

STUDIES ON THE BACTERIAL FLORA
OF THE
INFANT UPPER RESPIRATORY TRACT

by

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ABSTRACT

Observations were made on 160 newborn babies delivered in hospital, and on 36 babies delivered at home. Two or three per-nasal and throat swabs were obtained from each baby during its first two weeks of life. Further consecutive swabs were obtained, at one to four week intervals, from 34 of the hospital-born group, and from 28 of the home-born group. The hospital-born babies were observed for six to twelve months where possible, and the home-born babies for four to six months. An additional eight hospital-born babies were observed for shorter periods, not necessarily from birth. The swabs were inoculated routinely on blood agar plates, with or without gentian violet, and incubated aerobically. For certain organisms (e.g., *Haemophil*, fusiforms) special cultural methods were used.

Staphylococcus aureus was present in both nasal and throat cultures of 99 per cent of the hospital newborns (106) and 72 per cent of the home-born group (36). Ninety-seven per cent of the hospital newborns (34) and 18 per cent of the home-delivered newborns (28) acquired penicillin resistant strains. Many babies ceased to be nasal carriers of staphylococci after about the first month, but continued to be throat carriers. Phage typing demonstrated that the strains neonatally acquired may be carried for as long as

a year. New strains seemed not to become established during the later months of life, and home-delivered babies who had not acquired staphylococci neonatally remained negative during the later period of observation.

Pneumococci were found in less than one per cent of the hospital newborns (160). They occurred in 14 per cent of the home-delivered group (36), but seemed not to persist. In both groups pneumococcal carriage began in the late neonatal period, and within four to five months about half the babies were carriers. The particular pneumococcal type acquired usually persisted for many weeks.

Carriers of beta haemolytic streptococci, including Group A, were seen occasionally in the newborns, as well as in the older babies of both groups. H. influenzae was never found in any of the newborns, and was rare among the older babies.

Coliform bacilli often became established in the throats of the hospital newborns, and usually persisted for at least six months. Fusiforms were present in most babies' mouths before tooth eruption.

Note - Figures in brackets indicate the total number of babies observed for the particular organism.

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INTRODUCTION

Investigations on the normal body flora usually are undertaken with reluctance because it is regarded as both complex and variable. Its variability may have been overemphasised, for knowledge gradually accumulating indicates it to be a precisely balanced ecological system. Better knowledge of this system should contribute much to our concepts of immunity and resistance, but cannot be obtained without fuller understanding of its component parts. The normal flora is most easily studied in young infants where the sequence of its establishment may be observed. That of the upper respiratory tract was chosen for the present investigation because 1) the upper respiratory tract presents a fairly well defined region toward which some bacteriological work, mainly on adults, has been directed, 2) material from different sites of the upper respiratory tract can readily be obtained, and 3) information on the normal flora of this region should be useful for the advancement of dental bacteriology in which the author has been interested.

PART I. REVIEW OF THE LITERATURE

CHAPTER 1.

ESTABLISHMENT OF THE INFANT
UPPER RESPIRATORY FLORA

The upper respiratory tract of the foetus in utero is believed sterile. Bacteria have never been cultivated from the oral or nasal cavities of infants delivered by Caesarian section, although the sum total reported to have been examined is rather small. It is thought that bacteria do not normally cross the placental barrier. As evidence of its effectiveness, Neter (1950) reported a case of paratyphoid occurring in late pregnancy. Although numerous Salmonella cholerae-suis were cultured from the placenta, blood and mouth cultures of the baby were sterile. Transmission of bacteria, viruses, and spirochaetes may occur across the damaged placenta (McKhann and Kapnick, 1938), but it is improbable that bacteria often reach the infant upper respiratory tract by this route.

Just after birth the oral and nasal cavities are frequently sterile. The incidence of positive cultures reported by different investigators has varied considerably, due, no doubt, to the use of different culture techniques, or to differing circumstances of labour. Salomon (1923)

pointed out that sterility of the newborn mouth was partly related to the duration of labour, the time of rupture of the foetal membranes, and to delivery manipulations. This was later confirmed by Cornelison et al. (1946), who found that the incidence of positive oro-nasal cultures was directly related to the time at which the foetal membranes had ruptured prior to delivery. Snyder (1936 a,b) believed that membrane rupture, with consequent ingestion of bacteria, explained the positive cultures he obtained from meconium specimens collected immediately after normal birth or at still-birth.

Nearly all investigators have found that infants whose noses or mouths are sterile at birth begin yielding positive cultures not later than the second or third day of life; frequently colonisation begins within the first day. As a rule, throat cultures become positive before nose cultures. The organisms usually reported include Staphylococcus aureus, Staphylococcus albus, viridans streptococci, gamma or "indifferent" streptococci, Gram negative cocci, diphtheroids and coliforms.

Most workers agree that staphylococci are predominant in the nose, and streptococci are predominant in the mouth. Moniliae are often found in the mouth when appropriate media are used. Air saprophytes are rarely present, in either the nose or mouth, and never in significant numbers; it is believed that they are only transients (Bloomfield, 1922).

Although there is general agreement as to the composition of the primary flora, there has been considerable dispute as to its derivation. Early investigators thought it was derived principally from the maternal vagina and perineum, and regarded the frequent presence of Döderlein's bacillus and coliforms in the infant oropharynx as evidence of this (Schweitzer, 1919). Their argument, however, has been weakened by more recent recognition of the ubiquity of these organisms. Döderlein's bacillus, although originally cultivated from the vagina, is now usually considered identical with Lactobacillus acidophilus, a common component of the adult oral flora. Wide dissemination of coliforms in nursery

air, dust, and bedding has now become apparent during investigations on the transmission of E. coli associated with infantile gastro-enteritis (Rogers, 1951; Stevenson, 1952; Taylor and Charter, 1952). Nurses attending newborn infants may carry coliforms on their hands (Torrey and Reese, 1944).

The best evidence that the newborn oro-nasal flora may be derived, at least partially, from the vagina is the transfer of vaginal infection to the newborn upper respiratory mucosa. Candida albicans infection is an example probably of most common occurrence. Anderson et al., (1944) found a statistically significant correlation between the presence of C. albicans in the vagina of mothers and the mouth of newborn infants. Transmission may also occur by other routes, however, as demonstrated by Ludlam and Henderson (1942) who cultured C. albicans from infants hands, and the hands and throats of nursing personnel. Viral "epidemic stomatitis of the newborn" may be derived from infection of the maternal vagina, as shown by Buddingh (1946) and by Dodd (1946). Again,

however, infection may also originate from other sources, as indicated by recovery of the virus from the mouths of adult hospital staff.

Some investigators have felt that most of the bacteria which the newborn acquires from the vagina are only transient (Torrey and Reese, 1944; Smith and Bloomfield, 1950). As evidence they cite the high frequency of sterile oronasal cultures obtainable during the first half day of life. However, if only a small number of bacteria were acquired from the vagina it is doubtful whether they would multiply sufficiently during this time to be detected by the usual culture methods. Torrey and Reese also pointed out that streptococci of the newborn upper respiratory tract are more closely related in fermentation reactions to those of the adult respiratory flora than to those of the vagina, but this is not a very acceptable basis for comparison. Indication that at least some vaginal bacteria may be eliminated is found in the rapid disappearance of gonococci from the mouths and nasopharynx of babies whose mothers have

gonorrhoea (Salomon, Torrey and Reese, 1944).

A sharp increase in the number of positive cultures observed to follow the first feeding of newborns indicates that some of the primary flora may be derived from the mothers breast (Bloomfield, 1922a; Kneeland, 1930; Torrey and Reese, 1945). It has also often been suggested that the bacteria of the mother's upper respiratory tract are passed on to the infant. This has been considered true particularly of certain pathogens, discussed in Chapter 3. The literature thus indicates that newborns may derive their upper respiratory flora from several sources. The relative importance of these is difficult to decide for it may differ for different organisms. Also, as pointed out by Smith and Bloomfield (1950), the initial inoculum need not necessarily be large.

Numerous observations have been made on the upper respiratory flora of the newborn (i.e. babies less than two weeks old), but few have been made during the late neonatal period (i.e. the last weeks of the first month). It is generally presumed that no change occurs during this

time. Torrey and Reese (1944) seem not to have noted any difference in the flora of the 37 premature babies they examined between the 5th and 25th day of age. The only study on the upper respiratory flora in older infancy is that of Kneeland (1930) who compared the cultures of 28 healthy babies four to eight months old with those of 30 newborns. Their throat flora differed from that of the newborns mainly in that the viridans streptococci and Gram negative cocci had increased at the expense of the "indifferent streptococci" and staphylococci. The nasal flora showed a similar decrease in staphylococci, while diphtheroids and "large Gram positive cocci" had increased in numbers. By eight months of age the flora seemed entirely comparable to that of adults.

It is often stated in texts that there is a definite change in the oral flora at the time the deciduous teeth erupt. This is supposed to be principally the appearance of anaerobes, believed to require the gingival crevice for their habitat. They include such organisms as fusiforms, spirochaetes, and "leptothrix".

Few actual studies have been made and these, cited by Appleton (1950), contain too little data to give much support to this contention. For example:

Brailovsky-Lounkevitch (1915)

examined the oral flora of an unstated number of newborns, and of children aged one to fourteen years. Strict anaerobes were said to occur rarely in the newborns, and because leptothrix, spirochaetes, vibrios, etc., were present only in the older children it was suggested that they appeared with the eruption of teeth.

Kostečka (1924) claimed to have observed anaerobes to appear only after tooth eruption, and to disappear in the edentulous mouth. He gave no information on the number of cases observed, nor his methods.

Andre (1934) reported that he had been unable to cultivate anaerobes from the mouths of 26 infants under one week old, or from 14 infants eight days to six months old.

Because he cultivated fusiforms, actinomycetes, and leptothrix from five of eight infants aged seven to nine months, as well as from older children, he concluded that these organisms had appeared with tooth eruption. Although his cultural methods seem adequate, his conclusion is based on circumstantial evidence.

It is conceivable that the requirements of these anaerobes could be met, prior to tooth eruption, in symbiotic growth with the aerobic members of the oral flora. Their possible presence before tooth eruption requires further investigation.

Summary

The infant upper respiratory tract, often sterile at birth, becomes colonised within the first few days. Streptococci become predominant in the newborn's throat;

in the nose staphylococci are most numerous. Other bacteria sometimes found in either site include "indifferent streptococci", diphtheroids, Gram negative cocci, and coliforms. It is difficult to determine the exact source of these because they are common to the upper respiratory tract, skin, alimentary canal, or vagina of the mother and other adult contacts.

It is presumed that there is little change in the flora during the rest of the neonatal period, or in the first few months of life. The only study on older babies indicates that some change occurs by the fourth to eighth month. Viridans streptococci and Gram negative cocci increase at the expense of "indifferent streptococci" and staphylococci in the mouth; in the nose staphylococci also decrease while diphtheroids and "large Gram positive cocci" become more numerous. There is a general belief that obligate anaerobes, including fusiforms, spirochaetes and "leptothrix" appear in the infant mouth at the time the teeth begin to erupt. However, few actual studies have been made, and those reported contain insufficient data to support this conclusion.

CHAPTER 2

COMPARISON OF THE INFANT FLORA WITH THAT OF CHILDREN AND ADULTS

SECTION A

STAPHYLOCOCCI

Staphylococci almost invariably have been found predominant among the upper respiratory flora of newborn infants, and present also, although in lesser numbers and frequency, in older infants. The occurrence of potentially pathogenic strains cannot be accurately estimated from the early reports because, in these studies, the species were designated according to pigmentation only. In recent years, with acceptance of coagulase production as an index of potential pathogenicity, many reports have confirmed that the infant upper respiratory tract is highly susceptible to colonisation by coagulase producing strains to which the names Staphylococcus aureus or Staphylococcus pyogenes are applied. Non-coagulase producing strains, usually white pigmented and designated as Staphylococcus albus or "white micrococci", have received little attention from recent investigators. The figures of Kneeland (1930), who did not test coagulase production, suggest that they are more numerous than Staphylococcus aureus in both the nose and throat throughout the first seven

months. Torrey and Reese (1944), who differentiated Staphylococcus aureus by coagulase and haemolysin production, also reported Staphylococcus albus more frequent in both nasopharynx and throat of premature babies during the first 25 days. Martyn (1949) reported non-coagulase producing staphylococci in the nasopharynx of 54% of the 130 newborn infants he examined. From these reports, it therefore appears that Staphylococcus albus and Staphylococcus aureus have a similar propensity for becoming established in the newborn upper respiratory tract.

Incidence of Staphylococcus aureus

Most infants born in hospital have Staphylococcus aureus in their upper respiratory tract by the end of the first week of life. Colonisation begins very soon after birth, and usually over half the babies are found affected as early as the third or fourth day. Positive nose cultures appear suddenly, the negative cultures of

the early days of life being followed by a heavy growth of Staphylococcus aureus (Parker and Kennedy, 1949; Cunliffe 1949). The throat, according to Torrey and Reese (1945) is colonised less rapidly than the nose, and they found that the incidence in the throat remained lower than that of the nose and nasopharynx throughout the first week. Among 63 babies aged four to eight days the incidence was 56% in the nose and 35% in the throat. Krag-Anderson (1943) whose figures are slightly higher for babies of comparable number and age, nevertheless found a similar difference between the throat and the nose. McFarlan et al. (1949), who investigated throat swabs only, reported the incidence to be as low as 27% among 41 babies aged four days. On the other hand, Duncan and Walker (1942) reported the throats of 91% of 75 newborn infants to contain Staphylococcus aureus within the first eight days of life. All other investigators have considered the findings of nasal swabs only, or of nose and throat swabs combined. In these reports, too numerous to mention, the figures cited vary from 60% to 100%.

It has been suggested that the incidence of upper respiratory Staphylococcus aureus among babies born at home is probably lower than that of babies born in hospital. Brailovsky-Lounkevitch, as early as 1915, observed that "pyogenic cocci" were only present in the mouths of babies born in the city hospital and not amongst those of babies born in the village. No observations have yet been made on newborn infants soon after home delivery. However, Cunliffe (1949) examining infants aged two to eight weeks found eight babies born at home to have a nasal carrier incidence of 12.5% in contrast to 84.6% among 26 babies born in hospital. Ludlam (1953), similarly examining two-week old babies, also found fewer nasal carriers among those born at home than among those born in hospital. The incidence among 54 babies born at home was 59.3%, whereas the incidence among 57 born in one hospital was 70.2%, and among 29 infants born in another hospital, 86.2%. In view of the prevalence of Staphylococcus aureus in the hospital nursery environment, discussed later, such findings may

be expected.

Cunliffe (1949) presented data suggesting that later infancy is characterised by a much lower nasal carrier rate of Staphylococcus aureus. In 78 babies aged six months to two years the rate was only 23%, and among 65 babies aged two to four years it was 29%. The observation was confirmed by Ludlam (1953) whose data also showed the nasal carrier rate to decline progressively from two weeks onwards until at 12 to 24 months it had reached 15-25%. The same trend was exhibited both by babies born in hospital and those born at home.

According to Cunliffe's figures the carrier rates of hospital and home-born babies had reached the same level by the age of 3-12 months. Ludlam, however, found a statistically significant difference still existent between these two groups at five months, although not at two months nor at 12-24 months. Such inconsistencies suggest that the nasal carrier rate may be affected by factors other than place of birth, and that comparisons of small samples drawn from different populations must be made with caution.

Cunliffe found that the nasal carrier rate of Staphylococcus aureus gradually increased up to 60-65% in the tenth year of age. Although the number of children sampled in each age group was small, his statistical analysis of the data indicated that the differences were significant. His figures for the nasal carrier rate of infants and older children compare closely with the 58.4% incidence Hallman (1937) reported in the anterior nares of 272 children aged two months to twelve years. Similar figures were obtained by Mathews, et al., (1949) when examining mouth washings of 194 school children aged five to twelve years. Coagulase positive staphylococci were found in 55.7% of the specimens. These reports indicate that the carrier rate of older children is about the same as that of adults.

In adults, the carrier rates reported for the general population vary from 30% to 60%, with about 50% the mode; among hospital patients and staff it is often higher. These figures usually are based on recovery of Staphylococcus aureus either from nasal swabs alone, or

from throat as well as nasal swabs. Most investigators regard the nasal mucosa to be the natural reservoir of Staphylococcus aureus and attach little significance to its presence in the throat. Packalén and Bergqvist (1947), however, reported that "staphylococcal findings in the throat reflect fairly faithfully the corresponding flora in the nose". They also noted that patients whose throat cultures contained staphylococci had a distinct tendency to retain them for long periods of time. Among 362 subjects suffering from tuberculosis or other medical disease they found it present in the throats of 63%; in 38.4% it represented 5% or more of the total throat flora. Vogelsang (1951, 1953) likewise found that Staphylococcus aureus was usually present in the throat as well as in the nose. It was numerous in the throats of 28.6% of 1,000 healthy adolescent school children aged 14-15 years. Blackburn (1945) examined excised tonsils of an unstated number of children and adults, and found coagulase-producing staphylococci in 30%. They were more frequent in the younger age group (under 20 years) than in the older age group (over 20 years).

TABLE 1

INCIDENCE OF COAGULASE PRODUCING STAPHYLOCOCCI IN THE THROAT

<u>Reference</u>	<u>Throat +</u>	<u>Nose + Throat +</u>	<u>Throat only +</u>	<u>Persons Examined</u>
Krag- Anderson (1943)	26% 18% 45% 29%			19 puerperal women 33 hospital staff 29 puerperal women 359 hospital staff Pemphigus outbreak, 1939 Pemphigus outbreak, 1941
Packalén & Bergqvist (1947)	63%	58%	7.8%	362 patients with tuberculosis or other medical 77 " (143 cultures) disease
Colbeck (1949)		23% 12% 30% 19%	10% 12% 4% 16%	48 staff, maternity ward 42 " " " A- free of pemphigus 118 old staff, " " B- pemphigus outbreak 32 new staff, " " C- " " 50 healthy adults C- " "
Martin & Whitehead (1949)	18%			
Vogelsang (1951)		47.3%	17.1%	1000 healthy school children, aged 14-15 years
Vogelsang (1953)		39.9%	17.3%	764 hospital ward & domestic staff, medical students
Laurell (1953a)		70.5%*	9.6%*	166 hospital staff (*coagulase production not always tested)

Not all investigators have noted as high an incidence of Staphylococcus aureus in the throat, but inspection of the literature indicates that it is probably more common than generally realised. Data reported are summarised in Table 1. It may be significant that some of these investigators used Chapman's selective medium containing NaCl, or ordinary nutrient agar, either of which discourages growth of other components of the throat flora (Krag-Anderson, Colbeck, Vogelsang, Laurell). However, others used blood agar (Packalén and Bergqvist, Martin and Whitehead). It is, of course, debatable whether these throat staphylococci are only transients which have washed back from the nose. In those instances where they are present in the throat alone (Table 1) their possible colonisation of this site merits consideration.

Sources of Staphylococcus aureus Colonising the Newborn Upper Respiratory Tract

As colonisation of the newborn's upper respiratory tract begins about the time of the first feeding (Chapter

1), it might be thought that staphylococci are acquired from the mother's breast. Recent work, however, indicates that babies more often obtain their staphylococci from other sources. Duncan and Walker (1942) presented evidence that only a small proportion of babies acquired staphylococci from their mothers' milk, even though these organisms were frequently present in aseptically collected samples. Among 87 mothers and their babies, Staphylococcus aureus was present in the mother's milk before it was present in the baby's throat in just 15 instances. In 33 instances it was present in the baby's throat before it appeared in the mother's milk, and in 39 it occurred simultaneously in both. Thus, in only 18% of the cases was the baby likely to have received its staphylococci from its mother's milk, while in 38% the mother's breast appeared to have been infected by the baby. The observations of Buttiaux and Pierret (1949) later gave indirect evidence that the baby does not receive its upper respiratory staphylococci from the maternal milk. While the presence of these organisms in the milk supply could be correlated with their presence in the baby's stools, there was no correlation between their occurrence in the stools

and in the baby's nasopharynx. These workers also advanced some evidence that the skin of the mother's breast is not a common source of the baby's upper respiratory staphylococci. Only 23% of 22 breast-skin swabs were positive for staphylococci. This was also the experience of Marsh and Rodway (1954) who found only 32% of 57 mother's nipple swabs to yield Staphylococcus pyogenes on hospital admission, although 54% were positive in the second week after delivery. Knott and Blaikely (1944) reported that newborn babies whose mouths and hands carried Staphylococcus aureus quickly transferred them to their mother's breast. The various reports therefore indicate that although the mother's breast may occasionally transmit staphylococci to the baby, more frequently they are passed from baby to mother.

Since it is now known that adult nasal carriers of staphylococci are likely to carry them on their skin and clothing, it would seem probable that mothers who were nasal carriers would transmit them to their babies via this route. There are, however, two lines of evidence indicating that the babies do not commonly acquire their

mother's nasal staphylococci: 1) babies nasal carrier rates often cannot be correlated with the maternal nasal carrier rates. 2) babies strains often differ from their mother's nasal strains in serological type, phage type, or penicillin sensitivity.

Cunliffe (1949) drew attention to the fact that a comparison between nasal carrier rates amongst newborn babies and their mothers indicated that the mothers were not the major source of the babies nasal staphylococci. Although during each of the first four days of life he found the carrier rate to be higher among babies whose mothers had been nasal or skin carriers on admission than those whose mothers had been negative, his statistical analysis showed that the difference could have been due to chance. Rountree and Barbour (1950) likewise reported that the difference in carrier rates between babies and their mothers indicated the mothers to be an unlikely source of infection for their newborn babies. Ludlam (1953) also found carrier rates of newborn babies and mothers to be unassociated, but claimed that they were somewhat

associated during the babies' early months of life. He suggested that this was due to continued contact between mother and infant. Phage typing, however, showed a high proportion of the babies to carry strains distinct from those of their mothers. Moreover, later in their first year of life he found no association with the mothers' carrier rates. His hypothesis therefore finds contradiction in his data as well as support.

The application of serological^{has} and phage typing techniques have done much to clarify the connection between mother and baby nasal strains. This information has been derived mainly from investigations on the epidemiology of pemphigus neonatorum and other neonatal infections due to staphylococci. Since the incidence of nasal staphylococci among mothers and babies has not been found higher during outbreaks than in the absence of infection, these findings probably reflect the normal relationships between them. In some investigations attention has been given to the epidemic strain, but in others a comparison has been made between all coagulase-producing strains carried by mothers and babies.

Krag-Anderson (1943) appears to have been among the first to employ serological typing in the investigation of a pemphigus outbreak. She found the serological type responsible for it to be present in the noses of 54% of the 46 babies examined, but in the noses of only 24% of 29 mothers. It was also present in 54% of the babies throats, but in just 3.5% of the mothers' throats. The relative sequence in which the mothers and babies became infected was not investigated, but these observations demonstrated that the epidemic strain was not equally distributed between mothers and babies.

Allison and Hobbs (1947), also investigating the spread of pemphigus, compared the serological types of nasal staphylococci isolated from 15 babies with those obtained from their mothers. In only four instances did both mother and baby carry the identical serological type. Throat cultures were taken on the mothers only, but just two of these contained the serological type present in the baby's nose. The epidemic strain was isolated from the noses of only five of the mothers, and while it was also

obtained from the lesions of their babies it was not necessarily present in the baby's nose. Two of the mothers appeared to have been infected by their babies, as one had developed a septic finger, and the other mastitis, after their babies had become infected with pemphigus. Allison and Hobbs concluded from their observations that the mothers were not often responsible for infecting their infants.

Phage typing technique more recently has provided universal confirmation that mother's and baby's nasal strains are not frequently associated even when staphylococcal infections are present. Williams, Sims-Roberts and Cook (1949) investigating a pemphigus outbreak due to phage type 3A in an English nursing home, found it present in the nose of eight healthy infants and in the nine affected ones, but not in the nose of any of their mothers. Similarly, Cunliffe (1949) investigating an outbreak of neonatal impetigo due to phage type 47B, found it present in the nose of all six of the affected infants as well as in the nose of five of the six healthy ones. As only half of their mothers' nasal swabs yielded

staphylococci, he concluded "the odds would be very great against the chance occurrence of staphylococci of the same phage type in more than one of these".

McGuinness and Musgrove (1949) who investigated an outbreak of pemphigus in three Winnipeg hospitals, found the epidemic strains "W" common among the babies' nasal cultures, but rare among the nasal cultures of their mothers. Later, De Pape and McEwen (1951) reported on this same outbreak in greater detail, and supplied more complete data on the lack of association between mother and baby nasal carriage. Upon examination of the nasal swabs taken from 64 mothers and their babies, either the mother's swab or that of the baby was negative in 35; in 17 both were negative. In only one pair did both swabs yield the epidemic strains "W", while in four pairs staphylococci not phage type "W" were isolated but not further typed. Thus, in only 2% did mother and baby nasal swabs correspond, and yield the epidemic strain. In 92% there definitely was no correspondence in the nasal carriage of mother and baby, while in the remaining 6% the correspondence which may have existed was not

established with certainty.

Parker and Kennedy (1949) attempting to trace the source of a pemphigus outbreak at Carmarthen, did not find the epidemic type among the nasal swabs taken on 104 mothers on admission. Furthermore, they observed that there was little relation between the phage type contained in the mother's admission swab and that subsequently isolated from the nasal swab of her infant.

There are two reports in which the nasal staphylococci of mother and babies were compared when no severe staphylococcal infection was present. In both studies penicillin sensitivity was used in conjunction with phage typing for comparison of the strains. Rountree and Barbour (1950), in an attempt to determine the origin of nasal staphylococci among newborns in a Sydney hospital, swabbed 54 babies, aged six to seven days and their mothers at the same time. From only 13% of these pairs were Staphylococcus pyogenes of the same phage type isolated. In 24% the mother and baby carried a different phage type, while in the remaining 63% either a swab from mother or

baby was negative, or both were negative (5.5%). In a somewhat similar investigation in a London maternity ward, Barber et al. (1953) obtained comparable results. They took nasal swabs from the mothers both on admission and at discharge, and from the babies at discharge. Comparison of the swabs taken on 128 mothers and babies at discharge, usually on the tenth day, showed that in only 16% did both contain the same phage type. Strains of different phage type were isolated from mother and baby in 34%, and in the remaining 50% the swabs from either one or the other were negative, or else both were negative (17%). When the baby's nasal culture on discharge was compared with that of its own mother on admission, differences were obtained in 80%. Finally, when results of the two swabs from the mother were compared with the one from the baby the difference was very great. Of the 111 cases where all three swabs from mother and baby had been positive, in only three cases (3%) were staphylococci of the identical phage type and penicillin sensitivity obtained. These studies therefore indicate

that the mother as well as the baby acquire nasal staphylococci during hospitalisation. The chance of both acquiring the same strain is slight, and there seems little interchange between the two.

As newborn babies do not usually acquire their nasal staphylococci from their mothers, it might be supposed that they acquire them from the hospital nursery staff. In general, the probability of transmission from nurse to baby seems greater than from mother to baby. It has been found that the incidence of nasal carriers among adult hospital personnel is often somewhat higher than that of adults in the general population, and that nurses may persistently carry hospital strains over considerable periods of time. Moreover, since it has been shown that, in adults, nasal colonisation by hospital strains is related to the length of time spent there, the nurse is more likely to have acquired them than the mother, and therefore more likely to transmit them (Hobbs, 1944; Barber and Whitehead, 1949; Barber, Hayhoe and Whitehead, 1949; Rountree and Barbour, 1951; Wallmark and Laurell, 1952; Vogelsang, 1953).

In a comparison of nasal strains from 41 babies and 24 nurses in a neonatal nursery, Rountree and Barbour (1950) found 14 phage types among the babies, only 7 of which were also present in the nurses. The remaining 50%, therefore, seemed to have been acquired from other sources. On the other hand, when Barber et al. (1953) investigated staphylococci cultured from the hands and aprons of nurses in a neonatal nursery, the most common phage type was that also most common in the babies' nasal cultures. They concluded that the phage types which they isolated from babies, mothers and nurses indicated that the babies acquired staphylococci more readily from nurses than from their mothers.

During outbreaks of neonatal infection a considerable number of nurses are sometimes found to be carriers of the epidemic strain. Hobbs (1944) reported pemphigus neonatorum in a nursing home where, after two babies had become infected, five of the seven nurses were found to be carrying the pemphigus strain in their nose or throat. Allison and Hobbs (1947) found that at the outset of a pemphigus out-

break 37.5% of the nursing staff were nasal or hand carriers of the epidemic strain. In the course of two outbreaks in the same unit during the previous two years, the nurses' carrier rate had been about 30%. Colbeck (1949) investigating pemphigus and mastitis in three Winnipeg hospitals, all due to the same phage type, found the nasal carrier rate among staff personnel to be only 4% in one hospital, but 19% and 22% in the other two. Denton et al., (1950) in a Montreal outbreak of pemphigus, found only 13% of the nursing staff to be nose and throat carriers of the epidemic strain, but concluded that the nurses were at least partly responsible for the spread of the epidemic.

In none of the reports cited has it been clearly established that the nurses nasal carriage of the epidemic strain was the cause or the result of the outbreak. Elliott et al., (1941) published what seems the most suggestive evidence implicating a nurse to have introduced a pemphigus strain into a nursery, but even this may have been circumstantial.

There are, on the other hand, several reports of neonatal outbreaks in which 10% or less of the nursing staff were carriers of the epidemic strains (Krag-Anderson, 1943; Parker and Kennedy, 1949; Melin and Wallmark, 1949). Other vectors of infection therefore must be important.

The hospital environment is believed by many investigators to be the reservoir from which babies, mother and nurses alike derive their staphylococci. Allison and Hobbs (1947) showed that 28% of the staphylococci obtained from the nursery dust were the serological type currently causing pemphigus there. Similarly, Rountree and Barbour (1950) found nursery dust samples to contain large numbers of Staphylococcus pyogenes, even in the absence of any severe staphylococcal infection. Five of the seven phage types contained in the dust were those also isolated from the noses of babies and nurses. Coagulase-producing staphylococci, including the strains associated with neonatal infection, have also been cultivated from the infants blankets and baby oil, (Blair

and Carr, 1953), nurses' aprons (Barber et al., 1953) and the communal changing bench (Rountree and Barbour, 1950). In view of these findings, it is rather surprising that the staphylococcal contamination of the air of these nurseries sometimes has been reported rather low (Allison and Hobbs, 1947; Marsh and Rodway, 1954). Wallace and Duguid (1952) found the nursery air to carry 0.01 to 0.10 staphylococci per cubic foot during morning activity, and calculated that the infant there would inhale only about one staphylococcal-containing particle per day. On the other hand, Rountree and Barbour (1950) obtained a mean count of 1.8 staphylococci per cubic foot during morning activity, and 4 to 5 per cubic foot during sweeping and dusting. In some nurseries, therefore, the air may be a primary vector of infection, but in others fomites probably are more important.

The complex mode of staphylococcal spread in the neonatal nursery is further indicated by the extreme difficulty experienced in controlling pemphigus infection. Even the most drastic measures such as ward closure

accompanied by thorough cleaning, painting, and use of ultraviolet light have failed to prevent recurrences of this infection (McGuinness and Musgrove, 1949; Farquharson et al. 1952). Antibiotic treatment of the nurse nasal carriers, or their withdrawal from duty has also proved ineffective (Parker and Kennedy, 1949; McGuinness and Musgrove, 1949). It may be significant that the most successful pemphigus control measures claimed are those directed against control of staphylococcal carriage among the babies. McGuinness and Musgrove (1949) reported that aureomycin, prophylactically administered to the babies seemed to stop the outbreak when all other measures had failed. Farquharson et al. (1952) claimed remarkable success in bathing the babies in a compound (pHisoHex) leaving a long lasting bacteriostatic film on the skin. The success of these measures lend support to belief that the most important vector of staphylococcal spread is from baby to baby as some workers have suggested (Parker and Kennedy, 1949; Melin and Wallmark, 1949).

Significance of Staphylococcal Carriage in the
Infant Upper Respiratory Tract

Considering how readily the newborn's upper respiratory tract becomes colonised with staphylococci, and how easily it may spread them, it is apparent that the newborn may play an important role in the epidemiology of staphylococcal infection. These infections present serious problems in the maternity ward both for mothers and babies; in the babies they may prove severe and even fatal. Some paediatricians are concerned that such infections are occurring with increasing frequency.

Not only are the newborn staphylococcal carriers potentially dangerous to themselves and their mothers, but upon hospital discharge they become a source of infection to the general population. Colbeck (1949) cited examples of staphylococcal infection, due to the phage type of the hospital nursery, developing in family members as long as 10 to 15 months after the arrival of the new baby. Similarly, Denton et al., (1950), claimed to isolate from the noses and throats of the general population

the phage type causing pemphigus at the local hospital.

The hospital strains colonising the infant upper respiratory tract frequently are penicillin resistant. This is to be expected in view of the increasing prevalence of penicillin resistant strains in hospitals since the introduction of this antibiotic (Barber and Rozwadowska-Dowzenko, 1948; Vogelsang, 1953b). Reports on their occurrence in the newborn upper respiratory tract vary from 25% to 100% (Martyn, 1949; Rountree and Barbour, 1950; Wallmark and Laurell, 1952; Barber et al. 1953; Ludlam, 1953; Marsh and Rodway, 1954). It is therefore apparent that the newborn's dissemination of penicillin resistant strains among the general population may be of major importance to this alarming problem.

Summary

The newborn infant upper respiratory tract is readily colonised by Staphylococcus aureus and there is some evidence that Staphylococcus albus is also frequently present. Colonisation of the throat seems to occur more

slowly than that of the nose and nasopharynx. By the time of hospital discharge nearly all babies have acquired Staphylococcus aureus. It is believed that the incidence of Staphylococcus aureus among babies delivered at home is probably lower than that of babies born in hospital. Very low carrier rates have been observed among home-born babies during the first few months of life, while the carrier rates of hospital-born babies are still high at this age.

In the later months of infancy nasal carriage of Staphylococcus aureus decreases until, by the end of the first year, the carrier rate has fallen to about 25%. After the first year of life the nasal carrier rate progressively increases until, at about the tenth year, it reaches the 50% level of adulthood. Throat carriage in young infants and children has not been investigated. In older children and adults, however, positive throat cultures often accompany positive nose cultures.

There is considerable evidence that newborn infants do not usually acquire their staphylococci from the

mother's breast or upper respiratory tract. Staphylococci may be cultivated from the babies throats before they are present in the mother's breast milk and negative cultures are often obtained from the breast skin. Moreover, when the nasal carrier rates of mothers and babies are compared, they are found to differ, as do the serological and phage types of their strains. A closer connection has been found between the staphylococci carried by nurses and babies than between mothers and babies. However, it has not been ascertained whether the nurses transmit them to the babies, or whether nurses and babies both acquire them from the hospital environment.

The spread of staphylococci in the nursery probably is complex, for it is heavily contaminated. Large numbers of Staphylococcus aureus may be cultivated from the air, dust, blankets, gowns, etc. In view of the ease with which the infant upper respiratory tract becomes profusely colonised with staphylococci, it is likely that the infants themselves are important contributors to the nursery reservoir.

Staphylococcal infections among newborn infants and

their mothers are common, and in the infants they are sometimes severe. The newborn infant carriers therefore are a potential hazard to one another, as well as to their mothers; upon hospital discharge they become a potential hazard to the family. As penicillin resistant staphylococci are now prevalent in hospital nurseries, it is clear that the newborn infant may play a major role in disseminating these strains among the general population.

CHAPTER 2

SECTION B

COLIFORM BACILLI

Coliforms usually are not considered a component of the upper respiratory flora, although a review of the literature indicates that they are present more frequently than is generally believed. Early in the twentieth century several workers, cataloguing components of the upper respiratory flora, noted coliforms particularly among children. These very early reports have been reviewed by Laurell (1952a).

In more recent years, many investigators have found coliforms in the upper respiratory tract of newborn infants. The incidence figures reported are compiled in Table 2. They vary greatly, perhaps as a result of the different cultural methods used. For example, Gundel and Schwarz, who obtained the highest incidence, used broth for primary cultivation. Others, whose figures are lower, used blood agar plates, while Cornelison et al. used MacConkey's agar.

