

AN INVESTIGATION INTO THE AEROBIC
AND ANAEROBIC BACTERIAL FLORA OF
NORMAL AND ILL/LOW BIRTHWEIGHT
NEWBORN BABIES

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Ph. D in the University of London

by

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ABSTRACT

The aerobic bacterial flora of the ear, upper respiratory tract, (nose and mouth), skin (hands), umbilicus, and rectum was studied for the first week of life in 37 normal infants and for the first month in 64 ill/low birthweight infants.

The numbers and types of organisms isolated from all sites immediately after birth were few, but in all infants the flora of the upper respiratory tract and rectum quickly became established. In normal infants streptococci predominated in the mouth and coagulase-negative staphylococci in the nose. In the first week of life, the faecal flora changed from an initial gram-positive flora to a gram-negative flora with a high incidence of Escherichia coli.

Gram-negative bacilli were significantly more common in the upper respiratory tract and rectum in ill/low birthweight infants than in normal infants, and the types of organisms acquired varied with the treatment of the infant. In 14 infants receiving antibiotic therapy, Pseudomonas aeruginosa and Klebsiella species predominated: 17 infants, not receiving antibiotics but nursed in incubators, were colonised with Ps. aeruginosa, Klebsiella species and E. coli; 33 infants were nursed in open cots and E. coli was the predominant gram-negative bacillus.

A ward regime including use of hexachlorophene was followed throughout this study and only 5.3% of normal infants and 1.6% of ill/low birthweight infants became carriers of Staphylococcus aureus.

Pyocine typing of Ps. aeruginosa and serotyping of E. coli

indicated that once acquired, specific types were usually retained by the infants for the duration of the study. The isolation of certain common types suggested that some form of cross colonisation occurred.

The anaerobic faecal flora of ill/low birthweight infants was also investigated, and the incidence of lactobacilli and bifidobacteria in relation to that of E. coli was much lower than that found in either bottle fed or breast fed normal infants.

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PART I

SECTION 1

REVIEW OF THE LITERATURE

COLONISATION OF THE NEWBORN INFANT

During interuterine life, the foetus is protected against infection by the foetal membrane and the chorionic villi; towards the end of pregnancy, the foetal membrane thins out and becomes an ineffective barrier to infection (Morison, 1963; Prevedourakis, Papadimitiou and Ioannidou, 1970). This physical barrier plus the wide anti-bacterial activity of amniotic fluid (Galask and Snyder, 1968; Bergman, Bercovici and Sacks, 1972), maintain a low incidence of interuterine infection.

Once the membranes rupture, organisms may ascend the genital tract and infect the foetus. The longer the membranes have been ruptured before birth, the greater the incidence of contamination of the infant (Glynn, Gain and Gillespie, 1967; Habel et al., 1972; Oman et al., 1972). Organisms may also be acquired by the infant from the mother during birth (Gareau et al., 1959; Tessier et al., 1972). The carriage rate of potentially pathogenic organisms such as Group B streptococci in the female genital tract was determined (Bergquist et al., 1971b) and the acquisition of streptococci Group B by infants during birth was reported (Bergquist et al., 1971a; Franciosi, Knosterman and Zimmerman, 1973; McCracken, 1973; Prakash, Ravidrau and Sharma, 1973), as a cause of neonatal infection; Group A streptococci infections have also recently been reported. (Geil, Castle and Mortimer, 1970).

The development of the bacterial flora of newborn has been investigated but in most reports the work was confined to either one area such as skin or upper respiratory tract or to one particular organism. The literature is reviewed under the following headings -

ear, upper respiratory tract, skin, umbilicus and rectum.

EAR

Scanlon (1971) considered that swabs taken from the external auditory meatus would reflect the level of contamination of the infant in utero and during delivery and therefore could be used as an index of neonatal sepsis. Six infants diagnosed as septic had positive aural swabs but 15 (52%) of the control infants also had positive swabs. Evans, Akpata and Baki (1973) found that the number of positive aural swabs increased over the first four days of life and by day four, 70% of normal infants and 68% of premature infants were colonised. Staphylococcus epidermidis was the most common isolate; the incidence of potential pathogenic organisms was low, by day 3 only 4% of infants had acquired Staphylococcus aureus and none of these infants were clinically infected.

These two reports indicate that the isolation of bacteria alone is not an adequate criterion for the diagnosis of neonatal sepsis but that the isolation of potential pathogenic organisms might provide useful information in the early diagnosis and treatment if the infant became clinically ill.

UPPER RESPIRATORY TRACT

The sterility of the upper respiratory tract of newborn infants has been a subject of much discussion and the percentage of positive cultures reported in the literature varied widely. Organisms present in small numbers would not be isolated unless enrichment techniques were used and then it would not be possible to make any quantitative assessment of the individual species. This technique

has not been used in most reports and the results report the predominant species isolated.

Bloomfield (1922) investigated the throat flora of eight infants at less than twelve hours of age and before they had been nursed, only one infant had a positive throat swab and this grew Staphylococcus albus and a diphtheroid, similar results were reported by Torrey and Reese (1945), Smith and Bloomfield (1950) and Ehrenkranz (1970). The throat flora of premature infants at birth was investigated by Torrey and Reese (1944) and Evans et al., (1970). In all reports the majority of infants had sterile upper respiratory tracts immediately after birth.

Colonisation of the throat occurred rapidly once the infant was nursed (Bloomfield, 1922; Kneeland, 1930; Torrey and Reese, 1945; Smith and Bloomfield, 1950); colonisation of the nose occurred but at a slightly slower rate than found in the throat.

Most authors agreed that the predominant organisms in the mouth were streptococci and in the nose, staphylococci. Little interest was shown in non-pathogenic organisms and the majority of reports were concerned with organisms such as Staph. aureus and gram-negative bacilli such as Escherichia coli and more recently Pseudomonas aeruginosa. A review of the early literature was made by Hurst (1954) who pointed out the difficulty in comparing results because the identification of Staph. aureus by the production of coagulase did not become a standard procedure until 1944 (Torrey and Reese). The identification of gram-negative bacilli also varied, some reports fully identified the organism but in other reports organisms were grouped together as coliforms or colon bacilli.

Results also varied with the exact site investigated (Box, Cleveland and Willard, 1961) and the time of year of the survey (Evans et al., 1970).

A low incidence of Staph. aureus was reported by Bloomfield (1922) who regarded the isolates as transients. Kneeland (1930) found a higher incidence of Staph. aureus in the nose than in the throat. Torrey and Reese (1944, 1945) reported incidence rates of over 50% in both normal and premature infants. The increase in the acquisition of Staph. aureus during the first week of life was investigated by Cunliffe (1949) who found that by days 7 - 14 the incidence rate in the nose was 96%. McFarland, Crone and Tee (1949) reported that in the mouth the rate was 27% by day 4. They also investigated the incidence of coliform bacilli in two units and found that a higher incidence was related to increased numbers of artificially fed infants and more crowded conditions.

This difference in incidence related to environment was reported for Staph. aureus by Hurst (1954) who found rates of 99% in infants in hospital but 72% in infants nursed at home. Coliform bacilli were isolated from more infants in hospital than infants at home and Hurst agreed with Laurell (1952b) that the presence of coliforms was primarily due to hospital cross-infection.

When antibiotics became available for general use in hospitals, there was a large amount of interest shown in the effect of these classes of compounds on the bacteriology of newborn infants.

Haffer, Neter and Rubin (1950) found that sulphonamides did not prevent the conversion of a gram-positive flora to a gram-negative one in children admitted to hospital. McCurdy and Neter (1952) investigated infants under 2 years of age and found that broad spectrum antibiotics reduced the number of infants acquiring a gram-negative flora. The effect of penicillin therapy on the colonisation of the throat in healthy newborn infants was reported by Smith and Bloomfield (1950) who found that although colonisation was delayed, penicillin did not alter the types of organisms later isolated except that non-haemolytic streptococci were not isolated. Stoppelman (1954) found that infants receiving antibiotics had a lower incidence of streptococci and gram-negative bacteria than infants not receiving antibiotics.

In the 1960s antiseptics such as hexachlorophene were widely used in the care of newborn infants. The use of chlorhexidine cream by the attendants and the wearing of gowns did not affect the colonisation rate of infants with Staph. aureus (Cook, Parrish and Shooter, 1958) but the introduction of hexachlorophene for bathing infants, and neomycin cream applied to the nostrils reduced the carriage rate to 8.1%. This decrease in incidence of Staph. aureus with the use of hexachlorophene has been widely accepted (Plueckhahn, 1973).

This decrease in the incidence of Staph. aureus was accompanied by an increase in the incidence of gram-negative organisms (Light et al., 1968) especially Ps. aeruginosa. Forfar, Gould and MacCabe. (1968) compared infection rates in two hospitals, one hospital used hexachlorophene for two periods of 1 year and 3 years over ten

years, the other hospital did not use hexachlorophene at all during this time. Their results agreed with Light et al (1968) that there was an increase in gram-negative infections.

The increased use of hexachlorophene, which selects a more gram-negative flora, and antibiotics which select a resistant flora, presents a potentially dangerous situation. Shallard and Williams (1965, 1966) found that infants admitted to hospital acquired a persistent gram-negative flora and this was more pronounced in infants receiving antibiotics. Gram-negative bacilli were found to be more common in premature infants than in term infants (Farmer, 1967). If these organisms were acquired in the premature baby unit they were more resistant than if acquired at birth and multiply resistant strains were not uncommon. A higher incidence of gram-negative organisms in ill/low birthweight infants was found in earlier work in this hospital (Davies et al., 1970) but in this report the majority of isolates of E. coli were sensitive.

In 1971 an increasing number of reports indicated that hexachlorophene was toxic to the central nervous system and the increased incidence of Staph. aureus when the use of hexachlorophene was limited by F.D.A. recommendations was reported by Dixon et al., 1973; Kaslao et al., 1973 and Kimbrough, 1973.

Staph aureus infections have also been controlled using bacterial interference (Shinfield et al., 1963; Ehrenkranz, 1970). In 1972 Houck, Nelson and Kay reported that 5.9% of

infants deliberately colonised with Staph aureus 502A (which inhibited colonisation with Staph aureus 52/52A/80/81) developed disease related to this organism and one (0.15%) infant died. However the authors thought that this technique represents a feasible method of controlling gram-positive infections.

