing and all proved to be 52/52A.

(2) A young woman died of septiciaemia in Cardiff Royal Infirmary and at autopsy a staphylococcus of this type was isolated from the blood, liver and spleen. Two doctors who attended the examination later developed boils from which the same phage type was grown.

These findings certainly suggested that staphylococci of type 52/52A were of greater virulence than others present in the area. But the presence of type 6/47 in St. David's Hospital made it difficult to obtain a clear picture of events. In the first place, it was so common that it could be isolated from nearly every baby whether other types were present or not. This made it, in my opinion, appear to be more virulent than it really was. Secondly, it was found to produce very little alpha lysin; this may have made it less virulent than the average, so that it was not a suitable strain to compare strain 52/52A with if one wanted to estimate the virulence of the latter. Another point which made me unwilling to jump to any conclusions as to the virulence of type 52/52A was that opinion at that time was against the possibility that one particular phage type might behave differently from the others. The current view was based on the observations of Williams, Rippon and Dowsett (1953) in this country and Rountree (1953) in Australia. They found

that patients with fulminating staphylococcal pneumonia following on infleuza were more likely to be infected with a group I strain and those with food poisoning would almost certainly have a group III strain. Rountree also found a preponderence of group II strains among boils and deep infections. Otherwise it was stated that "no particular staphylococcal type appears to be unduly common in any disease," (Williams et al. 1953). In 1953 also, Levy, Rippon and Williams examined 254 strains from human sources for alpha and beta, haemolysin production, pigment and fibrinolysin in order to see if there was any difference in the amount of these substances produced in the different phage groups. They reported that there was no significant association between phage type and other properties and no differentiation within the groups of any particular strain which happened to be epidemic. As the paper of Rountree and Freeman was not published until the summer of 1955, it may be seen that there was little support for any unusual theories about staphylococcal virulence.

In May, 1955, when work was started in the new Maternity Hospital in Cardiff, the phages 80 and 81 became available for routine phage typing. I was very interested to find that they lysed the strains 52/52A specifically, at the routine test dilution. I had kept cultures of these strains from the

1953/4 epidemic and found that these also belonged to the new type 80/81 (later phage 81 was omitted from the routine typing set and this strain became known as type 80). In Rountree and Freeman's paper the possible origin of this strain is discussed. It may have arisen by mutation from a previously existing strain of lower virulence. It was unlikely to have been present in Australia undetected for any length of time because of its striking behaviour. It was not the one that was responsible for the Canadian (Colbeck 1949 and Webb, 1954) or the Norwegian (Oeding 1952) outbreaks, and Williams stated, in a personal communication, the phage 80 lysed specifically few of the strains responsible for severe sepsis in Britain. It was first recognized in Australia in June 1953; in December 1953 it was responsible for an outbreak of severe sepsis in Cardiff. Did the strain arise spontaneously at opposite ends of the world and was it transferred by some means from one to the other? I found that none of the nursing staff at St. David's had come from Australia at that time, but no information was available about patients.

INVESTIGATIONS IN CARDIFF MATERNITY HOSPITAL.

In the study begun in May 1955, this strain was grown from a settling plate in one of the nurseries on the first day that the building was opened. After that it was not seen for

several months, as has been described in the section on epidemiology. When a pupil midwife who was a healthy carrier entered the unit in July, three babies and the mother of one of them acquired this organism on the floor where she worked. All developed sepsis, the mother a breast abscess and the babies unusually severe spots. When the infection broke out on the newly-opened floor C, it became obvious that this strain was indeed very dangerous, so a report was made to the clinicians concerned. All the adult carriers of type 80 were removed from duty and treated, and infected babies were isolated. As already described, these measures were effective and the outbreak was controlled. A continual watch was kept on all the new staff and any carriers of type 80 were kept away from the nurseries until they had been treated. A number of babies were infected, however, before these measures took effect and there were several cases of severe sepsis: - one of osteomyelitis, one of cellulitis, two breast abscesses in mothers and one in a baby.

The number of babies carrying the different phage types which developed sepsis are shown in the following tables. As in the investigation at St. David's the results are shown in two ways (Tables 14 and 15). In this hospital also there were two strains which produced less alpha lysin than usual, and infection rates with these are recorded separately.

TABLE 14.

The Distribution of Phage types among 388 babies with and without Sepsis.

	Total		80		52A/79		52A/6/7/54/73/81		Others	
Babies without sepsis	253	6	2·3%	35	13·7%	52	21·5%	160	62·5%	
" with sepsis	135	18	13·3%	11	8·1%	13	9·6%	93	69·0%	
" " "	80	14	17·4%	7	8·7%	5	6·2%	67	83·7%	

The third row of figures refers to strains received from the hospital laboratory.

TABLE 15.

The Percentage of Strains in Each Phage type Grouped According to the Subsequent History of Infection in the Babies carrying them

Phage type	Total	No. developing lesions	Percentage
80	24	18	75
52A/79	46	11	24
52/52A/6/7/5/73/81	65	13	20
other strains	253	93	37

Apart from the two weakly lysogenic strains, 253 staphylococci were isolated which produced average amounts of alpha lysin. So the apparent high virulence of type 80 was not due to comparison with an avirulent strain. The results show that when compared with fully toxigenic, coagulase positive staphylococci, strains of this phage type still appeared to have greater virulence. Although only 24 babies were infected,

two-thirds of them developed sepsis which was in most cases more severe clinically than that caused by other strains. What this difference might be due to has been studied in some experiments described in Appendix II.

DISCUSSION

"It would appear that Staph. aureus of phage type 80 is highly infective and possessed of more than ordinary virulence... Strains of this type present a challenge and an opportunity to the bacteriologist for fundamental investigation on the mature and mechanisms of pathogenicity of the staphylococci." (Blair and Carr, 1958).

This is by no means a new challenge. Bacteriologists have been interested in this problem for almost as long as staphylococci have been known. No one can fail to be puzzled by the difference between the harmless symbiosis in which these organisms can live with man, and the devastating diseases and epidemics which they can cause. If the fundamental reason for this difference could be found, not only would we gain insight into the mechanism of staphylococcal virulence, but the discovery would be of enormous practical value in preventive medicine. If only a simple laboratory test for virulence could be made available, the main difficulty in the prevention of staphylococcal cross-infection would be removed. We would be able to practice what Brodie, Jameson and Sommerville (1955) call

'species sanitation' - (this name is derived from the prevention of malaria which was made possible once the identity of the particular mosquito vector was known).

To consider the practical aspect of the question first; the difficulty is that the coagulase test, which is used to identify the species Staph. aureus, is too inclusive, as many workers have remarked:- Schwabacher et al. (1945); Colbeck, (1949); Brodie, Sommerville and Wilson, (1956). Certainly coagulase negative organisms are not likely to be a cause of trouble (although such strains - probably degraded coagulase positive ones - have sometimes been grown from closed and long-standing infections such as osteomyelitis and brain abscess). But within the coagulase positive group, strains seem to vary so much in virulence, that other properties have frequently been investigated as possible alternative criteria. The production of alpha lysin seemed to be the obvious one, and several workers have advocated its uses in preference to the coagulase test:- Christie, North and Parkin, (1946); Marks, (1952); Jackson, Dowling and Lepper, (1955). In my own investigations, I found that the three epidemic strains which were poor alpha lysin producers could virtually be ignored as a cause of disease. I have also found the routine use of sheep blood agar plates of great value for the early

identification of Staph. aureus colonies. Many people use the phenolphthalein phosphate plates described by Barber and Kuper (1957) for this purpose (this test gives a close correlation with coagulase). However, none of these tests gives an indication of the kind of differentiation we are looking for - the difference between a dangerous and invasive strain such as phage type 80 and the average staphylococcus with which we expect to be able to hold our own.

The more fundamental problem, the actual mechanism of staphylococcal virulence, has stimulated a great deal of research ever since the discovery of coagulase in 1903 and of alpha lysin in 1894. First there was the question as to whether the lysin for rabbit cells and the lethal and necrotizing factors were separate (Parker, 1924) or the same (Burnet, 1929). Then the Neisser-Wechsberg leucocidin was differentiated by Panton and Valentine in 1932 from that acting on human cells. Later, other lysins were described, beta by Bryce and Rountree in 1936, gamma by Morgan and Graydon in 1936 and Smith and Price in 1938, and delta by Williams and Harper in 1947. The enzymes which attack fibrin and hyaluronic acid were also discovered, and the mysterious substances which break down egg-yolk and serum. A series of investigations were carried out in which these various

properties of the staphylococcus were compared with its virulence. The latter was estimated in two ways:-

(1) By finding the source of the strain in question and comparing the properties of staphylococci from lesions with those from carrier sites - Wright (1936), Fairbrother, (1940), Christie (1940), Schwabacher et al. (1945), Gillespie and Simpson (1948), Selbie (1953), Lack and Walling, (1954), and Jackson, Dowling and Lepper (1955). Most workers agreed that the strains from lesions possessed a more complete armamentarium of properties than saprophytic strains. Any of these might be lost, the most likely being the production of alpha lysin, and a fully degenerate strain would be avirulent. One criticism can be made of this type of investigation now that phage typing has shown how one or two strains may be endemic in a hospital at any given time; when these studies were made of strains from in-patients in one hospital, many samples of one phage type may have been tested instead of the large variety of strains that the investigator imagined.

(2) By determining the virulence for animals. Most of these studies led to the conclusion that, while none of the reactions of the usual experimental animals can be compared with those of man, the virulence of staphylococci for animals appears to vary according to the amount of alpha lysin produced - Chapman, Berens, Peters and Curcio (1934), Cruickshank (1937), Flaum (1938),

Chapman, Berens, Nilson and Curcio (1938), Christie, North and Parkin (1946), Selbie and Simon (1952), Howard (1954), Fisher and Thompson (1956), and Anderson (1956). Elek, concluding that animal tests were not reliable in the assessment of virulence for man, recently published a description of pathogenicity tests carried out on human volunteers (1956). In summary, he said, "No difference in the virulence of known pyogenic strains and nasal strains from unselected carriers could be demonstrated by accepting pus formation in man as the criterion of virulence. In surgical infection the foreign body reaction and possible other mechanisms of delayed defense appear to determine whether clinical disease occurs."