The newborn's acquisition of coliforms may depend on several factors. These include the conditions of labour, and the degree of coliform dissemination in the nursery, as mentioned in Chapter 1. It has also been suggested that coliforms in the infant throat are

Table 2. Incidence Of Coliforms In The Upper Respiratory Tract Of Newborn Infants

<u>Investigators</u>	<u>Number of Individuals</u>	<u>Age</u>	<u>Incidence</u>	<u>Site</u>
Schweitzer (1919)	37	1st day 2nd day 2-9 days	8% 14% 0	M
Bloomfield (1922)	18	1-9 days	0	T
Salomon (1922)	19	1st day 8th day	20% 58%	M
Kneeland (1930)	30 (155 cultures)	1-14 days	3% 16%	N T
Gundel & Schwarz (1932)	52 (646 cultures)	3 days 12 days	40% 89%	N & M N & M
Witkowski (1935)	50	2 days 5 days	48% 30%	M
Torrey & Reese (1944)	85-premature	1st day 3 days	3.5% 13.5%	N.P. N.P.
(1945)	105-full-term	1st day	3%	N, N.P., T
Cornelison et al. (1946)	100	At birth	21%	T
McFarlan et al. (1949)	24 41	1 day 4 days	9% 17%	T T
Levine & Tanimoto (1954)	232	2-5 days	17.2%	T

M = Mouth

T = Throat

N.P. = Nasopharynx

N = Nose

associated with the type of feeding. Gundel and Schwarz (1932) reported that they were present in 92% of newborn babies being breast fed, and in 60% of those who were artificially fed. There were, however, only five babies in their artificially fed group, whereas there were 47 in the breast fed group. Conversely, McFarlan et al. (1949) found a positive correlation between artificial feeding and coliforms in the newborn infant's throat. Examining the throat and rectal flora of newborns in two hospital units, they noted the incidence of throat coliforms to be 8% in Unit A and 33% in Unit B. The fact that the percentage of rectal swabs showing lactobacilli was lower in Unit B than in Unit A suggested that these differences might be related to feeding. A further analysis of their data, part of which is shown in Table 3, indicated this to be the case. In Unit B a greater proportion of babies were being artificially fed, and coliforms were most frequent among them. As the feeding ingredients did not contain coliforms, they concluded that when a baby's intestinal coliforms were increased by artificial feeding they were then introduced into the throat in greater numbers, either during feeding, or by

Table 3. Relation Between Coliforms In The Newborn Infant Throat, and Type Of Feeding (McFarlan, Crone and Tee, 1949)

<u>Feeding Regime</u>	<u>Unit A</u>		<u>Unit B</u>	
	<u>No. of Babies</u>	<u>Throat Coliforms</u>	<u>No. of Babies</u>	<u>Throat Coliforms</u>
Breast	65	0%	-	-
Breast after complement*	28	7%	42	29%
Breast and complement*	28	14%	66	36%
Artificial	24	17%	33	60%

*Complementary feeds consisted of re-constituted dried milk fed with a cup and spoon.

regurgitation. They also thought that overcrowding in Unit B might have contributed to the high incidence of throat coliforms among the babies there. Apparently, they did not believe that the coliforms actually became established in the throat.

Laurell (1952a) has suggested that premature babies are especially prone to acquire coliforms in their upper respiratory tract. In observations on hospitalised infants, he found the highest incidence of upper respiratory coliforms to occur among those under one month old, and particularly in those who were premature. It is conceivable that the factor of prematurity may have affected the observations of McFarlan et al. Although prematures were included among the infants studied, their distribution among the feeding groups was not taken into consideration.

Information on the incidence of coliforms in the upper respiratory tract of older infants, children and adults is meagre. The few figures reported, pertaining mainly to hospitalised individuals, have been assembled in Table 4. From Kneeland's figures, they seem about as common in the nose and throat of older babies as in the

Table 4. Incidence Of Coliforms In The Upper Respiratory Tract Of Older Infants, Children and Adults

<u>Investigators</u>		<u>Incidence</u>	<u>Site</u>
Kneeland (1930)	28 normal infants aged 4 to 8 months	7%, 7%	N, T
	21 infants, first cold-average age, 3 months	10%, 5%	N, T
	14 infants, recurrent colds-average age, 7 months	14%, 0%	N, T
Gundel & Schwarz (1932)	51 puerperal women (426 cultures)	90%	} Oro-naso-pharynx
	21 nurses (66 cultures)	38%	
Witkowski (1935)	50 puerperal women	86%	Mouth
Torrey & Reese (1944)	70 adults in infant wards	4%	Nose & Throat
Lipman et al. (1948)	10 arthritic children and adults	0	Throat
Smith & Bloomfield (1948)	6 hospitalised adults, free of upper respiratory infection	0	Throat
Laurell (1951)	346 hospitalised children (2,140 cultures) - 12 years	*21% *16%	Throat Nose & Throat
	151 adults, hospital staff (1,446 cultures)	* 6% *10%	Throat Nose & Throat

newborn. For adults, and older children, there is a wide discrepancy between the figures of various workers. It may be that factors affecting the upper respiratory coliform incidence vary considerably in different hospitals, as Laurell (1952a) has suggested. He believes these factors to include the invasiveness of the coliform strains concerned, the age of the patients, and their length of hospitalisation.

Scarcity of data on the incidence of coliforms in the adult upper respiratory tract seems due, partly, to the assumption that any coliforms cultured from this site must only be chance contaminants. Bloomfield, in numerous early publications on the ecology of the upper respiratory flora (1920a, 1921b, 1921c), repeatedly emphasised that certain bacteria were permanent inhabitants and others transients. He believed that coliforms were transients because of their irregular occurrence, and because he was unsuccessful in implanting them in the upper respiratory tract of four subjects. B. coli placed on the tongue, nasal mucosa, and into the tonsil crypts disappeared within 3 days or less. Later, Arnold and co-workers (1928) reported similar failure in

implanting B. coli on the adult nasal mucosa. They found that 90%-95% of B. coli sprayed into the noses of 42 volunteers disappeared within 10-15 minutes. At the time of these studies the fact that strains within a species may differ greatly in invasive power was not fully appreciated.

Feeding adults large numbers of E. coli isolated from infantile diarrhoea has not resulted in their implantation in the throat. Ferguson and June (1952) fed 46 prisoner volunteers milk which had been inoculated with three strains of E. coli serotype O 111, B4. Throat cultures taken on three consecutive days after the feeding were negative, although the stool cultures usually remained positive for seven to twelve days, and there were mild to severe gastro-intestinal symptoms. In a second series of experiments (June, Ferguson and Worfel, 1953) they fed 58 volunteers with three strains of E. coli O 55, B5. Except that the symptoms were milder, the results were similar to the first experiments, and the throat cultures remained negative. In each series, a small number of volunteers were also fed E. coli isolated from two healthy normal babies. No symptoms

Note - The terminology applied to the coliform bacilli by the individual authors is used throughout this review.
e.g., B. coli, E. coli

resulted, and the throat cultures were negative although the same organism usually could be recovered from the stools throughout the week of observation.

A possibly successful implantation of E. coli in an infant's upper respiratory tract has been reported by Neter and Shumway (1950). They fed a two-month old baby, having multiple congenital defects, with a culture of E. coli, serotype D433. On the following day, the strain^{was} cultured in small numbers from the throat and in large numbers from the faeces, while on the second day large numbers were present in the throat, nasopharynx and faeces. Diarrhoea with weight loss occurred, and the experiment was terminated by administering terramycin at the end of the second day.

Although artificial implantation of E. coli in the upper respiratory tract usually has not been successful, evidence is accumulating that coliforms may become a part of the upper respiratory flora under certain conditions. This suggestion is forthcoming from several different avenues of research: 1) Observations on the E. coli serotypes associated with infantile gastro-enteritis; 2) Studies on the occurrence of serologically specific

coliform types in the upper respiratory tract of hospitalised children; 3) Observations on the emergence of coliforms during antibiotic therapy.

In infantile gastro-enteritis, E. coli of the same serological type as that found in the stools have been cultivated from the nose and throat. Taylor et al. (1949), using blood agar, found strain D 433 in the nasopharynx of twelve out of eighty-eight babies ill with this disease. Similarly, Neter and Shumway (1950) found this serotype in the throat of one, and in the nasopharynx of three out of the four infants observed. Magnusson et al. (1950) cultivated it from the fauces and nose of six out of eight babies from whose stools it was also recovered. Laurell (1951), using Conradi-Drigalski's agar, examined sixty-two infants with the disease and found it in the nose and throat of twenty, and in the throat, alone, of five. Neither Neter and Shumway, nor Magnusson and co-workers stated the medium used.

There appear to be several possible explanations for finding these coliforms in the infant upper respiratory passages: 1) that they were deposited there during

vomiting, 2) that they were inhaled or ingested from the infants' heavily contaminated environment, 3) that, however introduced, they have actually colonised on the upper respiratory mucosa.

Vomiting, very obviously, might account for upper respiratory coliforms, but evidence appears to contradict this belief. For example, although vomiting was a symptom of all cases investigated by Taylor, strain D 433 was cultivated from the nasopharynx of only 13% of them. Neter and Shumway obtained positive cultures in the absence of vomiting, and Laurell cites cases in which negative nose and throat cultures accompanied several days of vomiting. That vomiting per se does not explain the presence of this organism in the upper respiratory tract, was pointed out by Neter and Shumway.

Laurell believes that the frequent presence of these strains in the nose and throat during infantile gastroenteritis indicates air-borne spread of the disease. He demonstrated the same strains to be present in the air, and others, likewise, have reported their wide dissemination in the hospital environment (Rogers, 1951; Taylor and Charter, 1952; Stevenson, 1952). Laurell did not find

them in the milk nor on the rubber teats used, suggesting that they were not being introduced with feeding.

Laurell (1952a,b,c) is of the opinion that the upper respiratory tracts of hospitalised children actually become colonised by coliforms. During his observations on infantile gastro-enteritis (1951) he found coliforms frequently present in the upper respiratory tract of the children hospitalised for other illnesses. Not infrequently, too, he cultured coliforms from the upper respiratory passages of the adult hospital staff. He does not specifically state their incidence, but the figures which may be calculated from his data are seen in Table 4. They suggest that the incidence, both in hospitalised children and in adult hospital personnel, may be quite high. Throughout his studies, both blood agar and Conradi-Drigalski's agar were used, and the swabs taken from both nose and throat.

In Laurell's further studies, he demonstrated by serological methods that particular coliform strains could be cultured repeatedly from the nose and throats of certain children. These children he therefore considered

to be carriers. As a rule they carried one or, sometimes, two serotypes throughout many weeks of hospitalisation. The highest carrier incidence was among infants under one year of age, and as previously mentioned, premature infants were particularly apt to become carriers. The majority of children acquired their coliforms after their admission to hospital, and carriers were most common among those hospitalised for more than two weeks. The latter fact, Laurell points out, partially may have accounted for the high incidence of carriers among the premature infants whose hospitalisation was long. Coliforms were most often present in both the nose and the throat, although it was not uncommon for them to be present in the throat alone.

The majority of coliform strains which Laurell isolated from these children belonged to three serotypes: O 6 (39%), O 18 (19%) and O X₁ (25%). In analyses of the hospital floor dust, O 6 was most common (21%) while O 18 and O X₁ were less frequent (5%, 10%). Several strains which were common in the floor dust, such as O X18 (11%) and O 15 (14%), were never found among the

children. The strains obtained from the adult hospital staff were, as a rule, different from those recovered from the children, and also different from those in the floor dust. Likewise, the majority of strains obtained from children visiting the out-patients department were different from those of the hospitalised children. Untypable strains were numerous among both adult hospital personnel and out-patient children, but not in the hospitalised children.

It may be argued that the repeated recovery of a single strain from an individual hospitalised child is not necessarily evidence of a carrier state. If the environment were heavily contaminated with the strain it might constantly be re-introduced into the child's upper respiratory tract. Laurell himself observed that the hospital environment often contains an extraordinarily high concentration of coliforms. However, the fact that different children were characterised by particular strains at the time when other strains were also numerous in the hospital environment, strongly suggests that these children had become carriers. It is interesting to note that two of the three out-patient children found to have

hospital strains had evidently retained them since their discharge from hospital a month earlier.

Laurell thinks that there occurs in a hospital, a selection of strains most likely to invade the child's upper respiratory mucosa. The facts supporting this are 1) the children admitted to hospital usually acquired the strains most commonly present among the other children, 2) the common infecting strains often replaced other strains present on the child's admission, 3) some of the strains numerous in the hospital dust were never found among the children, 4) the strains encountered among the out-patient children and adult hospital staff differed from those of the hospitalised children. He believes that penicillin therapy may favour both the carrier state and the spread of coliforms in hospital. Many of the children who became carriers did so after the commencement of penicillin treatment.

Laurell does not think that the number of upper respiratory coliform carriers among hospitalised children necessarily represents that among healthy, non-hospitalised children. However, it can be calculated from his data (1952a, 1952c) that the coliform incidence

among out-patient children was slightly higher (44%) than among the hospitalised children (34%) during the same years of investigation (Table 4). Also, the frequent occurrence of coliforms among the adult ward staff, although serologically distinct from those of the children, suggests that children are not necessarily unique in harbouring upper respiratory coliforms. Further observations on the upper respiratory coliform incidence among both children and adults of a healthy, non-hospitalised population are needed to evaluate the full significance of Laurell's findings.

It has been a general experience that coliforms may become predominant members of the upper respiratory flora during treatment with antibiotics, particularly penicillin. Long (1947) reported that in 312 patients with gingivo-stomatitis and treated with penicillin pastilles, Gram negative bacilli became numerous in 77% between the second and seventh days. The majority of strains examined were of the coli-aerogenes group. Lipman et al. (1948) found that of ten arthritic children and adults given oral penicillin for up to six months, nine developed a predominantly Gram negative flora,

including coliforms, in their throats. After the cessation of treatment, the coliforms became less numerous, but persisted for periods varying from seven days up to three months. In contrast, Smith and Bloomfield (1948) found coliforms frequently but not consistently present in the throats of six patients treated with penicillin, and that they quickly disappeared after treatment. These patients, however, had been given intramuscular penicillin in low dosage for not more than several weeks. Haffner et al. (1950) observed that all of 22 children, to whom penicillin was given with or without sulphonamides, developed a predominantly Gram negative flora in the nasopharynx and throat. In a second group of 68 children with upper respiratory infection, 30 acquired Gram negative bacilli during or following penicillin therapy. They noted this tendency to be greater among children receiving aerosol penicillin than among those receiving it by intramuscular injection alone. The majority of the Gram negative bacilli found were members of the B. coli-A. aerogenes group. McCurdy and Neter (1952) reported the same phenomenon occurring in 19 of 33 children given either penicillin or penicillin in combination with a broad spectrum antibiotic. On the other hand, only four

of 21 children receiving a broad spectrum antibiotic alone developed a predominantly Gram negative upper respiratory flora. They also made the interesting observation that coliforms were no more likely to appear in patients with upper respiratory infections than in those with other illnesses. Smith et al.(1952) observed 36 rheumatic children receiving oral penicillin tablets daily for three months. The incidence of E. coli in their throat cultures during this period was 7%, as contrasted with 0.2% in the throat cultures of the control group not receiving penicillin. It appears from these various reports that the appearance and persistence of the coliforms in the upper respiratory flora is somewhat dependent on the type of antibiotic used, mode of administration, the dosage, and the duration of treatment.

Unfortunately, not many observations have been reported on the frequency of coliforms in the upper respiratory passages prior to antibiotic therapy. Lipman and co-workers state that their ten patients were initially free of coliforms, although K. pneumoniae were found in two. Smith and Bloomfield cultured no coliforms from their six patients prior to therapy. On the

other hand, although Haffner et al. state that in one group of 22 children there were no Gram negative bacilli before treatment, in a second group they were present in 22 of 68 children prior to treatment. No explanation for this difference between the two groups is offered, but it may be significant that the media used for the second group included Endo agar and penicillin agar, in addition to the blood agar used for the first group. McCurdy and Neter did not determine the incidence of coliforms before therapy, but state that in their control series of 52 children receiving no therapy, nine children developed a Gram negative bacillary flora during the period of observation.

From the little data available, there is reason to believe that the coliforms becoming predominant during antibiotic therapy sometimes may have been present previously. Cultures negative before therapy may have contained coliforms in small numbers easily missed by the usual routine cultural methods. Thus, it is quite possible that the coliforms multiplying during antibiotic therapy often are of endogenous origin. As yet, no observations have been made on the serological types present before and after antibiotic therapy.

SUMMARY

Nearly all investigators have found coliforms present in the upper respiratory tract of newborn infants, although there has been no agreement on the incidence. Factors believed to influence their occurrence in newborns are the conditions of labour, prevalence of coliforms in the nursery environment, type of feeding, and perhaps prematurity. Coliforms have also been found in the upper respiratory tract of older infants, as well as older children and adults who were hospitalised. Unfortunately, no information is available on their incidence among the general, non-hospitalised population.

It is usually assumed that the coliforms occasionally cultured from the upper respiratory tract in small numbers are only chance contaminants. The only evidence that they can not become established in the upper respiratory tract is that they have never been successfully implanted there by feeding or other experimental methods. Laurell, however, has demonstrated that specific serological types not only become established in the upper respiratory tract of hospitalised children, but may be carried for long periods of time.

Evidence is accumulating that coliforms commonly occur in the upper respiratory tract under certain conditions. The work of Laurell suggests that, among children, a hospital environment is particularly conducive to the acquisition of certain serological types. In ~~infantile~~ gastro-enteritis, E. coli of the serotypes recovered from the stools are also found present in the upper respiratory tract, and do not necessarily accompany vomiting. During antibiotic therapy, coliforms frequently become predominant in the upper respiratory tract, and occasionally persist for a long time afterwards. It is not known whether they are of endogenous or exogenous origin. The various observations suggest that coliforms often do become established in the upper respiratory tract, although not always in large numbers.

CHAPTER 2

SECTION C

FRIEDLÄNDER'S BACILLUS

Friedländer's bacillus, or Klebsiella pneumoniae, is often stated to be a normal inhabitant of the upper respiratory tract. Reports on its actual incidence are few, and are difficult to assess because of the frequency with which it may have been confused with the coli-aerogenes group, also sometimes present. Recognition has depended mainly on its colonial appearance, said to be characteristically mucoid. However, non-mucoid variants have commonly been noted, particularly on secondary cultivation (Topley and Wilson, 1946). With loss of its mucoid character the organism becomes indistinguishable from the coliforms. Its biological activity is also variable, but resembles that of the coli-aerogenes group.

Osterman and Rettger (1941), in a comparative study, concluded that Friedländer's could not be distinguished from the coli-aerogenes group in either its biochemical or serological properties. Kauffmann (1949) later described K. pneumoniae and A. aerogenes to contain type-specific capsular K antigens apart from the somatic O

antigens which they shared with the E. coli group. He therefore advocated that A. aerogenes and K. pneumoniae be included in a single genus, Klebsiella, distinct from Escherichia. Very recently Pickett and Cabelli (1953) have described an "5m" antigen present in all mucoid and smooth strains of Kauffman's genus Klebsiella, and absent in E. coli. These new serological methods may prove useful in clarifying the actual frequency of Friedländer's in the normal upper respiratory tract. For the present review, the designation "Friedländer's", as given by the authors, has been accepted.

Most investigators have not found Friedländer's among the upper respiratory flora of the newborn infant (Torrey and Reese, 1945; Kneeland, 1930; Bloomfield, 1922). However, Gundel and Schwarz (1932) reported to have found it in 8% of 52 infants under twelve days of age, and Witkowski (1935) found it in 16% of 50 infants up to five days of age. Both of the latter workers used broth for primary cultivation in contrast to the other investigators who used blood agar plates. The organism

designated as "Friedländer's" was not described, but as coliforms were also mentioned in these reports, it is probable that the colonial appearance was distinctive.

The incidence of Friedländer's in the normal upper respiratory tract of older children and adults has been variously estimated by different workers. Thomson and Thomson (1931), who reviewed the literature up to that date, found the figures reported to range from 5% to 20%. Those reported subsequently have been compiled in Table 5. With the exception of the figures reported by Gundel and Schwarz, and Witkowski, they do not exceed 10%.

The tendency of Friedländer's to persist in the upper respiratory tract of certain individuals for long periods has been noted by several workers (Bloomfield, 1920b, 1921a; Blackburn et al., 1930; Straker, 1939). Bloomfield made a long term study of four adults carrying Friedländer's and found that they retained the organism for from five weeks to four months. It was usually present in the throat, with the tonsils being the apparent

Table 5. Incidence Of Friedländer's In The Normal Upper Respiratory Tract Of Older Children And Adults, As Reported Since 1930

<u>Investigators</u>	<u>Incidence</u>	<u>Group Sampled</u>	<u>Culture Medium</u>	<u>Site</u>
Wohlfiel & Jaffe (1931)	2.2%	115 Königsberg school children	Blood agar	Oral cavity and throat
	9.0%	286 Bonn school children	"	"
Gundel & Schwarz (1932)	18%	51 puerperal women (426 cultures)	Dextrose broth	Naso-oro pharynx
	8%	12 nurses (66 cultures)	"	"
Witkowski (1935)	34%	50 puerperal women	Dextrose broth	Oral cavity
Straker et al. (1939)	1-8%	111 adults, London School of Hygiene	Fildes' and Blood agar	Nose, naso-pharynx
	8%	106 adults, Min. of Health	"	"
	1-7%	100 boys, 11 to 15 years	"	"
	3%	200 Public School Boys, 13 to 18 years	"	"
	0	50 Orphanage children, 2 to 12 years	"	"
Torrey & Reese (1945)	7.1%	70 puerperal women	Blood agar	Nose, Throat

focus, and rarely in the nares. In some interesting artificial implantation studies on a few individuals, Bloomfield found that even with repeated introduction a non-carrier did not become a carrier of the organism. Moreover, it proved impossible to transfer a distinct Friedländer strain from one carrier to another carrier, and throat carriers could not be induced to carry their own strain in their nose.

SUMMARY

Friedländer's bacillus has not frequently been reported in the upper respiratory tract of newborn infants. In older children and adults it has been reported as a normal component of the upper respiratory flora of a small percentage of individuals, some of whom remain carriers for long periods. The persistence of Friedländer's bacillus in the upper respiratory tract is analogous to that observed for coliforms to which it is closely related.

CHAPTER 2

SECTION D

STREPTOCOCCI

Viridans and non-haemolytic streptococci are invariably present particularly in the mouth, almost from birth. Kneeland (1930) found the "indifferent streptococci" to be among the most predominant bacteria in the mouth during the first two weeks of life. Torrey and Reese (1945) did not find the indifferent streptococci to be more numerous than the viridans types during this age period, but both were abundant. In the adult mouth, viridans streptococci are sometimes stated to constitute the bulk of the flora, but this may be an illusion gained from their ready growth and easy recognition on blood agar.

The complex heterogeneity of the oral streptococci has discouraged their study, and biochemical reactions have proved unsatisfactory for their classification. Serological classification appears more promising, although it has not yet been worked out. Selbie and co-workers (1949) were able to divide 92% of 197 strains isolated from normal throats, extracted teeth, and endocarditis, into five serological groups distinct from those of

Lancefield. Farmer (1953) isolated from 25 healthy mouths the Lancefield Groups F, G, H and K, as well as the Group O of Boissard and Wormald. Both of these studies indicated that the streptococcal flora of the individual mouth is composite, often containing several serological types.

In recent years, Streptococcus salivarius has been proposed as a distinct species, although as Selbie and co-workers have pointed out, it is probably, more correctly, a group with homologous characteristics. It is usually characterised by its production of mucoid colonies on agar containing 5% sucrose, and by fermentation of salicin and raffinose. Torrey and Reese (1944, 1945) believed that this organism was one of several unable to become established in the newborn upper respiratory tract. In their study on 150 premature babies they found it present in the nasopharynx and throat of about 28% under four days old, but in only 8% of those who were two to four weeks old. In their later study on 70 full-term newborns, they found it in 17.6%, and stated that it did

not persist.

The literature contains no information on the occurrence of this organism in older infants. It is usually cultivated from the adult mouth. Data given on its incidence in children and adults are cited in Table 6.

Table 6. Incidence Of Streptococcus salivarius In The Upper Respiratory Tract Of Children And Adults

	<u>Site</u>	<u>Number of Individuals</u>	<u>Incidence</u>
Safford, Sherman and Hodge (1937)	Throat	20 adults (322 cultures)	88%*
Torrey & Reese (1944)	Nasopharynx and Throat	40 adults	75%**
Torrey & Reese	Nose & Throat Nose only	70 adults "	100% 4%
Shiere et al. (1951)	Saliva	58 adults 56 children	98%

* % of positive cultures

** approximate incidence, calculated from data cited

CHAPTER 2

SECTION E

GRAM NEGATIVE COCCI

Non-pathogenic *Neisseria* are nearly always present in the adult upper respiratory tract, particularly in the nasopharynx. Although they may be found in the newborn infant, their incidence is uncertain. Gundel and Schwarz (1932) claimed to have obtained them from the mouth and nose of 84% of their 52 infants under 12 days old, but this figure seems very high. The figures cited by Witkowski (1935) and Torrey and Reese (1944, 1945) were much lower, but their figures on the incidence among the mothers were lower than would be anticipated (Table 7). According to Kneeland (1930) they are more frequent among the throats of older infants than those under two weeks of age. In children aged 0-4 years Dawson and Zinneman (1952), who used Bordet-Gengou agar with penicillin, noted them in 32% of 468 nasopharyngeal swabs obtained from whooping cough suspects or contacts. The literature thus seems to suggest that these organisms occur less commonly in infants and young children than in adults.

Table 7. Incidence Of Non-pathogenic Neisseria In The Infant Upper Respiratory Tract

<u>Investigators</u>	<u>Designation</u>	<u>No. of Babies</u>	<u>Babies' Age</u>	<u>Site</u>	<u>Babies' Incidence</u>	<u>Mothers' Incidence</u>	<u>Site</u>
Kneeland (1930)	Gram negative cocci	30	< 2 wks.	N	1%	-	
				T	4%	-	
		28	< 4-8 mo.	N	18%	-	
				T	71%	-	
Gundel & Schwarz (1932)	M. catarrhalis	52	< 12 days	N, M	84%	86%	M
Witkowski (1935)	"	50	< 5 days	M	4%	12%	M
Torrey & Reese (1945)	N. catarrhalis	70	< 9 days	N, NP, T	1.4%	5.7%	N, NP, T

N = Nose T = Throat M = Mouth NP = Nasopharynx

Neisseria meningitidis has been found in the mouths of 6% to 25% of children (2 to 11 years) and adults. (Straker, et al., 1939). It was claimed by Wohlfeil and Jaffe (1931) that infants aged three to seven months do not harbour meningococci, but they did not state the number they investigated. They also did not find it in the nasopharynx or throat of 20 children aged three to six years, although they reported 43% to 47% in somewhat larger groups of older children aged six to fourteen years. The literature seems to contain no other report on meningococcal carriers in early infancy.

Anaerobic Gram negative cocci are believed to be a regular component of the normal oral flora. Lewcowicz (1901) noted "Micrococcus gazogenes" in the mouth of an eight day old infant, but Andre (1934) claimed it not present in the infant mouth until after the teeth erupt. Hall and Howitt (1925) found Micrococcus gazogenes in all mouths of 23 individuals (age not stated) which they examined. In present taxonomy this organism is described as Veillonella gazogenes. Morris (1954a) recently cultivated it and other Veillonella species from all mouths of 11 subjects aged 10 to 24 years.

CHAPTER 2

SECTION F

CORYNEBACTERIA

Diphtheroids are common among the upper respiratory flora of both infants and adults, although they are not always present. Kneeland (1930) found them more likely to be predominant in the nasal flora of babies four to eight months old than among newborns under two weeks old, but their incidence was similar in both. Figures which have been reported for their incidence in infants are shown in Table 8.

It is well known that Corynebacterium diphtheriae may be carried both by healthy children and adults. The carrier rate seems to depend upon the closeness and continuity of contact with infection, and varies accordingly between about 0.5% and 15% (Topley and Wilson, 1946). The literature seems to contain no reports on the carrier characteristics of young healthy infants. However, Cruickshank (personal communication) observed several newborns to become healthy nasal carriers during an outbreak of diphtheria in a neonatal nursery. Interestingly, the cord blood of the three babies found to be carriers had high antitoxin titres, whereas those

of the three babies who became ill had low titres.
Diphtheria in early infancy is not as rare as is thought
(Parish, 1949). Babies recovering from the disease may
carry the bacilli for 5-6 months (Liakka, 1947).

Table 8. Incidence Of Diphtheroids In The Infant Upper Respiratory Tract

	<u>No. of Babies</u>	<u>Age</u>	<u>Incidence</u>	<u>Site</u>
Kneeland (1930)	30	2 wks.	43% 7%	N T
	28	4-8 mo.	46% 7%	N T
Gundel & Schwarz (1932)	52	> 12 days	44%	N & M
Witkowski (1935)	50	> 5 days	16%	M
Torrey & Reese (1944)	22 premature	2-4 days	18%	N.P. & T
Torrey & Reese (1945)	17 full-term	1st day	17.6%	N.P. & T

N = Nose

T = Throat

M = Mouth

N.P. = Nasopharynx

CHAPTER 2

SECTION G

LACTOBACILLI

Rosebury (1944, 1952) asserts that lactobacilli present in the mouth of the newborn, disappear toward the end of the second week, and do not reappear until the time of dentition. This statement seems completely unsupported, however, for their incidence in this age group has been little studied. The only recorded report is that of Andre (1934) who, on the contrary, claims to have found aerobic "*Bacillus acidophilus*" in the mouths of 13 babies aged eight to six months.

Aerobic lactobacilli are found, almost invariably, in the mouths of children and adults, but their predominance varies greatly in different individuals. It has been claimed that large numbers are associated with dental caries and high carbohydrate diet, but the matter is highly controversial. While the majority of reports favour this view, there are also a substantial number which are contradictory (Cox, 1952). The subject is much confused by 1) the technical difficulty of quantitatively estimating the salivary bacteria, 2) inadequate criteria for classifying the lactobacilli and the closely related

streptococci, 3) unsatisfactory clinical definition of "active caries", 4) failure to assess adequate controls. The oral lactobacilli are very similar to the aerobic species of the intestinal tract, but identity of the two has been much disputed in a voluminous body of literature.

Lactobacillus bifidus, the anaerobic species of this genus, has long been known to be a major component of the breast fed infant's intestinal flora. It would be interesting to know whether it occurs in the infant mouth, as well. Unfortunately, its presence in the mouth has been little studied. Lewcowicz (1901) mentioned finding it in the mouth of nursing infants, and stated that it became more numerous in the mouths of older children. Although he stated the organism to be identical with that isolated by Tissier from the infant intestinal tract, the criteria for classification are not given. Howe and Hatch (1907) reported that L. bifidus was abundant in dental caries but, again, the classification is doubtful, because they do not state whether it was an obligate anaerobe on primary isolation.

CHAPTER 2

SECTION II

ACTINOMYCETES

Andre (1934) was unable to find actinomycetes in the mouths of 13 infants aged eight days to six months, but claimed to cultivate them (characteristics undescribed) from five of eight infants six to nine months old. This suggests that they are associated with dentition, but it is the only report available on the sequence of their establishment in the infant mouth. A number of workers have reported them present in the healthy adult mouth; those described include members of the aerobic Genus *Nocardia*, and the anaerobic Genus *Actinomyces*. It is probable that many of the *Nocardia*-like organisms described in the mouth by early workers were contaminants. The common occurrence of the anaerobic actinomycetes, on the other hand, is more firmly established. Much taxonomic confusion has arisen from the fact that many oral strains, morphologically resembling *A. israeli*, are facultative in their oxygen requirements and grow equally well aerobically (Garrod, 1953; Morris, 1954b). Their incidence in the mouth is difficult to estimate because some strains are highly

pleomorphic, and easily mistaken for diphtheroids and cocci (Hurst, 1950; Morris, 1954). Although they may be isolated from saliva, they are more abundant in scrapings from tooth surfaces.

CHAPTER 2

SECTION I

FUSIFORMS AND SPIROCHAETES

It has been stated that fusiforms and spirochaetes do not appear in the infant mouth until the time of dentition, but the evidence for this is meagre (Ch. 1). They are believed to be constant oral inhabitants of older children and adults. The numbers vary enormously in different individuals. While it is generally said that larger numbers are associated with inflammation, many exceptions to this may be found. Their study has been greatly handicapped by the difficulty of their isolation and maintenance. Recent reviews on the oral members of these two groups have been presented by Hoffman (1949, 1952) and Macdonald (1953).

CHAPTER 2

SECTION J

OTHER ANAEROBES

Little work has been done on the anaerobic members of the upper respiratory flora. It has been claimed that anaerobic bacteria are not present in the infant mouth until after the teeth erupt, but the subject has not been adequately investigated (Ch. 1). A few of the anaerobes which have been reported as normal components of the adult flora are briefly mentioned here.

Anaerobic Gram negative filter passing organisms have been cultivated from the healthy nasopharynx by a number of workers. Originally described as B. pneumosintes, they have been reclassified recently as Dialaster pneumosintes (Bergey's manual, 6th ed.).

A pigment producing organism described as Bacteroides melanogenicum which seems to be a normal parasite of the mucous membranes was cultivated from the throat and tonsils by Oliver and Wherry (1921) and others. Its pigment, originally thought to be a melanin, has now been shown to be a parahaematin. Andre (1934) reported that an aerobic organism, to which he applied this name, was absent in the mouth of infants, rare in young children,

but abundantly present in adults. His organism seems to have been improperly classified, however, for he describes it as a small short rod, with polar granules, probably related to the diphtheroids.

Clostridia, including Cl. tetani and Cl. welchii, were found by Hall (1925) in six saliva samples collected from 43 persons aged 10 to 54 years. They appeared to be only transients, however, as they were not consistently recovered from the same individuals. Hartles and McDonald (1951) claimed to have isolated Cl. welchii from 60% of 252 extracted carious teeth and 10% of 48 sound ones. However, they were unable to duplicate this in later work (Hartles, personal communication). These strains, studied by Evans and Prophet (1951), were classified as Type A.

The taxonomic position of the Leptothrix frequently mentioned by early students of the oral flora has never been resolved satisfactorily. It seems probable that some of these would now be classified as Fusiforms or Bacteroides, and others as Nocardia. However, Morris

(1954b) in a recent study of this complex and heterogeneous group, has concluded that some strains are sufficiently distinctive to warrant a separate generic rank, *Leptotrichia*.

CHAPTER 3

RESISTANCE OF THE INFANT UPPER RESPIRATORY TRACT TO CERTAIN POTENTIAL PATHOGENS

Some investigators believe that the upper respiratory mucosa of the newborn resists colonisation by potential pathogens such as pneumococci, haemolytic streptococci, and Haemophilus influenzae. The evidence for this is considered here, as well as the data on the occurrence of these organisms among older babies and young children.

SECTION A. PNEUMOCOCCI

The majority of investigators have failed to find pneumococci in the upper respiratory passages of newborns delivered in hospitals. Bloomfield (1922) made no mention of finding them among the 18 newborns he examined. The first report to draw attention to their absence, however, is that of Kneeland (1930). He was unable to find them in the noses or throats of 30 babies during their first two weeks of life. The observation was later confirmed by Torrey and Reese (1944, 1945). They found no pneumococci in the nasopharynx or throat of 62 premature infants up to ten days of age, despite the fact that five of their adult contacts were known to be throat carriers of Types 3, 19, 22 and 23. Among 70 full-term infants of similar age, pneumococci were present in only three (4.3%). They did not persist in any of the three babies for more than a few days. One baby's pneumococci were Type 9, and those of the other two were Type 29. These types were not harboured by

their mothers. Examination of the 70 mothers showed 22.8% to carry pneumococci in their throats (Types 3, 6, 8, 9, 15, 16, 22, 24, 28, 31).

Subsequent to the studies of Torrey and Reese, Cornelison et al. (1946) reported pneumococci absent in 150 babies examined immediately after birth. Likewise, Smith and Bloomfield (1950) failed to find them in eleven newborns up to ten days old.

Contrary to these findings are the reports of Gundel and Schwarz (1932) and Witkowski (1935) that pneumococci commonly occur in newborn infants. Gundel and Schwarz claimed to isolate pneumococci from the nose or oral cavity of 59% of 52 infants during the first 12 days of life. Type 3 was present in 11 (37%) of these, in some instances on several occasions. The other 19 babies harboured pneumococci of Group IV. Of the 51 mothers examined 71% had pneumococci in their throats, of whom 36% carried Type 3, and the remainder had Group IV. Gundel and Schwarz concluded that the mothers had infected their babies because Type 3 was isolated from all babies whose mothers carried it.

Witkowski reported that she found pneumococci in the mouths of 46% of 50 babies up to four days old. Eleven of these (48%) harboured Type 3. Type 2 was encountered in

one, and the other babies had members of Group IV. Sixty-eight per cent of their mothers carried pneumococci in their throats, and 56% of these had Type 3.