Studies on the sources of bacteria which colonise the infant have been confined to organisms which have adequate typing methods. Phage typing of Staph aureus indicated that some strains are acquired from the mother and some from other mothers or hospital staff (Hurst 1954; Anderson, Coulter and Keynes, 1961; Wolinsky, Gonzago and Mortimer, 1962). Laurell (1952b) investigated the mode of spread of coliform organisms and found that transmission at birth or after birth via staff or infected equipment were the minor routes and that the major route was via dustborne or airborne organisms.

SKIN

Interest in the bacteriology of the skin of newborn infants has been predominantly concerned with the isolation of pathogenic organisms. Hardyment et al., 1960 reported the reduction in staphylococcal skin disease following the introduction of hexachlorophene. This was also reported by Plueckhahn (1961) who also found that although the incidence of gram-negative bacilli remained stable there was an increase in the numbers causing skin lesions. Forfar et al., (1963) reported similar findings.

In order to evaluate the role of hexachlorophene in the

alteration of the skin flora, it was necessary to determine the normal flora of the skin of infants (Sarkany and Gaylarde, 1967a). Staph albus was the predominant organism and the skin flora remained remarkably stable despite routine handling and washing. Hexachlorophene significantly reduced the incidence of these staphylococci after the first day of life and had a similar but less marked effect on the diphtheroid bacilli (Sarkany and Gaylarde, 1967b).

With the increased interest in hexachlorophene toxicity, Sarkany and Arnold (1970) investigated the effect of a single bath using hexachlorophene compared with regular usage and a control group who were bathed using a hospital soap. They found that for the first four days, the single bath with hexachlorophene produced a skin flora comparable with that of infants bathed daily with hexachlorophene but that after four days there was a sharp increase in the numbers of diphtheroids and non-pathogenic staphylococci. The isolation of streptococci was not affected by the regular or single use of hexachlorophene.

The origin of the skin flora has been investigated (Sarkany and Gaylarde, 1968). Infants born by Caesarean section usually had a sterile skin at birth whereas infants born by vaginal delivery with or without the use of forceps had a flora resembling the vaginal flora of the mother. This result suggested that the flora of the birth canal played an important role in the initial colonisation of the skin of the newborn infant.

UMBILICAL CORD

Infection of the umbilical cord stump is now rarely seen in this country, but before the introduction of aseptic techniques this was one of the major causes of neonatal death (Hurst, 1965). The rate at which the umbilicus became colonised was noted by Fairchild et al. (1958) who found that by the third day all infants were colonised and Staph aureus was the most common isolate. Jellard (1957) considered that the necrotic tissue of the umbilical cord stump produced an ideal culture medium for this organism and that it acted as a reservoir of infection. This was disputed by Laursen (1963) who phage-typed Staph aureus from the umbilical cord and the nares; in only 30% of infants were the phage types identical and this was evidence against auto-infection.

The reduction in the colonisation with Staph aureus on the introduction of hexachlorophene was reported (Gluck and Wood, 1961; Plueckhahn and Banks, 1968; Evans et al., 1970) together with an increased incidence of gram-negative bacilli (Forfar et al. 1968). The most common isolate from the umbilical stump was Staph epidermidis (Evans et al., 1970; McHattie et al., 1974). Colonisation of the stump was higher in premature infants than in normal infants (Evans et al., 1970; Davies et al., 1970).

The use of topical antibiotics in reducing the colonisation of the umbilicus has also been investigated. Polybactrim (neomycin sulphate 495,000 i. u., polymyxin B sulphate 150,000 i. u., zinc bacitracin 37,500 i. u.) has been used to reduce the levels of post operative sepsis from both gram-positive and gram-negative

organisms (Gibson, 1958; Forbes, 1961; Lowbury et al., 1962). Laursen (1963) treated the umbilical cord stump with Topicin (0.5% neomycin sulphate, 1% bacitracin) or Hibitane powder (1% chlorhexedine). The isolation of both Staph aureus and gram-negative bacilli was considerably lower in the infants treated with Topicin and a topical antibiotic spray is now commonly used.

INTESTINAL TRACT

The acquisition of intestinal flora by newborn infants has been of interest to bacteriologists since the mid-nineteenth century. The vast amount of literature published up to the mid-twentieth century was reviewed by Olsen (1949) and a summary of this review is given.

The article stresses the important work of Escherich published in 1866 which defined a methodology for future work based on a gram-stained preparation of the faeces and the use of isolation techniques on solid media to identify the component organisms. Early papers agree that the meconium is generally sterile at birth. Escherich reported that by the third to fourth hour he would isolate gram-positive cocci and later gram-positive bacilli and gram-negative bacilli. He was unable to isolate the gram-positive bacillus which was presumably the obligate anaerobe Bact. bifidus but was able to isolate the aerobic gram-negative rod Bact. coli. By the end of the nineteenth century, advances in anaerobic bacteriology enabled the isolation and identification of the anaerobic gram-positive bacilli and Tissler described the organism Bact. bifidus and Moro isolated and identified Bact. acidophilus.

Tissler agreed with Escherich that meconium is generally sterile and he found that after ten to twenty hours, gram-positive cocci, then Bact. coli and Bact. bifidus could be isolated. Tissler later described the difference in flora of breast fed and artificially fed infants; breast fed infants acquire a flora which is composed mainly of Bact. bifidus whereas the artificially fed infant had a mixed flora of Bact. coli, Bact. bifidus, Bact. acidophilus, streptococci and sarcina. Moro initially thought that Bact. acidophilus was the predominant organism in the faeces of breast fed infants but later agreed with Tissler that Bact. bifidus was the main organism present.

Olsen reviewed many other reports written in the early part of this century concerned with the differences in faecal flora of breast fed infants. Most authors he cited agreed that Bact. bifidus was the predominant organism found in the faeces of breast fed infants and that Bact. coli, Bact. acidophilus and enterococci form the basis of the faecal flora of artificially fed infants.

Early workers also considered the source of these bacteria which colonised the gastro-intestinal tract. Olsen reports Tissler as of the opinion that infection occurred via the mouth except for Bact. coli which entered via the anus. Moro thought that Bact. bifidus also entered via the anus as he could not isolate it from milk or the mouth of infants. He isolated Clostridia, Bact. bifidus and Bact. coli before the organisms could have passed down the gut, therefore presume that colonisation must have occurred via the anus. This view was also held by Maassen and Albertson who could not isolate Bact. bifidus from the vagina, milk or nipples

of the mothers but did isolate in the faeces and this organism was transferred to the infant at birth or indirectly after birth. Olsen also reviewed the work of Lauter who isolated Bact. bifidus from the oral cavity of 2 out of 6 infants investigated immediately after birth and postulated that the intestinal flora was introduced via the mouth. Hall and O'Toole (1934) found that the percent of sterile samples of meconium decreased with the age of the infant and in a later report (1935) investigated the acquisition of a faecal flora by breast fed infants.

Snyder (1936) found that small numbers of bacteria could be isolated using enrichment techniques from infants after birth and were derived from the amniotic fluid which had become contaminated with the organisms of the vaginal flora. The foetus is surrounded by this fluid which enters the intestinal tract as shown by the presence of lanugo hair, epidermal cells and vernix caseosa in the meconium and therefore bacterial colonisation could have occurred if the amniotic fluid was no longer sterile. Snyder suggested that the length of time between rupture of membranes and birth would influence the isolation of organisms from the meconium. In a later paper (1940) he investigated the stability of 22 infants' faecal flora over the first year of life. He identified many species of bacteria but considered that only a few species were present consistently enough to be considered normal faecal flora. These were in order of occurrence: Bact. coli, Str. faecalis, B. wechii, B. tertius, Bacteriodes Group 1, Bact. aerogenes, Str. mitis, M. epidermidis, L. bifidus (present in all breast fed infants therefore isolation rate dependant on ratio of breast fed to artificially fed infants).

Shallard and Williams (1966) in an investigation of aerobic flora found that all infants had acquired gram-negative bacilli by day 3 and that 38.5% had more than one type. A similar result was reported by McAllister et al., (1974) who found that 86.9% of infants were colonised by the third day and E. coli, Enterobacter cloacae and Kl. aerogenes were the predominant gram-negative bacilli.

Mata, Jimenez and Mejicanos (1971) found that bacteria could be cultivated from meconium 4 hours after birth and that within 24 hours 50% of infants had E. coli; this increased to 100% by day 2 with viable counts of $10^5 - 10^{11}$ per gram of faeces. The incidence of Clostridium welchii was also similar to that found in breast fed infants by Smith and Crabb (1961); Olsen (1949) found an initial rise to the third day and then a decrease to a very low frequency of isolation. Streptococci were isolated with a frequency similar to that of E. coli. The main difference between these two reports was in the isolation rates of bacteroides. Smith and Crabb found that this was consistently high whereas Mata et al. found that the isolation rate of bacteroides never rose above 50% over the first four weeks of life.

The predominant interest in the establishment of faecal flora was the difference between breast fed and artificially fed infants. As stated previously, this factor had been investigated and the results agreed on about the turn of the century. The emphasis has now changed to the factors in human milk which produce this difference in faecal flora and the resistance of breast fed infants to gastro-enteritis.

Lactobacilli and bifidobacteria are the prominent organisms in the faeces of breast fed infants but may also be present in the faeces of infants fed on a cows milk preparation. Gyongy (1957) found that there was a specific bifidus factor in human milk which gave the physiological stimulus to the propagation of L. bifidus in the flora. Bullen and Willis (1971), assuming that both breast fed and bottle fed infants were exposed to the same bacterial contamination, proposed a mechanism based on the physical properties of the two milks which would account for the difference in bacterial flora of the two groups of infants. The effect of pH on cows milk preparations was investigated by Harrison and Peat (1972) and the addition of small amounts of alkali to the milk would produce bacterial flora similar to that of breast fed infants. Willis et al (1973) prepared a new milk feed which resembled human milk in its lactose, protein and phosphate content and in its buffering capacity. Infants fed on this synthetic milk had a stool flora which resembled that of breast fed infants.