Another type of animal experiment was carried out in an attempt to find out what actually happens in the course of a staphylococcal infection. Burnet, in 1929, for example, found that although rabbits immunized with a filtered toxin were unable to clear the blood of staphylococci injected intravenously, they survived longer than non-immunized ones. This suggests that there might be an aggressive factor in the bacteria themselves apart from the power to produce toxin. Lyons (1937) also studied the rate of clearance by staphylococci from the blood of normal and immunized rabbits. In both, there was rapid disappearance of organisms from the blood stream but a secondary rise after 5 hours occurred in the non-immunized

animals, and some of the immunized ones survived. He ascribed the difference to the protective action of the antibody to the capsules on the immunizing staphylococci. At autopsy, staphylococci were isolated from the kidneys of all the rabbits. Smith and Dubos (1956) studied the behaviour of coagulase negative and positive staphylococci (using mouse plasma) when injected into mice; a) by study of the lesions at autopsy; b) by survival times following intravenous injection, and c) by quantitative determination of numbers isolated from blood and various organs. In both cases, most organisms disappeared rapidly from liver, spleen and kidney, but a few living staphylococci always persisted for several weeks. Then they started to multiply in the kidneys, causing abscesses which killed the mouse in the case of coagulase positive staphylococci but became sterile with coagulase negative ones. Fisher and Thompson (1956) found that some strains of Staph. aureus were rapidly lethal for mice and others much less so - the effects being mainly in the lungs. This was not due to abnormal production of alpha lysin or coagulase, so a seardh was made for the lethal factor - without success.

No information has resulted from all this work as to how or why one strain of Staph. aureus should be more virulent than another. Some interesting discoveries in recent years, however, have a bearing on this subject. It was formerly

thought, as described earlier in this section, that no differences could be detected, either in laboratory tests or clinical behaviour, between staphylococci of different phage types. Where differences could be shown, as for example the tendency to be associated with a certain disease, or to become resistant to antibiotics (Group III), or to colonize the noses of staff in Maternity Hospitals (Group I) they affected the broad phage and serological groups, not individual phage types. Therefore any demonstration that lysis by a particular phage was associated with certain clinical and in vitro characters was of very great interest indeed.

In 1955, Parker, Tomlinson and Williams published a paper showing that strains from superficial infections such as pemphigus and impetigo were nearly always lysed by a new phage, 71. Tomlinson had noticed, in 1941, before phage typing was introduced, that staphylococci isolated from cases of impetigo inhibited the growth of C. diphtheriae on solid media and caused opacity in plates containing serum. These properties were found to be present in strains of type 71. Another empirical test had been described by Gillespie and Alder in 1952 - the production of opacity in broth containing egg-yolk. This property was seen most often in strains isolated from deep-seated closed lesions occurring in non-hospital patients.

Hospital staphylococci, which were mainly from superficial wound infections, were penicillin-resistant, egg-yolk negative and tended to belong to phage group III. It was suggested that strains which were egg-yolk negative and positive in the corynebacterium inhibition and serum opacity tests were likely to cause superficial infections, while strains which showed the opposite characters were likely to be more invasive and capable of causing deep lesions. Strains of phage type 80 come into the latter category. Although these observations do not tell us a great deal at the moment about the pathogenesis of staphylococcal lesions, they are very important in showing that in vitro differences between phage types can be linked up with differences in behaviour in the course of an infection. If similar observations could be made which would identify strains likely to cause epidemics, we would be on the way to solving our problem.

One method which has been used to detect such staphylococci is by the antibiotic resistance pattern. It can be assumed that strains resistant to more than one antibiotic substance are likely to be "hospital staphylococci" and for that reason and because they are difficult to treat, they should be regarded as unusually virulent. Shooter et al. (1958) tried the effect of barrier nursing in a side ward patients infected with tetracycline-resistant staphylococci. They found this

effective in preventing the spread of their infection.

This brings one to the rather academic question; are staphylococci nowadays really more virulent than those seen in the days before the introduction of antibiotics; or is the increase in staphylococcal disease simply due to the selection of virulent strains by the use of substances to which they are resistant? McDermott (1956) discussed this question and came to the conclusion that a) staphylococci are no commoner in the general population than they were 12 years ago; b) apart from empyema in infants, staphylococcal infections outside hospitals seem to be no commoner than before, and c) there is no evidence that "hospital strains" are more virulent than others, although there may be localised epidemics of strains which are more pathogenic. What we are seeing nowadays, he concludes, are signs that the hitherto satisfactory equilibrium between our hospital patients and staphylococci is becoming unbalanced - not because there is greater challenge from the staphylococci but because the patients are less well-equipped to deal with them. He blames the use of cortisone, X rays and "broad spectrum antibiotics" for this, and the survival of people such as diabetics who would formerly have died.

These theories do not explain the recent severe outbreaks in maternity hospitals, however. In my opinion, there is no evidence that staphylococci nowadays are more virulent than they used to be. The accounts of outbreaks of fifty and a hundred years ago describe the ravages of highly virulent organisms. What we do know now is that strains of certain phage types are more likely to cause outbreaks of infection than others; that some strains become epidemic or endemic without causing disease; and that as yet there is no way of telling the difference between them.

Several papers have been published recently which show how few the virulent and epidemic strains are. Baldwin, Rheins, Sylvester and Shaffer (1957) isolated 197 distinct strains in 7½ months from a nursery, but found that only 5 of them accounted for 75% of the colonisation, and one alone for 30%. Shooter et al. (1958) identified 186 different phage patterns among the staphylococci isolated from a general surgical ward in eight months. Only 13 of these caused disease and only three were responsible for disease in more than one patient. Williams (1959) in a survey of the staphylococci received for typing by the Staphylococcal Reference laboratory

at Colindale between 1954 and 1957, and of published reports of outbreaks, described a similar situation. "Over 50% of epidemic spread was due to six phage types - 80, 71, 7/47/53/54/75, 75/77, 52A/79 and 47/53/75/77. There appear, therefore, to be substantial differences in the pathogenic abilities of different phage types of staphylococci." Sometimes the occurrence of an outbreak has been ascribed to the presence of a dangerous carrier. Such a carrier is, in Williams' opinion, likely to be a person who carries a dangerous staphylococcus.

There is unfortunately at the moment no test for such staphylococci. We cannot even label a phage type as invariably pathogenic, as one can predict that S. typhi for example, will be more virulent than S. typhimurium. Not all the strains of phage type 80 are equally virulent, while others, such as the strains described by Colbeck (1949) and Webb (1954) seem to have been just as virulent as strain 80 at its worst. Gillespie found some strains of type 80 which had a different antibiotic pattern and clinical behaviour from the expected ones (1957). In each outbreak the causal organism must first be identified and then treated according to its virulence in that particular place at that particular time.

APPENDIX I.

Experiments on Resistance to Adverse Conditions.

Introduction.

In the investigations into the epidemiology of Staph. aureus in Cardiff Maternity Hospital, it was found that a number of strains became epidemic for periods of several months at a time. It is interesting to speculate on why these particular phage types could have behaved in this way and if they might have possessed some special qualities which made them more easily transferred than other strains or better able to resist the action of inimical agents in the environment. The first quality would be difficult to test, but the second is one which it seemed possible to estimate roughly by simple laboratory tests. Several workers who have studied the resistance of various species of bacteria have commented that different strains of Staph. aureus varied in their reaction:- Burtenshaw (1945) in the case of skin lipids, and Lowbury and Fox (1953) on the effects of drying. For this reason, there seemed a chance that there might be a variation in the resistance of certain strains of Staph. aureus which could explain their epidemiological behaviour.

Four agents were selected, and the resistance to them of strains which became epidemic and strains which did not, were tested. The agents were:

(1) Fatty acids. Burtenshaw (1945) found that ether extracts of skin, hair and nails were bacteriacidal and that the active

component was in the fatty acid fraction. Therefore two fatty acids, oleic and linoleic acids were used in an experiment to see if the epidemic strains were more resistant than others.

(2). Soap. Since ordinary soap is relied on every day for the removal of staphylococci from the hands, it was thought that it would be worthwhile to test the resistance of the two types of staphylococci under consideration to soap solutions.

(3) Acidity. Some workers have postulated that the self-sterilizing power of the skin is due to its acidity, which is mainly derived from lactic acid, but also from formic and butyric acids and the breakdown of keratin. Burtenshaw estimated that the skin all over the body, except the areas where there are apocrine glands, has a pH of about 5. I found that staphylococci survived pH 3 for 15 minutes but not much longer, so this degree of acidity was used to test the resistance of different strains.

(4) Drying. The survival of staphylococci in dust has been studied by Lowbury and his colleagues - Lidwell and Lowbury, (1950) and Lowbury and Fox (1953). In my own investigations, I found that 3,400 colonies of Staph. aureus could be isolated from 1 gramme of dust obtained from the vacuum cleaner used on floor C, after it had remained on the bench for 6 weeks. These may have been protected from the effects of drying by serum or pus, for drying from a drop of water or saline has been found in vitro experiments to be highly destructive to

most organisms. In fact, Norton and Novy (1932) considered that dessication was responsible for the death of organisms when a drop of a suspension was placed on the skin. Therefore, the epidemic and non-epidemic strains were tested for resistance to drying, both from the point of view of survival in dust for long periods and of survival on hands for long enough to be transferred from one patient to another.

Materials and Methods.

Sixty strains altogether were tested for resistance to all four agents; 30 epidemic and 30 non-epidemic. The first group consisted of 6 strains belonging to 5 of the epidemic phage types, each obtained from a different source. The other group was made up of staphylococci belonging to 18 different phage types, none of which ever became common in the hospital. One experiment was carried out every day for 6 days, testing 10 different types each time, so that any minor variation in conditions would be distributed over a series of different strains.

Before the experiment began, tenfold dilutions of the 10 strains to be tested were made in saline, and 3 drops of the 10^{-5} and 10^{-6} dilutions were allowed to fall onto agar plates. These were incubated, and the number of colonies counted the following day, giving the size of the inoculum used. Pasteur pipettes calibrated to deliver 50 drops per ml. were used throughout.

(1) Fatty Acids. Oleic and linoleic acids were used; the method was similar to that described by Ricketts, Squire and Topley (1951). Each was diluted in buffered peptone water to a concentration of 5 mg/ml. 1·8 ml. amounts of this were put into 10 test tubes, and 0·2 ml. of the 10^{-4} dilutions of each strain to be tested, was added. The tubes were incubated in a waterbath at 37°C for 30 minutes and then 2 drops from each tube allowed to fall onto an agar plate.

(2) Ten tubes, each containing 1·8 ml. of buffered peptone water at pH 3, were inoculated with 0·2 ml. of the same 10^{-4} dilutions of 10 cultures of Staph. aureus strains as in the previous experiment. They were incubated for only 15 minutes before a sample was withdrawn from each tube and two drops delivered onto plates.