The extraordinarily high figures obtained by these investigators may be partially explained by the cultural methods used. Gundel and Schwarz rinsed their swabs in 1% dextrose broth, which was incubated 6 to 10 hours and then inoculated into mice. It is now known that, as a single method, mouse inoculation yields the highest percentage of pneumococci, although the recovery is still not as high as when combined methods are used (Finland, 1942; Hedges and co-workers, 1946). Witkowski's swabs were rinsed in 1% dextrose broth also, but this was plated on blood agar after 24 hours incubation. This method, conceivably, may have given a higher recovery than if the swabs had been plated directly. Kneeland, as well as Torrey and Reese, inoculated their swabs directly onto blood agar plates. Torrey and Reese contend that direct plating gives the most accurate estimate of the actual

numbers of pneumococci present. According to their view, colonisation is indicated only by the presence of large numbers; small numbers may be regarded as transients. However, even if the argument of Torrey and Reese is accepted, the fact that Gundel and Schwarz repeatedly obtained Type 3 pneumococci from certain babies makes it unlikely that these were transients.

It is also possible that the infants observed by Gundel and Schwarz, and by Witkowski, were exposed to pneumococci in very high concentration. The report of Gundel and Schwarz contains some evidence of this, because many of the babies' mothers were found to acquire pneumococci during their stay in hospital. Several case histories cited indicate that respiratory infection of varying severity were common among both mothers and babies. It may be significant too, that Type 3 was prevalent in their study, as well as that of Witkowski. That Type 3 tends to become established on the healthy upper respiratory mucosa is suggested by its high incidence among the adult population (Webster and Hughes, 1931;

Smillie, 1933; Straker et al., 1939). However, in children the Type 3 carrier rates usually reported have been low (Neufeld and Etinger-Tulczynska, 1932; Blacklock and Guthrie, 1933; Andrews, 1937). Landsman (1953) found them infrequently among babies under one year old, and believed them to occur only in association with upper respiratory infection.

In a recent study on babies born at home, Landsman (1953) observed a pneumococcal carrier rate of 15.2% among 99 babies less than one week old; by their third week it had risen to 30%. This suggests that the newborn's apparent immunity is not complete, and may depend partly on the degree of contact with upper respiratory infection. The babies of Landsman's study were born into homes where "overcrowding and lack of care were manifest"; the pneumococcal carrier rate of their mothers was estimated at 36%. Grosse and MacKintosh (1953) have emphasised that babies born at home have a much greater risk of respiratory infection than those born in hospital. In an extensive clinical survey, they

found nasal discharge and respiratory infections more frequent among 1 to 10 day-old babies born at home than among those born in hospital.

The newborn's resistance to pneumococci has been suggested due to its transplacental acquisition of immune substances. Several pneumococcal antibodies have been demonstrated to be present in high titre in cord blood, but absent or low during the third to fifth weeks and in the second to fifteenth months of life (Burhans and Gerstenberger, 1924; Sutcliffe and Finland, 1932). However, the protective properties of these antibodies never have been satisfactorily demonstrated. It has also been proposed that the newborn's immature upper respiratory mucosa is nutritionally inadequate for the pneumococcus (Torrey and Reese, 1945), but there is no evidence to support this hypothesis.

Pneumococcal carrier rates among older infants have been variously reported. Robertson and Doyle (1942) found only 4% of 694 babies under six months old to have pneumococci in their nasal or throat cultures, while

Kneeland found 14% of 28 babies aged four to eight months to have them in their nasal cultures. The study of Landsman indicated that among babies living in crowded homes the per-nasal carrier rate may be as high as 35% to 40% during the first year of life. This is comparable to that of older children and adults among whom it is usually estimated between 25% and 60%. It would appear, therefore, that any existent immunity of newborn infants is of relatively short duration.

Pneumococci occur most frequently in the nasopharynx of infants, and less frequently in the throat. Adults, in contrast, sometimes are found to carry them only in the throat. This difference may be more apparent than real for, as pointed out by Mendes and Likar (1953), adequate swabbing of the nasopharynx via the nares is more difficult in adults than in children. Nasopharyngeal swabs obtained from adults by the West method have been reported to yield pneumococci as often as throat swabs, and more frequently than nasal swabs (Blackburn, et al., 1930; Straker et al., 1939).

There have been three pneumococcal carrier studies on infants in which the serological types were recorded. Neufeld and Etinger-Tulczynska (1932) examined 125 infants with colds, aged 1 to 20 months, and found Types 6 and 19 most frequent. Types 10, 14, 23 and 26 were next in frequency, while Types 11, 17, 18 and 20 were only occasionally encountered. Sutcliffe and Davies (1937) took weekly nasopharyngeal swabs from nine babies aged 6 to 13 months in a prison nursery. All had pneumococci most of the time during the fifteen months they were observed, 41.4% of which were Types 4, 6, 15, 17 or 19. Types 1, 2 and 3 were not found among these babies, nor among another group of 25 infants aged 5 to 25 months. Landsman (1953) obtained Types 6, 10, 11, 19 and 23 most commonly among the 99 babies she observed throughout their first year. Among older children (Nemir et al., 1936; Lindeberg, 1941; Yang, 1941) and adults (Straker et al., 1939; Mendez and Likar, 1953) Types 6, 19 and 23 are also among those frequently carried. From the available data there is no indication that the types carried by infants

and children differ greatly from those carried by adults. Paradoxically, Types 6 and 19 often carried by healthy babies, frequently cause infant pneumonia. On the other hand, Types 14 and 1, frequently responsible for pneumonia in babies, are rarely carried by healthy babies.

Cultures from healthy infants and children rarely contain more than one pneumococcus type. Neufeld and Ettinger-Tulczynska found two types present simultaneously in only two of the cultures obtained from thirty-five young babies swabbed two to five times. Hemir et al. (1936), observed that only 5% of 425 healthy children aged two weeks to thirteen years harboured more than one serological type in a single laryngeal swab. Multiple types were never found by Yang (1941) in cultures obtained from 38 two to eight year old children swabbed at intervals over a five month period.

It is not uncommon to find two or more serological types recurring in successive swabs taken on the same individual. Sutcliffe and Davies (1937) experienced this in eight of the nine babies on whom they obtained consecutive swabs, and Neufeld and Ettinger-Tulczynska found different types at different times among 14% of the 35 babies they examined. Landsman believes that babies who

become pneumococcal carriers during the neonatal period are more likely to retain a single type than those who become carriers later (personal communication). In Yang's group of 38 children aged two to eight years, different types appeared in 84% of the consecutive swabs. The frequency of this occurrence in adults has been variously reported by different investigators. Webster and Hughes (1931) rarely experienced it, but others (Bliss et al., 1934; Straker et al., 1939) have found it common.

It is well known that adults may remain pneumococcal carriers for very long periods of time. The few carrier studies on infants, mentioned above, suggest that this is true of this age group also. Some of the babies examined by Sutcliffe and Davies were carriers throughout the six months that they were observed.

SUMMARY

Usual failure to obtain pneumococci from babies born in hospital indicates the newborn upper respiratory tract to be resistant to their colonisation. This immunity seems not complete, for a 15% carrier rate has been reported among newborns delivered at home. It may be that the supposed immunity of the newborn is due partly to its relative isolation. The newborn in the hospital

nursery is not protected from upper respiratory infection entirely, but its dosage is probably often less than that of a baby born at home. It has been suggested, also, that the newborn is resistant because of passively acquired antibodies, or because its immature mucosa is nutritionally inadequate for pneumococcal growth. There is little evidence, however, for either hypothesis.

The newborn's apparent immunity seems of short duration, because carrier rates reported for older babies are only slightly lower than those of children and adults. Pneumococci most often become established in the infant nasopharynx, and sometimes in the throat. Like adults, babies may remain carriers for long periods of time. The types carried by healthy babies are similar to those commonly found in healthy adults. Types 6 and 19, frequently carried, may cause pneumonia in babies, while Types 1 and 14, likewise responsible for infant pneumonia, are rarely carried by healthy babies.

SECTION B. BETA HAEMOLYTIC STREPTOCOCCI

Torrey and Reese (1944, 1945) appear to have been among the first investigators to draw attention to the infrequent occurrence of beta haemolytic streptococci in the newborn upper respiratory tract. They did not find them among 150 premature infants up to 25 days of age, despite the fact that 5 of the adult contacts were throat carriers. Among 70 full-term infants up to eight days old, beta haemolytic streptococci were harboured by only one infant, although eight of the mothers were carriers. Previously, Kneeland (1930) had recorded them absent in 30 newborn infants during the first two weeks of life, and had commented on the relative resistance of newborns to these and other pathogens. Bloomfield (1922) had noted haemolytic streptococci as only transients in the newborn upper respiratory tract.

Contrary to these observations are the reports of Gundel and Schwarz (1932) and of Witkowski (1935). Gundel and Schwarz stated that "haemolytic streptococci" were present in the nose and mouth of 13% of 52 newborns by the third day, and in 63% by the 12th day. It has been

suggested by Torrey and Reese that these "haemolytic streptococci" may not have been of the beta variety. While it is true that no clear definition was given, the fact that "Mundstreptococci" were cited present in 90% of the babies, suggests that these "haemolytic streptococci" were not the usual commensals. However, these "haemolytic streptococci" were also claimed present in the cultures of 53% of the mothers. This unusually high incidence among adults free of infection makes their classification of these streptococci very uncertain, and it is difficult to accept the figures of these authors. Witkowski reported haemolytic streptococci in 8% of 50 infants 2 days old, and in 14% when they were 5 days old. It would appear that these were of the beta group, as members of the non-haemolytic and viridans groups were cited separately. The degree of haemolysis, however, was not noted. Similar haemolytic streptococci were present in the mouths of 14% of the mothers, but Witkowski was of the opinion that the babies derived them partly from the maternal vagina.

Cornelison et al. (1946) presented evidence that babies may acquire beta haemolytic streptococci from the vagina during delivery. Using sodium azide broth as a selective medium, they found beta haemolytic streptococci in the oronasal cultures of 50 babies examined immediately after birth. These seem to have been enterococci, because they were resistant to 60°C. They gave no reaction with Lancefield Group sera A, B or C; Group D serum was not available. It was not determined how long these streptococci persisted. Parker and Kennedy (1949), however, noted an instance where beta haemolytic streptococci apparently acquired by a baby during delivery became established. Nasal cultures on this infant persistently yielded Group B streptococci during its first eight days of life, and its mother was found to have profuse numbers of Group B in her vagina. As yet, there is no record of Group A streptococci being recovered from the upper respiratory tract of healthy newborn infants.

The newborn's supposed resistance to beta haemolytic streptococci has been suggested to be due to possession of maternal antibodies. It is true that antistreptolysin, antifibrinolysin and erythrogenic antitoxin are sometimes present at birth, although their actual protective power remains undemonstrated. It is known, however, that these are lost within the first few months of life. Antistreptolysin has been shown usually to remain at low titre during the first few years of age (Rantz et al., 1951; Laurell and Wallmark, 1953b). Erythrogenic antitoxin likewise is soon lost, and erythrogenic toxin sensitivity does not reappear during the first year, although this is probably because the infant's highly vascularised skin does not "fix" the toxin (Cruickshank, 1954). Babies under three months of age have been reported to have high titres of hyaluronidase-inhibiting substance, while those between 3 to 12 months have low titres (Friou, 1949).

Relatively few observations have been made on the incidence of beta haemolytic streptococci in the upper

respiratory tract of healthy older babies. Kneeland (1930) reported haemolytic streptococci present in the throats of 11% of 28 healthy babies four to eight months of age, although they were absent in the nasal cultures. They were not found in 35 babies with colds, aged 3 to 7 months. In a recent survey, Holmes and Williams (1954) found that the Group A carrier rate in babies and young children was related to their degree of community life. The carrier rate of those living at home was considerably lower than that among those attending day nurseries (Table 9). Other reports giving some index of the prevalence of beta haemolytic streptococci in babies are those citing the incidence among infants admitted to hospital for illness other than upper respiratory infection (Table 10). The data of these several reports indicate that beta haemolytic streptococci are not infrequently acquired at a fairly early age. As is to be expected, somewhat higher frequencies have been noted among infants and young children with upper respiratory infection (Kneeland and Dawes, 1932; Brooks et al., 1942).

Table 9. Nose And Throat Carrier Rates In Young Children At Home And In Nurseries (Holmes and Williams, 1954)

<u>Age (Yrs.)</u>	<u>Percentage Carrier Rates in Children of</u>			<u>"Community" Group</u>		
	<u>"Home" Group</u>					
	<u>No. of</u> <u>Children</u>	<u>Nose</u>	<u>Throat</u>	<u>No. of</u> <u>Children</u>	<u>Nose</u>	<u>Throat</u>
Under 1	166	0.6	1.8	19	10.5	10.5
1 and 2	62	1.6	4.8	122	6.6	23.0
3 and 4	35	0	9.1	201	14.4	33.8

Table 10. Incidence Of Beta Haemolytic Streptococci
Among Babies Admitted To Hospital With
Non-Upper Respiratory Illness

	<u>No. of Babies</u>	<u>Age</u>	<u>Site</u>	<u>Incidence</u>	<u>Serologic Group</u>
Long, McKhann, & Cheney (1939)	322	< 2 yrs.	NP	9%	--
del Mundo & McKhann (1941)	469	< 2 yrs.	N, T	6%	--
Robertson & Doyle (1942)	394	< 6 mos.	T	< 11%*	A

* approximate incidence inferred from data cited

NP = Nasopharynx

N = Nose

T = Throat

Rantz et al. (1952) who investigated 204 upper respiratory infections occurring in babies under one year, found that 5.9% were apparently caused by Group A streptococci, and 3.4% by groups other than A. Among 92 babies observed at intervals during their first fifteen months of life, 30.5% had definite evidence of streptococcal respiratory infection at some time.

The throat carrier rate for haemolytic streptococci is generally said to be higher among school children than among older persons. Holmes and Williams (1954) found it to be higher among pre-school children, aged 3 to 4, than among older children. They believed this due to the greater number of tonsillectomies among the older age group.

In adults, the haemolytic streptococcal carrier rate has been variously estimated. Straker et al (1939) found it to range between 0 and 20% in different groups observed in London and South-East England. From surveys in different parts of the world, Hare (1940) estimated the average Group A carrier rate in healthy adults to be

7%, but Pike and Pashena (1946) from later literature estimated it to be two or three times higher.

Streptococcal infections in infants are distinctly different in character from those of older persons. In infants under two or three years, streptococcal infection tends to be a low grade process, frequently accompanied by suppurative complications. Streptococcal osteomyelitis is a disease of infancy almost entirely, while rheumatic fever and nephritis rarely occur in infancy (Dingle, 1954). It is presumed that these differences are in some way related to the antibody responses of infancy, which are quite different from those of adults. Seventy to eighty per cent of adults have significantly higher anti-streptolysin O titres following infection, but in infants the increase in titre is usually negligible and not sustained (McCarty, 1954).

SUMMARY

The evidence as to whether beta haemolytic streptococci can colonise the upper respiratory tract of the healthy newborn baby is somewhat equivocal. Some investigators have reported them to be absent, but others have stated them to be present in a significant proportion of newborn babies. Unfortunately, in the early reports citing them present the organisms are inadequately described. More recent reports suggest that newborns may

acquire beta haemolytic streptococci, other than Group A, from the vagina during delivery. Establishment of Group A streptococci in the newborn infant's upper respiratory tract has not yet been described.

It appears that beta haemolytic streptococci may be acquired rather early in infancy for they have been recorded present in 11% of a small group of healthy babies four to eight months old. There is no evidence that the incidence among healthy older babies and young children differs from adults. The streptococcal diseases of infancy are of different character from those of adults, however. This is believed to be related to the fact that streptococcal antibodies present at birth are soon lost, and are not rapidly reformed during early childhood.

SECTION C. HAEMOPHILUS INFLUENZAE

It was suggested by Torrey and Reese (1944, 1945) that the haemophilic bacteria are incapable of growth in the newborn upper respiratory tract. This belief was based on their failure to find them in the throat and nasopharynx of 150 premature babies up to 25 days old, and 70 full-term babies up to 8 days old. Haemophilus influenzae was found in the throat of three mothers and hospital staff contacts of the premature babies, and in 10% of the full-term babies mothers. Haemophilus haemolyticus frequently was present in the adults of the first study, and occurred in 20% of the mothers of the second. Previously, Kneeland (1930) also had noted H. influenzae (*B. pfeifferi*) and H. haemolyticus ("Bacillus X") to be absent in the noses and throats of 30 newborns up to two weeks old. However, Gundel and Schwarz (1932) reported H. influenzae ("Influenzabacillen") present in the mouths of 18% of 52 babies by their 12th day of life. They also found them in 43% of the mothers. Unfortunately, they did not describe the organism, and the method of isolation is not entirely clear. Primary

cultivation was in dextrose broth, later subcultured on a variety of media not named. It therefore is doubtful whether their organism was truly haemophilic. The other investigators used blood agar plates.

The only observations on the incidence of H. influenzae during the early months of life are those of Kneeland. He found "B. Pfeifferi" in the nose of 7% and in the throat of 53% of 28 babies four to eight months of age. Among infants with colds, they were somewhat more frequent, and he concluded, perhaps on insufficient data, that "B. Pfeifferi" probably plays a secondary role in infants' colds.

Observations on the incidence of H. influenzae in the upper respiratory tract of young children have been few, and attention has been directed mainly to those being admitted to hospitals, or living in institutions where upper respiratory infection was high. In the figures which have been reported (Table 11) the incidence varies from less than 10% to 80%. The highest figures were obtained by investigators using media permitting maximum development of the colonies, such as Fildes' agar (Straker et al.) or Bordet-Gengou agar with penicillin (Dawson and Zinnemann). The majority of H. influenzae recovered from

Table 11. Incidence of H. influenzae in the Upper Respiratory
Tract of Young Children

<u>Investigator</u>	<u>Number of Children</u>	<u>Age</u>	<u>Incidence</u>	<u>Site</u>
Knoland (1930)	50 in an institution	< 3 years	10 to 50% (seasonal variation)	Nose and throat
Straker (1939, 1945)	50 in an orphan home	2-11 "	{ 54% (8% "smooth" strains) 78% (10% " " ")	Nose Nasopharynx
Yang (1941)	40 in a day nursery	2-8 "	26%	Nose
del Mundo and McKernan (1941)	469 hospital admissions, with and without upper respiratory infection	< 2 "	8%	Nose and throat
Brooks (1942)	293 hospital admissions, with upper respiratory infection and other illnesses	< 2 "	9.8%	Nasopharynx
Alexander (1943)	847 hospital admissions, with or without infection	Children (age not stated)	25% non-encapsulated 5% encapsulated	Nasopharynx
Dawson and Zinnemann (1952)	650, whooping cough suspects, or contacts	{ 0-4 years < 1 "	82% non-encapsulated 4.7% encapsulated 6.7% encapsulated	Nasopharynx
Hendes and Likar	72 children 79 "	< 5 " > 5 "	3% encapsulated	Nose and throat

healthy children are non-encapsulated strains. Capsulated strains occur infrequently (3% to 7%) and only a small proportion of these are of serological Type b which causes influenzal meningitis in children (Alexander, 1943; Dawson and Zinnemann, 1952; Mendez and Likar, 1953). Studies on the familial spread of Type b have suggested that healthy children become carriers only through direct contact with meningial infection (Good, et al., 1943; Silverthorne and Paterson, 1943).

In healthy adults the incidence of H. influenzae in the upper respiratory tract usually is estimated between 30-80%, although it may be demonstrated in all if penicillin is added to the medium (Fleming and Maclean, 1930). Smooth, capsulated strains occur in only a small percent of adults, and Type b carriers have never been found even among adults in contact with children having Type b meningitis.

It is well known that influenzal meningitis is a disease of infancy, entirely. Few cases occur among infants under two months. The incidence increases to a maximum between the sixth to ninth months, and then gradually falls to a low level between the second and third years (Fothergill and Wright, 1933). It has been suggested that this age-morbidity relationship parallels

the absence of immune substances. Fothergill and Wright demonstrated that cord blood, and the blood of babies less than two months old, had considerable bactericidal power for a virulent strain of H. influenzae. The blood of children between two and three years old had almost none, but after the third year the bactericidal power increased until it reached a high level in adulthood. Whether the absence of non-encapsulated strains of haemophili in the upper respiratory tract of newborn infants may be related to their possession of immune substances acquired from the mother, is not known.

SUMMARY

H. influenzae seems not to occur in the newborn upper respiratory tract. Although one report claimed it to be present, the organism was inadequately described, and may not have been haemophilic. In the single study on its occurrence in later infancy, it was reported present in the throats of 53%. Various studies on older children indicate it to be fairly common. Smooth encapsulated strains, including serological Type b, have been found infrequently in healthy children.

CHAPTER 4

FACTORS CONTRIBUTING TO DIFFERENCES
BETWEEN THE FLORA OF THE INFANT AND
THAT OF THE ADULT

In the preceding chapters it has been shown that the upper respiratory tract of the very young infant differs somewhat from that of the adult both with respect to its colonisation by certain commensals, and in its resistance to certain pathogens. These differences probably are due to a complex mosaic of factors, difficult to investigate. A few which may be contributory, are considered briefly here, eg., relative isolation, anatomical differences, and newborn immunity.

ISOLATION OF INFANCY

It is often postulated that the apparent immunity of the young infant is due partly to its relative isolation. Undoubtedly the infant's quantitative exposure to many infections is less than that of older children and adults. It is well known that many infectious diseases become most frequent at school age, and are also more common among institutionalised infants and children than among those living at home. The degree of protection afforded by the infant's home environment is, of course, dependent on the hygienic conditions there,

and on the number of school-age siblings in the family. Because infection risk in the home environment is determined by a multitude of factors, it is impossible to assign any absolute value to the degree of protection the home affords.

Although "home isolation" may reduce the infant's opportunity to acquire infections directly from the general population, at the same time the intimacy of the home increases its contact with family infections. Yet it is well known that young infants often escape the infectious diseases of other family members. Moreover, the young infant does not always share the common commensal flora of its adult contacts. As mentioned in the previous chapters, the infant frequently does not acquire the pneumococcal type of its mother, nor does it necessarily carry her phage type of Staphylococcus aureus. Obviously, infant resistance does not depend entirely upon exposure, and must be controlled partly by its inherent characteristics.

ANATOMICAL DIFFERENCES

It has been stated, aptly, that "the human infant

must not be regarded as a miniature man" (Cruickshank, 1946), for its physiological and anatomical systems are not yet comparable with those of the adult. The anatomy of the infant upper respiratory tract differs from that of the adult in several important respects. The infant mucosa, like the skin, is thinner than the adult's, and is more vascularised. According to Peter et al. (1938), the upper respiratory epithelium of the newborn is only three to four cell layers thick, and lacks the basal membrane and lymphoid layer which do not begin to form until the fourth month. It has been suggested that the anatomical configuration of the infant nose favours infection (Pfaundler and Schloemann, 1935). Narrowness of the nasal entrance, which is more turned up than in older children, promotes obstruction by crusts. Moreover, the infant choanae are extremely small and easily closed by even a moderate degree of swelling. It may be that anatomical peculiarities explain why organisms such as Staphylococcus aureus, pneumococci, and C. diphtheriae are apt to become established in the infant's nose particularly.

The infant nasal sinuses are usually stated to be rudimentary, although some authors feel that they are

more developed than is generally recognised (Marantz, 1931; Pfaundler and Schlossmann, 1935). Most texts agree that the ethmoidal sinus is present at birth, though small, but disagree on the extent to which the maxillary, sphenoid, and frontal sinuses are developed. Adenoid tissue is said to be present at birth, but usually remains small until childhood.

The young infant's oral cavity differs from that of the adult in several ways. Most obvious of these is the absence of teeth which probably favour bacterial multiplication by the accumulation of food debris. Dentition probably also increases certain components of the oral flora such as the ill-defined filamentous organisms abundant on enamel surfaces, as well as fusiforms and spirochaetes. However, as previously mentioned, the changes attendant with dentition have never been investigated adequately. Saliva in the newborn is scanty due to the poor development of the salivary glands, but in older infants salivary secretion may become extremely copious. Whether the infant saliva differs in character from that of the adult seems not to have been studied. The palatine tonsils are present at birth, but because they rarely protrude beyond the pillars their

importance as a site for bacterial multiplication perhaps is underestimated. In fact, the crypt system in infant tonsils may be considerably developed (Arey, 1946).

NEWBORN IMMUNITY

The newborn's resistance to infection is often attributed to passive immunisation across the placenta. It is true that some maternal antibodies may be demonstrated present in cord blood and during the early weeks of life; in later weeks they gradually disappear. These include such antibodies as antistaphylococcal, antistreptococcal, pertussis agglutinin, diphtheria antitoxin, pneumococcal substances, etc. Yet, with the exception of diphtheria antitoxin, the protective properties of these antibodies remain undemonstrated. Their contribution to newborn immunity, therefore, is difficult to comprehend.

Susceptibility of the young infant to infection may be associated with underdevelopment of its reticulo-endothelial system, and inadequacy of its antibody-producing mechanism. This has not been investigated adequately, and the available literature is conflicting

(Parish, 1949). Experimental work with animal embryos has shown them to be remarkably unresponsive to foreign proteins, and it is thought that this principle operates in the human foetus and newborn as well. However, it is also known that infant antibody response may be underestimated because it is "blanketed" by antibody passively acquired from the mother. In the absence of maternal antibody, good antibody response may be obtained. It is also thought that young infants sometimes may appear to give a poor antibody response because, unlike older individuals, they have not had opportunity for previous sensitisation. The subject of newborn immunity is a fascinating one with wide opportunity for research work important to the epidemiology of childhood infections.

PART II

OBSERVATIONS ON HOSPITAL-BORN INFANTS
FROM BIRTH TO ONE YEAR

The literature contains numerous observations on the upper respiratory flora of the newborn, but little attention has been given the later months of infancy. No doubt this is due to the greater accessibility of the newly born infant in hospital. The Child Welfare Centres of Great Britain, however, afford an excellent opportunity for observing older infants. Moreover, they make possible repeated examination of the same baby throughout its first 6 to 12 months of life. Only with such sequential cultures is the constancy of the individual flora revealed.

GENERAL PROCEDURE

The newborn infants observed were mainly those born in Paddington General Hospital (London, W.2.) between December 1952 and June 1953. A small number of babies born at St. Mary's Hospital (London, W.2.) were included to supplement some of the observations. For the purpose of obtaining sequential cultures, attention was focused on those likely to visit the two Child Welfare Centres where arrangements for collecting the "follow-up" swabs had been made.

As a rule, two to three swabs were obtained from each infant between the third day of life and the time of hospital discharge, usually about the tenth day. Thereafter, swabs from these same infants were obtained on each visit they made to their Welfare Centre. They usually began visiting the Centres by about one month of age, and then came weekly for the first several months. Toward the end of the six months they often were reporting only once a fortnight, and toward the end of the year only once a month. Many of them discontinued regular visits after the first six months, so that the number of observations which could be made in the second six months of life was somewhat small. The examinations at the Centres were continued until December 1953 when all the babies still reporting were at least six months old, and some were nearly a year old.

Nose and throat swabs were taken at each observation. In order to recover pneumococci, the nose swabs were always taken by the pernasal method. A very fine cotton wool swab, wound on flexible wire, was inserted back as far as possible into the post-nasal space. Ordinary swabs were used for the throat where, with the aid of a

sterile tongue depressor, an effort was made to sample the entire faucial area.

Both in the hospital, and at the Centres, the swabs were immediately plated onto first, a blood agar plate, and second, onto a blood agar plate containing gentian violet 1:500,000. These plates were returned to the laboratory within a few hours time. The nose swab was streaked on one-third of each plate, and the throat swab on the other two-thirds.

All swabs obtained from the newborn infants were taken by the author. In each case, the hospitalised mother was personally interviewed to gain her consent, and her co-operation in bringing the baby to the Welfare Centre for continued swabbing was solicited. Although these interviews were time consuming, they were considered essential because the success of the carrier studies depended upon regular visits to the Centre. Also, it was deemed important to reassure the mother on the harmlessness of the swabbing procedure, to allay fears which might jeopardise her favourable relations with the Centre. Thus, Centre co-operation upon which the success of the project likewise depended, was insured.

The maternity unit of Paddington Hospital consists

of three wards, each with its adjacent nursery. The nurseries are all of similar design accommodating 26 to 29 bassinets spaced about $2\frac{1}{2}$ feet apart. Mothers are not permitted to enter the nursery, and at feeding time the babies are taken to their mothers who nurse them unmasked. Although the sisters are permanently assigned to each ward, the rest of the nursing staff and the domestic staff are often transferred from one nursery to another. During the six months of observation, no outbreak of infectious disease occurred among the Paddington newborns, apart from a mild one of thrush in Ward 10 in March, 1953 (see Part IV, D.). As no isolation unit was available, babies having mild infections of any kind were removed from the nursery into the mothers' ward. "Sticky eyes", which were quite common, were often treated in the nursery.

The nursery at St. Mary's Hospital rarely houses more than twelve babies, and is less crowded than those at Paddington. Mothers enter it, masked, to collect their babies for feeding. No outbreak of infectious disease occurred during the limited number of observations made there, mainly in February 1953.

It was regrettable that of the 130 newborns swabbed in hospital, only one third later reported to the Welfare Centre according to their mother's expressed intention. The waste of labour and materials on the newborns was therefore very great. However, encouragement was found in that mothers who once reported to the Centre usually continued to do so with faithful regularity, and only two eventually decided against having the swabbings continued.

The "follow-up" swabs were obtained at two Welfare Centres: one at Marylebone Town Hall (London, W.1.), and the other at Lisson Grove (London, N.W.8.). At each Centre, all swabs were taken by a single person; the district health visitor. These two women were instructed in the swabbing procedure at the beginning of the survey, and supplied with a small electric torch to aid in taking the throat swabs. Both Centres were kept adequately supplied with sterile swabs, sterile tongue depressors, and fresh blood agar plates.

The plates were examined first after overnight incubation, and once again after standing for two to three days on the laboratory bench. The latter was

found useful for detecting staphylococcal and coliforms, particularly when present in small numbers, and for recognition of diphtheroids and Neisseria, the colonies of which were sometimes still very small after overnight incubation. Other procedures used for studying the various groups of organisms are described in the following sections.

SECTION A

STAPHYLOCOCCUS AUREUS

Previous studies have indicated that the newborn upper respiratory tract is highly susceptible to colonisation by Staphylococcus aureus. That of older infants appears uniquely insusceptible, for it has been found that by one year of age the nasal carrier rate is much lower than that of older children and adults. Whether the throat carrier rate declines as abruptly as the nasal carrier rate during early infancy is not known. It is also not known whether the newborn retains the staphylococci acquired from the hospital for any length of time, or whether these are soon replaced by those of the home environment. The present prevalence of penicillin-resistant strains in the hospital maternity ward and nursery makes the latter a question of considerable epidemiological importance. Answer to this question was therefore a major objective of this investigation, for it was believed that an answer could be obtained through application of the recently developed technique of staphylococcal phage typing. This technique also offered a method for determining whether the staphylococci of the throat are only transients, as sometimes suggested (Bloomfield, 1921b; Thomson and

Thomson, 1932), or whether they are permanent residents.

METHODS

The plates were examined for staphylococci after two to three days at room temperature following overnight incubation. This permitted full development of pigment, and facilitated easier recognition if only a few colonies were present in the confluent area of growth. The number of colonies was recorded as follows: +++ - more than 50, or predominant; ++ - 10 to 50; + - less than 10.

One golden pigmented colony was always picked from each nose culture, and one from each throat culture. Although it was realised that a single colony could not be a representative sample if more than one strain were present, previous workers' observations on both babies and adults had indicated that only a single phage type is usually encountered in the nose (Williams and Rippon, 1952; Rountree, personal communication). In the instances where more than one colonial type was present, one of each was picked. To avoid overlooking coagulase-producing strains of pale cream or white pigment, one

such "Staphylococcus albus" colony was also always picked from each culture.

Well isolated colonies were planted directly into chopped veal broth. If not well isolated, they were subcultured onto nutrient agar plates from which a single colony was similarly transplanted after pigmentation had fully developed at room temperature. The chopped veal broth cultures were incubated overnight and tested for coagulase production by Fisk's tube method, using rabbit plasma diluted 1:10 in normal saline. Results were read after two hours incubation in a 36° water bath. Coagulase negative cultures were recorded but discarded. Coagulase positive cultures were stored in the chopped veal broth until they could be phage typed. The latter cultures are hereafter referred to as Staphylococcus aureus. As it could not be predicted which of the newborns would later report to the Centre, several hundred cultures had to be collected from them which proved of no subsequent value, but this could not be avoided.

Phage typing was done by the author at the Colindale Public Health Laboratory, under the direction of Dr.

Joan E. Rippon and Dr. R.E.O. Williams, according to the Colindale method (Williams and Rippon, 1952). The phages used included: 3A, 3B, 3C, 6, 7, 29, 42D, 42E, 44, 47, 52, 52A, 53, 54, 55, 70, 73, 75, 77, 79.

Staphylococcal strains failing to lyse with the routine test dilution of these phages were then retested with them undiluted. At the same time, they were also tested with the following phage pools:

Pool A - 31B, 52B, 42F, 47A	} in routine test dilution
" B - 31, 44A, 69, 51, 71	
" C - 57, 58, 75A, 75B, 78	
" D - 42B, 42C, 47B, 47C, 76	
" X - Pool A and B, undiluted	
" Y - Pool C and D, undiluted	

Strains lysing with any pool were, of course, retested with the separate components.

Few of the cultures could be phage typed before at least several months of storage, because during the period that the newborn material was being collected time was insufficient. This was disadvantageous in that 1) the survey was well under way before the general trend of the results was known, and 2) many of the cultures became "ropy" with storage so that the rough, granular growth obtained on the typing plates made weak lytic reactions difficult to read. However, beginning the

phage typing toward the end of the survey did have the advantage that a larger sample of the cultures collected from any one baby could all be typed on the same day. Identical strains give more consistent typing results if typed on the same day, thereby simplifying the interpretations (Williams and Rippon, 1952).

Nearly all of the hospital cultures collected from the babies who reported to the Centres were phage typed. Next, about half the cultures collected from each baby while visiting the Centre were also phage typed. Finally, as many additional cultures were phage typed for each baby as seemed necessary to give a more nearly complete picture of its carrier characteristics. The total number of cultures phage typed was approximately 600.

Conventionally, the "phage type" of any particular Staphylococcus aureus strain is described by the phages which lyse it. More correctly, it is referred to as a "phage pattern" (Williams and Rippon, 1952). These patterns may be very complex when the strain is lysed by many phages. To simplify interpretation of the results obtained in this study, a system of coding these patterns was developed. The 21 distinct phage patterns

encountered were first divided into the three broad Groups I, II and III suggested by Williams, Rippon and Dowsett (1953). Each pattern within a group was then coded with a lower case letter, as follows: Ia, Ib, etc. Patterns which could not be assigned to any of the three groups, and which Williams and Rippon would call "unclassifiable", were assigned the upper case letter N and coded with a lower case letter subscript: e.g. Na, Nb, etc. As there is considerable gradation between the Group III and the unclassifiable phage patterns, the distinction between them, in some instances, was somewhat arbitrary (e.g. IIIe might perhaps have been regarded as an unclassifiable strain). A few strains lysed by several phages of both I and III, and thus possibly intermediately related, were designated NX. Strains not lysed by any phage, even undiluted, were recorded as "untypable", using the letters UT.

The complete code system adopted was as follows:

- Ia - 52A/79
- Ib - 52/52A
- Ic - 29/52
- Id - 29/52/52A/53/79

- II - 3C/55
- IIIa - 42D/42E/54/70
- IIIb - 7
- IIIc - 47/53/75/77
- IIId - 7/42D/70
- IIIe - 42C/47/57/75A
- IIIf - 42E/47/53/54/77
- IIIg - 7/47/53/54/73/75/77
- IIIh - 53/77/79 (usually undiluted phages only)
- IIIi - 42E/53/79

- Na - 31
- Nb - 44/52/52A/70 (undiluted phages only)
- Nc - 29/77 (undiluted phages only)
- Nd - 29/42D/44/54 (undiluted phages only)
- Ne - 7/29/53/54/77, sometimes plus 42C, 42E, 52,
52A or 79
- Nf - 47B/47C/57, sometimes plus 42B, 42E, 58 or 75
- NX - multiple reactions suggesting relationship
to both Groups I and III. e.g.,
29/42E/52/52A/53/54/79, or
29/42D/42E/52A/53/79
- UT - Untypable

Interpretation of the similarity between phage patterns of different strains was made according to the conventions suggested by Williams and Rippon (1952): strains differing in strong lysis by two or more phages are considered distinct. Slightly more variation was allowed, however, in the patterns within some III and N subgroups when it was found that the same strain if typed on different days gained or lost two or even three strong reactions. For example, a IIIa strain might give the following patterns if typed on different days:

42D/42E/54/70
 42E/54
 42E

However, two IIIa strains from the same baby, having a different number of IIIa reactions usually gave the identical pattern if typed on the same day and if they had not become "ropy" with storage. The slightly modified convention adopted for judging similarities within certain III and N subgroups therefore seems valid. However, the relation between strains within these subgroups cannot be considered as certain as the relation between strains within the I subgroups or in Group II.