Breast milk also contains antibodies (Michael, Ringerback and Hoffenstein, 1971; Gindrat et al., 1972; Goldman and Smith, 1973). The level of IgM, IgG and IgA immunoglobulins in the clostrum was at the peak immediately after delivery and then declined over the first four days. Michael et al (1971) observed a direct relationship between the concentrations of immunoglobulins and reduction in the numbers of coliform bacilli present in the faeces of breast fed infants. Goldman and Smith (1973) reviewed the host resistance factors which as well as the bifidus factor and immunoglobulins included an anti-staphylococcal agent, lysozyme

lactoperoxidase, lactoferrin, macrophages and lymphocytes and indicated that little is known about the effect of these defence factors upon the infant. However the presence of these factors determines that the formulation of artificial milks can only resemble human milk in a limited range of components.

Although most research has been concerned with the question of breast versus bottle feeding, Davies et al (1970) compared the colonisation of ill/low birthweight infants nursed in a Premature Baby Unit with normal infants nursed in lying-in wards in the same hospital. The results indicated that the percentage colonisation was slightly lower in the sick infants and that these infants had a slightly higher colonisation with gram-positive organisms on day 3 but by day 7, the two groups of infants had a similar faecal flora.

SECTION 2

MATERIALS AND METHODS

1. MATERIALS

Commercially prepared media made in accordance with the manufacturers' instructions were used in this study. The media used are listed below.

Agar plates

Blood agar	Oxoid Nutrient Broth + Agar + 4% horse blood
Lysed Blood agar	Oxoid D. S. T. Agar + 4% horse blood lysed with saponin
Cetrimide agar	Difco-Bacto Psuedomonas Agar F. +0.03% Cetrimide + 15ugm Nalidixic Acid per ml.

Liquid Media

Carbohydrate fermentation tests	- Oxoid Bacteriological Peptone + 0.5% Sodium Chloride 0.5% Carbohydrate 1% Andrades indicator
Peptone Water	Oxoid Peptone + 0.5% Sodium Chloride
Simmons Citrate	Oxoid
Urea	Oxoid Urea Broth Base + 0.002% Urea
Hugh and Leifsons OF Medium	Difco - Bacto OF Basal Medium
Nutrient Broth	Oxoid No. 2
<u>Storage Media</u>	
Dorset Egg	80% egg + 20% of 0.9% Sodium Chloride

2. TECHNICAL METHODS

a) Specimen Collection

Albumin coated swabs made by Medical Wire and Equipment Co. (Bath) Ltd., Corsham, Wilts. were used throughout this work. All swabs were taken by the author except for a few swabs taken by the resident medical staff when an infant arrived in the Premature Baby Unit (P.B.U.) during the night and the two swabs taken from the mother immediately after delivery by the nursing staff.

1) Normal Infants and their Mothers

A high vaginal swab and a rectal swab were taken from the mother immediately after delivery. The following six sites of the infant were swabbed: mouth, ear, nose, umbilical cord, rectum and hands (palms and interdigital spaces). The swabs were taken as soon as possible after birth and before the infant was bathed. The same six sites were swabbed on the second, third, fourth, fifth and eighth days of life if the infant was still in the hospital, In addition, the mouth, nose, hands and fingers of the mother were swabbed daily; if the mother was breast feeding her infant a breast swab was also taken.

2) III/Low Birthweight Infants

The mouth, ear, nose, umbilical cord, rectum and hands were swabbed on admission when possible and daily for the first ten days of life, then at regular intervals until the infant reached 32 days of age or had left the unit. In some patients, indwelling tubes in the mouth or nose or umbilical catheters prevented the collection of a specimen.

3) Environment

Swabs of the surfaces in the wards and the P.B.U. were taken, also incubators and sinks in the P.B.U. were swabbed. Settle plates were exposed for eight hours and slit sample studies were carried out.

b) Treatment of Specimens

Each swab was placed in a sterile bijoux containing 2.5 mls. of nutrient broth, and immediately shaken on a rotary shaker for 20 minutes: 0.5 mls. of the suspension (10^0) was pipetted into 4.5 mls. of sterile peptone water and the dilution (10^{-1}) shaken. A range of dilutions from 10^0 to 10^{-6} were prepared in this manner for each swab and 0.1 mls. plated on solid media. By experiment, it was possible to predict the range of dilutions required to give single colonies on at least one dilution. The ear, umbilicus, and hand swabs did not require further dilution of the 10^0 suspension and the nose swab was diluted to 10^{-1} . Three serial dilutions of the mouth and rectum swabs were plated out, the exact dilutions used each day varied with each infant and ranged from 10^0 to 10^{-3} for the mouth swab and 10^0 to 10^{-6} for the rectal swab. As the 10^0 dilution of these two swabs was not always plated out, a cetrinide plate was included in the list of media in order to isolate Ps. aeruginosa if present in small numbers. The media and dilutions used are given in Table 1.

Table 1

<u>Swab</u>	<u>Media and Dilutions Used</u>		
	<u>Dilution Range</u>	<u>Blood Agar & MacConkey Agar</u>	<u>Cetr imide</u>
Mouth	$10^0 - 10^{-3}$	$10^0 - 10^{-3}$ or $10^{-1} - 10^{-3}$	10^0
Ear	10^0	10^0	
Nose	$10^0 - 10^{-1}$	$10^0 - 10^{-1}$	
Cord/ Umbilicus	10^0	10^0	
Rectum	$10^0 - 10^{-6}$	3 dilutions	10^0
Hands	10^0	10^0	

Each plate was spread using a wire spreader and incubated at 37°C . overnight.

c) Calculation of Viable Count

After incubation, the plates were examined for bacterial growth. When more than one dilution had been plated, the dilution which gave about 100 colonies per plate was selected and counted to give the total bacterial count. Each colonial type present on any of the dilutions was also counted. The counts were multiplied by the dilution to give a semi-quantitative analysis of the flora and relate to the number of organisms present in the suspension of that swab in 2.5 mls. of nutrient broth (10^0 dilution).

d) Identification of Isolates

One colony of any gram-positive organism or gram-negative coccus was plated on a half blood agar plate. Three colonies of organisms tentatively identified on colonial morphology as E. coli

or Klebsiella-Enterobacter were isolated on a half blood agar plate; any Proteus species or organism contaminated with Proteus were plated on a half MacConkey plate. The isolates were incubated overnight and then examined for pure growth. Any mixed cultures were respread and incubated.

Primary identification was made on the basis of colonial morphology and the gram stain. Organisms were divided into the following groups:

- 1) Gram-Positive Cocci
- 2) Gram-Positive Bacilli
- 3) Gram-Negative Cocci
- 4) Gram-Negative Bacilli
 - a) Enterobacteria
 - b) Pseudomonas species

The identification of Medical Bacteria (Cowan and Steel, 1965) was used as a basis for the identification of these organisms.

1) Gram-Positive Cocci

Organisms were tested for the production of catalase (see Appendix) and placed in one of these two sub-groups a) Staphylococci/Micrococci - catalase positive, b) Streptococci - catalase negative. The catalase positive organisms were further tested for the production of coagulase, and the ability to ferment a carbohydrate (see Appendix).

The identification system used for this group of organisms is given in Table 2.

Table 2

Identification of Gram-Positive Cocci

	Catalase Production	Coagulase Production	Fermentation of Carbohydrate
Staph. aureus	+	+	+
Staph. coagulase negative	+	-	+
Micrococci	+	-	-
Streptococci	-	Not tested	Not tested

Any haemolytic streptococci were grouped using the Formamide Extraction and the Lancefield Grouping Sera for groups A, B, C, D and G.

2) Gram-Positive Bacilli

No further identification of this group of organisms was made.

3) Gram-Negative Cocci

One colony was inoculated into a tube of nutrient broth and a tube catalase test was performed (see Appendix). Neisseria were classified as catalase-positive gram-negative cocci.

4) Gram-Negative Bacillia) Enterobacteria

One colony was inoculated into a tube of peptone water and this suspension was used to inoculate a set of biochemical reagents; glucose, lactose, Simmons citrate and urea. The tests were incubated at 37°C for seven days except for the peptone water which was used to test for the production of indole after overnight incubation. Any citrate positive organism was reinoculated on to a fresh citrate slope to ensure that growth was due to the utilisation

of citrate as the sole carbon source and not to any carbon carried over in the original inoculum. If the organism failed to ferment lactose within seven days an ONPG test was performed.

The identification system used is shown in Table 3.

Table 3
Identification of the Common Enterobacteria Isolated

	Glucose	Lactose	ONPG	Indole	Citrate	Urea
<u>E. coli</u>	A+G	+	+	+	-	-
Klebsiella- Enterobacter	A+G	+	+	-	+	+
<u>Proteus mirabilis</u>	A +G	-	-	-	-	+

A = Acid production. G = Gas production

Any organism which could not be identified using this scheme was further tested for the ability to ferment sucrose, salicin, dulcitol and mannitol, growth in potassium cyanide, production of hydrogen sulphide and the Methyl-Red and Voges-Proskauer test. Identification was made using the system of Cowan and Steel (1965), and further tests were performed when the organism was found not to be a member of the enterobacteria.

b) Pseudomonas species

All Pseudomonas species were tested for the ability to grow on cetrimide agar and the production of oxidase. Pigmentation was also noted. Ps. aeruginosa was defined as positive for all three tests.

SECTION 3

CLINICAL INFORMATION

CLINICAL INFORMATION

A comprehensive form with information of the birth and progress of each infant was completed by Dr Pamela Davies of the Department of Child Health, Hammersmith Hospital. The classification of new born infants was made in accordance with hospital policy (Davies et al, 1972) and is summarised below:

Pre-term	Born before 37 completed weeks of pregnancy estimated from first day of last menstrual period
Post-term	Born at 42 weeks or more of pregnancy
Small for dates	Birthweight below the 10th percentile for gestational age
Large for dates	Birthweight above the 90th percentile for gestational age.

a) Normal Infants

Thirty-seven infants born at Hammersmith Hospital between November 1971 and June 1973 were included in this study. Thirty-five infants were born on a Monday and 2 infants were born on a Tuesday: the selection of infants in this manner enabled investigations to be carried out on the first five days of life and the eighth day if the infant was still in hospital.