(3) Soap. A 1 in 10,000 (w/v) solution of the soap used for handwashing in Cardiff Maternity Hospital was used in this experiment. 1·8 ml. was put into each of 10 tubes and inoculated as before. Two-drop samples were removed after incubation for an hour.

(4) Drying. 1 drop from a 10^{-5} dilution of each of the 10 cultures to be tested was allowed to fall onto each of 4 sterile cover slips. These were stored in sterile petri dishes with

the lid half open. When the drops were dry, two of the cover slips were inverted onto agar plates, left there for 15 minutes, and carefully removed. The same was done with the remaining two next day.

After overnight incubation of the plates, a series of small areas were obtained with a number of staphylococcal colonies on each, two for each strain in each test. These were counted and an average taken of the two. After the number of staphylococcal colonies in the inoculum was counted, the percentage survival in each test was estimated. The results are shown in Table 16.

Results.

TABLE 16.

The Average Percentage Survival of Epidemic and Non-Epidemic Strains.

	Epidemic strains	Non-epidemic strains	Difference	S.E. of Difference
Linoleic acid	7•558	10•154	+2•596	±2•463
Oleic acid	29•568	34•675	+5•107	±2•971
pH 3	1•007	2•768	+1•761	±1•317
Soap 1 in 10,000	20•153	28•586	+8•433	±6•154
Drying after 1 hour	41•637	44•703	+3•066	±5•084
" after 18 hours	5•400	8•850	+3•450	±2•179

The average percentage survival of the 30 epidemic and 30 non-epidemic strains are shown in Table 16. It may be seen that in every case, the non-epidemic strains showed greater resistance than the epidemic ones. Although the results in individual tests were not significant, when those of all six are added together, the total difference is significant. This is the opposite of what I had expected to find, which was that strains which spread round the hospital would be more resistant to inimical agents than those which did not spread. I, therefore, asked the advice of Dr. C. C. Spicer, who is statistician at the Central Public Health Laboratory. He reviewed the statistical analysis of this section and came to the following conclusion: "The weighted mean difference is $2 \cdot 832 \pm 0 \cdot 885$. As the weighted mean difference exceeds zero by about three times its standard error it can be taken that spreading strains survive less well than non-spreading ones."

Discussion.

As may be seen from the large figures for the standard errors, the results of these experiments were very variable. But since a significant difference was found in the sum of the

mean percentage survival rate of epidemic strains, possible reasons for this must be considered.

The most interesting of these is simply that the ability to spread round a hospital does not depend on resistance of staphylococci to such things as drying, soap and the self-disinfecting power of the skin, but on the amount of multiplication which takes place in the host. If a strain is able to grow rapidly and to colonize the host successfully, it will be able to spread through a hospital because of the large numbers of organisms present. Many more will be disseminated than with a less-successful colonizer, so even if the survival rate is poorer outside the body, this is relatively unimportant. One comes again to the difference between the importance of the 'reservoirs' of infection and the places where organisms are deposited but cannot multiply. If a strain forms large reservoirs from which spread is continually occurring by rapid transfer between baby and baby, the survival rate outside the host is unimportant.

It is well known that the more pathogenic organisms which are well-adapted to growth in the host are often not well-equipped for survival outside the body. Examples of this may be seen within the genus Neisseria, where the non-pathogenic species

survive well in dust and air, while N.meningitidis and N.gonorrhoea are amongst the most sensitive bacteria we know. Within the group of Micrococci, Ricketts, Squire and Topley found that Staph. aureus was more sensitive to the action of fatty acids than the other cocci tested; and it often occurs that a micrococcus survives on a selective plate or in a medium containing an antibiotic when Staph. aureus is killed.

For this reason, these experiments which gave completely the opposite result to the expected one, may be taken as a demonstration of a fact which we are coming to appreciate more and more:- the importance in hospital cross-infection of sites where Staph. aureus can multiply, compared with places where organisms are deposited and remain visible but do not grow. If we can remove the first, the second can be ignored.

APPENDIX II.

Experiment on the In Vitro Properties of some Staphylococcal Phage Types.

Introduction.

This section is an account of a series of experiments which were carried out, using the staphylococci which were most commonly isolated during the epidemiological work at Cardiff Maternity Hospital. As many strains from different sources were used as possible. Since the clinical and epidemiological behaviour of these strains was known, I thought that it would be interesting to study their reactions to various laboratory tests. None of these was very elaborate, and all were designed to see if any in vitro differences could be detected between the strains which could be linked up with the clinical findings.

1. Haemolysins.

During the latter part of my epidemiological investigations about 24 representative strains were examined each week for alpha lysin by a simple plate test. It was thought that this would show if there was any variation in the amount of alpha lysin produced by different staphylococci of the same phage type at different times. It was also thought that it would be an advantage if the organisms were tested as soon as possible after isolation. When the plate method is used, it is possible to compare a number of strains at the same time, so that the medium and experimental conditions are the same. The medium used in these experiments appeared to vary slightly from week to week, judging by the average size of the zones of lysis obtained (possibly according to the amount of antitoxin in the sheep blood used) but as the same conditions applied to all the strains tested on that occasion, this variation was allowed for. As Marks pointed out in 1950, the estimation of toxigenicity by measuring the size of the zone of lysis round a colony made the differences between the zones appear smaller than the real differences in toxigenicity, for zone widths are measured linearly while toxin diffused into the agar through three dimensions. He considered, however, that the method was useful for purposes of comparison.

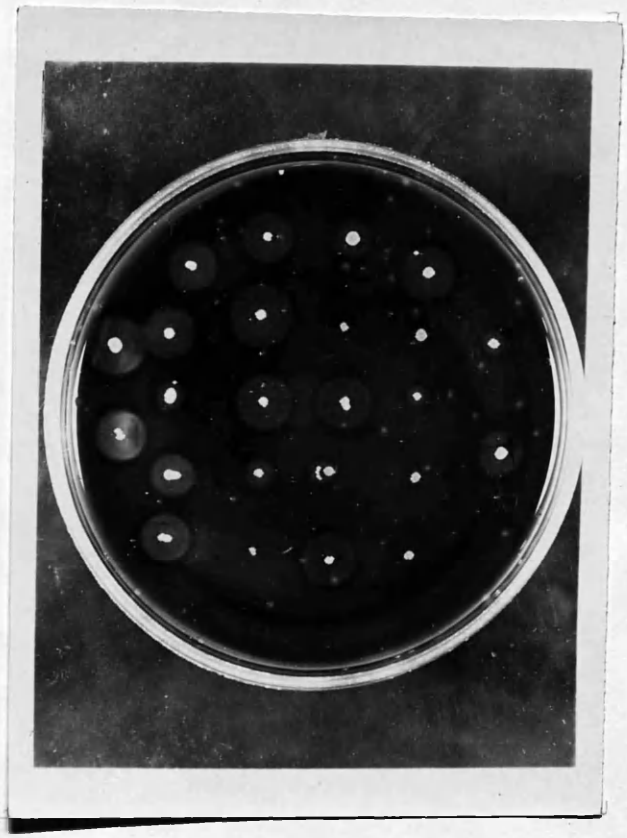


Plate III. A photograph of 24 colonies of Staph. aureus on a Sheep Blood Agar plate, showing the different zones of lysis produced.

Materials and methods:

Sheep blood agar plates: 5% sheep blood in nutrient agar
1 ml. 9% bovine albumin per plate.

Sheep cells were obtained each week from the Serum Research Institute, Carshalton and were used as soon as possible. They were not washed.

Bovine albumin fraction V was obtained from Armour Bros. and diluted in sterile saline. Its use was suggested by Dr. Marks as a method of suppressing non-specific lysis. Rabbit and human blood was obtained fresh by bleeding and defibrinated. Stab inocula were made in the plates, using a straight wire dipped into an over-night broth culture. The plates were incubated overnight in 20% CO_2. The zones of lysis were read the next day and recorded in millimetres. About 24 strains were tested on each plate, (plate III). In order to complete this study a series of strains were inoculated onto sheep, rabbit and human blood plates (5% concentration) in order to estimate the amount of beta and delta lysins produced. The sheep plates were incubated anaerobically, as Christie and North in 1941 showed that beta lysin was the only one which was produced under these conditions. The human blood plates were incubated in air without CO_2.

TABLE 17.

Frequency Distribution of the Size of Zones of Lysis produced by Staphylococci of Different Phage types.

Phage type	No. of strains	Size of Zones of Lysis in Millimetres. 0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8 -
80	95	3·2	1·1	4·2	34·7	33·9	16·9	6·3	-	-
52A/79	117	35·0	24·8	29·9	6·0	2·6	0·8	-	0·8	-
52/52A/6/7/-73/81	202	62·4	28·1	5·0	3·0	1·0	0·5	-	-	-
52A	84	16·7	36·9	26·3	16·6	2·4	1·2	-	-	-
6/47	42	4·8	7·1	9·5	21·4	26·2	19·1	7·1	4·8	-
7/54	143	0·7	3·5	1·4	11·2	17·5	30·0	18·2	14·7	2·8
6/7/47/53/-54/75	42	2·4	4·8	7·1	9·5	11·9	14·3	19·0	24·0	7·1
42D/77	112	0·9	2·7	10·6	8·9	24·1	25·9	8·1	16·1	2·7
47/53/75/77	14	35·8	-	-	-	7·2	14·0	43·0	-	-
3A	33	24·2	9·0	3·1	21·2	8·2	21·2	3·1	-	-
42E/77	31	-	-	3·2	6·4	64·5	9·7	9·7	6·5	-
71	23	-	-	8·5	17·3	39·0	31·0	-	4·2	-

Results

a) Table 17 shows the distribution of zones of lysis among large numbers of staphylococcal strains isolated week by week during the epidemiological investigations. In each phage type, while occasional strains appeared with unusually small or large zones, the majority of zones were about the same size. In some phage types - 3A, 7/54 and 47/53/55/70 - two varieties were present in the hospital, one producing an average amount of lysin (the lemon yellow one in the case of type 7/54) and one very little. But when the results are taken as a whole, it is apparent that strains of some types were more actively toxigenic than others. Strains of phage types 52A/79, 52/52A/6/7/54/73/81 and 52A consistently produced very little. Of special interest is the amount of toxin produced by staphylococci of phage type 80, since they were found clinically and epidemiologically to be unusually virulent. There is no evidence that they formed more toxin than other strains, in fact if anything they appeared to be slightly less actively toxigenic than the average. It is interesting to compare the results of these investigations with the carrier-lesion ratios given on pages 99 and 104.

b) The results of plate tests for lysis by ten strains on three different bloods are as follows:

TABLE 18

The Size of the Zone of Lysis Produced by 10 Strains in the Blood of 3 Species.