It might be thought that allowing as many as three

strong differences would result in confusion between such subgroups as:

IIIa - 42D/42E/54/70
IIIf - 42E/47/53/54/77
IIIi - 42E/53/79

However, despite variations, the strains within these subgroups were always lysed by at least one phage which marked them as distinct from the others. Further, IIIi was nearly always penicillin sensitive, whereas IIIa and IIIf were usually penicillin resistant. Where several strains were carried by the same baby, they were usually of different Groups, so that confusion between them was unlikely (see discussion).

The cultures were tested for penicillin sensitivity at the time that they were phage typed. This was done by "spotting" one loop of the freshly transferred overnight broth culture onto Levinthal agar plates containing 0.25 μ /ml and 5.0 μ /ml penicillin, and one with no penicillin as a control (May and Morley, 1952). The penicillin activity was controlled by spotting a known sensitive strain onto all three plates. Strains partially or completely inhibited by 0.25 μ /ml penicillin were recorded as sensitive, while those not inhibited by this concentration were recorded as

resistant. The majority of resistant strains were also not inhibited by 5.0 μ /ml.

RESULTS

a) NEWBORN INFANTS -

Incidence of Staphylococcus aureus in the newborn:

The incidence of Staphylococcus aureus was observed among 106 newborn infants in the Paddington nurseries between December 1952 and June 1953. It was found that 99% of them left the hospital with Staphylococcus aureus which they had acquired there. Only two failed to acquire this organism in either the nose or throat before their discharge from hospital. In one of these, a single swabbing was obtained on the fifth day so had additional swabs been taken before discharge a few days later they might have been positive.

Nearly all babies had Staphylococcus aureus in both their nose and throat by the time they left hospital. One baby had a positive throat culture only, on the sixth day, but just one swabbing was obtained from it. Five babies had positive nose cultures only, but single swabbings were obtained from three of these, and the throat cultures of the other two were overgrown with a

spreading Proteus species. It was noted that nasal cultures were usually positive as early as the third day, and once they had become so they remained positive throughout the period of hospitalisation. The throat cultures often became positive later than the nose cultures, and subsequently were not always regularly positive.

Nose cultures invariably contained very large numbers of Staphylococcus aureus. The throat cultures were more apt to contain numbers moderate to few, but large numbers were not uncommon.

During August 1953, incidental to a study of Streptococcus salivarius, the prevalence of Staphylococcus aureus was again assessed among the newborns in the Paddington nurseries. All of the 23 babies examined had Staphylococcus aureus, usually in both their nose and throat, before discharge. The incidence in the autumn therefore appeared still as high as during the first half of the year.

Multiple phage types of the individual newborn:

When phage typing was begun it was found that, contrary to expectation, the hospital cultures of many of

the newborn babies contained more than one distinct strain. For example, the nasal cultures obtained from one baby might contain type Ia on the third day of life, IIIa on the seventh day, and Ia again on the tenth day. This implied either that these babies were carrying two or more strains simultaneously, or that their staphylococcus population changed from day to day. To determine which was the case, five to nine colonies from two or three successive nasal cultures taken on five babies were phage typed. The results were as follows:

Baby 121 - 7th day - 3 colonies: Ia; 2 colonies: Ic
 10th day - 3 colonies: Ia; 3 colonies: Ic

Baby 127 - 3rd day - 6 colonies: UT
 7th day - 2 colonies: UT; 3 colonies: Ib
 13th day - 5 colonies: Ib

Baby 129 - 5th day - 5 colonies: Ic
 8th day - 6 colonies: Ic

Baby 130 - 2nd day - 6 colonies: Ia
 5th day - 5 colonies: Ia; 4 colonies IIIh

Baby 132 - 3rd day - 5 colonies: UT
 6th day - 2 colonies: UT; 4 colonies Ia

It was apparent that a single culture could contain two strains in nearly equal ratio. This might obtain in another culture from the same baby, or one strain might then be present either alone or predominantly.

In this study it was also noted that colonial differences were not necessarily useful in distinguishing different phage types within any one culture. Colonies which seemed identical were often of different phage type. Conversely, colonies of distinctly different pigment were sometimes the same phage type. This was further confirmed later in typing 30 pairs of distinct colonies isolated from 30 cultures. Seventeen of them proved to be of the same phage type, and 13 of them were different.

It was now evident that to determine accurately whether a baby carried more than one strain, a very large number of colonies would need to be typed from each culture. This was impractical. On the other hand, the original plan of typing single colonies from sequential cultures on the same baby might give a general, if not exact, index of its strains. That this proved to be the case will be seen in Table 12 (cover pocket) listing all the phage types encountered among the 34 babies swabbed both in hospital and at the Centres. Typing colonies obtained from the throat as well as the nose gave more complete information than would have been obtained from the nose alone. All babies demonstrated some consistency

in the phage types obtained from them.

Phage types occurring in the newborn group:

The hospital staphylococci of the 34 newborns who later reported to the Centres were phage typed. They contained 14 distinctly different types. The incidence of the different types is shown in Table 13. Most of the strains had phage patterns belonging to Group I, III, or were untypable. Apart from the untypable strains, those of Ia and IIIa were by far the most common.

The incidence of the various phage types seemed about the same in all three wards, as would be expected, with the constant interchange of the hospital personnel between them. However, the number of babies examined per ward was small, since the group of 34 was divided in a ratio of 12:11:11 between wards 9, 10 and 11 respectively. There was no indication that any phage type was more frequent at any time during the six months in which these newborns were observed.

Penicillin sensitivity of the newborn's staphylococci:

Surprisingly few penicillin sensitive strains were encountered. These belonged to phage types Ia, Ic, II

Table 13. Incidence Of The Staphylococcal Phage Types Present Among 34 Newborn Babies

Phage type (Code)	Number of babies	Percentage of babies*	
		Type	Group
Ia	18	53	
Ib	3	9	74
Ic	4	12	
II	2	6	6
IIIa	9	27	
IIIb	1	3	
IIIc	1	3	
IIId	1	3	50
IIIe	2	6	
IIIf	1	3	
IIIh	1	3	
IIIi	1	3	
Non-classifiable:			
Nb	1	3	12
NX	3	9	
UT	16	47	47

* as many babies harboured more than one phage type, the total percentage exceeds 100.

or IIIi, of which there were also penicillin resistant strains. Penicillin sensitive untypable strains also occurred. The various penicillin sensitive strains were found among seven of the 34 newborns examined (20%). However, six of the seven also harboured, in addition, at least one penicillin resistant strain of another type (See Table 12 - pocket cover). The seventh baby (No. 87) was unusual in that it carried penicillin sensitive strains only. All other 27 babies harboured penicillin resistant strains only. When the six babies with both penicillin resistant and penicillin sensitive strains are added to these, it is apparent that 97% were leaving the hospital with penicillin resistant strains.

b) OLDER INFANTS -

Incidence of Staphylococcus aureus among babies seen at the Centres:

The babies observed at the Centres included the 34 who had been swabbed at birth and an additional three not swabbed until the third or fourth week of age. The incidence of Staphylococcus aureus among them, in relation to their age, is given in Tables 14 and 15 and

Table 14. Incidence of Staphylococcus aureus
In The Nasal Cultures

Age- Weeks	No. of Babies	Cultures Positive	%	Age - Weeks	No. of Babies	Cultures Positive	%
1/2	21	17	81	24	7	2	20
1	31	30	97	25	7	3	
1-1/2	20	20	100	26	6	0	
2	20	20	100	27	5	0	
3	19	18	95	28	5	1	7
4	28	25	90	29	3	0	
5	27	22	82	30	5	0	
6	26	20	78	31	2	0	
7	27	18	67	32	3	0	23
8	19	13	69	33	3	1	
9	25	16	64	34	4	2	
10	15	7	47	35	3	0	
11	17	13	77	36	2	0	13
12	16	12	75	37	2	0	
13	16	9	56	38	2	1	
14	17	8	47	39	2	0	
15	14	6	43	40	0	0	
16	16	3	19	41	2	0	
17	11	4	37	42	2	0	
18	8	3	38	43	3	0	
19	13	5	38	44	2	0	
20	12	5		45	0	0	
21	7	2		46	0	0	
22	10	1	24	47	0	0	
23	8	1		48	0	0	
				49	1	0	

(The results of two consecutive swabbings on the same baby are sometimes included in the incidence calculated for the four-week periods.)

Table 15. Incidence Of Staphylococcus aureus
In The Throat Cultures

Age-Weeks	No. of Babies	Cultures Positive	%	Age-Weeks	No. of Babies	Cultures Positive	%
1/2	21	9	43	24	7	2	60
1	31	26	84	25	7	7	
1-1/2	20	16	80	26	6	3	
2	20	17	85	27	5	3	
3	19	17	90	28	5	2	53
4	28	19	68	29	3	2	
5	27	21	78	30	5	4	
6	26	18	70	31	2	0	
7	27	18	67	32	3	2	78
8	19	14	74	33	3	2	
9	25	16	64	34	4	3	
10	15	10	67	35	3	3	
11	17	11	65	36	2	2	75
12	16	9	56	37	2	1	
13	16	8	50	38	2	1	
14	17	10	59	39	2	2	
15	14	11	79	40	0	0	43
16	16	9	56	41	2	1	
17	11	7	63	42	2	1	
18	8	4	50	43	3	1	
19	13	5	38	44	2	1	66
20	12	7	60	45	0	0	
21	7	4		46	0	0	
22	10	5		47	0	0	
23	8	6		48	0	0	
				49	1	1	

(The results of two consecutive swabbings on the same baby are sometimes included in the incidence calculated for the four-week periods.)

shown graphically in Figures 1 and 2.

The percentage of positive nose swabs began a steady decline between the second and third week until it had fallen to about 15% at the end of the year. The percentage of positive throat cultures declined more slowly, reaching about 55% by the end of the babies first year of age. After the second month, therefore, the incidence in the throat was higher than in the nose. Many babies whose nose cultures became negative continued to have positive throat cultures. The rising frequency of negative nose swabs in conjunction with positive throat swabs is seen in Figure 3.

Carrier characteristics of the older babies:

The frequency with which the older babies tended to harbour Staphylococcus aureus in their throats rather than in their noses was evident when their individual records were examined. Sixty percent of them eventually lost their nasal staphylococci while continuing to harbour them in the throat. As about half of the others were not observed for more than a few months, they too might have become throat harbourers had they been observed longer. The one baby whose throat swabs were negative

FIG. 1.

STAPHYLOCOCCUS AUREUS : NASAL AND THROAT CARRIER RATES IN 1ST MONTH OF AGE.

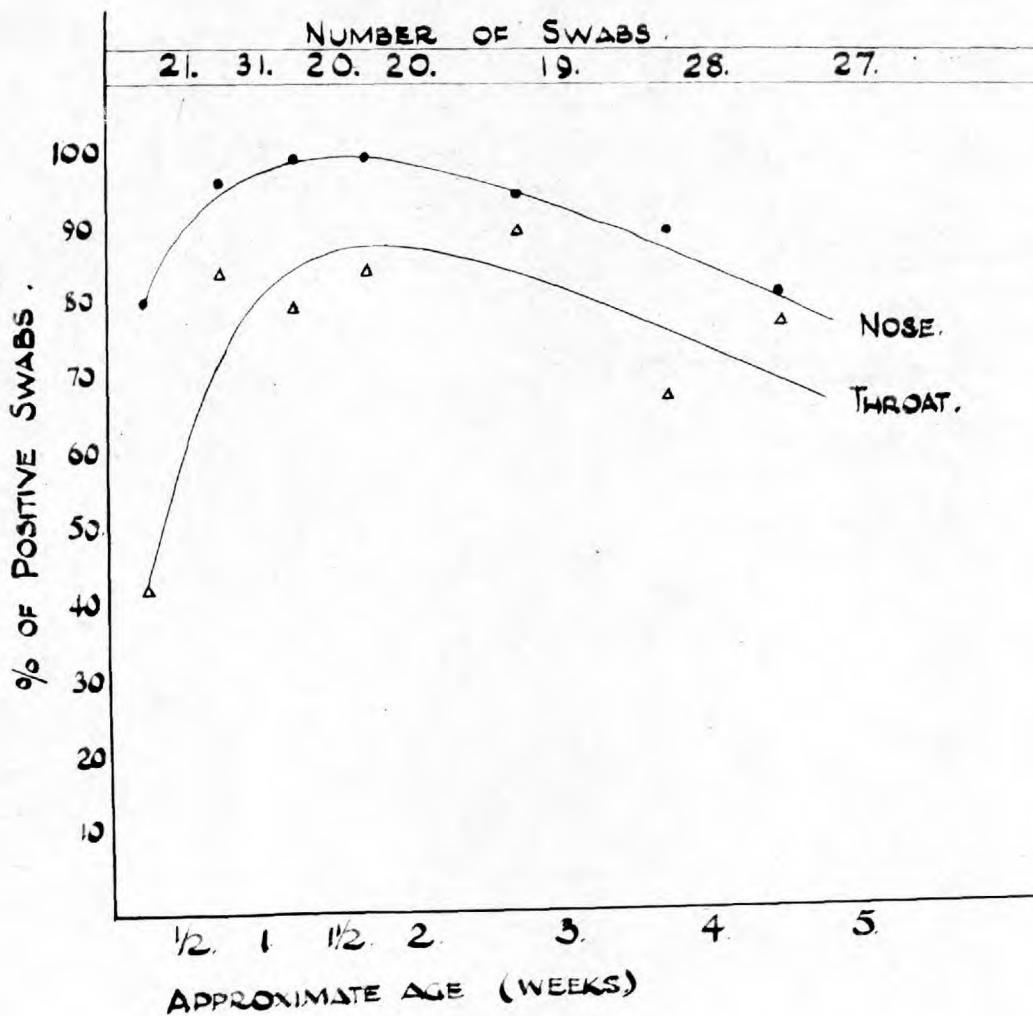


FIG. 2. STAPHYLOCOCCUS AUREUS :
NASAL AND THROAT CARRIER RATES FROM 1 TO 12 MONTHS OF AGE.

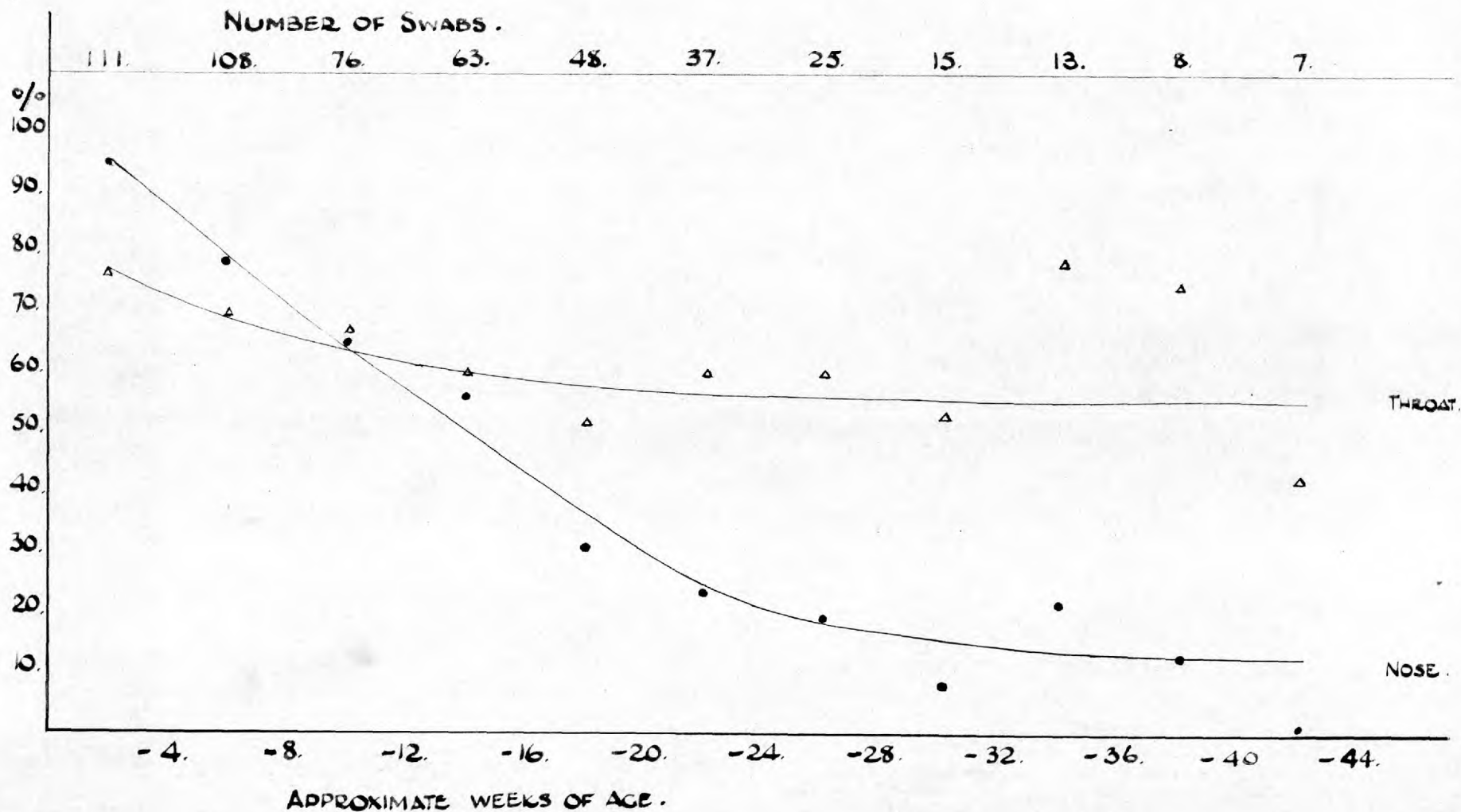
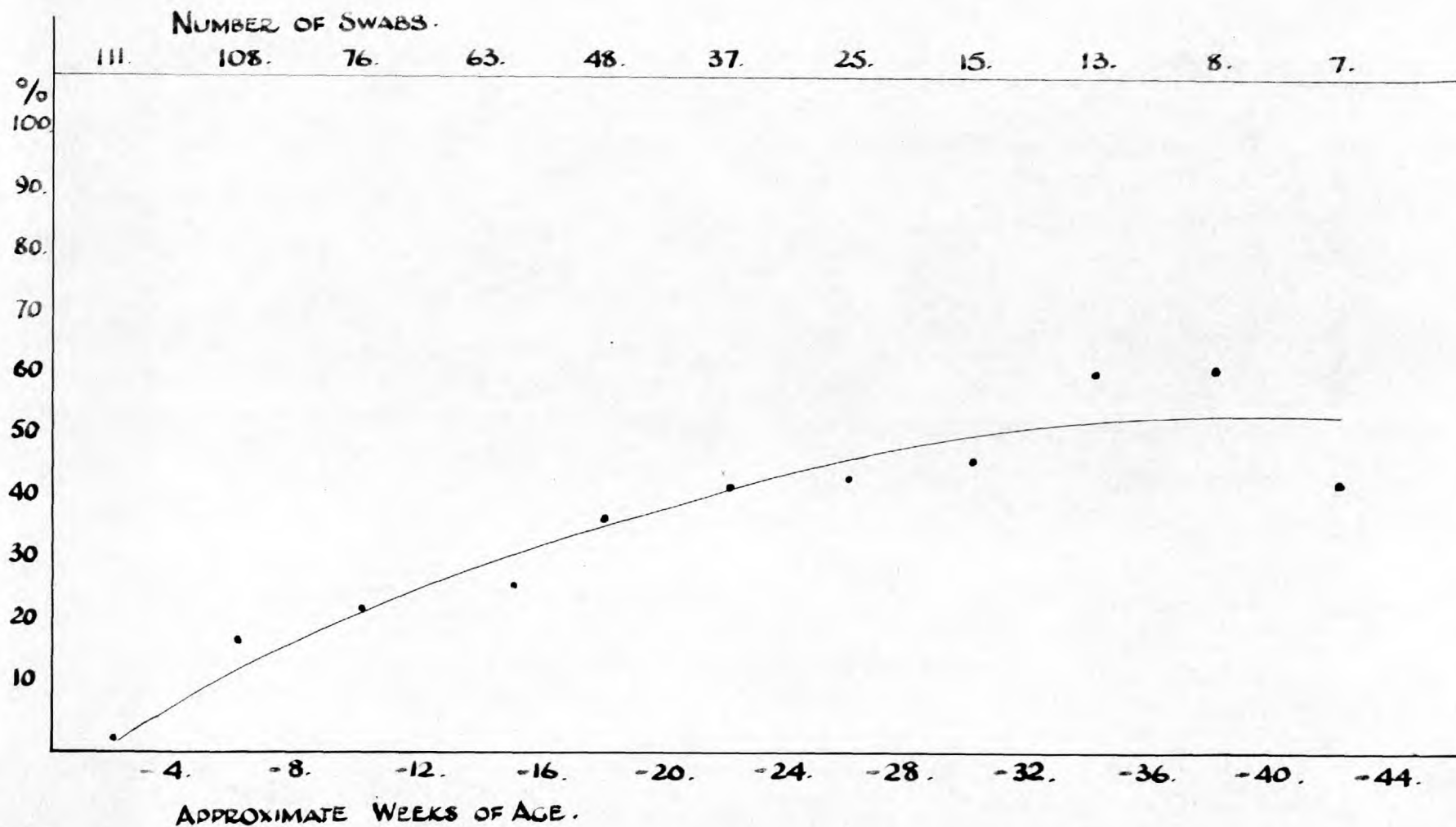


FIG.3. STAPHYLOCOCCUS AUREUS :
INCIDENCE OF NEGATIVE NOSE : POSITIVE THROAT CULTURE - PAIRS.



in hospital (No. 132) continued to have negative throat cultures during the twelve weeks it was observed. It was the sole example of a nasal carrier.

Loss of Staphylococcus aureus from the nose sometimes was quite abrupt, quantitatively, for a positive culture containing moderate or large numbers often preceded the series of negative cultures. After the nasal cultures became negative, the throat cultures usually continued to contain about the same numbers as they had previously. An occasional throat culture contained large numbers during the period that negative nose cultures were being obtained.

Phage typing clearly established that the babies harbouring Staphylococcus aureus maintained the same strains persistently (Table 12, cover pocket). It also demonstrated that the staphylococci in the babies' throats were not transients; their throats were actually colonised by the hospital strains. This was particularly evident when the hospital strains continued to appear consistently in the throat cultures long after the nose cultures had become negative. Hospital strains were carried longest, therefore, when they colonised the throat.

Each individual baby's staphylococcal population remained remarkably constant throughout its period of observation. The carrier period usually was of many weeks duration, as shown in Tables 12 and 16. Of 27 carriers whose strains were almost certain to have been of hospital origin, 24 carried at least one typable penicillin resistant strain. Ten of the 24 were observed to carry such strains for at least six months, and one was still a carrier when nearly a year old. An additional six babies (6, 8, 12, 19, 23, 125) whose hospital strains were untypable likewise continued to yield these for long periods - in three cases for over six months. Thus, there is little doubt that the majority of Staphylococcus aureus strains acquired in hospital were retained for several months or more.

The hospital strains did not invariably become permanently established, for an occasional one was never recovered again after hospital discharge. These included phage types such as Ia and IIa carried most commonly, as well as NX which was more seldom carried.

Sometimes phage types similar in pattern to the hospital strains were recovered for the first time from

Table 16

Length of Carriage of Known Hospital Strains

<u>Baby</u>	<u>Hospital Strain(s)</u>	<u>Carrier Period</u>	<u>Period Observed</u>
5	Ia	49 weeks	49 weeks
9	IIIa	20 "	22 "
	UT	5 "	
13	II*	14 "	15 "
	UT	13 "	
14	IIIa	9 "	9 "
16	IIIa	27 "	
	UT	41 "	44 "
25	IIIb*	17 "	32 "
29	Ia	23 "	42 "
	IIIa	39 "	
	UT*	21 "	
38	Ia	32 "	32 "
40	IIId	8 "	19 "
55	Ia	26 "	26 "
67	Ia	23 "	27 "
	IIIe	17 "	
	Nb	4 "	
78	Ia	24 "	34 "
	IIIc	13 "	
80	IIIa	34 "	34 "
81	Ia	25 "	34 "
	IIIa	34 "	
82	Ia	7 "	10 "
83	IIIf	30 "	30 "
84	Ic	9 "	24 "
87	IIIi*	26 "	30 "
89	Ia	38 "	38 "
	IIIe	16 "	

Table 16 (continued)

<u>Baby</u>	<u>Hospital Strain(s)</u>	<u>Carrier Period</u>	<u>Period Observed</u>
92	Ia	20 "	22 "
	IIIa	20 "	
93	Ia	24 "	33 "
	UT	33 "	
95	Ic	8 "	8 "
103	Ia	9 "	9 "
109	IIIh	19 "	28 "
118	Ib	4 "	25 "
131	Ib	25 "	25 "
132	Ia	12 "	20 "

* penicillin sensitive strains

(Babies who had only an untypable strain in their hospital cultures have not been included in this table; their records may be seen in Table 12).

a baby's Centre cultures (e.g., Babies No. 8, 12, 13). It is possible that these may have been present earlier but not revealed in the random sampling of a single colony per culture. Such an explanation seems particularly probable when they appeared in the early weeks of age, and were carried thereafter. However, strains similar in phage pattern to the hospital ones sometimes made their first appearance in the later months. It would be very difficult to determine whether they were identical to the hospital strains or not. Generally they did not become established, and it is conceivable that they could have been temporarily acquired from other babies attending the Centre on that day.

Five strains obtained from the babies' Centre cultures were never present among the hospital cultures and therefore probably were not hospital strains. These were: Id (Baby No. 14), IIIg (Baby No. 95), Na (Baby No. 23), Nc and Nd (Baby No. 72). Strains IIIg, Na and Nc seemed to become established in their hosts, as they were recovered in more than one swabbing. Strains Id and Nd appeared not to become established as they occurred only once.

"Transients" - strains never obtained more than once from an individual baby - were quite uncommon on the whole. While they were observed in nasal cultures of ten babies, they appeared in throat cultures of only two babies.

Individuality of the carrier state:

It was a striking fact that the babies often could be characterised by the staphylococcal strains they were carrying. There were seventeen distinct strains carried among them, and since 70% of the babies carried at least two strains, the chance of more than one baby having the same set was not great. When the different phage patterns were considered together with penicillin sensitivity, it was found that no more than two babies held the identical combination of strains. The 31 strain combinations which occurred among the 37 babies may be seen in Table 17. As a result, 25 (68%) of the individual babies could have been identified by the strains they carried; the remaining 12 babies could have been identified with 50% certainty.

Twins, despite their intimate contact, did not share the same types entirely. This was particularly true of pair No. 80 - 81. While one of them (No. 81)

Table 17. Strain Combinations of Staph. aureus Carried

One Strain:	Baby No.	Two Strains:	Baby No.
Ia	82, 103	Ia, UT	5, 93
<u>Ia</u>	132	Ia*, UT*	50
Ib	131	Ia, IIIa	55, 81
Ic	84	Ia, <u>IIIa</u>	38
IIIa	14, 80	Ia, IIIe	89
IIIf	83	Ia, Ne*	72
IIIh	109	II, UT	121
UT	19, 125	IIIa, UT	9, 16
		IIIi, UT	87
Three Strains:		Na, UT	23
Ia, Ib*, IIIa	92	UT, UT*	6
Ia*, Ib*, IIIi	40		
Ia, IIIe, <u>Nb</u>	67	Four to six strains:	
<u>Ia</u> , Ne, Nf*	75	Ia, IIIa, IIIe,	
Ia, Nf*, UT	76	NX	78
Ib, UT, UT*	118	Ia, Ib*, IIIa,	
Ic, IIIg, UT	95	NX, UT, UT*	29
II*, IIIa*, UT	13	Ic, IIIa,	
IIIa, UT, UT*	8	IIIb*, UT	13

* penicillin sensitive

— underscoring indicates lysis with undiluted phage only

carried phage type Ia and IIIa, the other (No. 80) carried only IIIa in all of its 23 positive cultures obtained during the 34 weeks of observation. The distinction between the strains carried by the other pair of twins observed was somewhat less clear. Nevertheless, Twin No. 75 tended to carry Ia, Ne and Nf, while his brother, No. 76, had mainly Ia, Nf and an untypable strain.

Relation between staphylococcal carriage and type of feeding:

It was conceivable that the babies who carried staphylococci in their throats only received them from their mother's milk supply which they might previously have infected. Analysis of the feeding records, however, indicated that this could not have explained most cases of throat carriage. (Table 18). Although three of the throat carriers received breast milk throughout most of their carrier period, the majority continued to be throat carriers long after they were fully weaned. There was no evidence that commencement of bottle feeding affected the carrier state in any way.

Table 18. Breast Feeding Of The Throat Carriers

<u>Baby</u>	<u>Age when weaned from breast</u>	<u>Observed throat carrier period*</u>	<u>Total period of observation</u>
No. 6	1 week	6 - 20 weeks	20 weeks
38	2 weeks	11 - 32 weeks	32 weeks
12	2 weeks	6 - 25 weeks	41 weeks
55	4 weeks	16 - 26 weeks	26 weeks
14	5 weeks	6 - 9 weeks	9 weeks
16	6 weeks	6 - 41 weeks	44 weeks
9	c.18 weeks	6 - 20 weeks	22 weeks
87	c.22 weeks	4 - 30 weeks	30 weeks
89	c.25 weeks	9 - 38 weeks	38 weeks
29	c.40 weeks	24 - 39 weeks	42 weeks
5	c.45 weeks	14 - 49 weeks	49 weeks

* Period during which the throat cultures remained positive after the nose cultures had become negative.

Anatomical distribution of strains carried by the same baby:

When it was found that most of the babies had carried several strains, it was thought that their different strains might be colonising different sites in the upper respiratory tract. Although it was known that the several strains of the same baby were found frequently in both its nose and throat, this might be due to the direct communication between these regions. However, since interchange between the right and left anterior nares is less likely, it was conceivable that separate swabs of these sites might yield different strains. Accordingly, right and left anterior nares swabs were obtained on three babies known to have previously carried several strains. Immediately after taking the anterior nares swabs, the usual pernasal swab was also taken as a control. Several colonies from all swabs were phage typed. In each baby the right and left anterior nares swab, as well as the pernasal swab, all yielded the same strain.

The results of this experiment do not preclude the possibility of selective colonisation, but indicate that it

is not readily demonstrable. Colonisation by any one strain could be limited to a very small area of the nasal mucosa which would not easily be sampled by the usual swabbing methods. However, it was felt that in the small babies it would be difficult to investigate adequately this interesting problem further.

DISCUSSION

Incidence Of Staphylococcus aureus In The Infant Upper Respiratory Tract

The very high incidence of Staphylococcus aureus found among the newborns' nasal cultures is in accordance with observations of previous investigators. Likewise, the somewhat less frequent occurrence in the throat agrees with other reports. The high frequency of penicillin resistant strains is in accord with the recent reports of Ludlam (1953) and Barber et al. (1953). As in the investigation of Barber, and co-workers, the most prevalent strains were of phage Group I, or were untypable. The Group III strains were more common than in their

experience, and the Group II strains less so. The non-classifiable strains encountered in the present study apparently were not found in theirs.

Mode of Staphylococcal Carriage in the Young Infant:

After the first several months of age the nasal staphylococci are often lost, while those of the throat persist much longer. In babies aged six to twelve months the throat carrier rate is similar to that of adults, while the nasal carrier rate is much lower. Babies are therefore unique with respect to their nasal carriage:—high in the early weeks, and low in the later months of their first year.

This apparent immunity of the nasal mucosa in late infancy cannot be correlated with any antibody mechanism understood at the present time, and has no known explanation. Anti-staphylolysin passed from mother to foetus is quickly lost after birth. While its formation begins near the end of the first year, not until the sixth year of age is the adult titre reached (Bryce and Burnet, 1932). Moreover, it seems a response which confers no protection against staphylococcal colonisation. In adults, high anti-staphylolysin titres are associated

with nasal and throat carriage (Packalén and Bergqvist, 1947; Bergqvist, 1950). For this reason, it has been suggested that the low anti-staphylolysin of late infancy results from the low nasal carrier rate (Cunliffe, 1949; Ludlam, 1953), but this hypothesis is incompatible with the present observation of a high throat carrier rate during this age.

It was clearly established, by phage typing, that the babies were persistent upper respiratory carriers yielding the same strains in sequential swabs. Until now, the infant carrier state has been largely a presumption based on the presence of Staphylococcus aureus in a single nasal swab per infant. Phage typing further demonstrated that the babies retained their hospital strains for very long periods of time, especially in the throat. With both babies and adults, there has been a tendency to ignore the occurrence of staphylococci in the throat, often on the assumption that they were probably transients there. In the present experience, transients were found to be extremely rare in the babies throats; possibly even more so than in the nose. Although the throat strains were often only intermittently recovered,

and in small numbers, there was no doubt of their persistence. The frequency with which negative throat cultures intervened between positive ones of any individual baby is not surprising if consideration is given the difficulty of obtaining adequate throat swabs in this age group.

This study demonstrated that the staphylococci which first become established in the newborn upper respiratory tract are not readily displaced by other strains. The finding may be analogous to Rountree and Barbour's observation (1951) that student nurses who are nasal carriers at the beginning of ward duty are least likely to acquire the penicillin resistant strains of the hospital. Although not understood, an ecological balance of the body flora probably does exist. There was some evidence in the present study that the babies occasionally did acquire new strains as they became older, but it happened too infrequently to draw conclusions about the circumstances.

Newborn infants in the hospital nursery are exposed to many strains of Staphylococcus aureus in large numbers. Therefore, it is perhaps not surprising that many babies

acquire several strains, with chance determining their particular set. Other investigators have noted recovery of more than one strain from newborns' nasal cultures (Allison and Hobbs, 1947; Parker and Kennedy, 1949), but the probability of their continuing to carry several strains has not before been considered. Perhaps their high susceptibility to staphylococcal colonisation is a factor in their maintenance of several strains. However, that babies differ from adults in this respect cannot be stated with certainty. The multiple strain carriers among the babies probably would not have been noted if frequent sequential swabs had not been examined. Such frequent sequential swabs have not often been taken on adults over long periods and it is possible that multiple strain carriers among them have been overlooked. When Rountree and Barbour (1951) phage typed up to ten colonies in each of 280 nasal cultures from nurse trainees, they found that 4.5% of the cultures contained two strains. They estimated from their data that the number of multiple carriers among the nurses would not exceed 10%. Nine of 38 student nurses yielded new strains after they began ward duty; whether the new strains were carried in

addition to the previous ones was not investigated Barber et al., (1953) studied 769 nasal swabs obtained from mothers, babies and hospital staff in a maternity ward. In 42 instances they found penicillin resistant and penicillin sensitive strains in the same swab. These were of different phage reactions in ten cases, and in twelve cases one strain was untypable. Such reports suggest that the hospital environment, heavily saturated with many strains of staphylococci, may induce multiple carriage in babies and adults alike.

Epidemiological significance of the infant upper respiratory carrier:

The finding that nearly every newborn baby carries at least one penicillin resistant strain of Staphylococcus aureus at hospital discharge is of considerable epidemiological importance. Clearly, the newborn may be a major source for dissemination of penicillin resistant strains among the general population. The potential hazard of their carrier state is increased by the facts that 1) they carry these penicillin resistant strains in profuse numbers, and 2) they may carry them in the nose for several months; in the throat for as long as a year.

It could be argued that the period in which the babies carry Staphylococcus aureus in their throats has little epidemiological significance, for it has been shown that certain organisms are more widely disseminated by nasal carriers than by throat carriers (Hamburger et al., 1945; Laurell, 1952c). However, young babies may distribute their saliva much more widely than do older children and adults. The possible significance of infant throat carriers, therefore, perhaps should not be minimised.