Six infants were born by Caesarian section; six infants were born with the aid of forceps and seven mothers had artificial rupture of membranes; thirty-three infants were born at term, two were pre-term and two were post term. The birthweights ranged from 2561 gms. to 4840 gms. with a mean of 3380 ± 42 gms. Only one infant was small for dates and four were large for dates. Thirty-five infants were single births and there was one set of twins.

The group consisted of 18 male infants and 19 female infants. Sixteen infants were breast fed entirely throughout the investigation and 21 were fed on prepacked half cream Cow & Gate milk either as a supplement to or in place of breast milk.

b) III/Low Birthweight Infants

Sixty-four ill/low birthweight infants admitted to the P. B. U. within the first twenty-four hours of life were included in this study. The gestational age ranged from 28 to 41 weeks and the birthweight from 980 gms. to 5340 gms. with a mean of 2330 ± 68 gms., this was significantly lower than the birthweight of normal infants ($p < 0.001$). Thirty-seven infants were male and 27 were female. Fifty-eight infants were single births and there were three sets of twins.

Table 4 summarises the data on normal and ill/low birthweight infants.

Table 4
Comparison of Normal and III/Low Birthweight Infants

	<u>Normal</u>	<u>III/Low Birthweight</u>
Number	37	64
Male: Female	18 : 19	37 : 27
Gestational Age (weeks)	36/7 - 42	28 - 41
Birthweight Range (gms.)	2560 - 4840	980 - 5340
Mean	3380 ± 42	2330 ± 68

The ill/low birthweight infants were divided into three groups on the basis of treatment received in the P. B. U.

Group 1. 14 infants who received antibiotics and were nursed in incubators

Group 2. 17 infants who were nursed in incubators

Group 3. 33 infants who were nursed in open cots.

Group 1 Infants

The gestational ages of the 14 infants in this group ranged from 29 to 40 weeks and the birthweight from 1400 to 5400 gms. with a mean of 2540 gms. Ten infants were preterm (71.4%) and 4 (28.6%) were term deliveries. No infants were classified as low birth-weight and two infants were large for dates. Nine (64.3%) were male infants and 5 (35.7%) were female.

The clinical details are given in Table 5. Six infants had prolonged ventilation therapy and five infants were intubated for up to 6½ hours. Three (21.4%) infants died, one of which was born at Hammersmith Hospital. The other two were born elsewhere. Five infants were born by Caesarian section, two by forcep delivery, one by vacuum and five by normal vaginal delivery; 7 (50%) were born at Hammersmith Hospital and 7 (50%) came to the Unit from outlying hospitals.

Group 2 Infants

Seventeen infants admitted to the Unit were nursed in incubators until considered well enough to be transferred to a cot. The gestational age ranged from 28 to 41 weeks. Ten (58.8%) infants were preterm and 7 (41.2%) were term deliveries. The birthweight ranged from 980 to 3500 gms. with a mean of 2170 gms. Four (23.4%) infants were small for gestational age; 13 (76.5%) were male and 4 (23.5%) were female.

Eight (47%) infants were born by Caesarian section, 4 (23.5%) by forceps delivery, 1 (5.9%) by breech presentation and 4 (23.5%) by normal vaginal delivery. Fifteen infants were single births and there was one set of twins.

Table 5. Clinical Details of III/low Birthweight Infants Group 1

Patient Number	Sex	Gestational Age (weeks)	Birth Weight gms.	Weight for Dates	Birth		Antibiotics	Clinical Details
26	Male	34	2340	Normal	HH	Forceps	Penicillin + Day 1 - 8 Kanamycin	Intubated 3 - 25 minutes Rhesus
47	Male	39	3500	Large	HH	C.S.	Penicillin + Day 2 - 3 Kanamycin	Intubated - Died 51 hours
61	Male	31	1980	Normal	E	Normal	Penicillin + Day 1 - 2 Kanamycin	Ventilated - Died 33½ hours
67	Male	37½	2400	Normal	WM	Vacuum	Penicillin + Day 2 - 10 Gentamicin	Ventilated 6 - 45 hours
69	Female	40	3100	Normal	HH	Normal	Penicillin + Day 1 - 8 Kanamycin	RDS - ? Infection
73	Female	36	1980	Normal	HH	Breech	Penicillin + Day 1 - 8 Gentamicin	RDS
84	Female	32	1700	Normal	CM	Normal	Pencillin + Day 1 - 4 Gentamicin	Ventilated - Died 4 days
86	Male	38	3220	Normal	HH	C.S.	Penicillin + Day 1 - 14 Gentamicin	Pneumonia
89	Female	29	1400	Normal	HH	Normal	Penicillin + Day 1 - 15 Gentamicin	Ventilated 0 - 5 days
94	Male	36	3180	Normal	HH	C.S.	Penicillin + Day 1 - 9 Gentamicin	Intubated 35 minutes
98	Male	36	5400	Large	WM	C.S.	Penicillin + Day 1 - 19 Gentamicin	Intubated 6½ hours. Rhesus Hydropic
106	Male	33	1700	Normal	WM	C.S.	Penicillin + Day 3 - 11 Gentamicin	Ventilated 0 - 6 days
115	Male	29½	1560	Normal	Av	Forceps	Penicillin + Day 1 - 15 Gentamicin	Ventilated 0 - 8 days
110	Female	31	2170	Normal	F	Normal	Penicillin + Day 1 - 5 Gentamicin	Apnoeic attacks

Table 5 - Key

HH	Hammersmith Hospital
WM	West Middlesex Hospital
Av	Avenue Clinic
P	Perrivale Hospital
CM	Central Middlesex Hospital
E	Edgware General Hospital
C.S.	Caesarian Section
RDS	Respiratory Distress Syndrome

The clinical details of these infants are given in Table 6. Only one infant had ventilation therapy and he died at 60 hours: 13 (76.5%) were born at Hammersmith Hospital and 4 (23.5%) were transferred from other hospitals.

Group 3 Infants

Thirty-three infants were nursed in open cots throughout their stay in the P.B.U. The gestational age ranged from 33 to 41 weeks, 17 infants were preterm (51.5%) and 16 (48.5%) were term deliveries. The birthweight ranged from 1770 to 3460 gms. with a mean of 2320 gms. Fourteen infants were small for gestational age. Fifteen (45.5%) were male and 18 (54.6%) were female infants. Eleven (33.3%) were born by Caesarian section, 2 (6%) by forceps delivery, 2 (6%) by a breech presentation and 18 (54.7%) were normal vaginal deliveries. There were two sets of twins, the remaining 29 infants were single births. Thirty-one infants were born at Hammersmith Hospital and 2 (6%) were born at home and transferred to the Unit immediately after birth. In all cases progress was uneventful and no clinical details of these infants is given.

Table 7 summarises the data on the three groups of infants in the P.B.U.

Table 6. Clinical Details of Infants in Group 2

Patient Number	Sex	Gestational Age (weeks)	Birth Weight gms.	Weight for Dates		Birth	Length of time in incubator	Clinical Details
22	Male	38	3440	Small	HH	C.S.	6 days	Rhesus 2 exchange transfusions
29	Male	38	2560	Small	HH	C.S.	6 days	RDS
38	Female	38	3500	Normal	HH	C.S.	2 days	
44	Male	36	2740	Normal	HH	C.S.	2 days	
49	Male	31 3/7	1020	Normal	HH	Normal	32 days	Birth asphyxia
52	Male	37 5/7	3120	Normal	HH	C.S.	3 days	RDS
70	Female	41	2650	Small	H	Normal	4 days	Rhesus 2 exchange transfusions
82	Male	28	980	Normal	Av	Forceps	3 days	Died 60 hours
87	Male	33	2146	Normal	HH	Forceps	9 days	1st Twin
88	Male	33	2160	Normal	HH	Breech	9 days	2nd Twin
91	Male	32 4/7	2010	Normal	HH	C.S.	4 days	
92	Male	32	1340	Normal	HH	C.S.	32 days	
101	Female	28	1210	Normal	P	Forceps	14 days	
105	Male	32	2180	Normal	HH	C.S.	13 days	
107	Female	39	2190	Small	HH	Normal	2 days	
114	Male	31	2030	Normal	Av	Forceps	13 days	
122	Male	40	2760	Normal	HH	Normal	8 days	Congenital heart disease

Key

HH - Hammersmith Hospital
C.S. - Caesarian Section

H - Hemel Hempstead Av - Avenue Clinic
RDS - Respiratory Distress Syndrome

P - Perivale Hospital

Table 7
Comparison of Infants in the P.B.U.

	<u>Group 1</u>	<u>Group 2</u>	<u>Group 3</u>
Number	14	17	33
Male: Female	9 : 5	13 : 4	15 : 18
Gestational Age (weeks)	29 - 40	28 - 41	33 - 41
Birthweight range gms.	1400 - 5400	980 - 3440	1770 - 3460
mean gms.	2540	2170	2320
Born Hammersmith Hospital	7	13	31
Born elsewhere	7	4	2
Preterm	10	10	17
Term	4	7	16
Small for dates	0	4	14

2. Duration of stay in Hospital

a) Normal Infants

Infants and mothers were selected, when possible, so that they could be investigated on days 1, 2, 3, 4, 5 and 8 after birth. Two infants were discharged after 48 hours and 2 at 72 hours; 10 infants were discharged on day 7, so that only 23 of the original 37 (62.2%) infants were investigated on day 8.