Phage type	Rabbit	Sheep *	Human
80	4 mm.	-	1 mm.
52A/79	1·5	-	1·0
52/52A/6/7/54/73/81	1·0	-	1·0
52A	1·5	-	2·0
6/47	4·5	-	2·0
7/54	5·04 and 1·25	-	2·0
6/7/47/3/54/75	4·5	-	0·5
52D/77	4·5	3 mm.	1·0
47/53/75/77	4·0 and 1·0	-	2·0
3A	3·0 and 1·0	-	2·0

The distribution of alpha toxin production follows much the same pattern as in the previous test with sheep blood plates. Only one strain was found to produce beta lysin, and this and the production of delta lysin showed no association with pathogenicity.

* incubated anaerobically for beta lysin production.

2. Coagulase Production.

It is reasonable to expect that the production of coagulase might have some association with virulence, for this property has been generally accepted as the criterion for inclusion in the species Staph. aureus. Organisms belonging to this species are assumed to be pathogenic, while Staph. albus and Staph. citreus are regarded as being non-pathogens. The actual mechanism by which the production of coagulase could make an organism virulent is not clear. The subject has been investigated most thoroughly by Smith, Hale and Smith (1947), who carried out experiments with guinea-pig plasma, which does not clot in the presence of coagulase. If staphylococci were injected into these animals along with a clottable plasma from another species, the resulting infection was found to be more virulent on the whole, than when a non-clottable plasma only was present. This suggests that the staphylococci may be protected by the fibrin formed in the clotting process. Smith showed in 1956 that when purified coagulase was injected intravenously into rabbits, their plasma fibrinogen fell steeply and fibrin clots were found post-mortem in the blood vessels, especially of the lungs. On the other hand, it has been suggested that coagulase might be held to protect the host by limiting the spread of infection - but this has not been demonstrated. It was studied in 1935 by Menkin and Walston, but they found that the principle which 'fixed'

the staphylococci at the site of invasion was not the same as that which caused coagulation in vitro.

The investigations to be described were carried out on six strains of different origins, belonging to ten phage types, as in the previous experiment. Their aim was to discover if any difference could be shown in the amount of coagulase produced, which might explain the different clinical and epidemiological behaviour of these strains in the hospital - particularly in the case of phage type 80. The two kinds of clotting mechanisms called "bound" and "free" coagulase which were described by Duthie in 1954, were investigated separately.

It was noticed by chance that when the broth cultures used in phage typing were left on the bench for several days, some tubes began to show the characteristics of 'roughness' - a granular deposit at the bottom of the tube and a pellicle on the surface of the culture, so that a ring of growth was left on the glass when the tube was emptied. It was observed that cultures of phage type 80 seemed to show this change less often than those of other types. This is an interesting point, because the work of Smith, Morrison and Lominski (1952) showed that rough variants on a plate were more active coagulase producers than organisms which grew in smooth colonies. In order to compare the roughness of these strains with their ability to produce the two kinds of coagulase, a system of 'points' was worked out, in which an arbitrary number was given for the amount

of a) free coagulase b) bound coagulase and c) the degree of roughness. In each case, the percentage gained by each strain of the total number of 'points' possible was recorded.

As described below, it appeared from Table 19 that strains of phage type 80 produced less free coagulase than those of other phage types investigated. In order to study this further, a titration was carried out on all the strains being investigated, in which diminishing amounts of culture were used. It was hoped that an accurate end-point could be reached which would give more detailed information about the amount of free coagulase produced by each strain.

Methods.

a) Free coagulase: Plasma obtained from the same donor was used throughout. 1 ml. of plasma diluted 1 in 10 was inoculated with ten drops from an overnight broth culture of the strain to be tested. 4 x $\frac{1}{2}$ in. tubes were used, and these were incubated in a waterbath at 37°. They were examined after they had been incubated for 1, 2, 4 and 6 hours. The amount of clotting was indicated by a number. The system of numbering was as follows: 1 = granular; 2 = small clot= 3 = moderate clot; 4 = solid clot.

In the titration of the amount of coagulase produced, 1 ml. of plasma diluted 1 in 10 was used as above. Overnight broth cultures were diluted 1 in 10 and 1 in 1000. 10 drops of each

dilution were added to 1 ml. of the plasma (using a pipette made to drop 50 drops to 1 ml.) so that final dilutions were about 1 in 50, 500, 5000 and 50,000. The tubes were examined at 2, 4, 6 and 24 hours but in this case only the presence or absence of a clot was noted.

b) Bound coagulase: The method used was that described by Duthie (1954). Cells from 24 hour broth cultures were washed three times in saline and resuspended to a standard density in saline. 0•2 ml. of this suspension was added to 0•2 ml. of 0•2 bovine fibrinogen in 4 x $\frac{1}{2}$" tubes. The rack of tubes was shaken in a Kahn shaker for 15 minutes and the degree of clumping then read, using a hand lens and direct lighting. It was recorded by an arbitrary system of numbers, 1, 2, 3 and 4.

Saline was the diluent used in these experiments.

Results.

TABLE 19.

To Compare the Amounts of Free and Bound Coagulase produced by Ten Strains of Staphylocci with the Degree of Roughness

Phage type	Free Coagulase 11 tests - 44 points possible		Bound Coagulase 12 tests - 44 points possible		Roughness 9 tests 36 points poss.	
80	16	36•3%	28	63•6%	5	13•9%
52A/79	21	47•7	25	56•8	16	44•4
52/52A/6/7/54/73/81	23	52•2	28	63•6	28	77•8
42D/77	34	77•3	26	59•1	24	66•6
7/54	35	79•5	21	47•7	24	66•6
6/7/47/53/54/75	30	68•1	22	75•0	25	69•5
6/47	36/36	100	26/36	72•2	25/32	78•2
52A	24	55•8	18	41•0	28	77•8
47/53/75/77	42	95•0	29	65•9	25	69•5
3A	43	97•7	32	72•7	20	55•5

Table 19 shows the total number of "points" given to each strain, indicating the amount of free and bound coagulase produced and the degree of roughness developed in broth cultures which had been left on the bench for 7 days. It sppears that the strains of phage-type 80 tested produced less free coagulase than the other strains and showed the change to roughness less often: the amount of bound coagulase produced was average.

If the strains which clotted first in the titration of coagulase were recorded, the results are:-

80		nil
52/52A/77/54/73/81	-	nil
52A/79	-	nil
42D/77	-	4
7/54	-	4
6/7/47/53/54/75	-	4
6/47	-	6
52A	-	nil
47/53/75/77	-	5
3A	-	2

I noticed that the strains which appeared to produce free coagulase most slowly and in the smallest amount belonged to Group I. I did not realise the significance of this until I read the paper by Barber and Wildy (1958) who remarked on the same phenomenon. They found that if serum or plasma were added to the growth medium, staphylococci of this group produced average amounts of coagulase.

Conclusions.

If the smaller amount of free coagulase produced by strains of phage type 80 can be accounted for by the lack of plasma in the medium, there is no remarkable difference in strains of this type as coagulase-producers and other strains. Certainly staphylococci of phage type 80 did not appear to form

unusually large amounts which could be taken to be responsible for its clinical virulence. The only difference found which might be of interest was the failure of broth cultures to become rough when compared with other strains. This was not due to the length of time since isolation, since this was much the same in all cases. One wonders if it could have been associated with an additional antigen on the surface of the organism, or with some sort of capsule.

3. The Number of Diffusible Antigens.

This technique, which was adapted in its present form by Elek and Levy in 1950, is used here to show the number of different diffusable antigens produced by one strain of Staph. aureus. As the antitoxin is that developed to the strain known as Wood 46, the antigens measured are only those which are also produced by that strain. Elek and Levy found that the number of flocculation lines produced by the interaction of antigen and antibody gives a rough measure of the amount of toxin production. Howard (1954) compared the number of lines produced by various strains with their pathogenicity for mice, and found them to be related. In 1956, Anderson carried out a similar investigation on 60 strains freshly isolated from a casualty department. He added pigment, fibrinolysin, hyaluronidase, alpha lysin, haemolysin pattern and coagulase production to the tests performed.

He found that the production of hyaluronidase, alpha lysin and mouse pathogenicity showed the closest correlation with the number of flocculation lines.

The present investigation is designed simply to find out if the virulence of strains of phage type 80 can be shown to be due to the production of an unusual number of diffusible antigens.

Methods

(1) the medium used was that described by Elek and Levy (1950)

(2) Antitoxin was Burroughs Welcome Standard Antitoxin globulins, at a strength of 1200 units/ml.

A strip of filter paper soaked in this was incorporated in the medium in a 4" petri dish. When the agar was set, the strains of staphylococcus were streaked across the plate at right angles to the strip. Plates were incubated in 30% CO_2 for 48 hours and read after they had been left of the bench for a further 48 hours.

Results

Seven plates were put up, with four different strains on each, but strains of phage type 80 on each plate. The number of flocculation lines are shown in Table 20. The average number of lines produced by 11 strains of phage type 80 was 4·9, while

that produced by the 17 other strains taken together was 5·5 (Difference 0·6, S.E. difference - 2·03%). It is obvious that strains of phage type 80 did not produce an abnormal number of diffusible antigens when tested against the anti-toxin to Wood 46.

TABLE 20.

To Compare the Number of Flocculation Lines produced by 13 Strains of Staph. aureus

Phage type	No. of flocculation lines	Phage type	No. of flocculation lines
(1) 80	5	(5) 80	5
"	4	"	4
3C	5	6/7/47/53/54/75	4
7/54	7	6/47	3
(2) 80	5	(6) 80	5
"	5	52	7
47/53/75/77	4	6/47/75	6
52A/79	7	52/52A/79	5
(3) 80	5	(7) 6/47	5
"	5	42D/77	7
29	3	3B/3C	6
6/47/53	5	79/3A/3B/7/42E/54/70	8
(4) 80	5		
80	6		
42D/77	6		
7/54	6		

4. Hyaluronidase.

The production of this enzyme by an organism may increase its virulence because of its action as a "spreading factor", allowing the organism and its products to penetrate further into the tissues than would otherwise be possible. Hyaluronidase is thought to add to the virulence of certain clostridia in this way, and it seems possible that if an unusual amount of this substance were produced by a particular strain of staphylococcus the result might be an apparent increase in virulence. This was investigated in 1945 by Schwabacher, Cunliffe, Williams, and Hale, who compared the amount of hyaluronidase, coagulase and alpha lysin in 814 strains from healthy carrier sites and from clinical infection. They came to the conclusion that "although hyaluronidase production is often associated with virulence, it is not a major factor in determining the virulence of an organism." Lack and Walling (1954) also included it among the tests of virulence they investigated and came to a similar conclusion. Anderson (1956) in his comparison of various characteristics, found that there was a significant correlation of hyaluronidase and alpha lysin production and mouse pathogenicity. The method of estimating the amount of enzyme produced which was used in both these studies was the Mucin Clot Prevention Test, described by McLean and Hale (1941) and McClean (1943). This technique was also used in the present investigation.