Recent studies indicate that not only may the hospital-born baby be a major vector for disseminating penicillin resistant strains in the general population, but that its mother may also carry them at the time of her hospital discharge (Ludlam, 1953; Barber et al., 1953). The alarming spread of penicillin resistant strains on the hospital maternity ward has led several investigators to suggest abolition of the communal nursery, and isolation of the baby with its mother (Allison and Hobbs, 1947; Parker and Kennedy, 1949; Barber et al., 1953). Unfortunately, this would require complete reconstruction of most maternity wards and their nurseries. It is possible that isolation of just

the babies, in individual units with filtered air and more strict aseptic nursing technique, might materially reduce not only their acquisition of staphylococci but, simultaneously, that of their mothers. Except for mothers in maternity wards, relatively few hospital patients acquire penicillin resistant strains during a week's stay (Barber and Whitehead, 1949; Barber et al., 1953). As there is now considerable evidence that the spread of Staphylococcus aureus in the hospital nursery is mainly from baby to baby, and from baby to mother (Chapter 2), the infant vector seems most important to control.

Applicability of Phage Typing to Long Term Carrier Studies

Phage typing proved a definite asset to the present investigation, and gave information not otherwise obtainable. As yet, phage typing in connexion with sequential swabbings has not been widely used. The experience of this investigation indicates that phage typing may be advantageously applied to long term carrier studies even where several strains are carried simultaneously by the same host.

Williams Smith (1948) predicted that phage typing might be inapplicable to long term epidemiological investigations, because the phage pattern of a given

strain may become altered. It is now believed that:

- 1) Two strains of different phage type may become identical by acquiring resistance to the phages carried by ^{one} another.
- 2) A single strain may change its phage type by developing variants unable to absorb certain phages to which it was previously sensitive.

The progeny resulting from either kind of alteration remains constant in its new phage pattern.

Lowbury and Hood (1953) found that considerable variation in phage pattern results from acquired resistance. However, they drew attention to the fact that the ability to acquire resistance is somewhat dependent on the phage pattern. Most members of Group III (6/47) readily acquired resistance, while members of Group II (3A) seldom did. Some strains of Group I (29/52) acquired resistance, but others did not.

If acquired resistance did occur in the present study, the consequence was not great enough to interfere seriously with the overall interpretations. There may be two reasons for this:

- 1) Attention was focused on the carrier state of the individual babies only. Acquired cross-resistance, as a possibility, could only occur between the

relatively small number of strains per baby. The strains of any one baby soon may have reached equilibrium in acquiring resistance to one another's phages.

- 2) The objective was to determine whether an individual baby's strains persisted over a period of time. As the phage pattern of a strain altered by acquired resistance remains constant, the persistence of an apparent new strain still would be determinable. The significance of a baby carrying several strains was just as great as one carrying a single strain. Alterations in phage type due to acquired resistance seem to have occurred very infrequently in the present study. This may have been because:

- 1) Babies who have carried several strains usually carried ones belonging to different phage groups. According to present belief, strains of different phage groups are unlikely to acquire resistance to one another's phages.
- 2) Seventy-four per cent of the babies carried Group I strains which are believed to be relatively stable.
- 3) Although 50% of the babies carried a Group III strain, most likely to be unstable, 47% of them carried their Group III strain only in conjunction with members of other groups.

Acquired phage resistance may have accounted for some of the untypable strains encountered in the present study. This is particularly likely with strains of Group I and Group II having a relatively simple phage pattern. Lowbury and Hood (1953) have shown that strains characterised by lysis with a single phage become untypable on acquiring resistance.

It is conceivable that acquired resistance may have occurred among some of the non-classifiable strains encountered, particularly NX. The NX strains exhibited phage patterns intermediate between Group I and Group III. Should an NX strain acquire resistance it might then have the phage pattern of either a Group I or a Group III strain. For example, an NX strain lysed by typing phages 29/42D/42E/52/52A/54/70/79 might, by acquired resistance, develop a phage pattern 52A/79 (Group I) or a phage pattern 42D/42E/54/70 (Group III).

Strains having NX patterns were encountered once among each of three babies' hospital cultures. All three of these babies also carried Group I and Group III strains either of which could have been derived from the NX strain. Two additional babies yielded NX strains among their Centre cultures on more than one occasion, but not in their hospital cultures. They too carried

Group I and Group III strains. The fact that their NX strains did not appear until their later cultures raises the question whether they could have resulted from cross resistance between the Group I and III strains previously present. This seems unlikely for two reasons:

- 1) Group I and III are believed not to develop resistance to one another's phages.
- 2) In all observations reported to date, acquired resistance has resulted in the strain's phage pattern becoming more simple rather than more complex. It seems more probable that the NX strains were actually present in the earlier swabs from these two babies, but were missed in the random sampling of a single colony per culture. The cross-resistance studies necessary to determine the possible relationships between the NX, Group I and Group III strains were not undertaken because of the great expenditure of time required. Moreover, the NX strains seemed to have been of too infrequent occurrence to have seriously affected the main conclusions.

SUMMARY

Nose and throat cultures of 106 newborn babies were examined for staphylococci. Ninety-nine percent acquired coagulase-positive Staphylococcus aureus during their stay in the hospital nursery. Strains isolated from 33 of 34 babies (97%) proved penicillin resistant. Phage typing demonstrated that both the nose and throat of an individual baby often became colonised by several strains. Strains having phage patterns of Group I were most common, while Group III and untypable strains were also frequent.

Sequential cultures were obtained from 37 babies visiting the Child Welfare Centres, 34 of whom were observed from birth. As they became older, their nasal carrier rate declined steadily, reaching about 15% by the end of their first year. Their throat carrier rate decreased much less, and was still 55% at the end of the year. Examination of the individual babies' carrier records showed that 60% of them eventually lost their nasal staphylococci while continuing to harbour them in the throat. No evidence could be found that throat

carriage was related to breast feeding.

Phage typing showed that the babies sometimes retained their hospital staphylococci for six months or more, especially in the throat. Hospital-born babies, therefore, may be important disseminators of penicillin resistant strains.

The staphylococcal population of each baby remained remarkably constant throughout its carrier period. Acquisition of new strains seemed infrequent, and "transients" were rare. Many babies who had acquired several strains from the hospital nursery could be characterised by these particular strain combinations which they continued to carry. It appeared that the strains which chanced to become established first determine the individuality of the baby's staphylococcal flora.

PART II

SECTION B

COLIFORM BACILLI

Early in the survey it was noted that coliforms frequently were present in the throat cultures, and sometimes in the nose cultures, of the newborn infants. Although the nose cultures seldom contained more than a few colonies, moderate to heavy growth was often obtained in the throat cultures. Subsequently, this was always recorded, and a single colony from each culture was saved for further study. They varied from flat, greyish, to slightly raised, creamy colonies, but never were of the large mucoid type said to be typical of Friedländer's bacillus.

Coliforms among the newborn babies:

The incidence of coliforms in the nose and throat cultures of 128 newborn babies is shown in Table 19. As coliform contamination of the infant upper respiratory tract could easily occur in the nursery environment, a single positive swab may not necessarily have significance. On the other hand, two or more consecutively positive swabs suggest the possibility of a carrier state, as described by Laurell (Chapter 2). For this reason, babies whose swabs were positive only once have been considered separately in the Table.

Table 19. Incidence Of Coliforms In Newborn Infants

<u>Nursery</u>	<u>Number of Babies</u>	<u>Positive Swabs</u>		<u>Total</u>
		<u>One</u>	<u>Two or more</u>	
Paddington				
Feb. - May '53	67	21%	27%	48%
August '53	22	9%	5%	14%
*February '53	18	17%	33%	50%
St. Mary's				
*February '53	21	0	10%	10%

* Primary cultivation in broth plus 20% blood and gentian-violet 1:500,000. Otherwise, blood agar plates with or without an additional blood plate containing gentian-violet 1:500,000 were used.

The incidence of upper respiratory coliforms among the babies of the Paddington nurseries during the spring of 1953 was high, and comparable to figures reported by some of the very early workers (Chapter 2). In August it was lower, and in St. Mary's Hospital nursery the incidence was also low during the observations made there in the spring. Unfortunately, the amount of data is too small to permit statistical evaluation of these differences. That such differences may occur at different times in different nurseries, is suggested by the results obtained by the various workers already quoted in Chapter 2.

Accurate feeding records could not be obtained for many of these newborn infants. The majority of the 128 observed were breast-fed, and all were fed Hartmann's solution during the first day of life. Of 25 for whom the method of feeding was ascertained, 19 were breast fed, and six were bottle fed. Only seven of the breast fed babies yielded positive cultures, while five of the bottle fed group had coliforms. This indicates that bottle feeding may favour coliforms in the newborn infant's throat, as suggested by McFarlan and co-workers (1949), but the amount of data is insufficient for statistical

consideration.

In view of Laurell's observation (1952a) of a high incidence of coliforms among premature infants, it should be mentioned that all but three of the 128 newborns observed were full-term. All three of the premature babies harboured coliforms; two were bottle fed, and one was breast fed.

Coliforms obtained from the babies as they became older:

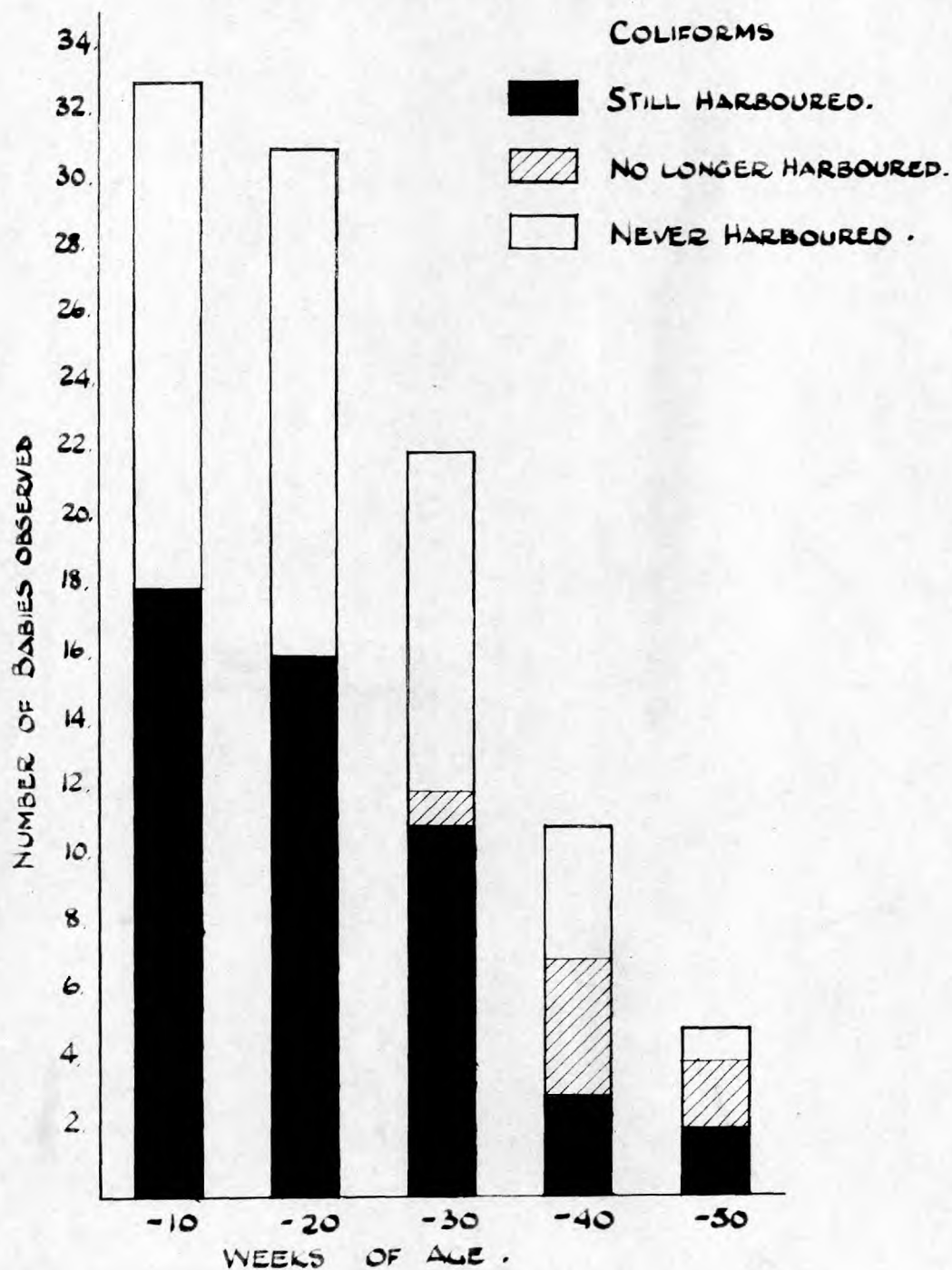
Thirty-three babies were observed for coliforms until they had reached 10 to 49 weeks of age. Of this group, 18 (55%) had at least one positive culture shortly after birth. Most of them continued to harbour coliforms for considerable periods of time. The term "harbourer" has been applied to them rather than "carrier", because no serological typing was done and a carrier state, in the strict sense, was not determined. Only those whose cultures were always, or nearly always, positive were considered to be harbourers. The cultures of the other babies were always negative or only positive on rare occasions.

The proportion of babies who continued to harbour coliforms is shown in Table 20, and graphically, in Figure 4. It was found that all babies who had harboured

Table 20. Persistence Of Coliforms Among The Older Babies

<u>Weeks of Age</u>	<u>No. Babies Observed</u>	<u>Coliforms</u>		
		<u>Still Harboured</u>	<u>No longer Harboured</u>	<u>Never Harboured</u>
0 - 10	33	18	0	15
10 - 20	31	16	0	15
20 - 30	23	11	1	11
30 - 40	11	3	4	4
40 - 50	5	2	2	1

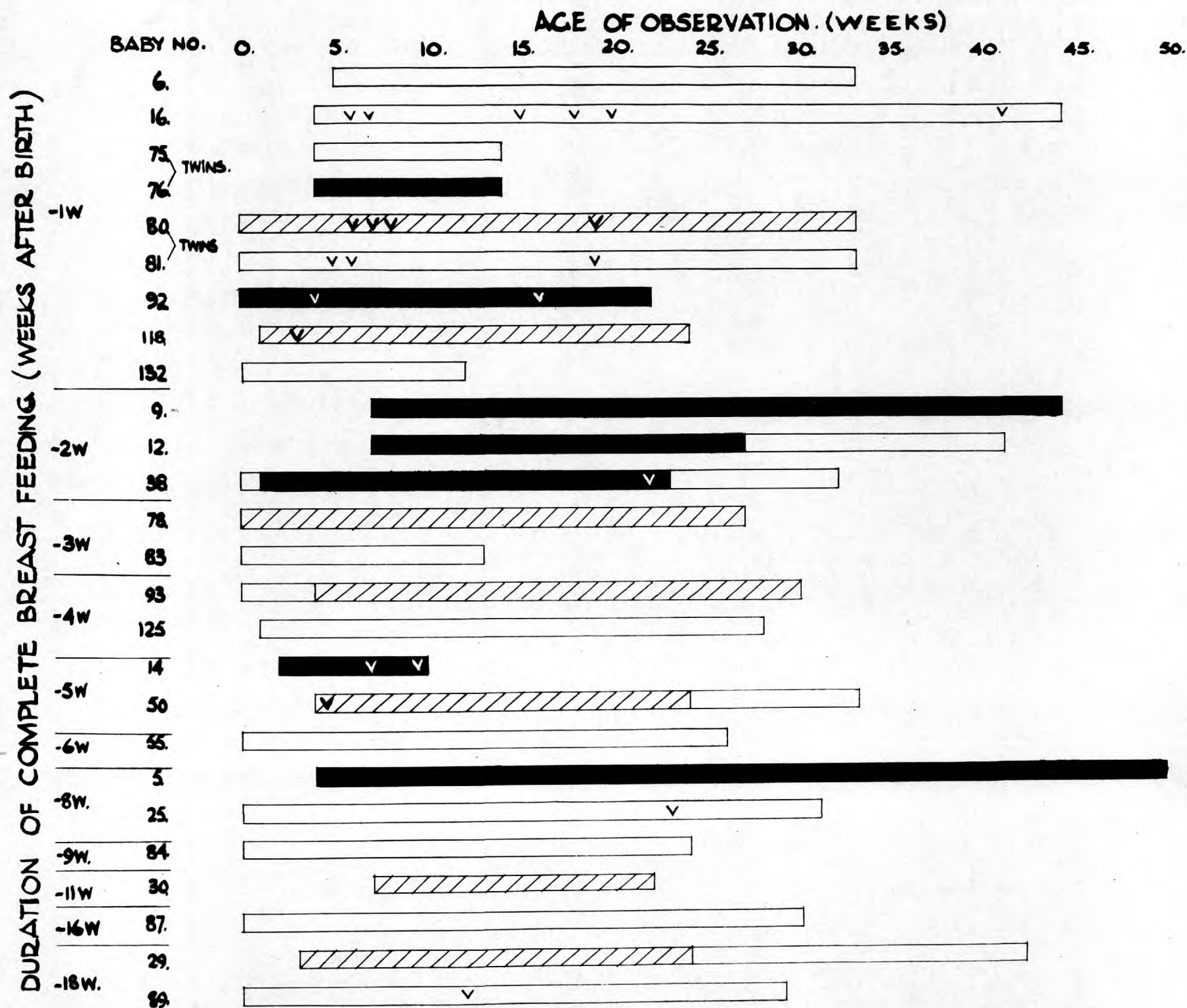
FIG.4. PROPORTION OF OLDER BABIES CONTINUING TO HARBOUR COLIFORMS .



coliforms in their early weeks of life, continued to do so until about their sixth month of age. Some of the babies still being observed after their sixth month of age lost their coliforms, but about half of this group continued to harbour them.

Examination of the clinic records available for 28 of the 33 babies suggested little relationship between the occurrence of upper respiratory coliforms and the type of feeding, or vomiting (Figure 5.). Among the twelve babies who were completely breast fed for two weeks or less, seven harboured coliforms and five did not. Of two pairs of twins (Babies No. 75 and 76, 80 and 81) breast fed for less than one week, only one of each pair harboured coliforms. Six of eleven babies breast fed for three to eleven weeks harboured coliforms, as did one of three babies breast fed for 16 to 18 weeks. In two instances, the appearance of coliforms may have been associated with the introduction of bottle feeding. In one, (Baby No. 38) cultures taken on the first and fourth day were negative, but were found positive on the ninth day of age when bottle feeding had begun. In the other, (Baby No. 93) three cultures taken during the first two weeks were

FIG.5. OCCURRENCE OF COLIFORMS IN THE UPPER RESPIRATORY TRACT IN RELATION TO TYPE OF FEEDING AND VOMITING.



negative, but positive cultures were obtained the fourth week when bottle feeding had commenced a week previously. With these possible exceptions, all of the babies who harboured coliforms acquired them during the first weeks of life irrespective of the type of feeding. In general, these observations conflict with the report of McFarlan et al. (1949) that, among newborn infants, there is a definite correlation between the occurrence of coliforms in the throat and artificial feeding (Chapter 2.).

Vomiting, in the present observations, did not seem to account for the occurrence of coliforms in the infant upper respiratory tract. Four of the ten babies whose clinic records noted vomiting did not harbour coliforms at any time. Conversely, of the fourteen babies harbouring coliforms, no vomiting was noted in eight (Figure 5). This is in harmony with the belief of Neter and Shumway (1950) that vomiting per se does not explain coliforms occurring in the throat during infantile gastro-enteritis. It is also in accord with the experience of Haffner et al. (1950) who found no relation between vomiting and the appearance of coliforms in the throats of children receiving antibiotic therapy. No

diarrhoea was noted in the clinic records of any of the infants.

Although it seems reasonable that the presence of coliforms in the upper respiratory tract may be related to environmental hygiene, this did not appear to be true in the two pairs of twins observed (Babies No. 75 and 76, 80 and 81). In the first pair, observed from four to fourteen weeks of age, one twin harboured coliforms while the other did not. In the second pair, one twin had a few coliforms in the throat on the sixth day, in the nose on the tenth day, and in the nose and throat at the fifteenth week, but otherwise remained negative. The other twin's throat cultures constantly contained numerous coliforms, and its nose cultures often had a few, throughout the 34 weeks of observation.

Properties of the coliforms isolated:

Approximately 200 of the coliforms isolated from 35 of the babies were examined for their biochemical reactions. These included those of the 33 babies observed for two months or more, and represented at least 50% of the strains obtained from any one baby. The properties tested were the formation of indole, the methyl-red test, Voges-Proskauer reaction, and the ability to grow on citrate agar.

These are the usual tests applied to coliforms, and for which Parr (1939), listing them in that order, coined the mnemonic "IMViC". Employing the "IMViC" convention, members of the Escherichia coli group have a reaction pattern of ++00, and those of the Aerobacter aerogenes group have the pattern 00++. Most of the strains were tested for lactose fermentation, and some, for the production of hydrogen sulphide. The details of the methods and media used are given in the Appendix.

It was found that 33% of the strains examined had an IMViC pattern of 00++ or 000+, and therefore would be classified as members of the Aerobacter aerogenes group. The Voges-Proskauer test was variable for many of them when re-tested, and single colonies picked from the same strain also varied. Such variability of the Voges-Proskauer test was reported by Koser (1924), and Parr (1939) considered such strains, which otherwise had the characters of A. aerogenes, to be members of that group. The IMViC reactions of 56% were those of true Escherichia coli, ++00. The remaining 11% gave IMViC reactions intermediate between the two, and therefore should probably be classified as "intermediates" according to Parr. All strains tested for lactose fermentation did so, although a small proportion fermented it only slowly without the production of gas. None produced hydrogen sulphide.

Forty-two of the strains found to resemble Aerobacter aerogenes were investigated further. In the sixth edition of Bergey's manual, the genus Aerobacter is divided into two species, A. aerogenes and A. cloacae. Characteristics distinguishing them are the liquifaction of gelatin and the failure to ferment glycerol by the latter species. Recently, Brooke (1953) reported that fermentation of inositol and production of urease were also useful in characterising the two species. In an examination of 411 strains, he found that A. cloacae did not ferment inositol, and usually utilised urea slowly if at all. A. aerogenes, on the other hand, fermented inositol and rapidly utilised urea. In regard to the taxonomy of this group, perhaps it should also be noted that Kauffmann (1951) states that no clear distinction can be made between Bergey's genera, Aerobacter and Klebsiella, and they therefore should be grouped into a single genus, Klebsiella. He cites the fermentation of inositol, and failure to liquify gelatin as characteristics of it.

It was found that ten strains (obtained from five babies) resembled A. cloacae in the most characteristic properties ascribed to this species by Bergey's manual and by Brooke. The other 32 strains (obtained from 17 babies) seemed intermediate between A. aerogenes and

A. cloacae because they exhibited some of the characteristics of both (Table 21). In respect to gelatin liquefaction, they more nearly resembled A. aerogenes because 72% failed to liquefy it. However, 91% did not ferment glycerol, 63% did not ferment inositol, and none rapidly attacked urea, suggesting a closer relationship to A. cloacae. According to Kauffmann all but three of the 42 strains would be excluded from his *Klebsiella* group because of their failure to ferment inositol, and their ability to liquefy gelatin. They are also unclassifiable in any other group he describes among the Enterobacteriaceae (Kauffmann 1951).

In Figure 5 it is seen that of the fourteen babies who harboured coliforms, the cultures of seven often contained members of the A. aerogenes-cloacae type. Other investigators have also found that the coliforms isolated from the infant upper respiratory tract are not always true E. coli. For example, Torrey and Reese (1944) indicated that 6% of 31 coliform strains they cultivated from newborn babies were Aerobacter aerogenes. In a recent report by Levine and Tanimoto (1954) it is mentioned that of 40 newborn babies having coliforms in their throats, only 26 (65%) harboured Escherichia strains; presumably the others harboured the "aerogenes" type.

In the course of mouse inoculation tests for pneumococci (Section C) it was noted that the coliforms present in the babies' nose and throat swabs were sometimes

Table 21. Properties Of The Coliforms Intermediate Between Aerobacter aerogenes And Aerobacter cloacae

Baby No.	Culture	Gelatin	Glycerol	Inositol
25	11N	a	c	c
25	11T	a	c	c
29	16N	a	c	c
29	23T	a	c	c
30	4N	c	c	a
30	4T	c	c	a
50	1T	a	c	c
50	2T	c	a	a
50	5T	a	c	c
63	2T	a	c	c
69	1T	a	c	c
69	3B	c	c	a
71	1T	a	c	a
72	2T	a	c	a
78	1T	c	c	a
78	10N	a	c	c
78	11N	a	c	c
80	2T	c	c	a
80	5T	c	c	a
80	8T	c	a	a
80	13N	a	c	c
80	13T	a	c	a
80	15N	c	a	a
81	12N	a	c	c
81	12T	a	c	c
82	4T	a	c	c
83	8T	a	c	c
93	10N	a	c	c
93	11N	a	c	c
118	20N	a	c	c
127	2T	a	c	c
131	5N	a	c	c

Gelatin a - not liquified, like A. aerogenes
 c - liquified, like A. cloacae

Glycerol a - acid and gas promptly produced, like A. aerogenes
 c - acid alone produced slowly, if at all, like A. cloacae

Inositol a - fermented (sometimes slowly), like A. aerogenes
 c - not fermented, like A. cloacae

N - Nose culture

T - Throat culture

virulent for mice. Only a few of these strains were examined for their IMViC properties, but there was no suggestion that virulence was associated with a particular coliform group.

Although serological investigation was beyond the scope of the present study, it seemed worth while to determine whether any of the strains were of the serological types which have been isolated from infantile gastro-enteritis. Sera for slide agglutination tests were kindly supplied by Dr. Joan Taylor of the Colindale Public Health Laboratories.

None of the strains were agglutinable in antisera for O 26, O 55 or O 111, the E. coli serotypes associated with infantile gastro-enteritis. The majority of them were agglutinated by acriflavine in saline 1:500, a property not exhibited by the strains of gastroenteritis (Charter and Taylor 1952). It was noted that those strains not agglutinated with acriflavine usually lost this property when subcultured, and this, too, is uncommon among gastro-enteritis strains (Taylor, personal communication). Frequently, these proved of the A. aerogenes-cloacae type. All evidence, therefore, indicated that the coliforms encountered in this study were not related to those associated with infantile gastro-enteritis.

DISCUSSION

The persistence of upper respiratory coliforms in certain babies makes it unlikely that they were present as mere contaminants. Although it is a temptation to regard the babies in whom they were consistently present as "carriers", this would not be justifiable without a demonstration of the serological identity of their strains. It is for this reason that the term "harbourer" has been used throughout this discussion. Carriers, if present, would most likely be those babies who persistently harboured coliforms having the biochemical reactions of true E. coli. However, the other babies whose coliforms sometimes included "aerogenes-cloacae" types cannot be excluded as possible carriers. They may have been carriers of several strains, so that the single colony picked from each culture was not representative of all types present. In Laurell's experience, some children carried more than one serological type, and although the majority were true E. coli, a small percentage had biochemical reactions of Aerobacter aerogenes. Moreover, he found that strains of the same serological type did not necessarily have the same biochemical behaviour.

The relatively frequent occurrence of the "aerogenes" type of coliform in the infant upper respiratory tract is

surprising in view of the general belief that they are not common in the intestinal tract. The literature on this subject is controversial, however. Hall and O'Toole (1935) reported B. aerogenes to be present in the faeces of breast fed infants up to ten days old, but Snyder (1940) was unable to corroborate this. According to Snyder, B. aerogenes was present in the stools of 44% of 22 infants under one year of age, but rarely before they had been weaned. Olson (1949) reported both B. aerogenes and B. cloacae to be rare in the infant intestinal flora. In adults, the incidence in the intestine is also disputed. Koser (1924) reported that coliforms of the "cloacae-aerogenes" type were rare in human or animal faeces, but common in soil. Cruickshank and Cruickshank (1931) stated that B. aerogenes is present in the majority of adult stools, although only in small numbers. However, Parr (1938) found them in freshly passed stools of only 33% of 100 adults. In 14% of these stools, Parr found "gelatin liquifying aerogenes" presumably A. cloacae. From these conflicting reports, it is difficult to decide whether the infant's upper respiratory coliforms are acquired from faecal contamination, or from other sources.

It may be significant that Long (1947) in a study of 300 strains of Gram negative organisms isolated from the mouths of gingivo-stomatitis patients being treated with

penicillin, found 40% of them to be E. aerogenes Type I. This suggests that the aerogenes type of coliform may easily establish itself in the mouth under certain conditions, and may mean that they are not uncommonly present in the normal oral flora in small numbers.

SUMMARY AND CONCLUSIONS

Many of the newborn babies harboured coliforms in their upper respiratory tract. They continued to do so after their discharge from hospital, usually for at least six months, and sometimes for nearly a year. Among the newborn babies there seemed a slight correlation between the presence of coliforms in the upper respiratory tract and artificial feeding, but this was not true among the older infants. Babies bottle fed within the first weeks of life were found no more likely to harbour coliforms than those completely breast fed for a longer time. No apparent relation was found between vomiting and the occurrence of upper respiratory coliforms.

Of fourteen babies observed to be persistent harbourers, the coliforms isolated from seven were found to be true E. coli. Those of the other seven contained, in addition to E. coli, members of the A. aerogenes group. Many of the latter liquified gelatin, and appeared intermediate between A. aerogenes and A. cloacae.

A few resembled A. cloacae in all properties tested.

That the babies who persistently harboured coliforms were probably carriers is suggested by the studies of Laurell. It was not possible to undertake the serological work necessary to establish this point.

None of the babies' E. coli were agglutinated by antisera for the serotypes commonly associated with infantile gastro-enteritis. These serotypes have been reported to occur in the infant upper respiratory tract during this illness, but it has not been determined whether they remain there after recovery. The frequent persistence of coliforms in the infant upper respiratory tract, as observed here, together with the studies of Laurell, suggest that upper respiratory carriers of these serotypes could occur. This possibility deserves more attention in future investigation of the epidemiology of the disease.

PART II

SECTION C

PNEUMOCOCCI

The upper respiratory mucosa of the newborn infant seems relatively resistant to pneumococcal colonisation. It is not known whether this is due to some form of inherent immunity, or to infrequent pneumococcal contact. Resistance appears to be of short duration, because carrier rates reported for older babies are not much lower than those of children and adults (Chapter 3.). The observations reported here were intended to investigate further the pneumococcal carrier characteristics of early infancy.

METHODS

Four methods were used for the cultivation of pneumococci:

- A. The swabs were inoculated directly onto blood agar plates containing gentian violet 1:500,000.
- B. The swabs were inoculated directly onto blood agar plates containing no gentian violet.
- C. The swabs were inoculated into broth containing 20% blood and gentian violet 1:500,000 which, after six hours incubation, was plated on cooked blood agar also containing gentian violet.

D. The swabs were inoculated directly onto blood agar plates containing gentian violet 1:500,000 and 5% sucrose.

Methods A and B were employed routinely for all swabs obtained from the babies being seen at the Welfare Centres. All four methods were used, at different times, for the swabs obtained from the newborn babies.

In addition, mouse inoculation tests were used for some of the cultures obtained from the newborn babies. The nose and throat swabs were inoculated into separate bijou bottles containing broth with 20% blood, and gentian violet 1:500,000. After overnight storage in the refrigerator the broths were incubated for 6 hours in a 36° water bath. Mice were then injected intraperitoneally with 0.5 ml., and one loopful was also streaked onto a cooked blood agar plate (Method C.). The mice were observed frequently within the first 48 hours, and were kept under observation for five days before the tests were considered negative. Mice which became ill were sacrificed for autopsy, and mice which died during the night were autopsied the following morning. Peritoneal exudate and heart blood were plated onto blood

agar, and films of the peritoneal fluid were Gram stained for microscopic examination.

All plates were examined for pneumococci after overnight aerobic incubation. Colonies of typical morphology were typed directly from the plates. Those whose morphology was not entirely characteristic were transplanted into Todd-Hewitt broth, and tested for bile solubility after 18-24 hours incubation. Early in the study, optochin sensitivity was tried as a method of identification, but it was abandoned when it proved not to detect some strains which were bile soluble, although untypable (see Appendix).

The typing sera were obtained from the State Serum Institute of Copenhagen. They included Types 1-45, excluding Types 26 and 30 which are identical with Types 6 and 15. When typing was done directly from the plates, ten or more colonies from each culture were mixed together. This mixture was first examined for capsule swelling in the group "pools" A-I, and then tested with all type sera within the group giving the reaction. This method insured recognition of all types contained within the random sample taken.

Before beginning the study, it was thought that swabs made of calcium alginate wool would facilitate the recovery of small numbers of pneumococci, as well as other organisms. The advantage of calcium alginate wool swabs is that they can be completely dissolved in 1% sodium hexametaphosphate, thus freeing them of all organisms entrapped within the fibres. (Higgins, 1950). Preliminary tests, however, indicated that sodium hexametaphosphate is somewhat bactericidal or bacteriostatic for pneumococci, so the method could not be used (see Appendix).

FINDINGS

Incidence of pneumococci among the newborn babies:

Pneumococci were not found, using Methods A and B, among 42 newborn babies during their stay in the Paddington Hospital nursery. This first series of plates was examined with particular scrutiny: to avoid overlooking any pneumococci, one or more smooth, alpha haemolytic colonies of over 0.5 mm diameter were picked

from each culture and tested for bile solubility.

To confirm the absence of pneumococci among the newborns, the mouse inoculation test was applied, in conjunction with Method C, to an additional group of babies, 24 of whom were born in Paddington Hospital, and eight of whom were born in St. Mary's Hospital. Since two or three consecutive swabs were obtained from each of the babies during their stay, a total of 154 nose and throat swabs were examined by this method. Illness or death occurred in 8% of the mice, but usually appeared due to coliform infection because coliforms were obtained in pure culture from both the heart blood and peritoneal fluid (Section B.). A mouse-virulent viridans streptococcus, not Lancefield grouped, was obtained from the swabs of one baby on two occasions. It was bile insoluble, and optochin resistant - properties which were not altered by mouse passage. No pneumococci were obtained from any baby of this group.

During the search for H. influenzae and coliforms, a small group of 13 babies born in St. Mary's Hospital was also examined for pneumococci using Method C alone.

A pneumococcus, Type 19, was obtained from the nasal swab of one baby on its sixth day of life. Its nose and throat cultures on the third day had been negative. Unfortunately, this baby was discharged from hospital on the seventh day, before additional swabs could be obtained.

Incidental to the investigation on Streptococcus salivarius, 22 newborn babies were examined simultaneously for pneumococci using Methods A and D. No pneumococci were recovered.

After so many newborn babies had been found not to harbour pneumococci using the various methods, all except Method B (blood agar plates) were discontinued. No pneumococci were found among the 51 newborn babies investigated by method B alone.

Pneumococci therefore appeared to be absent among a total of 159 of the 160 newborn babies observed between the months of December 1952 and August 1953.

Incidence of pneumococci among the older babies:

Forty-five babies reporting to the Centres were observed until they were from five to 49 weeks of age. Thirty-eight of these babies were observed from the time

of their birth; observations on the remaining seven began between their fourth and fifteenth weeks of age.

Unfortunately, the baby from whom pneumococci had been obtained while it was in hospital was not among those seen at the Centres.

The incidence of pneumococci, according to weeks of age, is shown in Figure 6. It was found that the incidence rose sharply in the first month, and had reached its maximum by the end of the second month. During the remaining ten months there was considerable fluctuation, but the average incidence was about 40%.

The pneumococcal types encountered may be seen in Table 22. There were fourteen different types, in addition to the untypable strains. Types 6, 21, 23 and the untypable strains were most frequent.

Carrier characteristics of the older babies:

The term "carrier" has been applied to every baby yielding pneumococci of the same type on at least two, and usually more, occasions. Twenty-five of the forty-five babies (56%) were carriers at some time during the period they were observed; the others never became carriers. The age at which twenty-two of the twenty-five

FIG.6. INCIDENCE OF PNEUMOCOCCI IN THE FIRST YEAR OF LIFE.