b) III/low Birthweight Infants

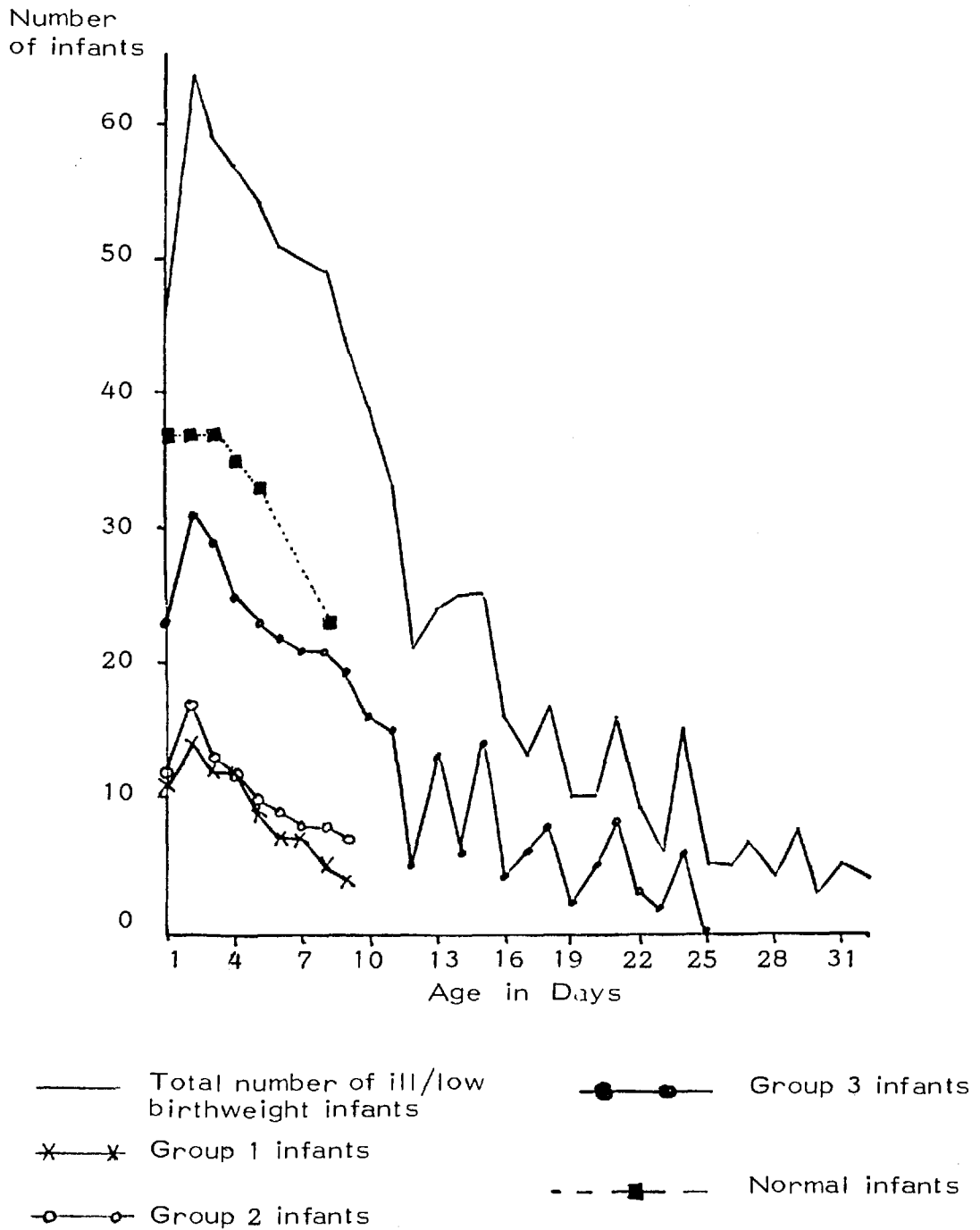
The duration of stay in the P.B.U. varied greatly with the clinical condition, the gestational age, birthweight, and social conditions of the infant. Infants were placed in one of the three subcategories dependant on initial treatment. If the treatment was altered, for example antibiotics

stopped or the infant moved into a cot, the infant was then removed from that group, although the data was still incorporated into the general classification of ill/low birthweight infants.

The variation in numbers of each group of infants is shown in Figure 1.

This figure indicates that the numbers of infants in each group declines rapidly with the age of the infants. Therefore the results obtained towards the end of the period of investigation when the numbers of infants in the group were considerably less than at the beginning are less significant.

Figure 1 Number of Infants in each Group investigated
on each day



SECTION 4

BACTERIOLOGICAL RESULTS

1. EAR

a) Normal Infants

Thirty-seven ear swabs were taken immediately after birth and 11 (30%) had bacterial growth. The incidence of positive swabs decreased slightly by day 2 but no significant change occurred over the first week of life.

The results are shown in Table 8.

Table 8

Colonisation of Ear of Normal Infants

Day of life	1	2	3	4	5	8
No. sampled	37	37	37	35	33	23
No. positive	11	10	12	12	12	7
% positive	30	27	32	34	36	30
Mean bacterial count	$10^{0.5}$	$10^{0.5}$	$10^{0.7}$	$10^{0.8}$	$10^{0.7}$	$10^{0.5}$
Organisms						
Staph. coagulase negative	7(19%)	9(24%)	9(24%)	10(29%)	10(30%)	6(26%)
Streptococci	3(8%)	1(3%)	2(5%)	2(6%)	1(3%)	2(9%)
Micrococci Gram-Positive Bacilli	2(5%)	1(3%)		1(3%)	2(6%)	1(4%)
<u>E. coli</u>	4(11%)	2(5%)	2(5%)			1(4%)
Klebsiella		1(3%)	2(5%)	1(3%)		

These results indicate that the level of colonisation remains low both in number of organisms isolated and the number of positive cultures. Coagulase negative staphylococci were the most common organisms

isolated, in 7 of 11 positive ear cultures on day 1 and rising to 6 of 7 by day 8. E. coli was isolated from four infants on day 1 but none of the 37 infants investigated show any clinical symptoms of infection.

b) Ill/low birthweight infants

The results are summarised in Table 9.

In both normal and ill/low birthweight infants there was a slight decrease in the number of positive cultures. The first swab was taken before the infant was bathed and reflects the initial colonisation of the ear. All normal infants had been bathed by day 2 and babies in the P.B.U. had a "top and tailed" bathing with hexachlorophene; this might be a contributing cause of the decrease in positive cultures.

The number of positive cultures did not increase markedly with time and there was little increase in the number of infants carrying Gram-negative bacilli in the ear. Coagulase negative staphylococci remained the predominant organisms isolated in 9 of 12 positive cultures on day 1, 7 of 7 positive at 2 weeks in all 3 positive cultures at 3 weeks and 4 of 5 (80%) positive cultures at 4 1/7

Table 9. Colonisation of Ear of III/Low Birthweight Infants

Day of Life	1	2	3	4	5	6	7	8	14	21	29
Number Sampled	46	60	58	56	53	51	50	49	25	16	8
Number Positive	12	7	17	17	16	22	15	23	7	3	5
% Positive	26.7	11.7	29.3	30.4	30.2	43.1	30.6	46.9	28	18.8	62.5
Mean Count	$10^{0.5}$	$10^{0.2}$	$10^{0.5}$	$10^{0.6}$	$10^{0.6}$	$10^{0.9}$	$10^{0.6}$	$10^{1.1}$	$10^{0.5}$	$10^{0.4}$	$10^{1.1}$
Organisms											
Staph-coagulase negative	9 (19.6%)	7 (11.7%)	13 (22.4%)	14 (25%)	14 (26.4%)	21 (41.2%)	12 (24%)	17 (34.7%)	7 (28%)	3 (18.8%)	4 (50%)
Streptococci	1 (2.2%)		1 (1.7%)	2 (3.6%)	2 (3.8%)			6 (12.2%)		1 (6.3%)	1 (12.5%)
Micrococci-Gram Positive Reds	2 (4.4%)	2 (3.3%)	2 (3.4%)	3 (5.4%)	1 (1.9%)	2 (3.9%)	3 (6%)	3 (6.1%)		1 (6.3%)	
<u>E. coli</u>	2 (4.4%)		2 (3.4%)	2 (3.6%)	1 (1.9%)	1 (2%)	1 (2%)	1 (2%)			
Klebsiella- Enterobacter	1 (2.2%)				1 (1.9%)			1 (2.0%)			
Pseudomonas				1 (1.8%)				1 (2.0%)			

DISCUSSION

The number of positive swabs found at birth (30% of normal infants) was lower than the 51.7% reported by Scanlon (1971) but was higher than reported by Evans et al (1973) who found 7% positive cultures in normal infants. This latter report also found 6% positive in premature infants in comparison to the 27% found in this study. The predominance of coagulase-negative staphylococci in the ear cultures reported in the earlier studies was also found in this study.

E. coli was isolated from both normal and ill/low birthweight infants but no clinical symptoms of infection were noted. This is in agreement with the two previous studies and indicated that the isolation of potential pathogenic organisms from the ear is not proof of clinical infection in the absence of clinical symptoms or a positive blood culture.

SUMMARY

Ear swabs were taken from 37 normal infants immediately after birth and organisms were grown from 11 (30%) swabs. The percentage of ears colonised remained at this level during the first week of life.

Ear swabs were taken from 47 ill/low birthweight infants within the first twenty-four hours of birth and 12 (26.7%) were positive. The percentage positive varied during the first month of life and reached 62.5% by day 29.

In all infants coagulase-negative staphylococci were the predominant organisms and gram-negative bacilli were isolated with low frequency in both normal and ill/low birthweight infants

This incidence of positive ear cultures at birth could not be considered indicative of perinatal infection.

UPPER RESPIRATORY TRACT

a) Normal Infants1) Mouth

Five (13.5%) infants had positive mouth swabs immediately after birth but by the fifth day of life all infants were colonised. Viable count studies indicated that the number of organisms became stable at about 10^5 organisms per swab. The data is shown in Figure 2.

Analysis of the predominant organisms in the mouth flora both in numbers and incidence is given in Table 10.

Table 10

Colonisation of the Mouth in Normal Infants

Day	Staph. coagulase negative		Streptococci		Gram-negative bacilli	
	GMC	% positive	GMC	% positive	GMC	% positive
1	0.16	10.8	0.12	5.4	0.03	2.7
2	1.0	35.1	1.30	29.7	0.14	5.4
3	1.16	35.1	3.43	73.0	0.21	8.1
4	1.96	45.7	3.97	80.0	0.17	8.6
5	2.25	57.6	4.20	87.9	0.40	12.1
8	2.90	69.6	4.74	100	0.60	17.4

GMC = Geometric Mean Count

The mouth quickly became colonised with streptococci and by day 8 all infants had acquired these organisms in high numbers. The second most common isolate was the coagulase-negative staphylococci and although the incidence increased with time it was lower than that of the streptococci reaching only 69.6% by day 8. The number of coagulase-negative staphylococci isolated also remained consistently lower (10^3)

than those of streptococci ($10^{4.7}$)

The isolation of gram-negative bacilli remained low throughout the eight days of study reaching only 17.4% positive by day 8 and E.coli was the predominant organism.