Methods.

Hyaluronic acid was prepared from fresh umbilical cords, as described by McClean in 1943. Overnight broth cultures of the organisms to be tested were centrifuged and the supernatants diluted 1 in 10, 20, 30, 40, 60 and 80 in distilled water. 0·5 ml. of this enzyme dilution was added to a row of tubes, each containing 1 vol. hyaluronic acid solution, 1 vol. 1% serum albumin solution and 2 vols. distilled water, totalling 1 ml. The rack was incubated for 20 mins. at 37°C. and then quickly cooled in ice-cold water. After 5 mins. 0·2 ml. 2N acetic acid was added to each tube. A typical mucin clot appeared in the control tube and any which contained no enzyme. The end point of the titration was the highest dilution of culture showing no clot or threads.

Results.

In Table 21 are shown the reciprocal of the highest dilutions of culture supernatant at which the formation of a mucin clot was prevented. 10 strains belonging to phage type 80 were compared with 10 other strains, mostly different and mostly from lesions.

TABLE 21.

A Comparison of the Amount of Hyaluronidase Produced by Strains of Different Phage Types

Phage type	Titre	Phage type	Titre
52A/79	15	80	20
7/54	-	"	15
"	-	"	10
"	15	"	-
42D/77	-	"	15
"	-	"	15
6/47	80	"	15
47/53/75/77	30	"	<10
6/7/47/53/54/75	80	"	15
3C	25	"	15
29	30	"	15
6/47/53	60	"	15

Conclusions.

From this small series of strains, it appears that hyaluronidase production is not closely associated with clinical virulence, at least where strains of phage type 80 are concerned. They did not appear to produce abnormally large amounts of this enzyme in vitro, and two strains, 7/54 and 42D/77, which were able to cause typical staphylococcal lesions in vivo, produced very little.

5. The Reduction of Methylene Blue.

This test is little used nowadays, but Lesbre and Jansion, who described it in 1926, claimed that it had some association with virulence. They compared the activity in the reduction of methylene blue of strains of staphylococcus derived from lesions and from carrier sites, and found that the former reduced the dye more quickly. McBroom, in 1937, found that this property showed a close correlation with the power to lyse rabbit cells. It was, therefore, thought to be worthwhile reviving the test, and comparing the behaviour of strains of phage type 80 with that of other strains isolated in Cardiff Maternity Hospital.

Methods.

(1) Methylene Blue was diluted 1 in 1000 in distilled water, and autoclaved. In the test, 0·5 ml. of 1 in 2000 dilution was added to each tube.

(2) In order to obtain a standard inoculum, 10 ml. of broth was inoculated with 0·5 ml. of broth culture and incubated for 24 hours. 0·5 ml. of this was added to each tube of Methylene Blue solution.

(3) The tubes were tightly stoppered and incubated in a waterbath at 37°C. They were examined after $\frac{1}{2}$ hour, 1 hour and

2 hours. The amount of reduction was recorded by numbers -4 = not reduced, 3 = slightly reduced, 2 and 1 = greater degrees of reduction.

Results.

Table 22 shows the results of two tests set up on eight strains of phage type 80 and sixteen strains of eleven other phage types, read at $\frac{1}{2}$ hour, 1 hour and 2 hours. There is no evidence from these results that strains of phage type 80 reduce methylene blue more actively than other strains.

TABLE 22.

The Average Degree of Reduction of Methylene Blue by Strains of Different Phage types.

Phage type	No. of strains tested.	Average degree of reduction.		
		$\frac{1}{2}$ hour	1 hour	2 hours.
80	16	3·9	3·6	2·6
6/47	4	3·1	2·1	2·0
3C	4	3·0	1·9	2·0
47/53/75/77	2	3·0	3·0	2·0
52A/79	2	3·0	2·5	2·5
42D/77	4	3·6	2·0	2·1
79/3A/3B/6/7/42E/70	2	3·5	2·5	2·0
52/52A/6/7/54/73/81	2	3·2	2·0	2·0
6/7/47/53/54/75	2	3·0	2·0	2·0
29	4	4·0	2·5	2·0

6. The Production of Fibrinolysin.

The amount of this enzyme produced by coagulase negative and positive strains from human and animal sources was studied by Christie and Wilson in 1941. They found that most of the coagulase positive strains which did not form fibrinolysin were strong alpha lysin producers; and also that this enzyme can be formed by coagulase-negative and alpha lysin-negative staphylococci. Christie, North and Parkin (1946) studied fibrinolysin production among the properties of the thousand strains they tested. Selbie and Simon (1952) and Anderson (1956) carried out similar investigations; the former found a significant correlation between fibrinolysin production and mouse virulence, while the latter did not. Levy, Rippon and Williams compared this along with other properties, within the three phage groups and found no difference(1953).

Methods.

The method described in Christie, North and Parkin's paper (1946) was followed here. 1 part of plasma in 7 parts of nutrient agar was heated at 56°C for 5 mins. and a plate poured. When the agar was set, stab inocula were made from broth cultures of the various strains to be tested. The production of fibrinolysin was shown by a clearing in the opaque agar round the colony. Only one strain in each phage type was tested in this case.

Results.

The results are shown in Table 23. It is obvious that strains of phage type 80 do not owe their clinical virulence to an unusually high production of fibrinolysin.

TABLE 23.

To Show the Amount of Fibrinolysin Produced by Different Strains of Staphylococcus.

Phage type	Area of Clearing	Phage type	Area of Clearing
80	1 mm.	6/47	3 mm.
52/52A/6/7/54/73/81	1 mm.	52A	1 mm.
52A/79	1 mm.	47/53/75/77	3 mm.
42D/77 (lysin)	1 mm.	3A	-
79/3A/3B/7/42E/54/70	-	3B/3C (lysin)	-
7/54	3 mm.	71	1 mm.
6/7/47/53/54/75	1 mm.	3B	3 mm.

7. Resistance to Phagocytosis.

When considering the possible mechanisms by which one strain of organism may be more virulent than others, one cannot ignore the question of susceptibility or resistance to phagocytosis by polymorphonuclear leucocytes. This is a complicated subject, as the action of these leucocytes depends not only on their power of engulfing any foreign particles, but also on the presence or absence of antibodies to that particular species in the serum. It was the level of these antibodies which Almwroth Wright (1912) investigated in his "opsonic index" test. He used washed leucocytes from a healthy person and the serum to be tested, with a normal serum as a control. In the present investigation, as a number of strains were being compared, both leucocytes and serum were provided by the same donor. In each test, several strains were examined at the same time, using the same blood, one or two strains of phage type 80 being included in each batch. In this way, it was hoped that the effect of antistaphylococcal antibodies in the serum would be allowed for. Blood from as many different donors as possible was used. Whether antibodies could be phage type specific is not known, but the use of different bloods would make this effect less evident if it were the case.

Method.

This was adapted from that described by Almwroth Wright in 1912. Blood was taken from a donor into citrate. The staphylococci to be tested were grown overnight in broth, centrifuged and resuspended in saline. Equal volumes of blood and this suspension were drawn up into a Wright's pipette, blown out onto a sterile slide and mixed. The pipettes were then refilled, placed upright in plasticine and incubated for 15 minutes at 37°C. The contents were then blown out once more and a smear made which was stained with Leishmann's stain. The degree of phagocytosis was estimated as follows:- 100 polymorphonuclear leucocytes were counted, in areas widely separated on the slide, and the number of these which contained staphylococci was recorded as the percentage phagocytosis index,

TABLE 24.

To Show the Average Percentage Phagocytosis of Different Strains of Staphylococcus.

Phage type	No. of strains examined	Average Percentage Phagocytosis	Difference	S.E. Difference.
80	26	12•4	4•9	3•42
other types	30	17•3		

The results given in Table 24, show that while the percentage of leucocytes containing staphylococci was slightly lower in strains of phage type 80, the difference in the small number of strains examined was not significant. In the course of this work it was noticed that if overnight broth cultures were examined instead of 3 hour ones, the percentage phagocytosis was considerably higher. In 17 old cultures examined, an average of 44·2% leucocytes contained staphylococci, while the average was 10.3% with young cultures. When an old strain of Staph. albus was examined, 77% of leucocytes contained staphylococci, and 59% in the case of a young strain. These findings suggest that this method, although crude, is capable of demonstrating a difference in the resistance of different strains of staphylococci to phagocytosis.

8. The Growth Rate.

If it could be shown that organisms of one particular strain had as a characteristic the ability to grow faster, (i.e. to divide more often) than other strains, this would give them virulence when they invaded a host. It was thought worth while, therefore, to investigate the growth rates if organisms belonging to phage type 80 and to compare them with those of other strains.

Methods.

The turbidity of broth cultures was used as an indicator of growth, measured photo-electrically in a nephelometer.

10 ml. tubes of broth were inoculated with 0·5 ml. of an overnight broth culture of the strains to be tested. Growth curves of four strains were estimated at the same time. The tubes were incubated at 37°C. and examined in the nephelometer every hour for 7 hours. The nephelometer reading were recorded and are shown graphically in the accompanying diagrams. No attempt was made to estimate the actual numbers of bacteria present.