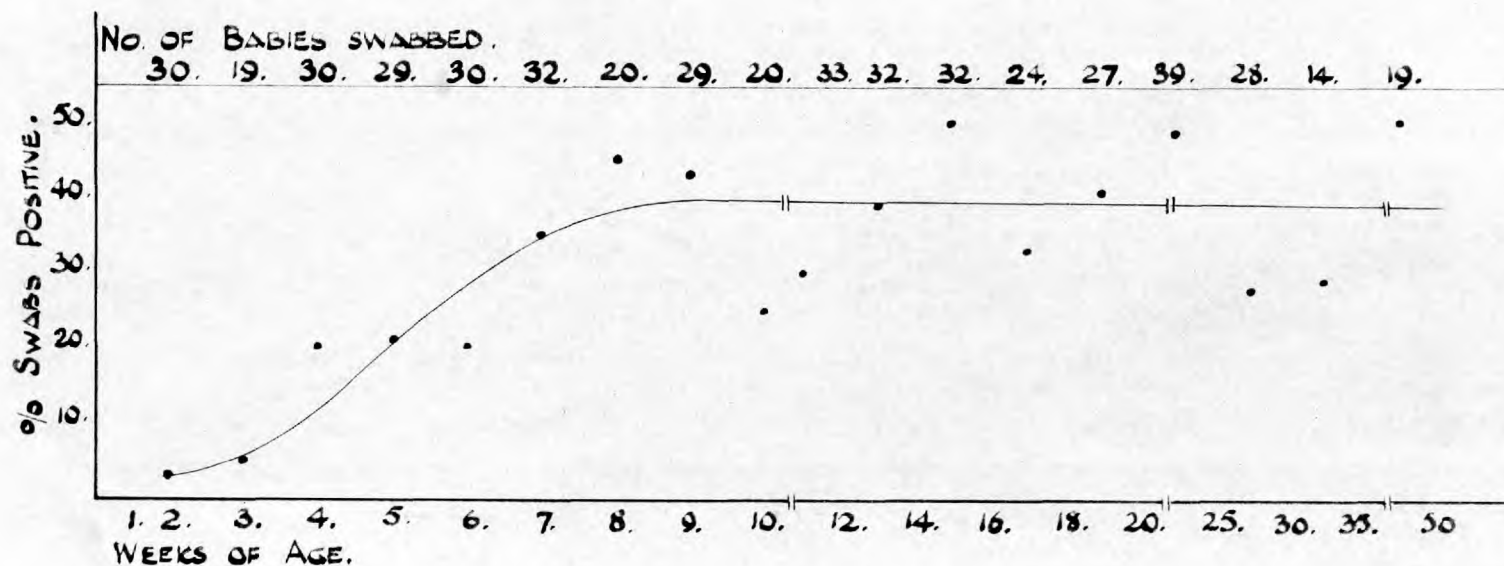


Table 22. Incidence of Pneumococcal Types

Carried: (Isolated at least twice per baby).

Type	No. of Babies.
2	1
6	3
7	1
9	1
10-39*	2 (twins)
12	1
13	1
14	2
20	2
21	3
22	1
23	3
UT	7

Not carried: (Isolated from only one culture per baby).

Type	No. of Babies
4	1
19	1

Isolated only from the last culture obtained:

Type	No. of Babies
6	2

* Strain giving cross-reactions between
Types 10 and 39

UT untypable strains.

became carriers is known within one to four weeks accuracy. Two were observed to become carriers within their first four weeks, over half had become carriers by the end of their eighth week, and nearly all had become carriers by the twentieth week (Figure 7.).

Most babies who were carriers harboured pneumococci in the nasal passages only. A few babies sometimes yielded them in their throat cultures as well, and one baby (No. 9) had them in its throat almost entirely during its carrier period. The nasal cultures usually contained moderate to large numbers of colonies; the throat cultures never contained more than moderate numbers, and frequently only a few.

Most babies were constant nasal carriers, although a few were "intermittent" in that an occasional negative culture appeared in a series of positive cultures. As these negative cultures were never obtained consecutively, it is likely that the pneumococci had accidentally been "missed" in the negative swabs. These babies, therefore, probably were also constant carriers.

The pneumococci were usually carried for long periods of time, in some instances for more than six months (Table 23). Some of the babies eventually lost

FIG.7. DEVELOPMENT OF PNEUMOCOCCAL CARRIAGE.

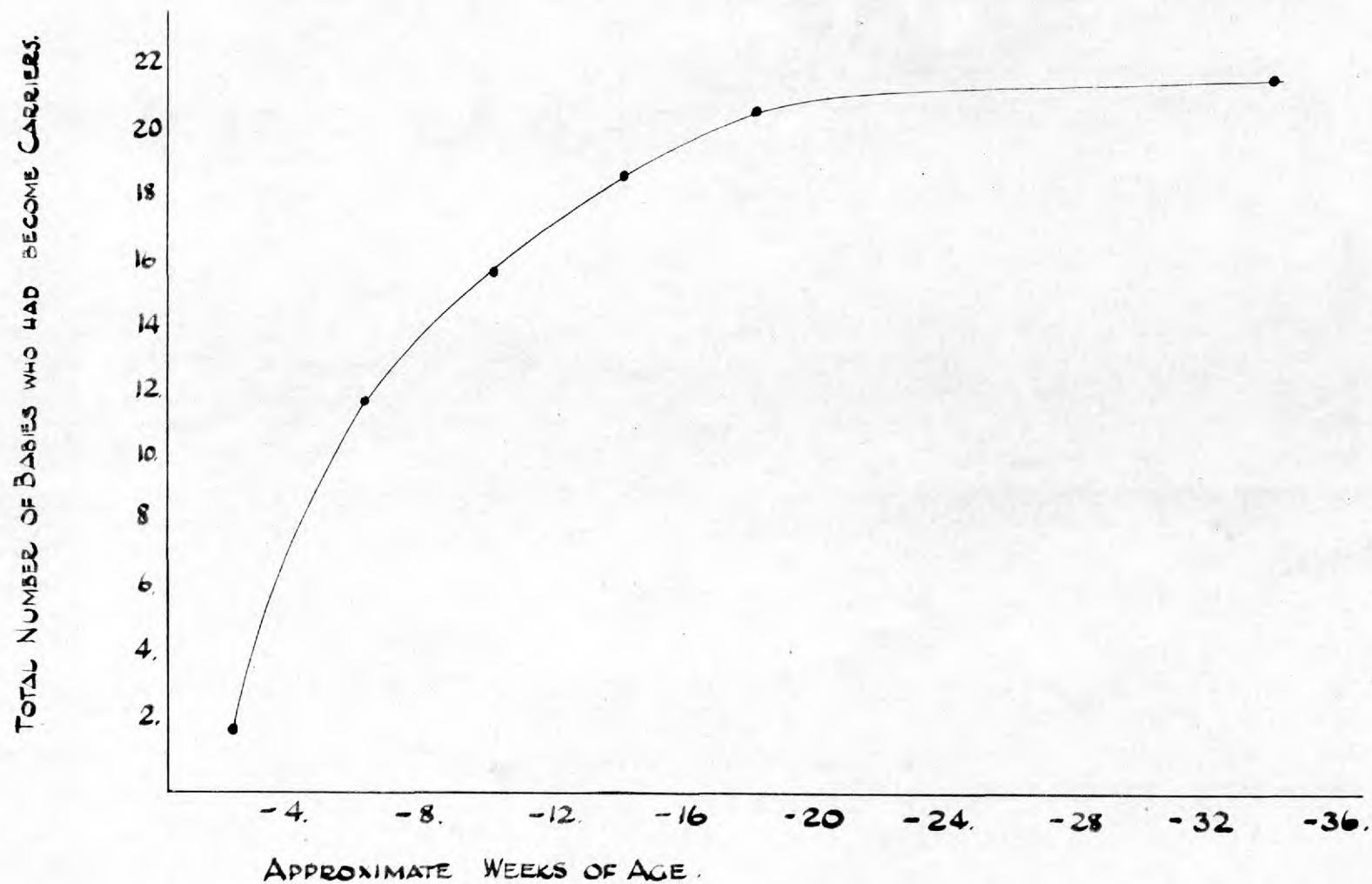


Table 23. Duration Of Pneumococcal Carriage

<u>Carriers:</u>				<u>Non-carriers:</u>	
<u>Baby No.</u>	<u>Known Carrier Period (Weeks)</u>	<u>Weeks of Observation</u>	<u>Type</u>	<u>Baby No.</u>	<u>Weeks of Observation</u>
75	35	43	21	76	43
40	31	38	9, 6	12	41
81	31	34	10-39*		
89	30	38	20	50	37
6	30	33	6, UT	83	30
38	29	32	21	125	28
29	24	42	7, 6	93	27
80	22	34	10-39*	55	26
9	21	44	UT	23	26
78	20	28	22	109	25
				72	25
84	18	24	23	132	20
30	16	16	14		
67	16	27	23	37	17
118	13	26	13	19	14
25	12	31	UT	130	13
66	12	12	23	8	11
101	12	12	UT		
13	11	16	21	14	10
				103	9
5	10	49	12	32	99
127	6	9	UT	95	7
82	4	10	2	11	6
10	3	5	20		
16	2	44	UT		
87	2	30	UT		
92	2	33	14		

10-39* = Cross reactions between Types 10 and 39

UT = Untypable strain

their pneumococci, but the majority continued to carry them throughout the period they were available for observation. There was insufficient data to determine whether any one type tended to persist longer than others. However, it is interesting to note that Type 6, which frequently causes pneumonia in babies, was among those carried longest.

The types carried remained remarkably constant for each individual baby, as may be seen in Table 24 (cover pocket). Only two babies changed types during their period of observation. One of these (Baby No. 29) changed from Type 7 to Type 6 between its 25th and 29th week. Type 6 was then recovered consistently up to the 42nd week, except when an untypable strain was obtained in a single culture on the 35th week. The other baby (No. 40) changed from Type 9 to Type 6 between its 14th and 19th week of age. No more swabs were obtained until its 38th week, but this swab also contained a Type 6.

In no instance was more than one type found in a single culture. One baby (No. 6), however, yielded an untypable strain from its throat several times while carrying Type 6 in its nose. From another baby (No. 9)

who had intermittently yielded an untypable strain in its throat, a Type 4 was obtained in one nasal culture. In all other babies the throat cultures, when positive, were of the same type as the nasal cultures.

Pneumococcal carriage was interesting to observe in the two pairs of twins included in the study. As would be expected, one pair of twins (No's. 80 and 81) shared a strain of the same type throughout their carrier period. The other pair of twins (No's. 75 and 76) were not identical in their pneumococcal carriage. One of them (No. 75) yielded Type 21 pneumococci in its nasal cultures with the onset of an inflamed throat and cough during its 9th week of age. These pneumococci persisted throughout the next five weeks. The other twin (No. 76) had no pneumococci during this time, although its clinic records noted "snuffles" at the 11th week of age. Further cultures were not obtained from this pair of twins until they were 43 weeks old. As this time, both were found to have Type 21 in their nasal cultures.

Relation between pneumococcal carriage and mild upper respiratory infection:

For indications of upper respiratory infection,

reliance was made upon the medical records of the babies, and upon the notes of the health visitors who took the swabs. Mild infections occurred among 17 (38%) of the babies at some time during the period that they were observed; there were no severe infections. In only three of the 17 babies was the onset of symptoms possibly associated with the appearance of pneumococci. In the others pneumococci either 1) were already being carried, usually for some weeks, 2) appeared some weeks later, or 3) were never present. The relation between the presence of pneumococci during the time of mild upper respiratory infections is seen in Table 25. Proportionally, pneumococci were somewhat more frequent among babies with infection than those without infection. However, application of the chi square test (with Yates' correction) indicates that the difference may have been due to chance.

Table 25. Relation Between Upper Respiratory Infection And Presence of Pneumococci In 45 Babies

		Pneumococci		Total
		Present	Absent	
Infection	Present	11	6	17
	Absent	16	12	28

$$\chi^2 = .0354$$

$$P = .85$$

DISCUSSION

The infrequent finding of pneumococci in the upper respiratory tract of the newborn babies is in agreement with several previous observations on hospital-born infants (Kneeland, 1930; Torrey and Reese, 1944, 1945). That the newborn's resistance to pneumococci is sometimes of short duration is evidence^d by the observation that over half of the babies who became carriers had acquired their pneumococci by their eighth week of life. Interestingly, this age period corresponds to that during which certain pneumococcal antibodies, present at birth, are reported to be lost. However, it is also the time when the babies have passed from relative isolation in the hospital nursery to greater communal life.

The fact that quite a few of the older babies did not become carriers at any time suggests that they may have possessed a resistance somewhat greater than is common in adults. Almost all adults can be demonstrated to be pneumococcal carriers at some time when cultures are taken repeatedly (Finland, 1942). Pneumococcal colonisation of the infant upper respiratory tract cannot be dependent upon contact with the organism entirely, for

in the twins observed the cultures of one remained negative for five weeks while the other's cultures were positive.

Except that the pneumococci were carried more frequently in the nose than in the throat, the babies' carrier characteristics seemed similar to those generally described for adults. Maintenance of single pneumococcal types by adult carriers has been reported, although recurrence of several types more often has been described (Chapter 3.).

SUMMARY

Using several methods for isolation, pneumococci were recovered from only one of 160 babies during their first two weeks of age. In further observations on 45 babies, over half were found to become carriers during their first year of life. Twelve are known to have become carriers by their eighth week, and 21 were observed to be carriers by their twentieth week. The pneumococci generally were recovered only from the nasal cultures, although sometimes they were also present in the throat

cultures. The carrier state, once established, persisted for long periods, often for six months or more. Usually each carrier maintained a single type throughout its carrier period. Fourteen different types were encountered in addition to untypable strains. The typable pneumococci included Types 6 and 14, which frequently cause pneumonia in babies. No relationship was ascertained between the presence of pneumococci and the occurrence of upper respiratory infection.

PART II

SECTION D

BETA HAEMOLYTIC STREPTOCOCCI

Some investigators have reported that beta haemolytic streptococci may become established in the newborn upper respiratory tract, although others have denied this. Recent work, employing serological classification, suggests that haemolytic streptococci other than Group A, may be acquired from the maternal vagina during delivery. Members of Group A have not yet been reported present in the upper respiratory tract of the newborn infant. According to the few observations reported, babies may become carriers of beta haemolytic streptococci, including Group A, within the first six months of life (Chapter 3.).

In the present investigation, a completely adequate study of the beta haemolytic streptococci was not possible. Practical considerations precluded the use of the special cultivation methods which would have given maximum recovery (Pike and Fashena, 1946). Also, serological classification could not always be performed because of the volume of other work. Nevertheless, the observations which could be made are of interest, and therefore are presented here.

METHODS

Only the routine blood agar plates, with or without the addition of gentian violet, were used for isolation. They had been incubated aerobically overnight. The relative number of beta haemolytic colonies were noted, as well as the degree of haemolysis. These were distinguished from Haemophilus haemolyticus by their colonial appearance, or in cases of doubt, by Gram stained films. Strains obtained in the early part of the survey were stored for subsequent serological classification, but unfortunately few of these survived. Later, as the volume of work with the newborn infants diminished, it was possible to perform the serological work soon after isolation. Antigen extracts were prepared by the Fuller formamide method. These were tested with antisera (Burroughs Wellcome) for Groups A, B, C, D and G, or in some instances, for Group A only.

Occurrence of beta haemolytic streptococci among the newborn babies:

The number of newborn babies whose cultures were examined for beta haemolytic streptococci during their

period of hospitalisation totalled 115. Beta haemolytic streptococci were noted in the cultures of 13 (9%).

The culture records of these babies are shown in Table 26. Strains obtained from five were classified serologically.

Three babies were found to have members of Group A. One of these (No. 117) had moderate numbers of colonies in the nose and throat cultures on two occasions. The second (No. 153) had profuse numbers in its last nasal culture alone, and the third (No. 91) had only a few colonies in just one of the three swabs obtained. An additional baby (No. 92), whose hospital cultures were not classified serologically, continued to harbour beta haemolytic streptococci when it was seen at the Centre from its fourth week onwards. As these proved to be Group A, it is probable that the hospital strains were also of this Group. It had been in the same hospital ward at the same time as Baby No. 91.

Two babies had members of Group C. In one of these (No. 144) they were present in small numbers on two occasions, and in the other (No. 155) only a few were present in the last culture obtained. Both babies

Table 26. Beta Haemolytic Streptococci Isolated from
Newborn Babies

<u>Baby</u>	<u>Age</u> <u>(days)</u>	<u>Site</u>	<u>Number of</u> <u>Colonies</u>	<u>Degree of</u> <u>Haemolysis</u>	<u>Serological</u> <u>Group</u>
No. 10	15	N	0		
		T	x	moderate	-
	9	N	x	"	-
		T	0		-
23	2	N	0		
		T	x	moderate	-
	5	N	0		
		T	0		
	9	N	0		
		T	0		
26	2	N	0		
		T	xx	moderate	-
	6	N	0		
		T	0		
	9	N	0		
		T	0		
	13	N	0		
		T	0		
85	1	N	0		
		T	xx	weak	-
	4	N	0		
		T	x		
91	1	N	0		
		T	0		
	5	N	x	moderate	A
		T	0		
	8	N	0		
		T	0		
92	3	N	x	moderate	(Probably A)
		T	xx	"	-
	7	N	0		
		T	x	"	-
	10	N	0		
		T	0		
115	4	N	0		
		T	xx	weak	-
	8	N	0		
		T	x	"	-

(continued on next page)

Table 26. (continued)

<u>Baby</u>	<u>Age</u> <u>(days)</u>	<u>Site</u>	<u>Number of</u> <u>Colonies</u>	<u>Degree of</u> <u>Haemolysis</u>	<u>Serological</u> <u>Group</u>
No. 117	4	N	0		
		T	0		
	8	N	XX	moderate	
		T	XX	"	A
	11	N	XX	"	A
		T	X	"	
127	3	N	0		
		T	XXX	weak	-
	7	N	0		
		T	0		
	10	N	0		
		T	0		
143	13	N	0		
		T	0		
	3	N	0		
		T	XX	moderate	-
144	7	N	0		
		T	0		
	5	N	X	moderate	C
144	6	N	0		
		T	0		
	8	N	X	moderate	C
153	5	N	0		
		T	0		
	8	N	XXX	moderate	A
155		T	0		
	3	N	0		
		T	0		
	5	N	X	moderate	C
		T	0		

* Group A obtained from later cultures

- Serological Group not determined

Number of colonies:

0 = none

X = 10

XX = 10-50

XXX = profuse growth

N = nose

T = throat

were hospitalised in the same ward at the same time.

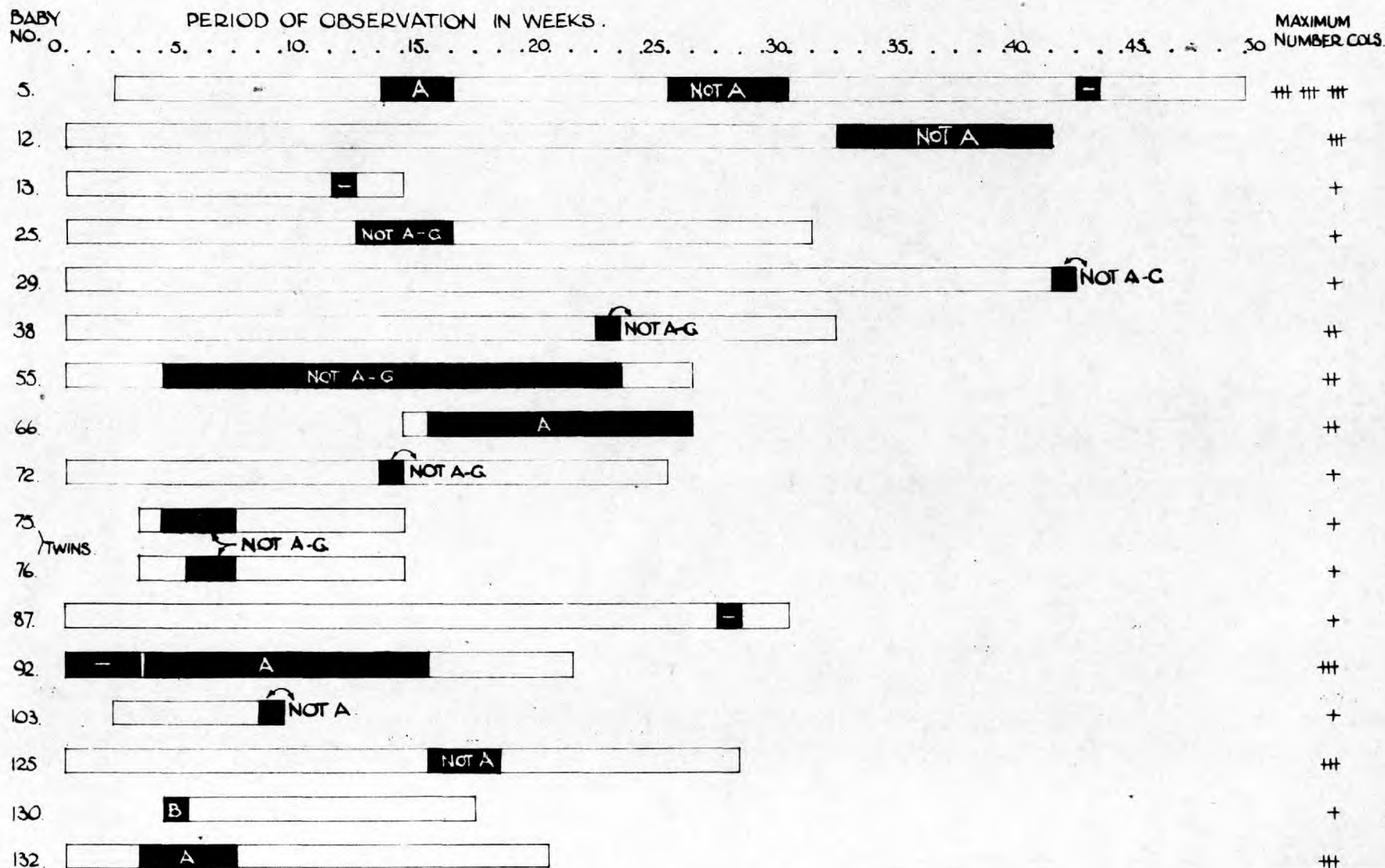
The strains of the other seven babies were not classified serologically. However, those obtained from three of them were only weakly haemolytic, and are believed not to have been members of Group A.

None of the 13 babies harbouring beta haemolytic streptococci exhibited signs of upper respiratory infection, or other illness.

Occurrence of beta haemolytic streptococci among the older babies:

Forty-five babies reporting to the Centres were observed for beta haemolytic streptococci. This group was the same as that observed for pneumococci, and has previously been described (Section C.). Beta haemolytic streptococci were obtained from 15 (38%) of the babies, the records of whom are shown in Figure 8. Ten babies (22%) were carriers in that they yielded these streptococci on more than one occasion. Members of Group A were recovered from four babies (No's 5, 66, 92, 132) and were carried for several weeks or more. One baby (No. 5) who was a Group A carrier, became a carrier of a strain other than Group A at a later date. The other

FIG. 8. BETA HAEMOLYTIC STREPTOCOCCI IN THE UPPER RESPIRATORY TRACT OF BABIES OBSERVED AT THE WELFARE CENTRES.



+++ = PROFUSE GROWTH. ++ = 10-50 COLONIES. + = <10 COLONIES.

A SEROLOGIC GROUP **-** NOT CLASSIFIED.

carriers did not have Group A strains; those of five are known not to have belonged to Groups B, C, D or G either.

The Group A carriers had moderate to profuse numbers in their throats, and sometimes in their nose as well. The other babies harboured few to profuse numbers in their throats, most frequently, but positive nasal cultures were common.

The presence of beta haemolytic streptococci in the 17 babies did not seem related to upper respiratory infection, as judged by their medical records. Six of them suffered mild upper respiratory infections during the period that they were observed, but the streptococci occurred either several weeks preceding or succeeding the infection. One baby (No. 55) who carried a serologically non-classifiable strain for 19 weeks had a history of recurrent colds during this period. It was interesting to note that four babies (No's 5, 25, 66, 92) carried haemolytic streptococci simultaneously with pneumococci. In two of these (No. 5, 66) the pneumococci and haemolytic streptococci first appeared at the same time.

DISCUSSION

The finding of Group A strains in the newborn upper respiratory tract is an observation not previously reported. However, studies employing serological classification have been few (Chapter 2.). The occurrence of haemolytic streptococci in five babies on more than one occasion suggests that the newborn upper respiratory tract is not resistant to their colonisation, as was thought by Torrey and Reese (1944, 1945) and Kneeland (1930). No attempt was made to determine the origin of five of these strains. However, there was no indication that they were acquired from the maternal vagina necessarily, because they were not always present in the first cultures obtained. Also, the few which were serologically classified did not belong to Groups B or D, normally most common in the birth canal (Lancefield, 1935). Twice, babies in the same ward at the same time harboured identical serological groups, suggesting that they may have acquired them by hospital cross-infection.

The incidence of beta haemolytic streptococci

observed among the older babies is somewhat higher than that observed by Kneeland who found them present in 11% of 28 babies aged four to eight months. Also, it is higher than that commonly observed among adults. This may have been due, in part, to estimating the carrier rate from consecutive swabs collected over a period of time.

That babies may become carriers of beta haemolytic streptococci, including Group A, in the early months of life is in confirmation of other reports. There was no evidence that the presence of beta haemolytic streptococci was directly related to upper respiratory infection.

SUMMARY

Beta haemolytic streptococci were found in the upper respiratory tract of 13 (9%) of 115 newborn babies. The strains of five babies were classified serologically. Three were Group A and two were Group C. Strains including Group A occurred in five babies on more than one occasion, indicating that they had become established.

Beta haemolytic streptococci were obtained from

38% of the 45 babies seen at the Centres, and 22% appeared to carry them. Group A strains, as well as others, were carried for several weeks or more.

PART II

SECTION E

HAEMOPHILI

Information on the occurrence of Haemophilus influenzae in the infant upper respiratory tract is meagre and unsatisfactory (Chapter 3.). Although it has been reported to occur in newborn infants, not all investigators have confirmed this. Torrey and Reese (1944, 1945) believed the newborn infant to be resistant to colonisation by most members of the Genus Haemophilus. They were unable to find H. influenzae in newborn infants, and H. haemolyticus was rare. This had also been the experience of Kneeland (1930). The few reports available on older infants suggest that non-encapsulated strains of H. influenzae are fairly common, but potentially virulent encapsulated strains, including Type b, seem rare in the healthy infant.

Further observations on the presence of H. influenzae in healthy infants, particularly the encapsulated strains, might contribute to better understanding of the epidemiology of Type b influenzal meningitis. It was desirable, therefore, that such observations be included in the present study. Unfortunately, they had to be somewhat subordinated to

attain the other objectives chosen for primary consideration. However, the observations which could be made, are reported here.

METHODS

For the most part, only the routine blood agar plates with and without gentian violet were used. During the special attempt to recover pneumococci from the newborns (Section C.) an additional method for cultivating H. influenzae was also employed. The primary cultures, in broth containing blood and gentian violet, were subcultured on cooked-blood agar also containing gentian violet (1:500,000). It was intended that the primary broth would increase the recovery of both pneumococci and H. influenzae if present in small numbers, and that the cooked-blood agar would facilitate recognition of H. influenzae in particular.

Between May and August, 1953, a more intensive effort was made to recover H. influenzae from the babies being seen at the Welfare Centres. As it was

undesirable to make a major change in the routine procedure, it was decided to incorporate a third medium in one of the two routine plates. Accordingly, the blood-gentian-violet plate was converted into a "wedge plate" containing Fildes' agar, with gentian violet, as well (see diagram). The swabs were inoculated, at the junction of the two media, and the streaking pattern used gave equal distribution between them. The theoretical advantage of the "wedge plate" was that it encouraged better growth of haemophili while permitting recognition of pneumococci and beta haemolytic streptococci at the same time. Experience later demonstrated that coliform colonies, common in the throat cultures, often were so semi-translucent on the Fildes' agar that they could easily be mistaken for non-encapsulated haemophili. The Fildes' agar was then replaced with cooked-blood agar, containing gentian violet, upon which the coliform colonies were more typical, and distinct from haemophili.

Colonies suspected of being H. influenzae or H. parainfluenzae were examined in Gram stained films. Those of typical cell morphology were suspended in normal saline to determine their "smooth" character (Pittman,

**"Wedge Plate" Used for Cultivation of H. influenzae,
Pneumococci, and Beta Haemolytic Streptococci**

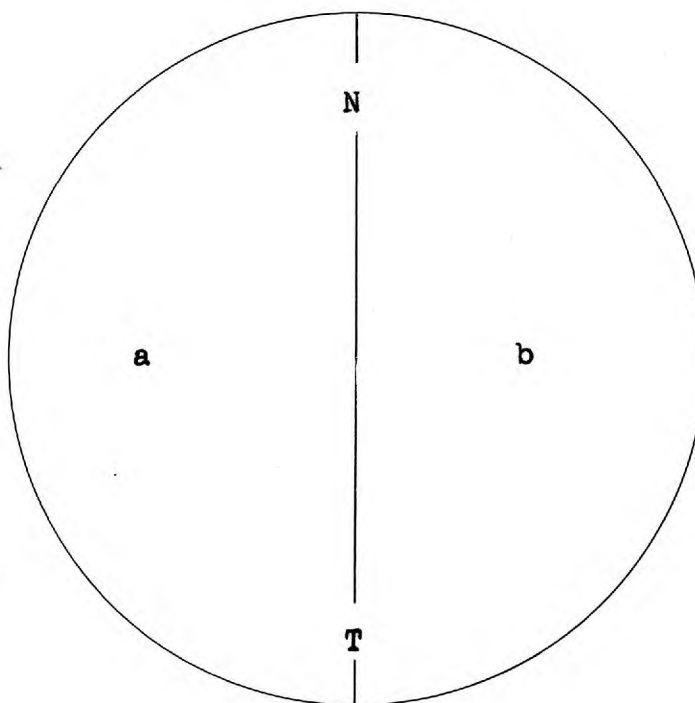


a=blood agar with gentian violet 1:500000.

b=Fildes' agar, or cooked-blood agar, with gentian violet.

N=site of inoculating nasal swab.

T= " " " throat "



1931). Antisera (Pittman a and b) were available for typing the smooth colonies, by a courteous gift from Lederle Laboratories, Pearl River, New York.

Haemolytic colonies of haemophili were distinguished from haemolytic streptococci by their colony morphology, and appearance in Gram stained films. They were recorded separately as "H. haemolyticus". This is the term suggested for most haemolytic strains by Bergey's Manual, although probably many workers would prefer to call them "haemolytic strains of H. parainfluenzae", as suggested by Topley and Wilson (1946).

OBSERVATIONS

H. influenzae and non-haemolytic strains of H. parainfluenzae were never found in any of the 160 newborn babies examined between December 1952, and August 1953. The distribution of the cultural methods had been:

	<u>No. of Babies</u>
Blood agar and blood agar with gentian violet	42
Blood agar only	51
Blood agar, plus 5% sucrose and gentian violet	22
Blood broth, subcultured on cooked-blood agar containing gentian violet	45

H. influenzae and non-haemolytic strains of H. parainfluenzae were extremely rare among the 45 older babies seen at the Centres. A non-encapsulated strain was found only once, in a baby nineteen weeks of age. Numerous minute colonies were present in the nasal swab which had been plated on blood agar; there were none in the throat swab. The strain proved haemophilic, but its X and V requirements were not determined separately. It agglutinated when suspended in normal saline. This was the only time that it occurred in this baby. Seven of the swabs previously obtained had been among those inoculated onto "wedge plates".

An encapsulated strain of H. influenzae was found in just one baby. It was first obtained when this baby was twenty-two weeks old. There were profuse numbers of colonies in both the nose and throat swabs which had been plated on blood agar. They were of atypical appearance in that they were pearly, very dome-shaped, and nearly 2 mm. in size. When subcultured on cooked blood agar they were more typical, and did not agglutinate in either normal saline or Type a antiserum. Rapid slide agglutination occurred in Type b antiserum. When the next swabs were obtained, in the baby's 25th week, a few similar colonies were present in the throat, but not in

the nasal culture. The baby therefore seemed to carry this strain for at least three weeks, although no illness was noted. Unfortunately, this baby was one of the few who reported to the Centre very irregularly, and no further swabs could be obtained from it. The only recent previous swab had been obtained in its 18th week.

"*H. haemolyticus*" was noted only once among the newborn babies. A few colonies were present in the throat of one baby on its second day, but were absent in later swabs. Among the older babies it was common after the fourth week of age, and was noted in 22 of the 45 babies at some time during the period they were observed. Usually it was present only intermittently, but six babies consistently yielded it for a period of seven or eight weeks. Most frequently it was present in the throat in varying numbers, and less frequently in the nose.

DISCUSSION

The absence of *H. influenzae* among the newborn babies is in agreement with the observations of Kneeland, and of Torrey and Reese. However, its almost complete absence among the older babies was surprising, as was the absence of non-haemolytic *H. parainfluenzae*. Although blood agar,

with or without gentian violet, is not ideal for their recognition, it is difficult to believe that they would have been overlooked constantly had they been present in moderate numbers. Moreover, they should have been recognised quite easily in the "wedge plates" containing Fildes' or cooked blood agar. Over half the babies were six to eight months old during the time the "wedge plates" were being used, and it was expected to find these haemophili among them. Kneeland (1930) claimed to have found H. influenzae in the throats of 53% of 28 healthy babies four to eight months of age, using blood agar plates. Of course, the incidence of H. influenzae may vary with different population groups and different seasons.

The tentative conclusion drawn from the present observations is that H. influenzae and non-haemolytic H. parainfluenzae occur infrequently in young babies, particularly during the first six or eight months of age. Confirmation with a more suitable and selective medium, such as Fildes' agar containing an appropriate antibiotic, would be desirable.

The absence of "H. haemolyticus" in the newborns, and its frequent presence among the older babies, lends further support to the belief of Torrey and Reese that

the newborn upper respiratory tract resists colonisation by most members of the genus Haemophilus.

SUMMARY

H. influenzae and non-haemolytic H. parainfluenzae were never found in the upper respiratory tract of the 160 newborn babies examined. They were uncommon among the 45 older babies upon whom swabs were repeatedly obtained. A non-encapsulated strain was found just once, and one baby carried an encapsulated Type b strain for three weeks. It is tentatively concluded that the non-haemolytic haemophili are rare in the infant upper respiratory tract, but further confirmation with more suitable media would be desirable.

Haemolytic haemophili ("H. haemolyticus") were almost never present in the newborns. They were common among the older babies and became established sometimes for considerable periods of time.

PART III

OBSERVATIONS ON INFANTS BORN
AT HOME

INTRODUCTION

In the preceding observations it was found that the upper respiratory tract of the newborn is colonised, in part, by bacteria derived from the hospital environment. Newborn babies quickly acquire the hospital's penicillin resistant strains of Staphylococcus aureus, and conceivably their upper respiratory coliforms also may be of hospital origin. A study of babies delivered at home promised to provide answers to several interesting questions:

1. Are home-delivered babies less likely to become upper respiratory carriers of Staphylococcus aureus?
 - a) What is the frequency of penicillin resistant strains among them?
 - b) Are the penicillin sensitive strains of the home environment perhaps less adapted to the upper respiratory mucosa, and therefore retained for shorter periods?
2. If the home-delivered babies do not acquire Staphylococcus aureus soon after birth, do they do so ultimately or, having passed the age of highest susceptibility, do they remain non-carriers?
3. Do coliforms colonise the upper respiratory tract of home-born babies as frequently as babies born in hospital?

4. Do home-delivered newborns acquire pneumococci earlier, because of their perhaps greater exposure to respiratory infection, as suggested by the recent report of Landsman?

The upper respiratory flora of babies delivered at home has never been studied by the sequential culture method, for the difficulties of collecting such material are obvious. The potential information to be gained from such a study, however, seemed to justify expending considerable effort towards its accomplishment.

Preliminary observations

It was first arranged to have the swabs on the newborns collected by the London County Council and Queen's Midwives. The "follow-up" swabs, as the babies became older, were taken by the medical staff of the Harrow Road Child Welfare Centre. They were inoculated directly onto blood agar, and the plates were returned to the laboratory within a few hours.

After a two-month trial, this arrangement proved administratively unwieldy, and seemed unlikely to provide the desired data. Only five babies had become available for observation, and only two were reporting regularly to the Centre. Although it had to be discontinued in favour of a more suitable plan, a small amount of interesting information had been obtained.

Results of the preliminary observations

The observations on Staphylococcus aureus are shown in Table 27. Only two of the five babies became carriers soon after birth (Nos. 162 and 164). One acquired a penicillin sensitive strain, and the other had a penicillin resistant strain. A third baby (No. 160) had a few colonies in both its nose and throat cultures in the second week, and again in the eighth week. These were penicillin sensitive, and proved to be of the same phage pattern (29/71/75). However, as all other cultures obtained up to its fifteenth week of age were negative, it is probable that this strain was a transient which did not become established. The other two babies were non-carriers (Nos. 161 and 163). One was swabbed only during its first two weeks of life, but the other was swabbed quite regularly until its sixth week, and again in its fifteenth and twenty-fifth weeks.