2) Nose

On day 1, 3 (7.9%) of infants had positive nasal swabs and by day 8, 17 (73.9%) infants had positive nose swabs with the geometric mean count reaching 10^2 . The data is shown in Figure 2.

Analysis of the predominant organisms both in numbers and incidence is given in Table 11.

Table 11

Colonisation of the Nose in Normal Infants

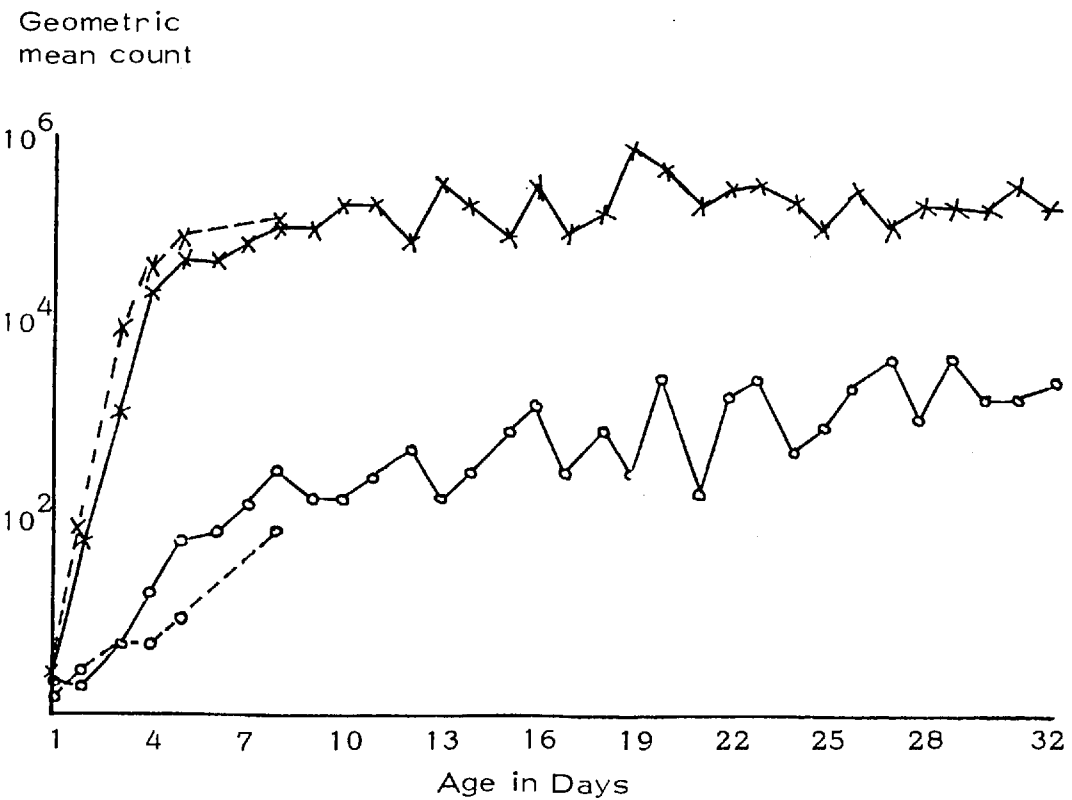
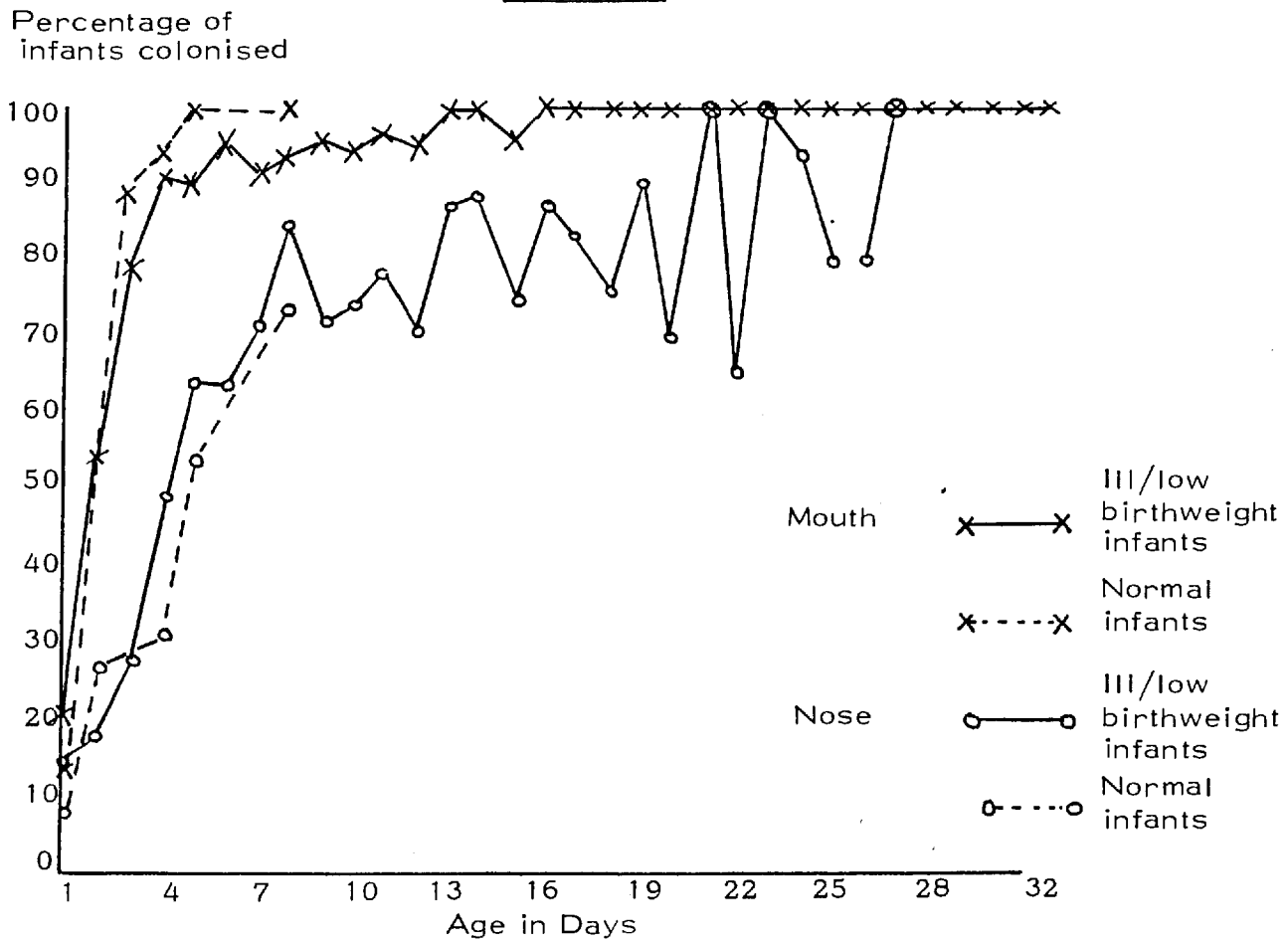
Day	Staph. coagulase negative		Streptococci		Gram-negative bacilli	
	GMC	% positive	GMC	% positive	GMC	% positive
1	0.18	5.3	0.06	5.3	0	0
2	0.35	24.3	0	0	0	0
3	0.47	25.7	0.09	5.7	0.03	2.86
4	0.46	22.9	0.22	11.4	0	0
5	0.76	42.4	0.22	15.2	0	0
8	1.38	56.5	0.10	4.35	0.09	0.08

Coagulase-negative staphylococci were the predominant organisms and by day 8 were isolated from 56.5% of infants, streptococci remained in low frequency throughout and Gram-negative bacilli were rarely isolated.

3) Breast fed and bottle fed normal infants

Normal infants were divided into two groups on the basis of their

Figure 2. Colonisation of the Upper Respiratory Tract
of Infants



feeding regime: infants fed entirely on breast milk and those infants fed on an artificial milk either as a supplement to or as a substitute for breast milk.

Colonisation of both the mouth and the nose occurred at the same rate in the two groups of infants throughout the first week of life but there was a significant difference in the incidence of coagulase-negative staphylococci in the mouth and nose of these two groups. The data is given in Table 12.

Table 12

Incidence of Coagulase-Negative Staphylococci in
Breast Fed and Artificially Fed Infants

Day	Mouth		Nose	
	Breast	Artificial	Breast	Artificial
1	13.3	9.5	12.5	0
2	56.3 *	19.0	43.8 *	9.52
3	50	23.8	46.7 *	10
4	60	35	26.7	20
5	34.3	52.6	64.3	26.3
8	100 **	50	66.7	50

* $p < 0.05$ ** $p < 0.025$

By day 2 breast fed infants had a significantly higher incidence of staphylococci in the mouth than bottle fed infants ($p < 0.05$). Although the difference was not significant except on day 8, the level remained higher throughout the first week of life. The incidence of these organisms in the nose was also significantly higher ($p < 0.05$) in breast fed infants on day 2 and 3 and remained higher for the rest of the period.

b) III/Low Birthweight Infants

1) Mouth

The percentage colonisation of the mouth is shown in Figure 2; this occurred at a slightly slower rate than found in normal infants and 100% positive was not achieved until day 13.

The predominant organisms both in incidence and numbers are shown in Figure 3. Initially coagulase-negative staphylococci were the predominant organisms and the incidence was similar to that found in normal infants. Streptococci were significantly less common than in normal infants ($p < 0.001$ on days 3, 4, 5 and 8) and the geometric mean count was also considerably lower than in normal infants. Gram-negative bacilli were consistently more frequent in the ill/low birthweight infants although this difference was never significant.

Over the first four weeks of life the relative incidence of these three groups of organisms varied but all three reached 75% incidence by day 28. Comparison of the geometric mean count indicated that the streptococci were present in higher numbers than either the coagulase-negative staphylococci or the gram-negative bacilli.