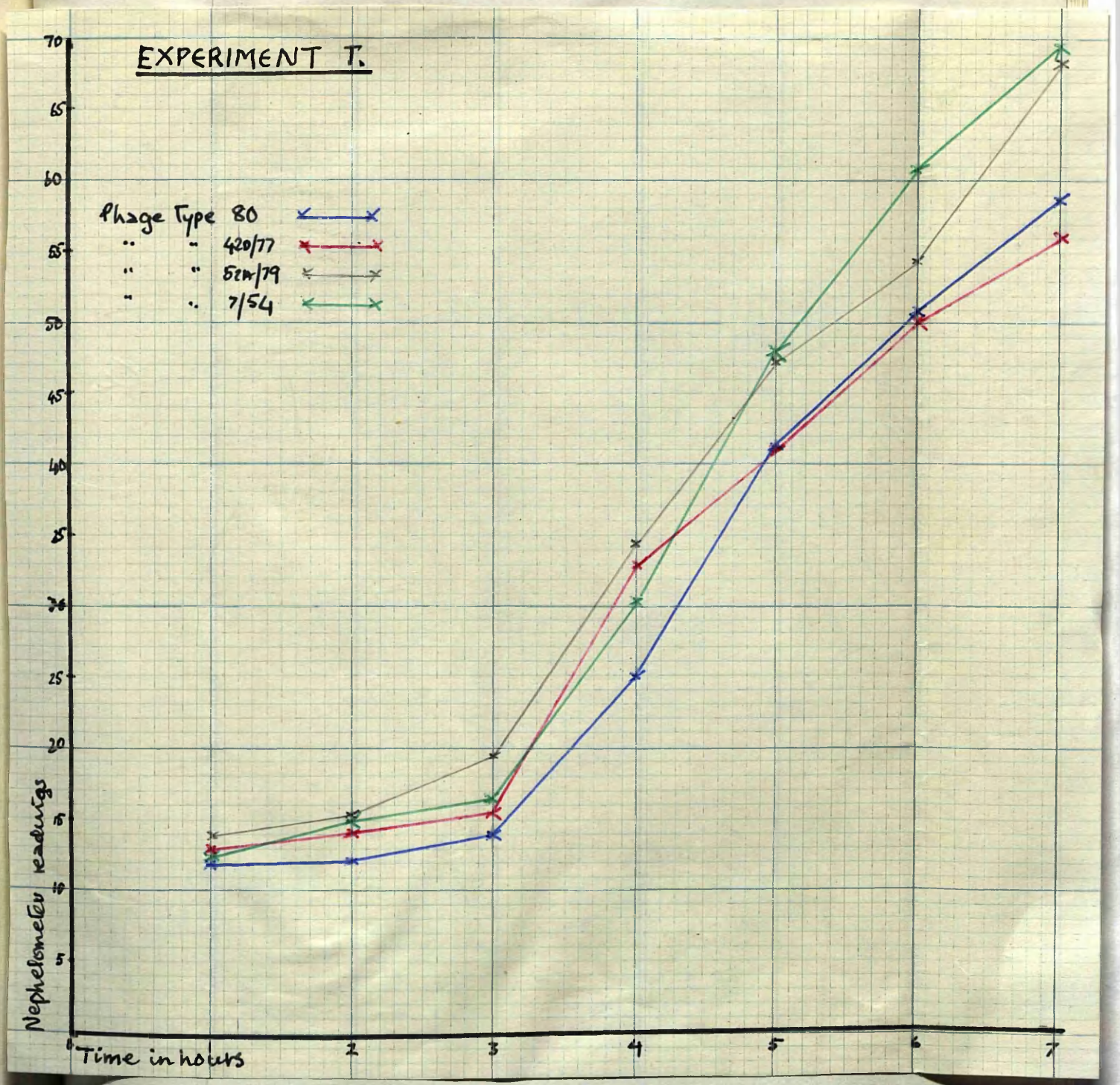


Diagram 7a. Growth Rates of 4 strains of Staph. aureus compared.

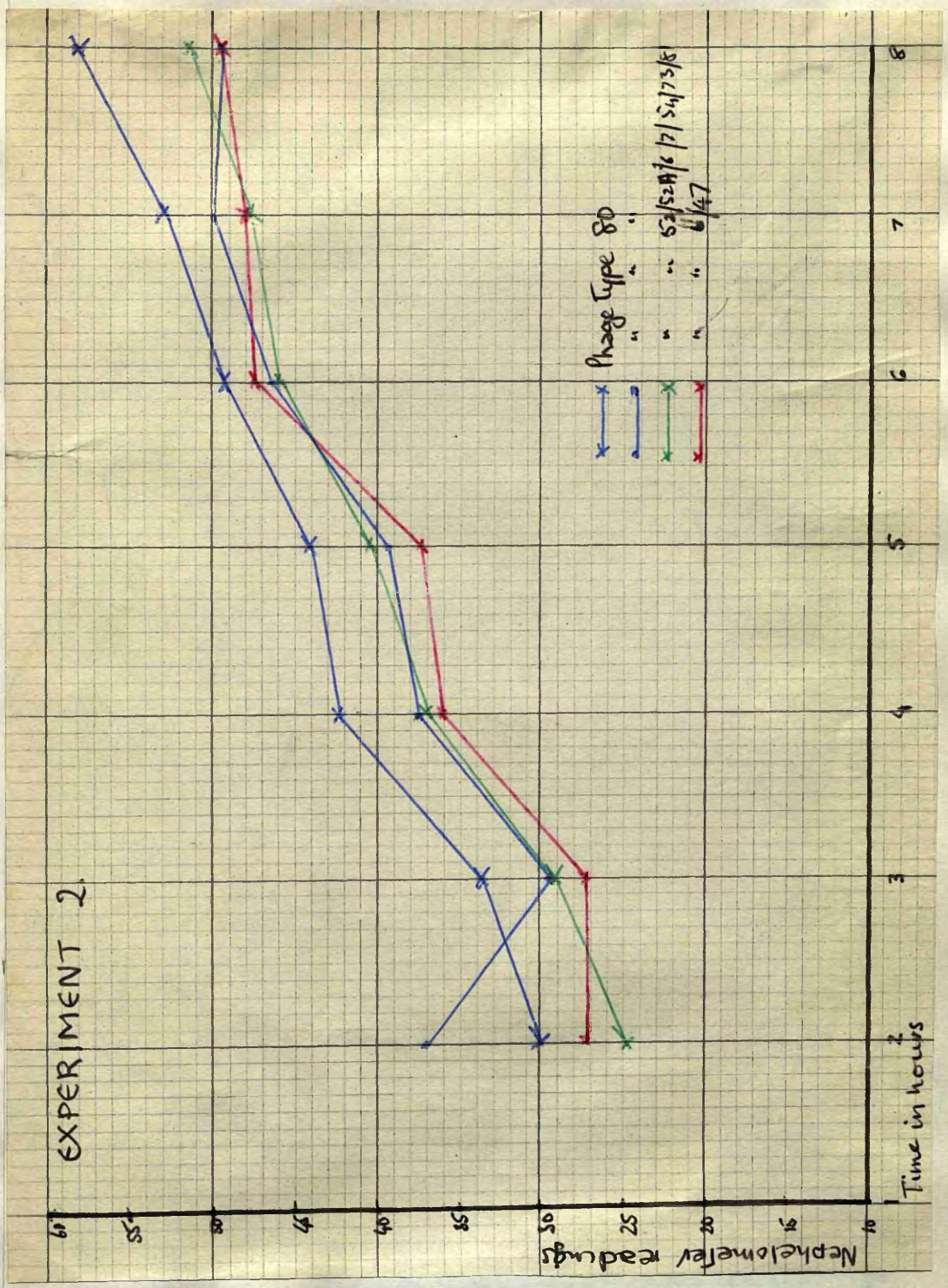


Diagram 7b. Growth Rates of 4 Strains of Staph aureus Compared.

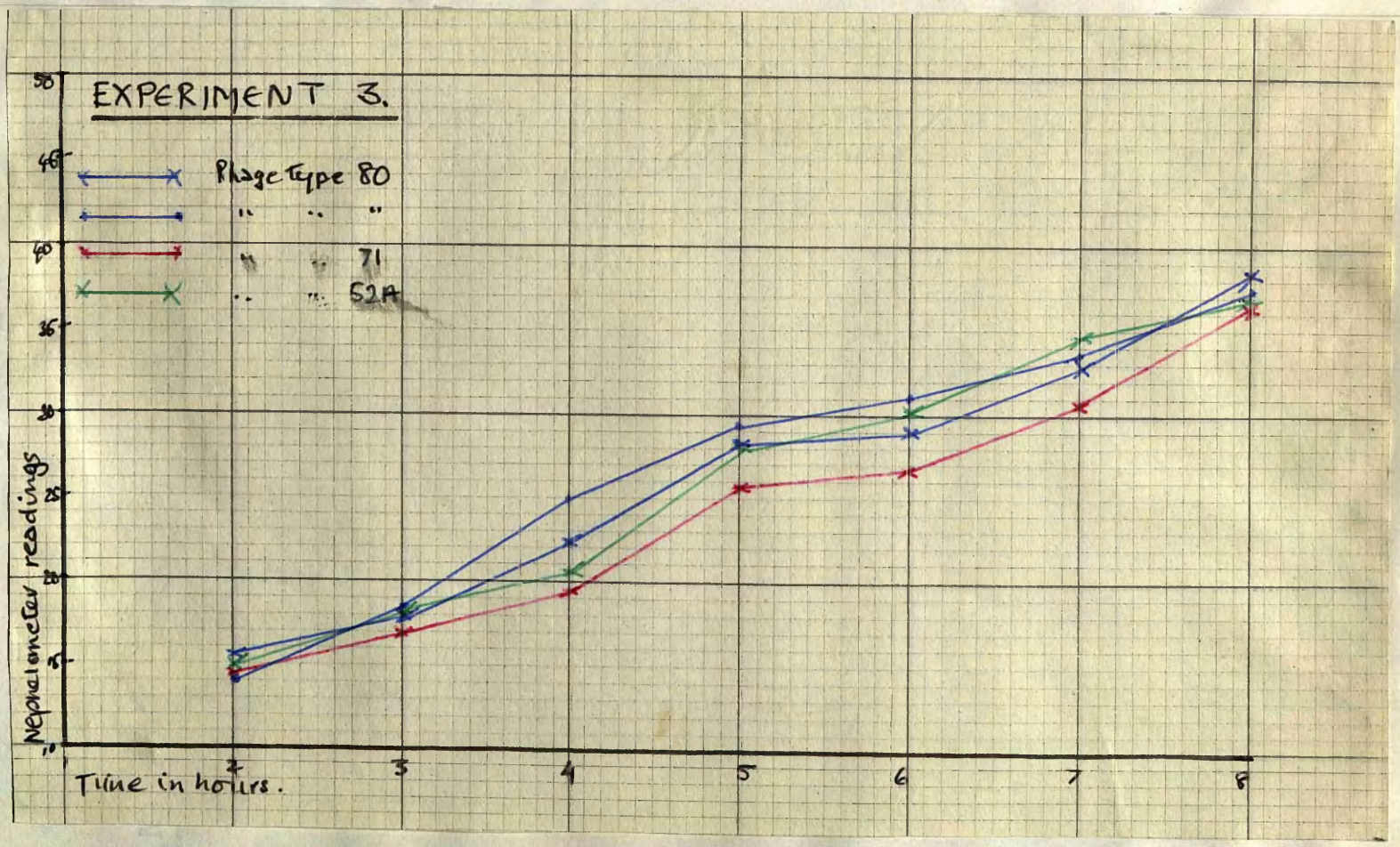


Diagram 7c. Growth Rates of 4 strains of Staph. aureus Compared.