Pneumococci were present among four of the five babies observed (Table 28). Two of them are known to have been carriers of Type 23 for at least six weeks. Of these, one had acquired them by its tenth day, and the other, by its sixteenth day. A third baby had a Type 14 pneumococcus on its eighth day of life, but as further swabs were not obtained it is not known for how long this strain was carried. The fourth baby had a

Table 27. Preliminary Observations on Staphylococcus aureus Among Babies Born at Home

Baby No.	- Weeks of Age -												Phage Pattern
	$\frac{1}{2}$	1	1 $\frac{1}{2}$	2	3	4	6	8	11	13	15	25	
160 N		0	0	S	0	0	0	S	0	0	0		29/71/75
T		0	0	S	0	0	0	S	0	0	0		
161 N	0	0	0		0	0	0				0	0	
T	0	0	0		0	0	0				0	0	
162 N	R	R	R			R	0						3A
T	R	R	R			R	R						
163 N	0	0	0	0									
T	0	0	0	0									
164 N	S	S											6/7/47/54/75+
T	S	S											

0 = culture negative
 R = culture positive; penicillin resistant strain
 S = culture positive; penicillin sensitive strain
 N = nose
 T = throat

Table 53. Pneumococci Present Among the Home-delivered
Babies Observed in the Preliminary Study

Baby No.	- Weeks of Age -											
	$\frac{1}{2}$	1	1 $\frac{1}{2}$	2	3	4	6	8	11	13	15	25
160 N		0	0	23	23	23	23	23	0	0	0	
T		0	0	23	0	0	23	0	0	0	0	
161 N	0	0	0		0	0	0				0	nt
T	0	0	0		0	0	0				0	0
162 N	0	0	23			23	23					
T	0	0	0			23	0					
163 N	0	0	0	0								
T	0	0	0	0								
164 N	0	14										
T	0	0										

0 = culture negative

Numbers indicate serologic type of positive cultures

nt = culture positive, but not typed

N = nose

T = throat

pneumococcus, not serologically typed, in the last culture obtained in its twenty-fifth week of age. None of the babies are known to have had any upper respiratory illness.

Coliforms were rarely found in the upper respiratory tract of these babies. When present, they never occurred in large numbers and did not appear to persist.

Beta haemolytic streptococci and H. influenzae were not observed.

Discussion of preliminary observations

The number of babies available for this preliminary study obviously had been too small to permit conclusions to be drawn. However, the failure of Staphylococcus aureus to colonise the upper respiratory tract of three of the babies suggested that unless it becomes established soon after birth, it is unlikely to do so later. This observation in particular, was the stimulus for a second attempt to collect further information.

OBSERVATIONS ON BABIES BORN IN HOMES OF THE
EALING AREA

The failure of Staphylococcus aureus to become established in the upper respiratory tract of three of five home-delivered babies seen in the preliminary study, suggested that a longer series of observations on a larger group of domiciliary deliveries would be worth while. Satisfactory arrangements for such a study finally were obtained through splendid co-operation from the Ealing Area Public Health Department, London, W.5., and the laboratory of King Edward VII Memorial Hospital, Ealing.

PROCEDURE

Thirty-six home-delivered babies were placed under observation between January 4 and June 23, 1964. Of these, eight were observed only during their first week or two of life, but the other 28 were observed until they were between 15 and 25 weeks of age.

All the swabs were collected by the midwives of the Ealing Area Public Health Department. Each baby was swabbed by the same midwife throughout its entire period of observation except when a long illness in one midwife necessitated another completing her task. Generally, swabs were collected three times between the third and twelfth day after birth, and then every two to four weeks thereafter. As in the study on the babies born in Paddington Hospital, both per-nasal and throat swabs were

obtained. They were inoculated directly onto blood agar plates, with and without gentian violet, which were delivered to the Ealing laboratory by late afternoon. After overnight incubation they were returned to the Wright-Fleming Institute for bacteriological examination.

All of the bacteriological technique was identical with that used for the study on the hospital-born babies, and described in Part II. Approximately 290 coagulase-positive staphylococci were collected, of which 254 were phage typed. The typing phages used were the same as before, except that phage 71 was now included in the primary routine test dilution set, and phage 44 replaced it in Pool B.

The staphylococcal phage patterns obtained were assigned the following code numbers:-

Group I:

IA - 52A/79 or 52/52A/79
IB - 29/52/52A/73/79 plus other weak reactions
IC - 29/52/52A/79
ID - 29/52
IE - 29/53/79
IF - 29/52/52A/79, "B" pool, plus other weak reactions.

Group II:

IIA - 3B/3C/55 or 3C/55
IIB - 3B/55/71 or 55/71
IIC - 55

Group III:

IIIA - 42E
IIIB - 53, plus other weak reactions (47/77)
IIIC - 70 (weak)
IIID - 6/54/70, plus other weak reactions (7/47/73/75)
IIIE - 6/7/47/53/54/75
IIIF - 47/75

Non-classifiable:

NC - 52/53+

Untypable: UT

To avoid an unnecessarily complicated code, the system adopted here is very similar to that used previously. It should be noted that these code numbers do not correspond entirely to those used to describe the strains encountered among the hospital born babies; to make this distinction more apparent, upper case letters have been assigned to the sub-groups in this second code system. Many of the strains obtained from the home delivered babies had phage patterns quite different from those present among the hospital-born group, but even in instances of similarity, no identity of strains can be assumed.

OBSERVATIONS

Section A. Staphylococcus aureus

Incidence of Staphylococcus aureus among the newborn babies:

Of the 36 newborn babies observed, 26 (72%) acquired Staphylococcus aureus in the nose, and frequently in the throat, within the first two weeks of life. Among the 28 babies who were later followed 18 (65%) were carriers during their first two weeks. The incidence of Staphylococcus aureus during their neonatal period is shown in Table 29 and in Figure 9. The nasal carrier rate remained somewhat higher than the throat carrier rate until near the end of the month at which time they were approximately the same.

Incidence of Staphylococcus aureus among the older babies:

The incidence among the babies as they became older is shown in Table 29 and in Figure 10. The nasal carrier rate declined steadily, reaching a level of about 10% by the twentieth week. However, the throat carrier rate continued to increase until about the eighth week, after which it decreased slightly, but did not fall below 40%.

Table 29. Incidence of Staphylococcus aureus in the
Home-born Babies

<u>Age - Weeks</u>	<u>No. of Babies</u>	<u>Nose positive</u>	<u>%</u>	<u>Throat positive</u>	<u>%</u>
$\frac{1}{2}$	27	11	41	6	22
1	27	16	60	9	33
1 $\frac{1}{2}$	21	10	48	9	43
2	15	12	80	5	33
3	21	12	57	12	57
4	22	10		12	
5	14	9		10	
6	11	5	45	6	54
7	18	7		7	
8	10	4		7	
9	17	8		9	
10	8	2	47	4	55
11	16	10		8	
12	9	1		5	
13	12	6		4	
14	7	2	27	4	47
15	13	2		6	
16	9	2		5	
17	2	0		3	
18	7	1	11	3	43
19	13	1		5	
20	9	1		4	
21	12	1		4	
22	10	2	10	4	39
23	8	0		3	
24	3	1		1	

(The results of two consecutive swabbings on the same baby are sometimes included in the incidence calculated for the four-week periods).

FIG.9. STAPH. AUREUS : INCIDENCE IN HOME-BORN BABIES DURING THE FIRST MONTH OF LIFE.

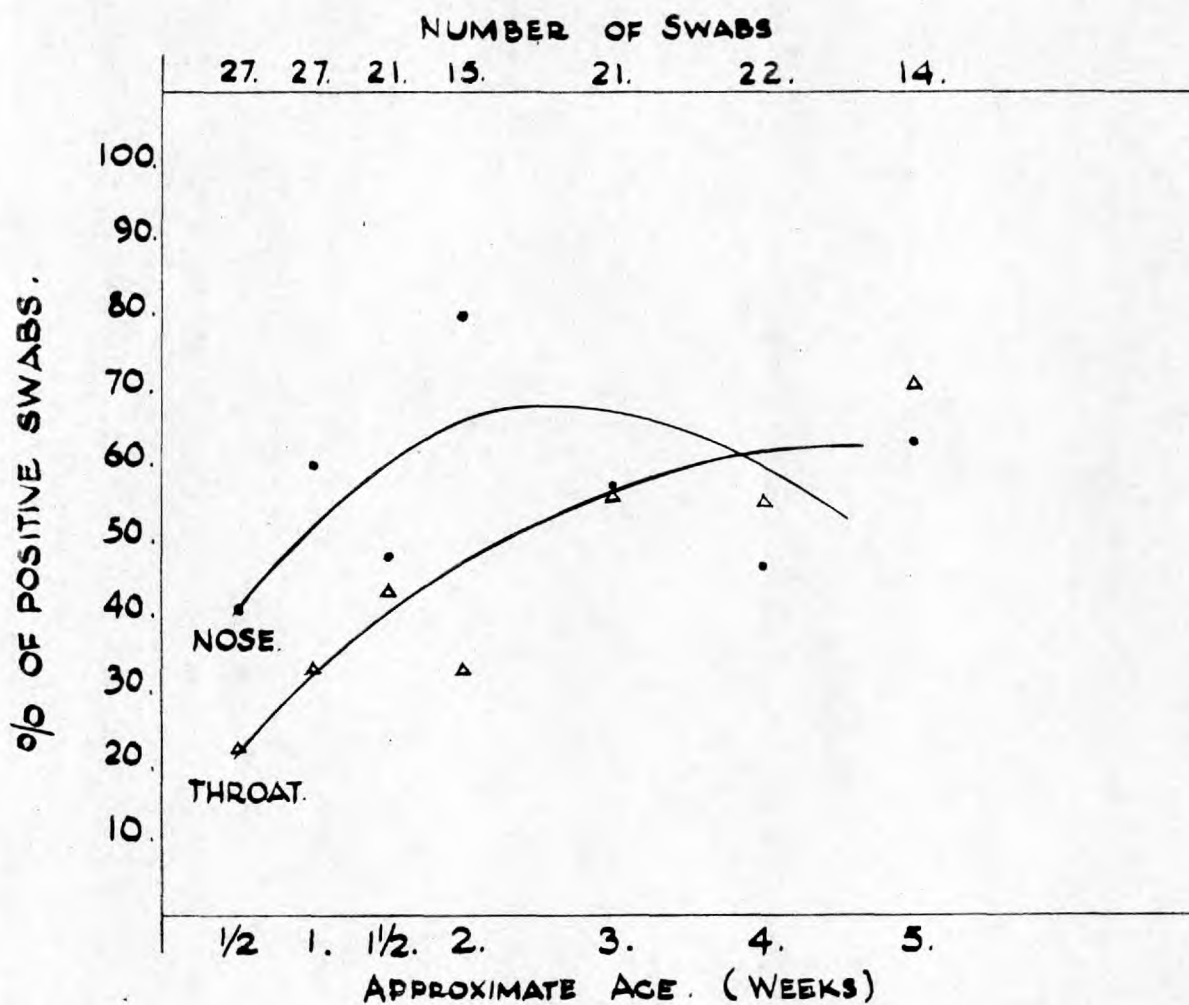
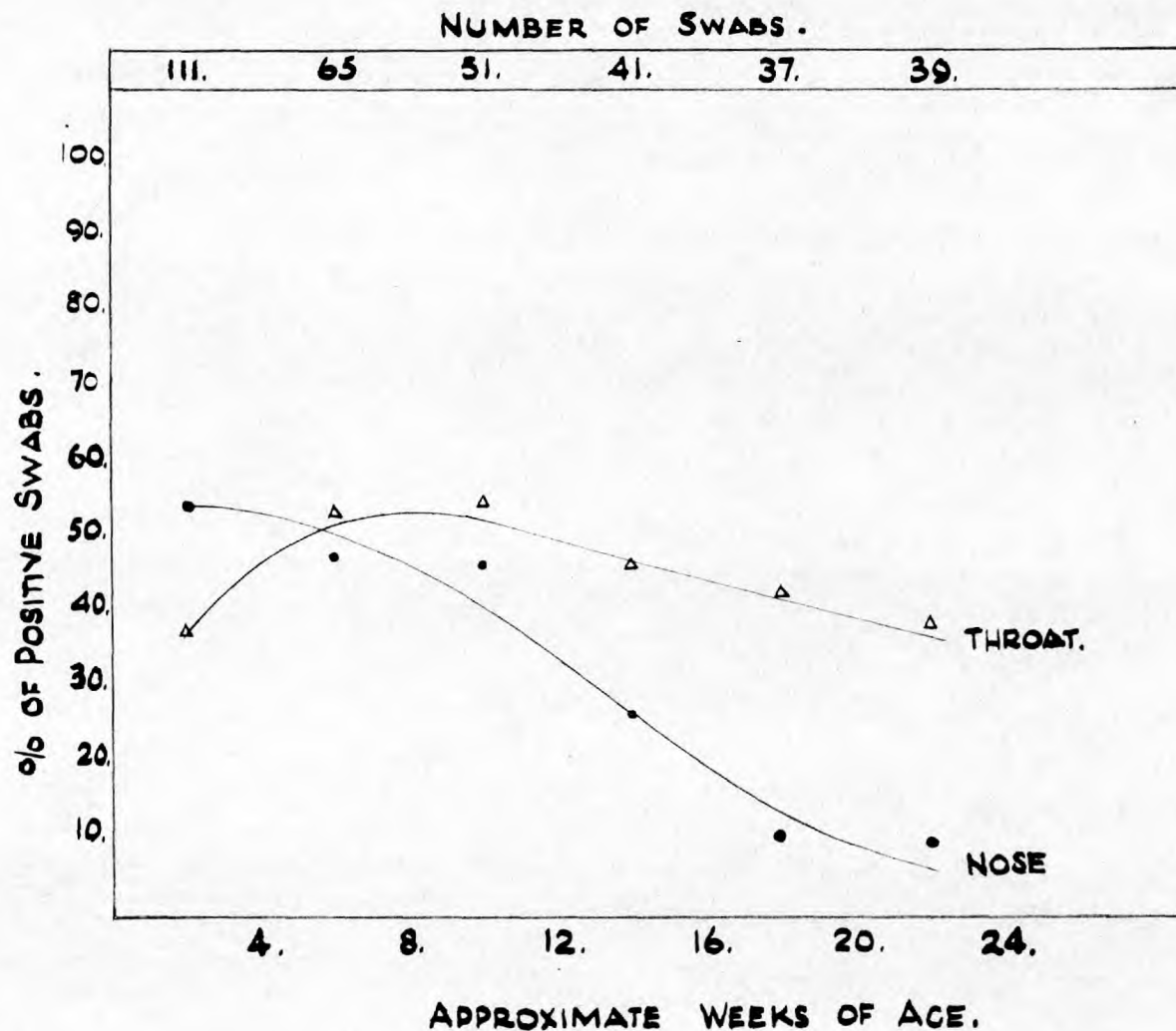


FIG. 10. STAPHYLOCOCCUS AUREUS : NASAL AND THROAT CARRIER RATES AMONG BABIES BORN AT HOME.



Carrier characteristics of the older babies:

Examining the records of the individual babies, it was found that the 28 who had been followed could be classified into three groups:

	Number	%
a) "early carriers" who acquired <u>Staph. aureus</u> within two weeks after birth, and continued to carry it.	18	64
b) "late carriers" who did not acquire it immediately, but became carriers within their first six weeks of life	5	18
c) "non-carriers" who never had <u>Staph. aureus</u> in their cultures, or only occasionally had a few colonies.	5	18

The records of these three groups of babies are shown in Table 30 (cover pocket). Most of the cultures collected from the 23 carriers were phage typed, as were all those occasionally recovered from the non-carriers.

The "early carriers" tended to follow the same carrier pattern as had the hospital-born babies.

Staphylococcus aureus usually was first acquired both in the nose and throat, and some babies later ceased to carry it in the nose while continuing to carry it in the throat. One baby (No. 201) appeared to be a nasal carrier entirely, but probably this was due to the fact that its throat cultures usually were overgrown with coliforms. Fourteen of the 18 "early carriers" carried a single strain, as judged by phage typing, throughout the period they were observed. The other four (Nos. 168, 175, 183 and 190) appeared to carry two strains, both of which were present

in the early weeks of life. It is probable that the two strains were carried simultaneously, although this was not detected because only one colony per culture was sampled.

The five "late carriers" were somewhat different from the early carriers in that two of them (No. 167, 182) had Staphylococcus aureus in their throat alone while their nasal culture remained entirely negative. One "late carrier" (No. 180) had just three intermittently positive cultures, and these contained only a few colonies. Except for the fact that the same phage type was present in all three, it might be argued that this baby should have been classed as a "non-carrier".

A few positive cultures occasionally occurred among four of the five babies classified as "non-carriers". When several were obtained from the same baby, they usually proved of different phage type, and therefore seem to have been transients, or contaminants. One baby (No. 191) had the same phage type in two consecutive swabs, but as only a few colonies were present in each, it is debatable whether the strain was carried for a short time, or was a chance contaminant occurring twice. The flora of the "non-carriers" did not seem to differ from that of the rest of the babies in respect to organisms other than Staphylococcus aureus.

Possible sources of Staphylococcus aureus

The study was not designed to determine the source of the babies' staphylococci, but the data permit some consideration of this subject.

To determine whether the positive cultures might be associated with breast feeding, the babies' carrier and feeding records were compared (Table 31). Thirteen of the carriers were partially or wholly breast fed throughout, so their staphylococci might have been derived continuously from the maternal breast. However, several facts indicated that the carrier state probably is independent of breast feeding:

- a) Four babies continued to be carriers for many weeks after they were fully weaned (i.e. no longer breast fed).
- b) Two of the carriers were artificially fed from birth.
- c) All of the non-carriers had been breast fed for nearly half the period they were observed, and one was breast fed the entire time.
- d) Four babies ceased to be carriers while still being breast fed.

It was possible, of course, that the carriers could have acquired their staphylococci from the midwives as suggested by Ludlam (1953). Although the midwives themselves were never swabbed, babies cared for by the same midwife might be expected to carry the same phage type had they obtained them from her.

**Table 31. Relationship between Staphylococcal Carriage
and Type of Feeding**

(a) Babies continuing to be carriers after being fully weaned:

<u>Baby No.</u>	<u>Approximate age when fully weaned</u>	<u>Known carrier period (weeks)</u>
172	3 weeks	22
173	2 months	23
189	3 "	19
195	6 weeks	16

(b) Carriers artificially fed from birth:

<u>Baby No.</u>	<u>Known carrier period (weeks)</u>
182	*5 - 22
190	24

(c) Non-carriers:

<u>Baby No.</u>	<u>Approximate age when fully weaned</u>	<u>Period observed (weeks)</u>
174	4 months	23
176	2½ "	23
177	2 "	23
185	20 weeks	21
191	3 months	20

(d) Babies ceasing to be carriers while being breast fed:

<u>Baby No.</u>	<u>Approximate age when fully weaned</u>	<u>Known carrier period (weeks)</u>
170	> 23 weeks	17
186	21 "	15
187	> 20 "	13
192	> 19 "	15

(Continued on next page)

Table 31. (continued)

(c) Babies breast fed during their entire carrier period:

<u>Baby No.</u>	<u>Approximate age when fully weaned</u>	<u>Known carrier period (weeks)</u>
166	23 weeks	24
167	> 23 "	4 - 23
168	> 24 "	22
169	> 23 "	22
176	> 23 "	23
178	> 22 "	6 - 20
179	> 22 "	22
180	16 "	6 - 14
183	> 22 "	3 - 22
184	? 8 "	? 9
186	13 "	17
200	> 16 "	16
201	> 15 "	16

* Carrier period began within the first two weeks of life unless otherwise stated.

The data (Table 32) contained no indication that the midwives were entirely responsible for the babies' staphylococci, for in no case did all of the babies swabbed by the same midwife have the same type. Usually when babies delivered by the same midwife had staphylococci with similar phage patterns these were also present among babies cared for by other midwives (e.g., IA, IIA, IIB). Phage types IB, IIC and untypable strains (UT) were found only among some babies delivered by midwives Sau, Wes., and Mur., respectively. While these strains therefore may have been transmitted by these midwives, the association could have been due to chance because IB and IIC are fairly common phage patterns.

Strains of Staphylococcus aureus present among the home-born babies

The incidence of staphylococcal phage types found among the babies is shown in Table 33. As judged by the differences in phage pattern and penicillin sensitivity, there were at least seventeen different strains in addition to those untypable. Four appeared only as transients. Strains having phage patterns of Groups I and II were most common, and IIA was the subgroup of most frequent occurrence.

The majority of strains were sensitive to 0.25 μ /ml. penicillin. These were obtained from 24 of 28 babies (87%), 21 of whom carried them. Two of the babies, and perhaps

Table 32. Distribution of Staphylococcal Phage Types
Among Babies Delivered by the Same Midwife

<u>Midwife</u>	<u>Baby No.</u>	<u>Phage Types Carried</u>	<u>Phage Types Not Carried</u>
Ald.	180	IIA	
	186	IIIC	
Btm.	166	IIA	
	170	IIB	
	175	IIB, IC	
	178	* IIB	
	179	IA	
	185	-	IIID, IA
Bft.	168	IIA, IIIA	
	174	-	* IA
	184	* IA, IA	
	190	* IA, IIA	
Mur.	167	IIA	
	176	-	
	177	-	IIIF
	182	UT	
	195	UT	
Sau.	169	IB	
	172	IB	
	189	IC	
	201	not typed	
Svg.	183	IIB	
	191	-	* IF, ID, IIB
	192	IC	
Wes.	173	IC	
	187	* IA	
	188	IC, * IIIE	
	200	IE	

* Penicillin resistant strains

Table 33. Incidence of Staphylococcal Strains Among
23 Home-born Babies

Strains carried:			Strains not carried:	
<u>Code</u>	<u>Number of carriers *</u>	<u>% of 23 carriers *</u>	<u>Code</u>	<u>Number of Babies</u>
IA	2	44	IA	1
**IA	3		**IA	1
IB	2		ID	1
IC	2		**IF	1
IE	1		IIB	
IIA	5	44	IIID	I
IIB	3		IIIF	I
IIC	2			
IIIA	1	17		
**IIIB	1			
IIIC	1			
**IIIE	1			
Non-classifiable	1	4		
Untypable	2	9		
Not typed	1	4		

* Several babies carried two strains (see Table 32)

** Penicillin resistant strains

a third (No. 184, discussed below) carried penicillin resistant strains in addition to the penicillin sensitive ones. Penicillin sensitive and penicillin resistant strains were also isolated from one non-carrier.

Penicillin resistant strains were obtained from 7 of the 28 babies (25%), only 5 of whom carried them (18%). Those isolated from two babies appeared to be transients. One baby (No. 184) had a penicillin resistant strain of phage pattern IA in its first cultures, but its later cultures of the same phage pattern were penicillin sensitive. These may have been different strains, but it seems possible that the original strain may have lost its resistance to penicillin. Interestingly, the penicillin resistant cultures from this baby retained their resistance during several months of storage (without subculture). All of the penicillin resistant strains belonged to four subgroups within Groups I or III; no penicillin resistant strains of Group II were encountered.

Comparison of staphylococcal carriage by babies born at home with those born in hospital

The incidence of staphylococci among the home-born babies, as compared with that of the hospital-born babies is shown in Table 34. During the first month of life the number of positive nasal and throat swabs was much lower among the home-born babies, the difference being

highly significant ($P = < .001$). Between the fourth and fifth month there still was a considerable difference in the number of positive nasal swabs, also significant ($P = < .01$). The difference between the number of positive throat swabs, however, was small and not significant.

Table 34. Comparison of incidence of Staphylococcus aureus in Babies Born at Home and in Hospital.

<u>Age Group</u>	<u>Place of Birth</u>	<u>No. of Swabs</u>	<u>Positive Nose Swabs</u>	<u>Positive Throat swabs</u>
0 - 4 weeks	home	111	61 (55%)	41 (37%)
	hospital	111	105 (95%)	85 (77%)
16-24 weeks	home	76	8 (10%)	31 (41%)
	hospital	85	24 (28%)	47 (55%)

$\chi^2 = 44.17$
 $P = < .001$

$\chi^2 = 33.94$
 $P = < .001$

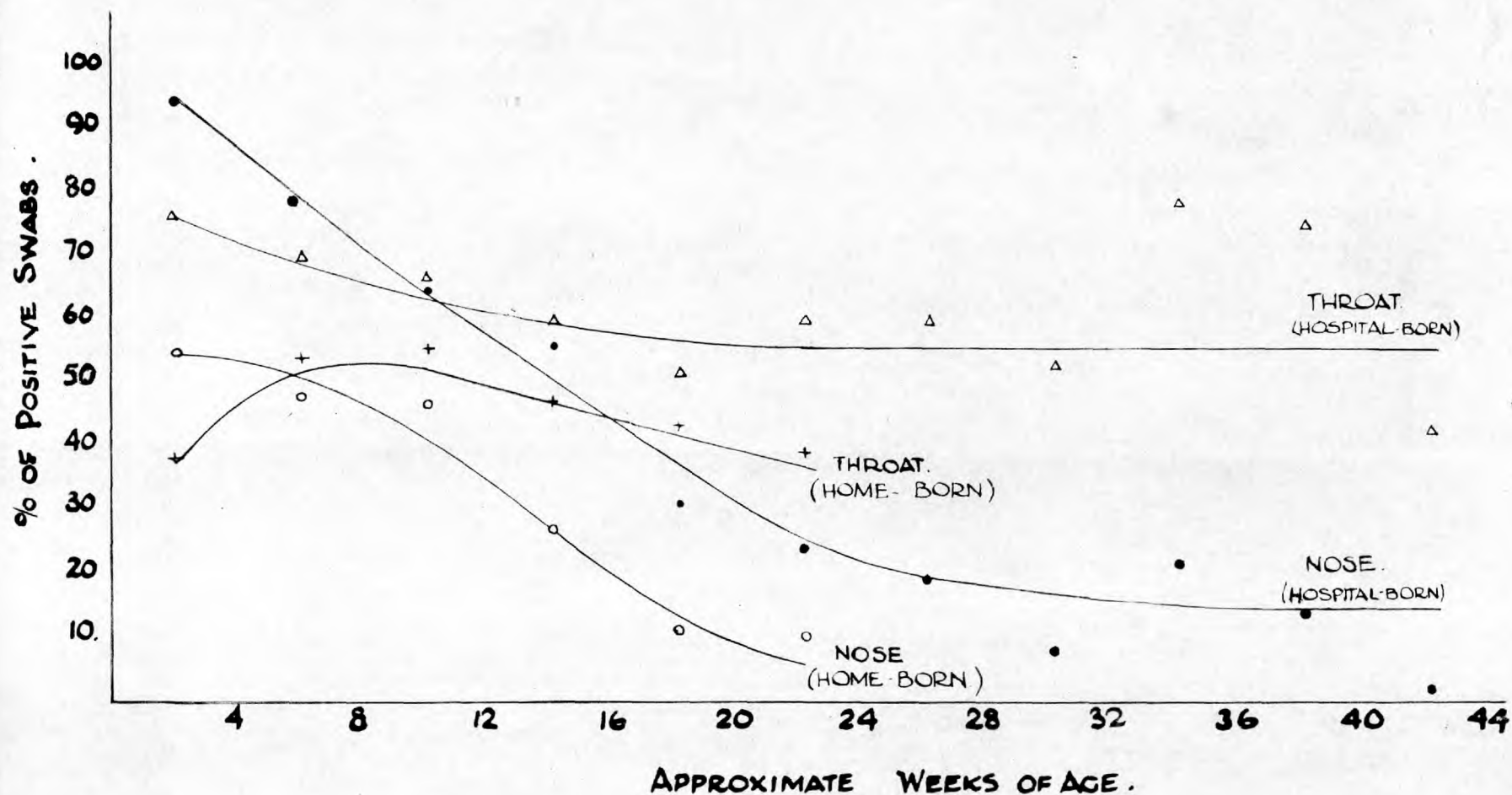
$\chi^2 = 6.80$
 $P = < .01$

$\chi^2 = 0.58$
 $P = \text{approx. } 0.80$

In relation to age, the nasal and throat carrier rates of the home-born babies seemed to follow the same general trend as those of the hospital-born group. This was particularly true of the nasal carrier rates which declined in nearly even proportion to one another between the fourth and twenty-fourth weeks (Figure 11). The throat carrier rate of the home-born babies reached its maximum more slowly than that of the hospital-born babies, but both tended to become relatively stable with only a slight decline after about the eighth week (Figure 11).

The incidence of penicillin resistant strains was

FIG. II. NASAL AND THROAT CARRIER RATES OF STAPHYLOCOCCI AMONG THE HOME AND HOSPITAL BORN BABIES.



markedly lower among the home-born babies than among those born in hospital. Whereas 97% of the hospital babies were discharged carrying penicillin resistant strains, only four (14%) of the home-born group were carriers of penicillin resistant strains within their first fortnight, and just five (18%) acquired them ultimately.

There were fewer carriers of multiple strains among the home-born babies than among those hospital-born (Table 35). Two different strains were obtained from only a few of the home group, and none carried more than two strains. Of the hospital group, the majority (70%) carried two or more strains although, as discussed previously, it was not always clear that each baby had acquired all its strains from the hospital.

Table 35. Incidence of Multiple-strain Carriers Among the Home and Hospital Groups.

	Home		Hospital	
	Number of carriers	% of Total (23) Carriers	Number of carriers	% of Total (37) Carriers
Two strains	4, perhaps 5	17-22%	14	38%
More than two strains	0	0	12	32%

Although no absolute comparison can be made between the strains of staphylococci encountered in the two studies, their distribution in the broad phage pattern categories were quite different (Table 36). The great majority of the hospital strains had phage patterns of Group I, III or were untypable and Group II were rare. Among the home-born babies, Group II were more numerous and Groups I and III were less frequent.

Table 36. Comparison of Staphylococcal Phage Groups Carried by Hospital and Home Born Babies.

Phage Group (Williams & Rippon) 1952	Incidence	
	% of 34 hospital-born babies	% of 23 home-born babies
I	74	44
II	6	44
III	50	17
Nonclassifiable	12	4
Untypable	47	9
Not typed	-	4

DISCUSSION

The relatively lower incidence of Staphylococcus aureus among home-delivered babies during their first few weeks corroborates the findings of other workers. Ludlam (1953) found the nasal carrier rate among 57 (59.3%) two-week old babies born at home to be very similar to that obtained in this study during the neonatal age. Cunliffe (1949) obtained a figure which was very low (12.5%) but it was based on a small group of just 8 babies. Neither of these reports, however, demonstrated clearly that the nasal carrier rate of home-delivered babies continues to be low during the later months of age. Although Ludlam found a significant difference between home and hospital-born groups aged five months, the difference in such groups aged two months, and 12-24 months was not significant. Such inconsistencies may be expected when small samples drawn from non-identical populations are compared. In the present study the nasal carrier rate of the home-born babies remained significantly lower than that of the hospital-born babies throughout the period of observation. This did not apply to the throat carrier rate which was not significantly lower than that of the hospital-born group after the first month of age. It was first thought that the predominance of throat carriers among the home-

born babies late to acquire their staphylococci might have explained the relatively high throat carrier rate after the first month. Examination of the data, however, showed that they had not affected it appreciably. The throat carrier rate curve of the home-delivered babies resembled that of the hospital-born group in that it tended to level off at the second month, with only a slight decline.

The babies who acquired their staphylococci early had essentially the same carrier characteristics as those born in hospital, and again demonstrated that after the first few months staphylococci tend to be lost from the nose, but retained in the throat. Although the number of "late carriers" and "non-carriers" was small, they seem to indicate certain important generalisations:

1. Babies who do not acquire their staphylococci until about the end of their first month are likely to acquire them in the throat alone.
2. Babies failing to become carriers within the first two months are likely to remain non-carriers during their first six months, and perhaps longer.

These tendencies are in harmony with those exhibited by the "early carriers" who have demonstrated that:

1. the nasal mucosa often seems to become refractory to staphylococci, beginning about the first month,
2. staphylococci acquired during the first month are more likely to persist in the throat than in the nose,
3. the throat does not seem to acquire new strains, in addition to those neonatally established, during later months.

It may be speculated that if babies could be protected from staphylococcal contact during their first six weeks they would not become carriers until much later in life. Cunliffe (1949) has presented data indicating that the nasal carrier rate of children increases only slowly with advancing years. It would be extremely interesting to know what proportion of the child population who are carriers are those who have retained them from early infancy.

The lower incidence of penicillin resistant staphylococci among the home-born babies was as to be expected. Ludlam also found relatively few penicillin resistant strains among home-delivered babies, although his figures were somewhat higher than that obtained here. Penicillin sensitive strains seemed to be carried just as long as the penicillin resistant ones, indicating that penicillin resistant strains

are not particularly adapted to establishment on the mucosa.

The staphylococcal phage patterns of the home-delivered babies, contrasted with those of the hospital group, correlates well with what is known, generally, of strains present in comparable environments in Britain. Williams and co-workers have found Group III strains to be associated particularly with hospital cross-infections, and Groups I and III most numerous in neonatal infections. It is thought that strains of Group I and III are prevalent in hospitals because a high proportion of them are penicillin resistant. Group III strains, in particular, seem unstable, and most likely to give rise to antibiotic resistant mutants. Group II strains are least likely to be antibiotic resistant, and are commonly carried in the nose of healthy adults (Williams, Rippon and Dowsett, 1953; Barber and Whitehead, 1949; Barbour and Edwards, 1953). For these reasons it was not surprising that strains of Phage Groups I and III were most common among the hospital-born babies, and that Group II strains were numerous among those delivered at home.

SUMMARY

The incidence of nasal staphylococci among the home-born babies was significantly lower than that of the

hospital group throughout the first four to five months of age. During this period the decline in the nasal carrier rate paralleled that which had occurred among the hospital babies. The throat carrier rate of the home-born babies, although initially lower than that of the hospital group, did not remain significantly lower. The throat carrier rate curve of the home-born babies resembled that of the hospital group in declining only slightly during the 2-5 month age period.

Examination of the individual babies' records disclosed that while the majority became carriers within their first fortnight, the others either tended to acquire them in the throat alone, or remained non-carriers. It is suggested that if babies had no staphylococcal contact during their period of highest susceptibility (approximately the first six weeks) they might remain non-carriers until much later in life.

As in the hospital-born babies, staphylococcal carriage did not seem related to breast feeding, nor was there any clear indication that the staphylococci were acquired from the midwives. The phage patterns of strains from babies under care of the same midwife were generally dissimilar.

The incidence of penicillin resistant staphylococci was much lower among the home-born babies (18%) than among those hospital-born (97%). The home-delivered babies' strains also differed from the hospital strains in that those having Group II phage patterns were much more numerous, and Group III, I and untypable strains were less frequent. There was no evidence that the strains from the home environment were carried for shorter periods than those acquired from the hospital.

COLIFORM ORGANISMS OF THE RESPIRATORY TRACT

Number of sputa producing coliform organisms: 62.

Number of throat, nose and antral swabs
producing coliform organisms: 12.

These coliform strains were distributed as follows:

Coliform Types	Material	
	Sputum	Throat, nose & antral swabs.
Bact. coli type I	16 (25.8)	3 (25.0)
" " " II	2 (3.2)	1 (8.3)
Intermediate type I	15 (24.3)	1 (8.3)
" " " II	4 (6.4)	0 (0)
Bact. aerogenes type I	5 (8.1)	0 (0)
" " " II	0 (0)	3 (25.0)
Irregular types	20 (32.2)	4 (33.3)
Totals	62	12 (100)

(Percentages in brackets)

Section B. Coliforms

Coliforms were present in the throat cultures of three of the 36 (8%) newborns examined during their first fortnight of life. This was in contrast to 107 newborns in the Paddington Hospital Nursery, 41% of whom had coliforms in their upper respiratory tract by the time they were discharged. Such an apparently lower incidence of coliforms in the home-delivered babies supports Laurell's view that upper respiratory coliforms represent, primarily, a hospital cross-infection.

Two of the three newborns harbouring coliforms retained them consistently throughout the entire period of observation (15 and 23 weeks respectively). One had been breast fed until about its seventh week, and the other was breast fed entirely. Vomiting was not noted earlier in either. The third baby was among those not observed after the first two weeks.

All of the coliforms isolated from these three babies gave "IMViC" reactions of true E. coli. They were "rough" in that they agglutinated when suspended in acriflavin.