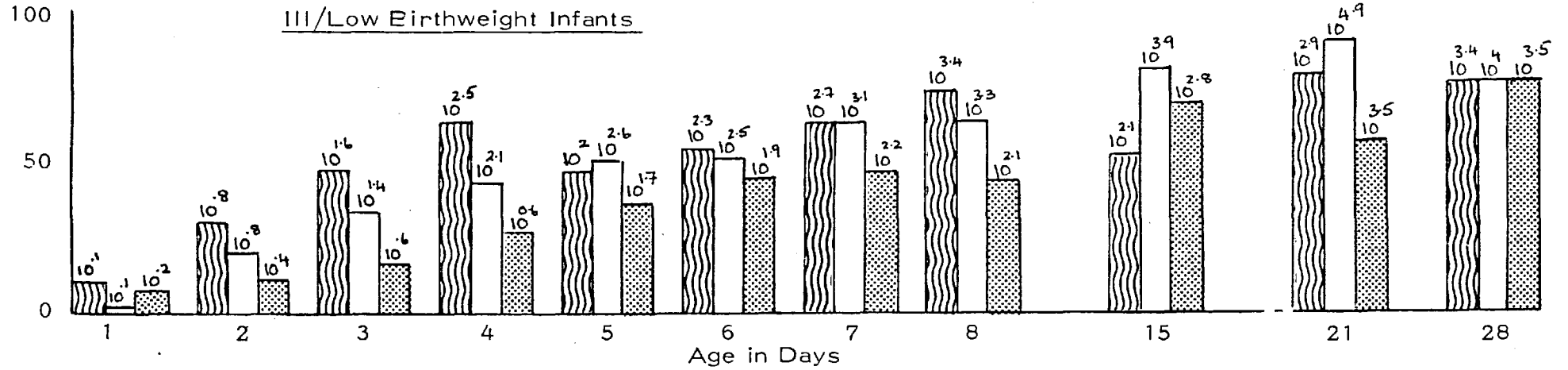
E. coli, Klebsiella and Ps. aeruginosa were isolated from the mouths of ill/low birthweight infants but only E. coli was isolated from normal infants.

2) Nose

The percentage colonisation of the nose was slightly higher than in normal infants (Figure 2) both in incidence and numbers of organisms. The predominant organisms are shown in Figure 4.

Figure 3. Comparison of the Mouth Flora of III/Low Birthweight and Normal Infants

Percentage of infants colonised



Percentage of infants colonised

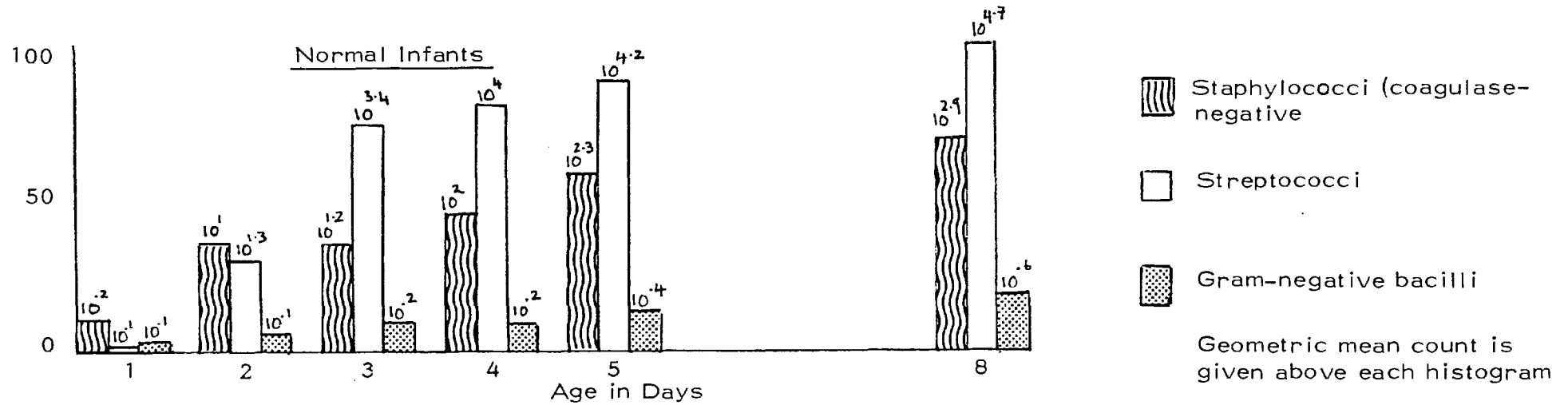
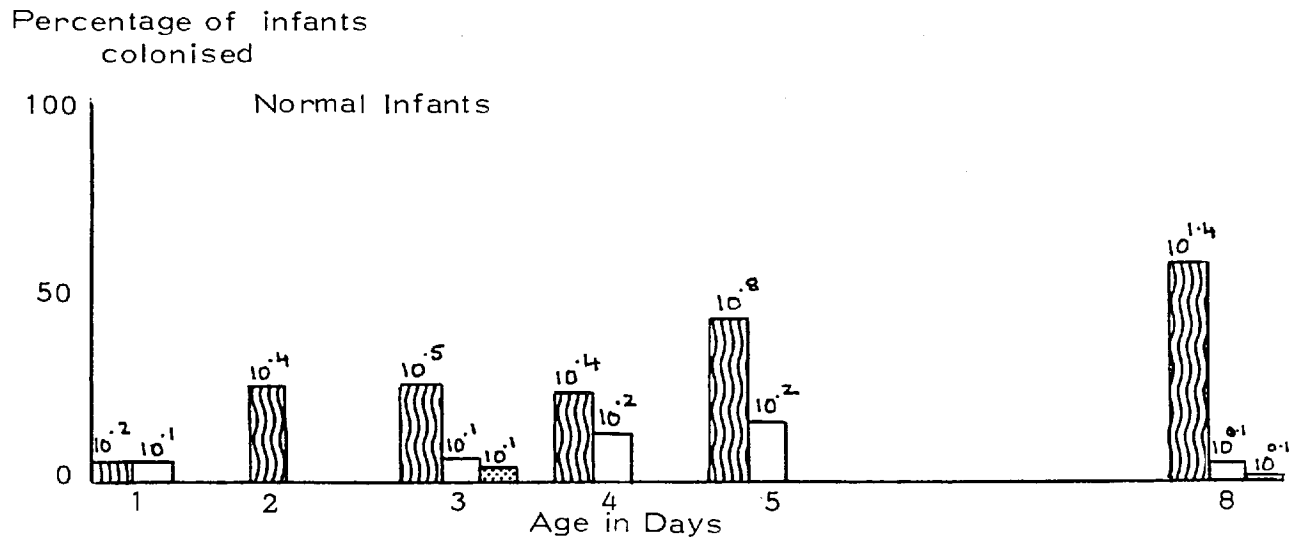
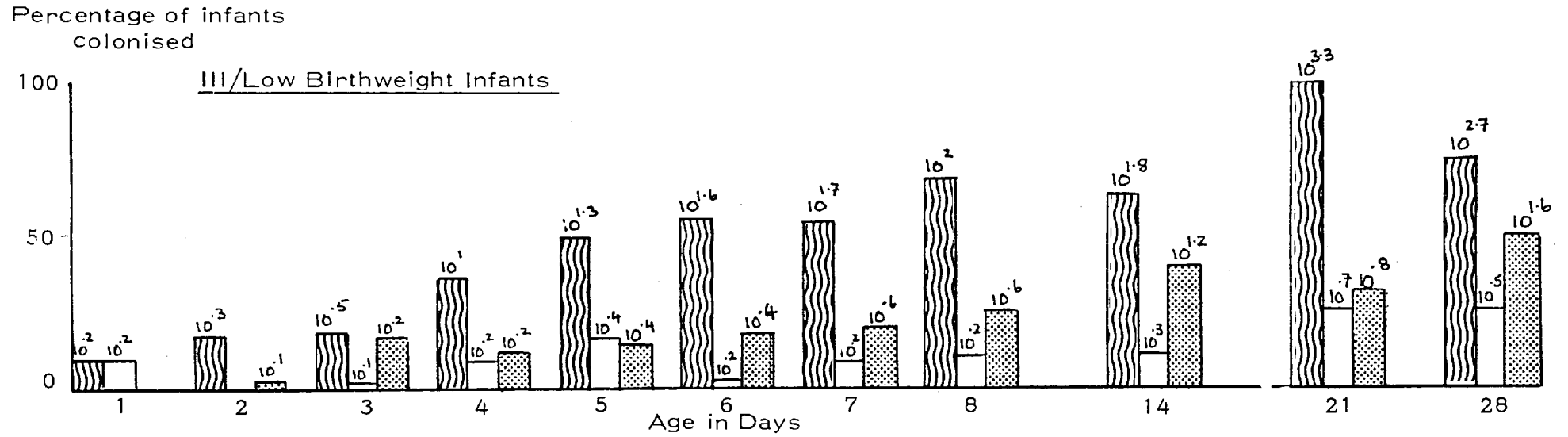


Figure 4. Comparison of the Nose flora of III/Low Birthweight and Normal Infants

Key - See Figure 3

Coagulase-negative staphylococci were predominant organisms throughout the period of study, the same result as found in normal infants. Gram-negative bacilli, which were not isolated from normal infants, were isolated with increasing frequency from the ill/low birthweight infants and reached an isolation rate of 50% by day 28 with a geometric mean count of $10^{1.6}$ organisms.

Streptococci were less common isolates than the gram-negative bacilli but again the incidence increased over the first four weeks of life reaching 25% by day 28 with a geometric mean count of $10^{0.5}$ organisms.

c) Comparison of infants in the P.B.U.

Infants nursed in the P.B.U. were then subdivided into three groups as defined in the clinical information (see page 34).

The rate of colonisation of the mouth and nose of the three groups of infants is given in Table 13.