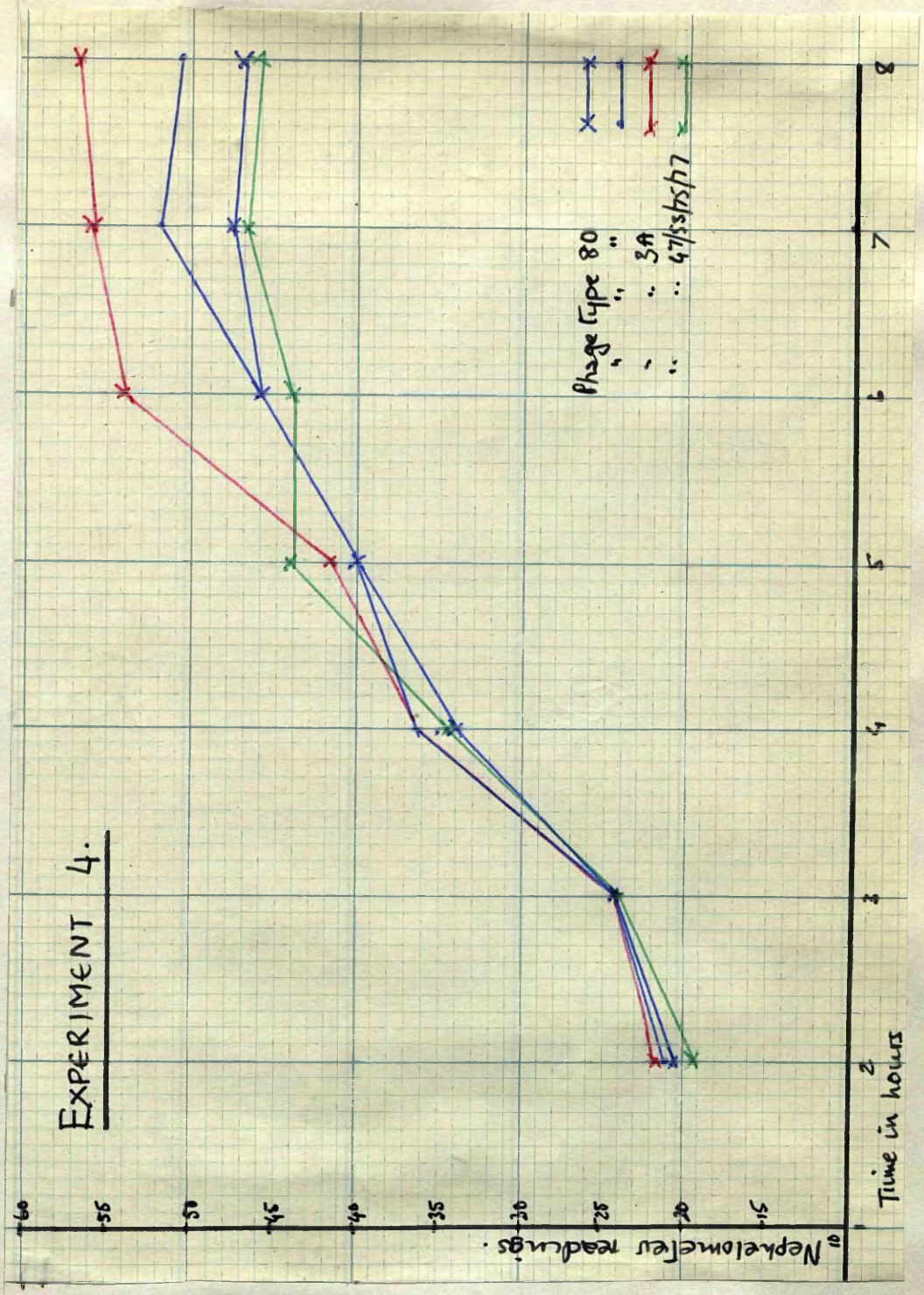


Diagram 7d. Growth Rates of 4 Strains of Staph. aureus compared.

Results.

It may be seen from the diagrams that straims belonging to phage type 80 gave no evidence of a more rapid rate of growth than the other strains tested.

9. Capsule Formation.

The staphylococcus is not commonly described as having a capsule, but several workers consider that they do occur, and a number of strains have been developed which are said to demonstrate them. Gilbert (1931) isolated an encapsulated staphylococcus which showed a well-defined capsule and grew in very mucoid colonies. It was highly virulent for guinea-pigs, while a non-capsulated varient which it produced, was not. The capsule in this case was best seen against a background of India ink. Price and Kneeland (1954) described a mucoid variant of Staph. aureus which appeared on passage through embryonated egg. Antiserum to this organism caused capsular swelling. There was no difference in the virulence for mice of this strain compared with others, or in any other properties. In 1956, an account was published by the same authors of a search for capsules in other strains. 39 were examined and 32 showed capsular swelling when tested with the anti-mucoid serum. The amount of capsular substance varied from strain to strain, but it seemed to be the same antigenically and quite unrelated to virulence. Organisms which did not produce coagulase or alpha lysin did not show capsular swelling and antisera to them did not cause it.

Lyons, who wrote a paper in 1937 on antibacterial immunity to Staph. aureus had quite a different view. He considered that the virulence of an organism is due to its invasiveness as well as its toxigenicity; as human white and red cells are known

to be immune to the effect of alpha toxin, the virulence of staphylococci for man is more likely to be associated with their ability to survive in the body, for example, by resistance to phagocytosis. Whether a staphylococcus is taken up by leucocytes depends, in his opinion, on whether it is encapsulated or not. Lyons was able to demonstrate capsules in young cultures of all the strains he examined, using hanging-drop preparations and stained smears. The organisms in these three hour cultures appeared as capsulated diplococci; the familiar grape-like clusters were only seen where there were no capsules - in old cultures, or strains grown in serum (which for some reason inhibited the formation of capsules). These agglutinated spontaneously in saline. When capsulated strains, toxigenic and non-toxigenic, were incubated along with whole blood, they were taken up by the leucocytes much more slowly than the non-capsulated strains. Non-toxigenic staphylococci were killed, while the toxigenic ones killed the leucocytes and survived to produce fresh capsulated forms. An anticapsular serum was developed, which was type-specific.

Since I had also found that old cultures were more rapidly taken up by leucocytes than young ones (using a method very similar to that described by Lyons), I was most interested to see if the latter could be shown to be protected by capsules. It also seemed that this might be a way in which strains virulent for man, such as phage type 80, could be shown to be different from less virulent ones.

Methods.

Several ways of demonstrating capsules were tried; starting with the two described by Lyons. He used a hanging drop preparation mixed with 1% "Collargol", (colloidal silver) and a strain for smears using undiluated Carbol Fuchsin, counterstained with Loeffler's methylene blue. I did not find these methods as satisfactory as the common India ink background technique, ("Handbook of Practical Bacteriology" by Mackie and MacCartney, 8th edition 1950, p.602), or Muir's capsule strain, (Ibid. p.97). These two methods were used on each slide in my investigation. Two other stains were also tried;

(1) Hale's method for staining acid muco-polysaccharides (1946) - modified by Berenbaum in 1955, who used "Ferrivenin"2 as a source of colloidal iron.

(2) The Periodic-acid Schiff stain for mucopolysaccharies - Kligman and Mescon (1950).

Neither of these methods shows any trace of a capsule round staphylococci. Films were made from three hour broth cultures of the strain to be tested and left to dry on the bench.

Results.

Obvious, well-defined capsules, such as were seen round pneumococci and Freidlander's bacilli, were never found with any of the stains used. It was common, however, to see 'haloes' round the cells, particularly in young cultures, in which the organisms were often diplococcal in arrangement. Old cultures and rough cultures did not show these haloes nearly as often; the cells in these were smaller and formed large clusters and a good deal of amorphous debris was present in the background. Occasionally the haloes were well-enough marked to deserve the name of capsule. Sometimes well-marked capsules were seen round a few organisms in a culture, while the majority showed no sign of them. I found it very difficult to decide in many cases whether capsules were present or not; the best I could do was to give an opinion on each film, not knowing whether it was young or old, or which phage type it belonged to. Cultures were described as having capsules, occasional capsules, haloes or no trace of either.

Table 25 shows the distribution of 22 strains of phage type 80 into these four categories compared with 42 strains of other types. There were 20 different phage patterns among these; most were obtained from the Routine Pathology department of Cardiff Royal Infirmary and came from lesions, as did the strains of type 80. All were examined as soon after isolation

as possible.

TABLE 25.

The Number of Staphylococci showing "Capsules". A Comparison of 22 straims of phage type 80 and 42 other Strains.

Phage type	Total No. examined	Capsules.	Occasional Capsules	Haloes.	Nil
80	22	2	1	16	3
others	42	9	9	18	6

Most of these strains were examined as well in the phagocytosis experiments already described. I was interested to see if 'capsulated' strains were more resistant to phagocytosis, as Lyons described, where all were young cultures.

Table 26 shows the percentage phagocytosis index of staphylococci grouped according to their appearance when stained for capsules.

TABLE 26.

The Average Percentage Phagocytosis of Young Strains according to the Degree of Encapsulation.

Presence of Capsules.	No. examined.	Percentage of W.B.C. containing staphylococci.
Capsules	11	11·95
Occasional "	15	13·23
'Haloes'	34	9·4
Nil	13	16·9

Conclusions.

The discovery of a layer of capsular material on the surface of certain staphylococci would have been most interesting, especially if it were shown to occur with a strain of unusual virulence. However, there was no evidence that any definite capsules were found more often in one strain than another. Nor was there any evidence that the strains which showed a kind of capsule were more resistant than others to phagocytosis. It is not at all certain that the haloes seen round the cells were in fact real capsules, as Butt, Bomynge and Joyce (1936) describing the capsules about haemolytic streptococci said, "We are not convinced that these zones represent in their entirety actual capsules. It is possible that the zones are evidence of surface tensions or propulsion forces which may be initiated in the reaction of the capsular material with the medium in which the India ink particles are suspended. But "non-encapsulated" bacteria do not produce these zones in spite of any of these procedures."

Summary and Conclusions.