Section C. Pneumococci

Pneumococci were present in the nasal swabs of 5 (14%) of the 36 newborns within their first fortnight of life. These included Types 23 (one baby), 34 (two babies), and untypable strains (two babies). Although present in considerable numbers, they never occurred in more than one swab. Therefore they did not appear to persist, although in two babies the same type later reappeared after a lapse of about a month. It was noted that family members of four of five newborns yielding pneumococci were suffering from "colds" and one of the babies had a "cold" itself. On the other hand, no pneumococci were obtained from four other newborns whose family members had "colds", nor from three babies who themselves had "colds".

The 28 babies observed as they became older became pneumococcal carriers at very much the same rate as had those born in hospital (Figure 12). Although a higher proportion had acquired pneumococci toward the end of their observation period, the difference probably was not significant (Table 37).

FIG. 12. COMMENCEMENT OF PNEUMOCOCCAL CARRIAGE IN RELATION TO AGE .

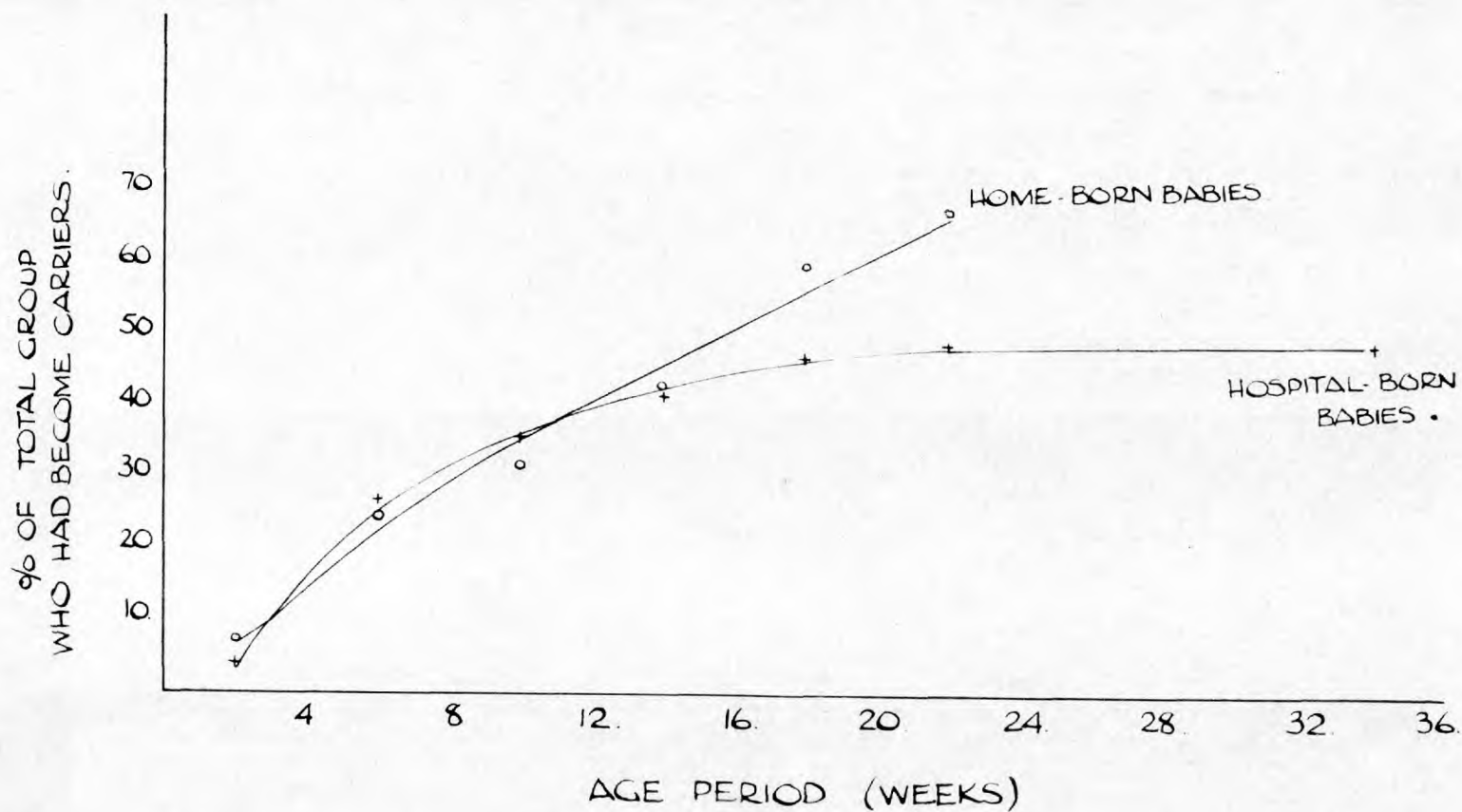


Table 37. Proportion of Babies Who Had Become Pneumococcal Carriers by 16-24 Weeks of Age.

<u>Place of birth</u>	<u>Carriers</u>	<u>Non-Carriers</u>	<u>Total</u>	
Home	19	9	28	$\chi^2=2.332$
Hospital	21	24	45	P=approx. 0.15

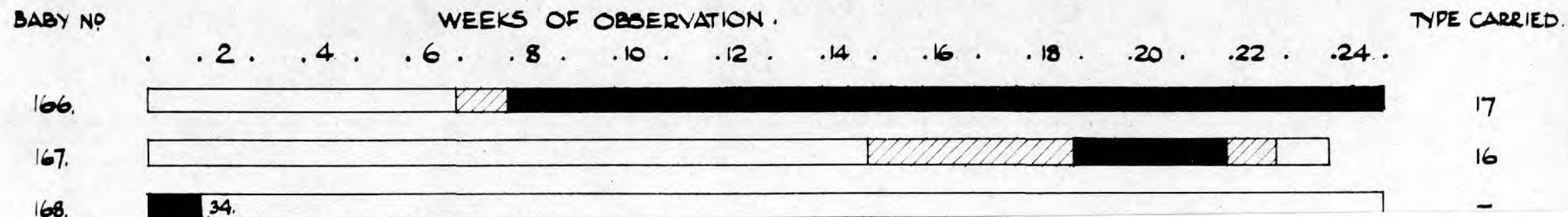
As in the hospital-born group, the older babies usually carried pneumococci for long periods, most commonly in the nose alone. The distribution of types encountered is shown in Table 38 and the carrier records may be seen in Figure 13.

Table 38. Pneumococcal Types Carried by the Home-born Babies

<u>Type</u>	<u>No. of Babies</u>	<u>Type</u>	<u>No. of Babies</u>
4	1	17	4
6	3	19	2
10	1	22	1
11	1	23	2
15	1	34 [*]	1
16	1	UT [*]	1
[*] untypable			

Except for types 6, 22 and 23, they were rather different from those obtained from the hospital-born group, as might be expected in small samples from different communities at different times. Information on the upper respiratory infections of these babies was fragmentary, unfortunately, but gave no indication of any particular association with

FIG.13. PNEUMOCOCCAL CARRIERS AMONG THE HOME-BORN BABIES.



their pneumococcal carriage.

DISCUSSION

The presence of pneumococci among 14% of these newborns agrees closely with the 15.2% incidence obtained by Landsman (1953) in her observations on home-delivered infants. However, she found pneumococci to become established in the newborns (personal communication), whereas in this study those obtained within the first week or two of life appeared to be transient. This may have been because 1) she had a larger group of newborns (99 babies) under observation, 2) the homes included in her survey were more crowded and unsatisfactory than those in this study. The present study contained some evidence that the presence of pneumococci among the newborns were associated with upper respiratory infections in the family. It seems likely that the newborn's acquisition of pneumococci depends, in part, upon exposure risk. This would explain the almost complete absence of pneumococci among hospital newborns, for as Crosse and Mackintosh (1953) have pointed out, home-delivered babies have more exposure to respiratory infection, through contact with unmasked visitors and small children, than do those in the hospital nursery.

SUMMARY

Pneumococci were obtained from 14% of the 36 home-delivered newborns, although they did not seem to become established until later infancy. There was some indication that the newborn's pneumococci were associated with respiratory infections among family members. As the babies became older, they acquired pneumococci at a rate very similar to that of the babies born in hospital.

Section D. Beta Haemolytic Streptococci and Haemophilii

Beta haemolytic streptococci occurred in only 2 of the 36 newborns examined, and in 8 (29%) of the 28 babies followed longer. The records of the babies in whom they were found are shown in Table 39. Group A occurred in just two, both of whom carried it for many weeks; one had acquired it during its first week of age. Strains not members of Groups A-G were carried for short periods.

Table 39. Beta Haemolytic Streptococci Occurring in the Home-Born Group.

<u>Baby No.</u>	<u>Known Carrier Period (Weeks)</u>	<u>Serological Group</u>	<u>Site</u>
167	10-12	Not A-G	Throat
172	7-9	A	Throat
	22-*	A	Throat
174	1-6	Not A-G	Throat
	10th only	G	Nose
176	21-23	Not A-G	Nose & Throat
185	21st only	Not A-G	Throat
187	21-*	Not A-G	Throat
188	1-9	A	Nose & Throat
191	18-20	Not A-G	Throat

* No further cultures obtained.

Non-haemolytic haemophili were never observed among the newborns, and were found in only three of the babies as they became older (Table 40). Only one baby appeared to carry them for a short time. Haemolytic haemophili were not found in any of the newborns. They seemed fairly common in the older ^{age} period, but their incidence was not recorded.

Table 40. Non-haemolytic haemophili occurring among the home-born group.

<u>Baby No.</u>	<u>Baby's age when isolated</u>	<u>Characteristics</u>	<u>Site</u>
166	8th week only	smooth, not Type a,b.	Nose
172	8th & 9th week; 20th week	rough rough	Nose Throat
187	5th week only	smooth, not Type a.b.	Nose

PART IV

MISCELLANEOUS OBSERVATIONS

SECTION A. FUSIFORMS

It is often stated that fusiforms are absent in the infant mouth, but the literature (Chapter 2) is not convincing. Therefore, in the period that arrangements were being made for the study described in Part II, nearly 70 babies' mouths were examined for fusiforms.

Procedure:

Babies of different ages were randomly sampled. They included newborns at St. Mary's Hospital nursery, foundlings of the Coram Nursery, and babies attending Welfare Centres in the Chelsea and Paddington districts of London. Swabs from the gums, tongue and buccal mucosa were either

- 1) rinsed in sterile broth which was inoculated into Robertson's chopped meat medium within a few hours, or
- 2) inoculated directly into the chopped meat. At intervals between two to seven days of incubation, Gram stained films of the meat medium were examined. When circumstances permitted, subculture of the fusiforms seen in the films was attempted. For this purpose, blood agar plates containing either gentian violet 1:20,000 or methyl violet 1:20,000 plus 0.1% cysteine hydrochloride, were incubated anaerobically for four to seven days.

At first, Gram and Giemsa stained films were prepared directly from the swabs, but they almost invariably proved negative even though another swab from the same baby yielded fusiforms in the chopped meat. Likewise, fusiforms were never recovered from swabs which were inoculated directly on plates incubated anaerobically. The general impression was that fusiforms usually are not numerous in babies' mouths, and that a primary enrichment medium is required for their successful cultivation. Cultivation of fusiforms from the adult mouth also is more successful if the mixed oral flora is first cultured in a liquid medium; strict anaerobiasis does not seem necessary when this procedure is used. (Pratt, 1927; Cruickshank and Cruickshank, 1931)

Results:

The results obtained are shown in Table 41. Fusiforms were cultivated from three newborn babies aged five, six, and nine days respectively. From one newborn, they were successfully isolated on an anaerobic plate. These were Gram negative rods, 10 to 15 μ in length, with sharply pointed ends, and appeared quite typical. Similar organisms were cultivated frequently from 16 babies under two months, and they nearly always were present among the babies over two months old. In some cases very long Gram negative filamentous forms were obtained in

the chopped meat medium. Subculture of either form was not always successful on the anaerobic plates, and the colonies were not always well isolated. The colonies varied in appearance, but in general corresponded to those which have been described in the literature. The cells varied from sharply pointed rods, to long slender filaments.

Table 41. Incidence of Fusiforms in Relation to Age and Dentition

<u>Dentition</u>		<u>Cultivated in</u> <u>Chopped Meat Broth</u>		<u>Subcultured on</u> <u>Anaerobic Plates</u>	
		<u>Number</u> <u>examined</u>	<u>%</u> <u>positive</u>	<u>Number</u> <u>examined</u>	<u>Number</u> <u>positive</u>
none	1-10 days	32	9%	2	1
	2 wks.-2 mo.	16	32%	5	2
	2 mo.-5 mo.	15	94%	12	8
1 to 3 incisors	6 mo.-8 mo.	6	100%	3	3

Discussion:

Elsewhere in this study (Part II, B) it was found that coliforms are common in the infant mouth. Some coliform strains, when grown under semi-anaerobic conditions

in liquid media, tend to produce long filamentous forms. The author now believes that Gram negative filaments, cultivated with the mixed oral flora in liquid media, should not be considered fusiforms unless 1) they are isolated and proved anaerobes, or 2) coliforms are demonstrated absent. Unfortunately, at the time this investigation on fusiforms was being made, the prevalence of coliforms in the infant mouth was not realised. Therefore, it is conceivable that some of the "fusiforms" found in the chopped meat, but not subcultured, may have been coliforms. That mistaken identity occurred frequently seems improbable. However, had it been a constant error, there can be little doubt about those isolated anaerobically from 58% of the babies in whom dentition was absent, nor from those with teeth.

Summary:

Gram negative rods and filaments, believed to be fusiforms, were cultivated almost invariably from the mouths of babies two to five months old, and sometimes from younger ones including newborns. Typical anaerobic fusiforms were isolated from the mouths of 58% of 18 babies without dentition, as well as from several having teeth.

SECTION B. DIPHTHEROIDS AND NEISSERIA

Kneeland (1930) observed diphtheroids and aerobic Gram negative cocci present in small numbers in the nose and mouth of newborn babies, and in greater numbers among those aged four to eight months. The general experience of the present study agrees with his observation. Large numbers of these organisms were not often noted until after the first month or two of age. Diphtheroids then tended to be most numerous in the nose, and Neisseria in the throat, but abundance of either in the alternate site was not unusual. The nasal diphtheroids often appeared to become most numerous after the nasal staphylococci had disappeared. It is possible, however, that they may have been numerous earlier, but were not observed because of the profuse growth of staphylococci.

SECTION C. NON-COAGULASE PRODUCING STAPHYLOCOCCI

When the cultures of Part II and Part III were examined for Staphylococcus aureus, white pigmented colonies considered "Staphylococcus albus" were also routinely recorded and tested for coagulase production. A few were found to produce coagulase, but the majority were negative. The non-coagulase producing strains tended to occur inconsistently, and in variable numbers, in both the nose and throat of the individual babies. In general, they tended to be more numerous among the babies during their first few weeks of life.

The nasal incidence among the babies born in hospital was about 50% during the first four weeks of life, and thereafter declined steadily to about 30% during the 20-24th weeks of their age. Among the babies born at home it was initially somewhat higher (65%) but thereafter was always lower and reached about 10% by their 20-24th week. At first, it was thought that the somewhat higher incidence among the home-delivered newborns might be associated with their lower Staphylococcus aureus carrier rate, but no evidence of this could be found in the data. Babies who failed to acquire Staphylococcus aureus, or who were late in acquiring them, did not seem to differ from the rest in the number or frequency of non-coagulase producing colonies. It is, of course, not possible to say that these strains were actually "carried" in the usual sense, particularly

in view of the heterogeneity of the group. The data definitely suggest, however, that the very young baby is more likely to harbour them than the infant approaching six months of age. Other workers have also noted non-coagulase producing staphylococci to be numerous in the upper respiratory tract of newborn babies (Chapter 2). Kneeland (1930), who defined "*Staphylococcus aureus*" and "*Staphylococcus albus*" on the basis of pigmentation only, reported the white pigmented strains to be present more frequently among babies under two weeks than among those 4 to 8 months of age.

SECTION D. MONILIAE

During the week of March 15, 1953 an outbreak of thrush began among infants in the premature nursery adjacent to Maternity Ward 10 at Paddington Hospital. On March 19, thrush was first noted in Ward 10's main nursery among twins who had been transferred from the premature nursery on March 9. Later in the same week two other babies developed thrush. All four babies' throat swabs, plated on Sabouraud's agar, yielded Candida albicans which produced chlamydospores in corn meal medium. It was also recovered from one baby's nasal swab. A fifth baby, just transferred from the premature nursery, yielded C. albicans. in its throat culture three days before clinical symptoms developed.

Upon recognition of their symptoms, all of these babies were removed from the nursery and treated with gentian violet. C. albicans was found in their Sabouraud plated swabs throughout the period of treatment. Neither the infection nor the gentian violet appeared to alter the rest of their oral flora, as judged by the growth on blood agar plates. C. albicans grew on the blood agar plates, but the colonies were small and few.

Three of the babies were discharged from hospital when their infections had cleared and before further swabs could be obtained. The twins, however, were observed to still harbour C. albicans three days

after their infection had subsided clinically. Eleven days later, when they reported to the Welfare Centre, no C. albicans were recovered from them, but their swabs were plated on blood agar only.

These observations, though limited, agree with those of Ludlam and Henderson (1942) that C. albicans may appear before the development of clinical thrush, and may persist after it has subsided.

SECTION E. STREPTOCOCCUS SALIVARIUS

Torrey and Reese (1944, 1945) found Streptococcus salivarius present infrequently in the newborn upper respiratory tract, and stated that it did not persist after the first few days. They believed that it was not "biologically adapted" for growth on the infant's immature mucous membranes. (Chapter 2.) As Streptococcus salivarius is present in the mouths of most adults, it was considered of interest to re-examine the report of Torrey and Reese, and to determine its incidence among infants somewhat older.

METHODS

The nose and throat swabs were plated on blood agar containing gentian-violet (1:500,000) and 5% sucrose. Large, pearly, mucoid colonies were recorded as Streptococcus salivarius.

OBSERVATIONS

Twenty-two newborn babies were examined for Streptococcus salivarius during their stay in the Paddington Hospital nursery. Fifteen, swabbed two or more times, had no Streptococcus salivarius. The other seven (32%) had moderate or large numbers in their throats, usually on both occasions they were swabbed. A few colonies were obtained from one baby's nasal culture on one occasion. The records of the seven babies yielding Streptococcus salivarius are shown in Table 42.

Table 42

Newborn Babies Having Streptococcus salivarius
in their Throats

Baby No.	Age (days)									
	3	4	5	6	7	8	9			
140				xx			x			
143	o			xx						
144			xxx			xxx				
145	xxx			o						
146	xx			x						
149	xxx						xxx			
155		x				xxx				

Number of Colonies

o = none

x = < 10

xx = < 50

xxx = many

Twenty-four older babies were examined for Streptococcus salivarius between their 15th and 49th week of life. It was present in the throats of all of them, usually in moderate or large numbers, and occasionally a few were also present in the nasal culture. Those babies on whom several or more swabs were obtained usually had them in every swab, although they were obtained intermittently from a few babies.

DISCUSSION

These observations concur with those of Torrey and Reese that Streptococcus salivarius is not always present in the newborn throat. However, it was found more frequently than they reported, and did not seem to disappear after the first few days as they claimed. Most of the babies in whom it was present yielded it at the end of their first week of life, as well as in the earlier swab.

The presence of Streptococcus salivarius in all of the 24 older babies examined, indicates that it always becomes established early in life, if not immediately after birth.

SUMMARY

Streptococcus salivarius was found in the throats of 7 of 22 (32%) of newborn babies, and in all of 24 older babies between 15 and 49 weeks of age. Sometimes it also was present in the nasal cultures, although in small numbers. Streptococcus salivarius therefore appears to become established very early in infancy, if not immediately after birth.

GENERAL DISCUSSION

and

CONCLUSIONS

These studies indicate that the upper respiratory flora first established in infants tends to remain relatively constant for a considerable period of time. Stability of the upper respiratory flora is not a new concept. Bloomfield, in numerous ^{early} studies, pointed out that although various bacteria constantly are being introduced into it, the majority are eliminated rapidly and only the normal residents remain. He stressed the necessity for distinguishing between "permanent residents" and "transients", defining "transients" as those recovered in small numbers, inconstantly.

Experience in these studies has shown that Bloomfield's definition of "transients" must be revised. Organisms permanently established are not necessarily recovered in large numbers, nor regularly, as evidenced by the few colonies of staphylococci often obtained intermittently from the infant throat carriers. These might easily have been considered as transients, or chance contaminants, had not phage typing showed them to be the identical strain in sequential cultures.

Sequential cultures on the same individual proved an invaluable aid in the present studies. For example, coliforms in the throat cultures might have been dismissed as contaminants had they not been seen to occur so frequently in certain babies. The sequential cultures did not always regularly demonstrate the commensals scantily present, but this is not surprising. Swabbing is but a crude sampling procedure, in which organisms not numerous may easily be missed. Moreover, bacterial species present in small numbers may be overgrown, in culture, by those predominant. Selective media, undoubtedly, would aid in their more consistent recovery. However, such media have not been devised for many commensals, and may prove difficult because they include groups heterogeneous in their growth requirements. Even if more selective media were available, their use in the present studies would have been limited by practical considerations:

- 1) collection of the swabs at places distant from the laboratory necessitated their immediate inoculation,
- 2) the volume of swabs being handled demanded a minimum

of manipulations with them, 3) the number of media on which a single swab may be inoculated directly is limited.

From experience here, it seems advisable to consider all organisms cultured from the upper respiratory tract as possible residents, for the chance of recovering, with a swab, a few temporary contaminants is small. As Topley and Wilson (1946) point out, no region of the body provides virgin soil in which any new-comer may flourish. "It is just as impossible to ensure proliferation of a particular bacterial species by introducing it into the mouth, as it is to ensure a crop of a particular plant by scattering seeds in a field already occupied by a pre-existing plant-association."

The flora first established in the infant upper respiratory tract probably determines, to a great extent, the organisms which may reside there subsequently. Thus, chance encounter after birth, may mould the future individuality of a baby's flora. Possible evidence of this was found here in that 1) different staphylococcal

strains were maintained by different babies, 2) the infant pneumococcal carriers rarely changed their serological types, 3) twins did not share the same organisms entirely, and 4) some babies persistently harboured coliforms or pneumococci while others remained entirely free of them. Of course, local tissue factors and passively conferred immunity also may be determinants of such individuality.

The newborn's sterile upper respiratory tract obviously is highly susceptible to any organisms adapted to multiplication there. With the present prevalence of antibiotic resistant strains in hospitals, this now becomes a fact of epidemiological importance. The data obtained here show that penicillin-resistant staphylococci which babies acquire from the hospital nursery may be carried, and perhaps disseminated by them, for as long as a year. If the spread of antibiotic resistant staphylococci is to be controlled, it is imperative that measures be found to prevent the newborn's infection with them. Antibiotics used in maternity hospitals may, by altering the flora to which the newborn is exposed, give rise to many other

problems in the future. A possible illustration of this was found in the high incidence of coliforms present among the hospital-born babies, in contrast to their infrequency among the home-born group.

Fortunately, persistence of staphylococci on the infant nasal mucosa is of limited duration. This was clearly evident from these studies, and also has been reported by other workers. However, the infant throat was observed here to be a site favouring very persistent staphylococcal colonisation. Such a high frequency of infant throat carriers may be an important consideration in staphylococcal spread. Similar occurrence of staphylococcal throat carriers among older children and adults has not been reported, but the literature contains some evidence that they are not uncommon. The observations on the home-born babies suggest that a baby not infected by staphylococci early is unlikely to acquire them in later infancy. This, if confirmed, may have practical value in controlling the infant vector of staphylococcal infection.

Newborn infants have been claimed to differ from adults in having greater resistance to some upper respiratory bacteria, such as beta haemolytic streptococci, Streptococcus salivarius, pneumococci, H. influenzae, and "H. haemolyticus". It was found in these studies, however, that beta haemolytic streptococci, including Group A, may become established in the newborn, and occur fairly frequently among somewhat older babies. Streptococcus salivarius, also, were found in the mouths of many newborns, and in all older babies.

Evidence of newborn resistance to pneumococci remained somewhat equivocal. In the intensive search among the hospital newborns, pneumococci were almost never found. Among the homeborn group they were encountered more frequently, but did not appear to persist. However, the fact that they sometimes became established during the neonatal age period in both groups, suggests that some babies must lose any existent "immunity" very early. Perhaps had the home-born group included a larger number of babies, pneumococcal establishment in the newborn might

have been observed. Recent data of Landsman suggest that this is probable. On the other hand, the failure of certain babies to acquire pneumococci over a long period of time, may mean that they retained some sort of immunity.

These studies failed to reveal H. influenzae among the newborns, as have those of other workers. However, selective media have never been employed. General failure to find H. influenzae among the older babies, even when Fildes' agar and cooked-blood agar were used, suggested that it is uncommon in infancy. As observed by other workers, haemolytic haemophili were extremely rare in the newborn babies, while among the older babies they were common.

The possible effect of tooth eruption on the infant flora merits further investigation. It has been claimed that fusiforme, spirochaetes, "leptothrix" and other obligate anaerobes are absent until the teeth appear. Yet, during these studies, fusiforme were nearly always found in the mouths of babies over two months old, and were sometimes present in the neonatal age period. It is probable that the literature contains other fallacies on this subject, never thoroughly investigated.

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APPENDIX

Comparison of the Optochin Sensitivity Test with the
Bile Solubility Test for Identification of Pneumococci

Optochin (ethylhydrocuprein) is markedly inhibitory for the growth of pneumococci, Moore (1915) suggested that its inhibitory action was sufficiently specific to distinguish pneumococci from bile insoluble streptococci present in the normal mouth. It therefore seemed that optochin sensitivity might provide a convenient method for recognition of pneumococci in the present study. However, as Moore's observations were confined to a few strains only, it was felt that a series of comparative tests should be done before the optochin sensitivity method was substituted for the conventional bile solubility test.

Method:

Optochin sensitivity - A small area of blood agar plate was heavily inoculated with the culture to be tested. A sterile filter paper disk of standard size (diameter 5 mm.), moistened with a standard sized loop (diameter 3 mm.) of Seitz filtered optochin 1:500, was placed in the center of the inoculated area. After overnight incubation, the zone of inhibition was measured.

Bile solubility - 0.9 ml. quantities of an overnight Todd-Hewitt broth culture were placed into each of

two tubes. To tube 1, 0.1 ml. of sterile sodium taurocholate (10% in normal saline) was added; to tube 2, 0.1 ml. of sterile normal saline was added. The test was read after incubation for 1/2 hour in a 36°C water bath.

Results:

	<u>Bile Solubility</u>	<u>Optochin - Zone of Inhibition</u>	<u>No. of Cultures; Source</u>
Viridans streptococci	0	0 to 3 mm.	86; 22 babies
<u>Pneumococci:</u>			
Types 6, 14, 21	+	8 to 10 mm.	9; 4 babies
Type 36	+	" " "	1; 1 mother
Types 2, 3	+	" " "	2; Type collection
Untypable	+	6 to 8 mm.	2; 1 baby
Untypable	+	0	8; 4 babies

Viridans streptococci, (bile insoluble) were not inhibited by optochin. Typable pneumococci, and one untypable strain, were inhibited. However, 8 cultures of untypable pneumococci (bile soluble), obtained from four babies, were not inhibited by optochin.

Conclusion:

Although typable pneumococci are inhibited by optochin, some untypable strains are not inhibited. In a study of the normal upper respiratory flora, recognition

of untypable pneumococci is of potential importance.
The optochin sensitivity test was therefore not
adopted for routine use.

Effect of Calcium Alginate Wool Solvents on the Recovery
of Pneumococci

Soluble calcium alginate wool swabs have been reported to give higher recovery rates of staphylococci than the usual insoluble cotton wool swabs (Higgins, 1950). It was therefore thought that the method might be used to obtain higher recoveries of the upper respiratory flora. Pneumococci were used to determine the possible effect of calcium alginate wool solvents on some of the less hardy members of the upper respiratory flora.

Method:

An overnight blood broth culture of a recently isolated Type 11 pneumococcus was used for the tests. Ten-fold dilutions of the supernatant were made in nutrient broth. From these, final dilutions were made in broth containing the required concentration of the solvent. Plate counts were done according to the method of Miles and Misra (1938)

Results:

Trial No.	Control Broth	1% Sodium Hexametaphosphate		0.5% Sodium Citrate	
		pH 5.5-6.0	pH 7.0-7.5	pH 7.0-7.5	
1	39	28	28%		
2	52	31	40%		
3	95	69	28%	55	42%
4	49			15	70%
5	22		18	18%	14
Average		32%	30%	45%	

Figures given in the table are the average count of six 0.02 ml. aliquots.

Percentages are the % reduction:

$$\frac{\text{Control count} - \text{test count}}{\text{Control count}} \times 100$$

Conclusion:

Calcium alginate wool solvents, such as 1% sodium hexametaphosphate and 0.5% sodium citrate, appear to have a slightly bacteriostatic or bactericidal effect on pneumococci. While a bacteriostatic effect might be overcome by dilution of the solvent after the swab had been dissolved in it, this additional manipulation would make the method impractical if a large number of swabs were being handled. The method was not considered further, therefore, for the present purpose.

Methods used for Testing the Biochemical Reactions of the Coliforms

<u>Test</u>	<u>Culture Medium</u>	<u>Reagent</u>	<u>Remarks</u>	<u>References</u>
Indole Production	Peptone water	Ehrlich's rosindole	Cultures incubated at 30°C. and tested at 5 days	Hickie and McCartney (1950) Topley and Wilson (1946)
Methyl-red test	Glucose-phosphate broth	0.04% methyl-red		
Voges-Proskauer (Barritt modification)		α -naphthol and 40% KOH		
Citrate utilization	Simmons' citrate agar	- - -	Plates examined after 48 hrs. at 37°C.	Simmons (1926)
Gelatinase & H ₂ S production	Ferro-chloride gelatine	- - -	Observed for 60 days (room temperature)	Kauffmann (1951)
H ₂ S production	Levine's iron agar	- - -	Used to re-check doubtful reactions obtained with FeCl ₂ gelatine; incubated at 37°C.	Levine, et al., (1952)
Urease production	Urea broth	- - -	Incubated at 37°C., and observed daily for one week	Kauffmann (1951b)
Sugar fermentations	0.5% lactose, inositol or glycerol in peptone water with Andrade's indicator			

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Table 30. Staphylococcus aureus Occurring Among Babies Born at Home

		- Weeks of Age -																									
		1/2	1	1 1/2	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
"early carriers"																											
Baby No. 166	N	O	IIA	IIA	IIA				O		O		O		O		O		O				O		O		O
	T	O	IIA	IIA	O				IIA		IIA		O		IIA		O		IIA				IIA		O		IIA
168	N	O	IIIA			O	O			O		O		O										O		O	
	T	IIA	O			IIIA	IIIA			IIIA		IIA		O									IIIA		O		O
169	N	IB	IB	IB	IB	IB	X		IB				IB		IB				O		O	O	O		O		O
	T	IB	IB	IB	IB	IB	X		IB				IB		IB				IB		X	IB	IB		O		O
170	N	O	IIB	IIB	IIB	IIB	IIB			O		IIB		IIB		O		O		O		O		O		O	
	T	O	O	IIB	O	IIB	IIB			O		IIB		IIB		O		O		IIB		O		O		O	
172	N	IB	IB			O	IB	IB	IB		O	IB	O		IB				O		O		O		O		O
	T	O	IB	IB		IB	IB	IB	IB		O	IB	O		IB				IB		X		IB		IB		O
173	N	O	IIC	O		IIC						IIC		IIC		IIC		O		O		O		O		O	
	T	O	IIC	IIC		IIC						IIC		IIC		O		IIC		IIC		IIC		IIC		O	IIC
175	N	IIB	IIB	IIB	IIB	IIB	NC	NC		IIB		X		NC		X		NC				NC	X		O		IIB
	T	O	O	O	O	O	IIB	IIB		IIB		IIB		X		O		IIB				IIB	X		O		IIB
179	N	O	IA	IA	IA	IA	X		IA				O		O		O		O				O		O		
	T	IA	IA	IA	IA	IA	X		IA				IA		IA		O		O				O		IA		
184	N		O		IA	IA	IA	IA		O		IA									O		O		O		
	T		O		IA	IA	X	IA		X		IA									O		O		O		
186	N	IIIC	IIIC	O	IIIC	X	IIIC		O		O								O		O		O		O		
	T	O	IIIC	IIIC	IIIC	X	IIIC		O		IIIC				IIIC		IIIC		IIIC		O		O		O		
187	N	IA	IA	IA		IA		X		IA	IA	IA		X		IA		O		O		O		O		O	
	T	IA	IA	IA		IA		X		IA	IA	IA		X		IA		O		O		O		O		O	
188	N	IIC	IIC	IIC	IIIE			IIIE		IIIE		O		IIC		IIC		O		O		O		O		O	
	T	O	O	O	O			IIC		O		IIC		O		IIC		IIC		IIC		O		O		O	
189	N	IC	IC		IC	IC	O	O		O			O	O	O	O	O	O	O	O	O	O	O	O			
	T	IC	IC		IC	IC	O	IC		O			O	IC	O	O	O	O	O	O	O	IC	O	O			
190	N	IA	IA	IIA	IIA		IA	IA	IA	IA	X	IA		IA		IA								IA		IIA	
	T	O	O	O	O		O	IIA	IIA	IA	IA	O		O		O							O		O		O
192	N	IC	IC		IC	O	IC		IC		O			IC		O		O		O		O					
	T	O	IC	IC	IC	X	IC		IC		O			O		O		IC		O		O					
195	N	UT	UT			UT		X			UT				O		O		O								
	T	UT	UT			O		X			UT				O		UT		UT								
200	N	O	O	O	X		O		O		O		O		O		O		O		O		O		O		
	T	O	O	IE	IE		X		IE		IE		IE		O		IE		IE								
201	N	X	X	X		O	X		X		X		X		X		X										
	T	O	O	X		O	O		O		O		X		O		O		O								
"late carriers"																											
Baby No. 167	N	O	O	O	O	O	O		O		O		O		O						O		O		O		O
	T	O	O	O	O	O	IIA		IIA		IIA		IIA		X						IIA		IIA		IIA		IIA
178	N	O	O	O	O	O	O		IIIB		O		IIIB		O		O		O		O		O		O		O
	T	O	O	O	O	O	O		O		O		IIIB		IIIB		IIIB		O		O		IIIB		O		O
180	N	O	O	O		O	O		O		O		IIA				O		O		O		O		O		O
	T	O	O	O		O	IIA		O		O		O				IIA		O		O		O		O		O
182	N	O	O	O		O	O		O		O		O		O								O		O		O
	T	O	O	O		O	O		UT		O		UT		O								O		UT		O
183	N	O	O	O	O	IIB	IIB	IIB		IIB		IIB		IIB		IIB		IIB		IIB		IIB		IIB		IIB	
	T	O	O	O	O	O	IIB	O		IIB		O		O		IIB		IIB		IIB		IIB		IIB		O	
"non-carriers"																											
Baby No. 174	N	O	O		IA	O	O		O		O		O		O						O		O		O		O
	T	O	O		O	O	O		O		O		O		O						O		O		O		O
176	N	O	O	O		O			O		O		O		O				O				O		O		O
	T	O	O	O		O			O		O		O		O				O				O		O		O
177	N	O	O	O		O			O		IIIF		O		O				O				O		O		O
	T	O	O	O		O			O		O		O		O				O				O		O		O
185	N	IIID	O	O		O	O	O		O		O		O		O		O		O		O		O		O	
	T	O	O	O		O	O	O		O		O		IA		O		O		O		O		O		O	
191	N	O		IF		O	O	O		O		O		O				IIB		IIB		O		O		O	
	T	O		O		ID	O	O		O		O		O				O		O		O		O		O	

O culture negative

X culture positive, but not phage typed

penicillin resistant strain

Phage Pattern Code

Group I:

IA - 52A/79 or 52/52A/79
 IB - 29/52/52A/73/79+
 IC - 29/52/52A/79
 ID - 29/52
 IE - 29/53/79
 IF - 29/52/52A/79; "B pool", +

Group II:

IIA - 3B/3C/55 or 3C/55
 IIB - 3B/55/71 or 55/71
 IIC - 55

Non-classifiable:

NC - 52/53+

Untypable: UT

Group III:

IIIA - 42E
 IIIB - 53, plus other weak reactions (47/77)
 IIIC - 70 (weak)
 IIID - 6/54/70, plus other weak reactions (7/47/73/75)
 IIIE - 6/7/47/53/54/75
 IIIF - 47/75