In none of the experiments described in this section has it been possible to show any difference in the behaviour of the phage types tested which could be associated with the differences noticed in the epidemiological study, except that two strains which produced less alpha lysin than usual were shown to have a lower infection/lesion ratio than normal. Strains of phage type 80 were not found to produce any more of the substances which are thought to be associated with virulence than other strains.

The aspect which I think would be most valuable to follow up would be that of resistance to phagocytosis. This could occur either because of the presence of a hitherto undetected layer of capsular material round the cell, or because of the production of an usually powerful leucocidin. Although no capsules were seen in the simple microscopic examination carried out, their presence is suggested by the persistant "smoothness" of strains of type 80 in old broth cultures when compared with other strains. Dr. G. P. Gladstone very kindly examined one strain of phage type 80 for the production of P.V. leucocidin by the method described by Gladstone and van Heyningen (1957). He reported (personal communication) that the strain examined produced abundant leucocidin, but as no comparison was made with other strains, it is not known whether an unusual virulence can be attributed to this.

Another study which might prove interesting would be that of the mutation rates of different strains. If clinically virulent strains could be shown to have a higher mutation rate than others, this might give them an advantage over other strains by virtue of their greater adaptability to a new environment.

FINAL CHAPTER

Since 1955, when the investigations described in this paper were completed, a great deal of work has been carried out in the staphylococcal field and several important advances made. One of the most striking differences has been in the amount of interest shown generally in the subject. This is a result of the increasing importance and difficulty of the problem, which may be seen from the number of conferences and seminars which have recently been held on staphylococcal cross-infection in hospitals. In 1956 there was a symposium on staphylococcal infections under the auspices of the New York Academy of Sciences. In 1957 one was held in London on Hospital Coccal Infections and another in Cleveland, Ohio, on Staphylococcic Infections. In 1958, again in the United States, there was a National Conference of Hospital-acquired Staphylococci.

It would be unwise to attempt to discuss all the recent work on the staphylococcal problem here. The best that can be done is to give an outline of the work that has been carried out since 1955 and indicate which, in my opinion, are the most important aspects.

(1) The Increase in Phage typing and Discoveries about the Behaviour of Different Phage types.

Phage typing was used at first more extensively in Britain and Australia than in other countries, but even there it was only carried out in certain centres. In the last few years it has been practised widely in the United States and has been introduced into many other countries and into regional laboratories in Britain. The availability of this invaluable method of identifying staphylococci has made the study of hospital outbreaks much more interesting and rewarding and has increased our knowledge of the behaviour of the different phage types. It is through the widespread use of this method, for example, that we have become aware of the pandemic of infection with phage type 80. In the last two or three years much more work has been published in America on staphylococcal infections than before, and the increase in the use of phage typing has probably contributed to this.

(2) The Spread of Hospital Staphylococci to the Community.

This is a subject which has been most thoroughly investigated in the United States. Colbeck 1949, in Canada, had noticed that a number of cases of boils and pustules arose in the families of patients who were in hospital during the epidemic which he had described. Hurst (1956) found that 14 out of 21 babies still carried the antibiotic-resistant staphylococci they had acquired

in hospital after they had been at home for six months. In Australia in the same year, Taft described the increasing incidence of pneumonia in babies, and showed that 10 out of 20 patients came from maternity units where staphylococcal infection had been epidemic. Several workers have commented on the increase in this disease:- Wallman, Godfrey and Watson (1955) and Disney, Wolff and Wood (1956) from this country and Beavan and Burry (1956) from New Zealand. Hutchison and Bowman (1957) and Shaffer et al. (1956) both drew attention to the danger of spread from infants sent home from hospital carrying antibiotic-resistant staphylococci. Ravenholt and his colleagues in Seattle - Ravenholt and LaVeck (1956) and Ravenholt, Wright and Mulhern (1957) made a study of this problem by instituting a survey by telephone of infection in mother and baby after they had left hospital. They found that a considerable amount of sepsis was occurring which would otherwise have been undetected. During one month there was a rate for infant pyoderma of 18% and for maternal mastitis of 4.3%. Five of the forty-one children born during this month and dying before the age of three months were proved to have died of staphylococcal disease.

The most recent investigation is that reported by Wentworth, Miller and Wentworth (1958). For five months they studied the families of 26 children infected with

type 80 during a nursery outbreak. 75% of the mothers and 33% of the fathers, brothers and sisters were found to acquire this strain and 11 families were found still to be infected at the end of the study. 40% of parents and 70% of siblings developed skin infections and abscesses. The authors comment that if these findings are accurate, a serious increase in the level of the community staphylococcal disease will follow any uncontrolled nursery outbreak. In this event, the "crude equilibrium between the public at large and the ubiquitous staphylococcus" referred to by McDermott (1956) as appearing to be relatively stable at that time, might well be shifted in favour of the staphylococcus and result in an absolute increase in the level of epidemic community disease.

These American workers all stress the importance of a) terminating nursery outbreaks as soon as possible for the benefit of the community as well as of the patients themselves, if necessary by the widespread use of antibiotics (Shaffer et al., 1956) and b) of making staphylococcal disease outside hospitals reportable, so that the occurrence of a hospital-derived outbreak in the community could be recognised and treated as a public health problem.

(3) New Studies of the Mechanism of Cross-infection.

A great deal of work has been done on this subject, particularly in this country. Much of it relates to hospital cross-infection in general, not specifically to infections in maternity hospitals. The work of Blowers, and Wallace (1955) on the disinfection of blankets may be mentioned, of Colbeck (1956) on mattresses and baths, of Frappier and Davignon-Frappier (1957) on bedding and of Schwabacher, Salsbury and Fincham (1958) on blankets. Studies specifically on the subject of nursery cross-infection were those of Hutchison and Bowman (1957), Wysham, Mulhern, Navarre, LaVeck, Kennan and Giedt (1957) and Baldwin et al. (1957). These workers came to the conclusion that the most important spread of Staph. aureus was from the babies themselves. Cook, Parrish and Shooter (1958) investigated the effect of various nursing techniques on the proportion of babies carrying staphylococci in their noses on the 12th day of life.

Many studies have been made of the carriage of staphylococci. A large proportion of these have been on the relative importance of nasal carriers among the staff in nursery outbreaks: Clarke, McGeogh and Sippe (1956), Hutchison and Bowman (1957), MacCartney and Yates, (1956), Munro and Markham (1957) and Baldwim et al. (1957). Opinions varied,

from those of MacCartney and Yates who considered that naso-pharyngeal carriers were the main source of epidemic staphylococci, to those of Burnett and his co-workers (1958) who came to the opposite conclusion. Several workers found that the application of ointments containing antibiotics to the nostrils of nasal carriers was a useful measure in the termination of an outbreak. Nasal carriage per se was the subject of papers by Gould (1955), Clarke (1957), and Hutchison Green and Grimson (1957). Attention was also drawn to other sites of carriage:- the faeces by Brodie, Kerr and Sommerville (1956), Mathias, Shooter and Williams (1957); and the perineum by Hare and Ridley (1958).

(4) Recent Developments in the Prevention of Staphylococci Cross-Infection.

The intensive work thus summarised was all designed to find out more about staphylococci cross-infection in order to discover how to prevent it. A number of new techniques are now used in some units as a result:- the use of disinfectants for bathing babies, treating umbilical cords and nurses' hands, and of individual gowns for the care of each baby. But the most important factor in the prevention of cross-infection with Staph. aureus in nurseries and elsewhere, which has been stressed again and again, is a return to the strict methods of asepsis which were essential before the discovery of

antibiotics. One of the most far-reaching results of the recent conferences has been to stress the fact that prevention of staphylococcal cross-infection is a challenge to hospital administrators, not only to bacteriologists. The setting up of Control-of-infection Committees; the appointment of a Control-of-infection officer, the keeping of accurate records, the reporting of all sepsis whether in patients or staff, are all administrative measures (May 1957). It is, as the authors of the recently published report of the Ministry of Health Subcommittee on Staphylococcal Infection in Hospitals (1959) point out, the duty of the hospital authorities to provide the necessary training of staff, the sterilizing equipment and materials, the clean air and adequate space, without which no bacteriologist or clinician can hope to prevent cross-infection. This new attitude to the problem is, in my opinion, one of the most important of the recent developments in the campaign to defeat this modern scourge of hospitals.

SUMMARY AND CONCLUSIONS

The epidemiology of Staph. aureus was investigated in two maternity hospitals between November, 1954 and January 1956. In the course of this work a number of conclusions were reached:

I. That colonization of babies occurs in two types of epidemic:- a) in which one particular strain is widely disseminated, mainly spreading from one baby to another; and b) in which the epidemic strain is introduced by a nasal carrier who infects babies directly. This type of epidemic ceases when the carrier leaves the unit, so it is likely that the spread is mainly from nurse to baby.

II. The most important single factor in baby-to-baby spread is the presence in the nursery of a reservoir of infection where staphylococci are multiplying freely. Cross-infection can easily take place both by contact and by airborne spread every time the baby is handled, but in my opinion the hands and clothing of the staff are the most common means of spread.

III. That all the strains within the species Staph. aureus are not equally virulent. The only way we have of detecting virulent strains at the moment is by finding out which phage type is responsible for the lesions in each separate epidemic. Carriers of this strain must then be looked for among staff and patients and treated or isolated.

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KEY to DIAGRAM 2.

Type I Epidemic Strains.

- Phage type 29/7/42B
- " " 6/7/42/53/54/75
- " " 77/52A/79/1/42E/54/70
- " " 42/53/75/77
- " " 42E/77
- " " 55
- " " 71
- " " 7/42/54/77
- " " 3A
- " " 3C
- " " 3C/71
- " " 75/77
- " " 53/54/75/77
- " " 53/75/77
- " " 42/70
- " " 29/52
- " " 52/77
- " " 6/42E/53/54/77

(This strain was not mentioned in the text. Its origin is not known.)

Type II Epidemic Strains.

- Phage type 80
- " " 80/52A/6/1/54/29/81
- " " 52A/79
- " " 42A/77
- " " 7/54
- " " 6/42
- " " 52A

If a strain was very common on the settling plates, it has been recorded 2 or 3 times on one day in the diagram.

FLOOR C.

Unit opened

Staphylococci isolated from babies:

Staphylococci isolated from vacuum cleaner dust:

Before unit opened:

September October November December January

FLOOR B.

Unit opened

Staphylococci isolated from babies:

Staphylococci isolated from the environment:

August September October November December January

FLOOR A.

Staphylococci isolated from babies:

Staphylococci isolated from the environment:

Unit opened

No staph. cols.

April May June July August September October November December January