Table 13

Day	<u>Percentage Colonisation of the Upper Respiratory Tract of III/Low Birthweight Infants</u>					
	Mouth			Nose		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
1	36.4	16.7	17.4	12.5	8.33	17.4
2	50	58.8	54.8	18.2	23.5	16.1
3	50	84.6	86.2	33.3	38.5	20.7
4	58.3	100	100	25	66.7	42.9
5	55.6	100	100	44.4	60	73.1
6	55.7	100	100	42.9	77.8	72.0
7	71.4	100	100	57.1	87.5	79.2
8	80	100	100	80	87.5	87.5
9		100	100		100	81.0

III/low birthweight infants and especially Group 1 infants, had various treatments, including ventilation therapy, to help overcome the

immediate clinical problems and these infants may not have been swabbed until much later in the first day of life than normal infants. The initial high rate of colonisation of the mouth found in Group 1 infants was not maintained on day 2 and by day 3 was significantly lower ($p < 0.05$) than that of either Group 2 or 3 infants. The percentage colonisation of the mouth of Group 1 infants remained significantly lower ($p = 0.001$) on days 4 and 5 but then the percentage colonised slowly increased over the next three days although it always remained lower than that of the other two groups of infants in the P.B.U. The geometric mean count for infants in Groups 2 and 3 rose to 10^5 by day 4 and remained stable over the rest of the period of study but by day 8 the geometric mean count for infants in Group 1 was only 10^3 organisms.

The rate of colonisation of the nose was also lower in Group 1 infants than in Groups 2 or 3 infants, although the difference was never significant. By day 8 all three groups of infants had a similar colonisation rate but the geometric mean count in Group 1 infants was only $10^{1.0}$ compared to $10^{3.1}$ and $10^{2.8}$ in Group 2 and 3 infants respectively.

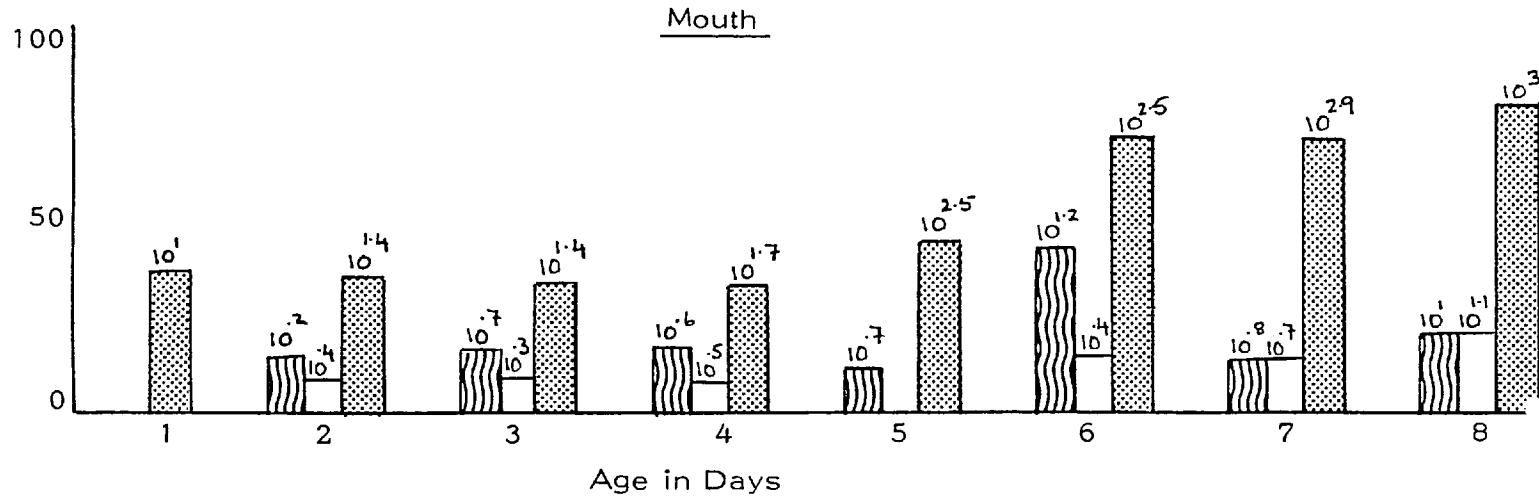
(i) Group 1 Infants

The predominant organisms isolated from the mouths of these infants are shown in Figure 5.

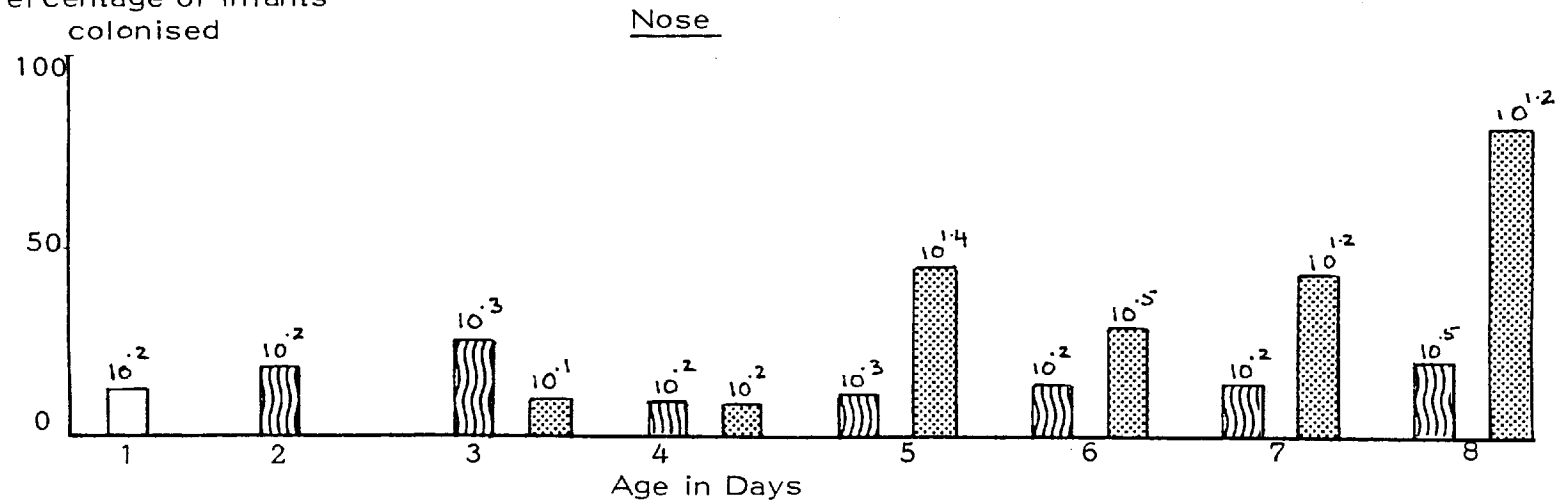
The mouth flora was predominantly Gram-negative in nature. On the first day 35% of infants were colonised with gram-negative bacilli and although the percentage decreased slightly to 35% on days 3 and 4 it rose to 80% by day 8 with a geometric mean count of 10^3 organisms. Streptococci were isolated from a few infants and the incidence reached 20% by day 8. Coagulase-negative

Figure 5. Upper Respiratory Tract Flora of Group 1 Infants

Percentage of infants colonised



Percentage of infants colonised

Key - See
Figure 3

staphylococci were also rarely isolated although a single high reading of 42% positive was recorded on day 6. The low level of isolation of the Gram-positive cocci is reflected in the geometric mean counts which reach about 10^1 organisms per swab by day 8.

The colonisation of the nose is also shown in Figure 6. Coagulase-negative staphylococci were initially predominant but by day 5 the Gram-negative bacilli had become established as the dominant organisms both in numbers and incidence, 79% of infants carried these organisms with a geometric mean count of $10^{1.2}$ organisms. Streptococci were isolated on day 1 but these organisms did not become established.

Ps. aeruginosa was the most common Gram-negative bacillus isolated and was found in 22.3% of all mouth and nose swabs.

Klebsiella species were the next most common isolate and were found in 12.2% of swabs, E. coli was the least common isolate, found in only 3.4% of swabs.

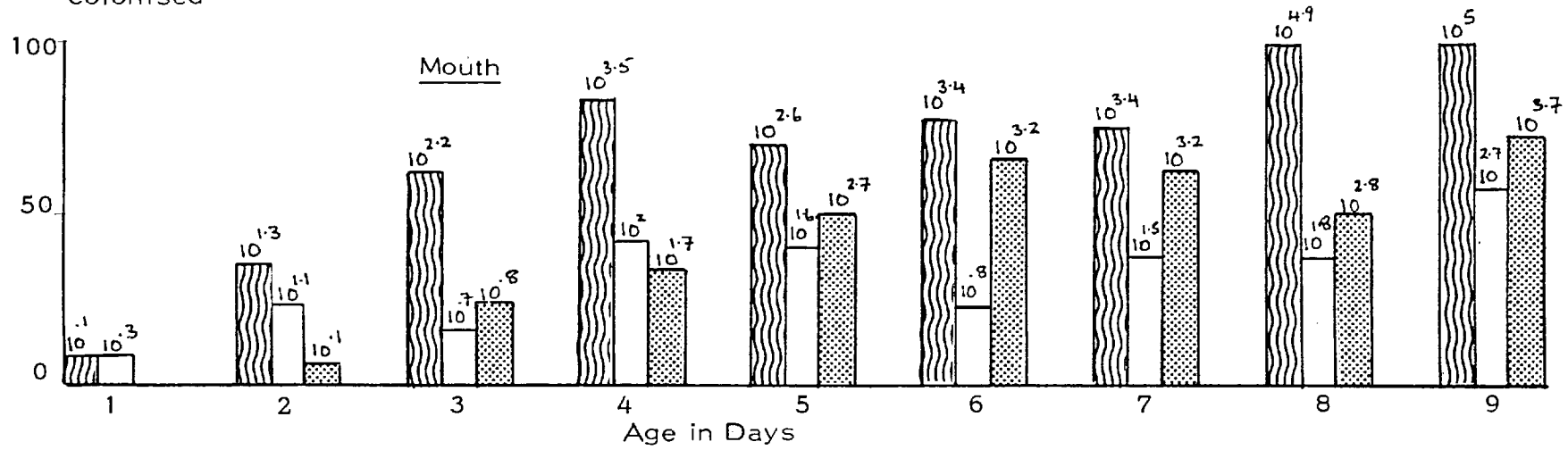
A difference was noted between infants on different antibiotic regimes, by day 3 all four infants treated with penicillin and kanamycin had positive mouth swabs but only four (40%) infants treated with penicillin and gentamicin had positive mouth swabs, and by day 8 this had risen to 75%. All infants treated with penicillin and kanamycin acquired Gram-positive cocci but only 10% of infants treated with penicillin and gentamicin acquired these organisms.

(ii) Group 2 Infants

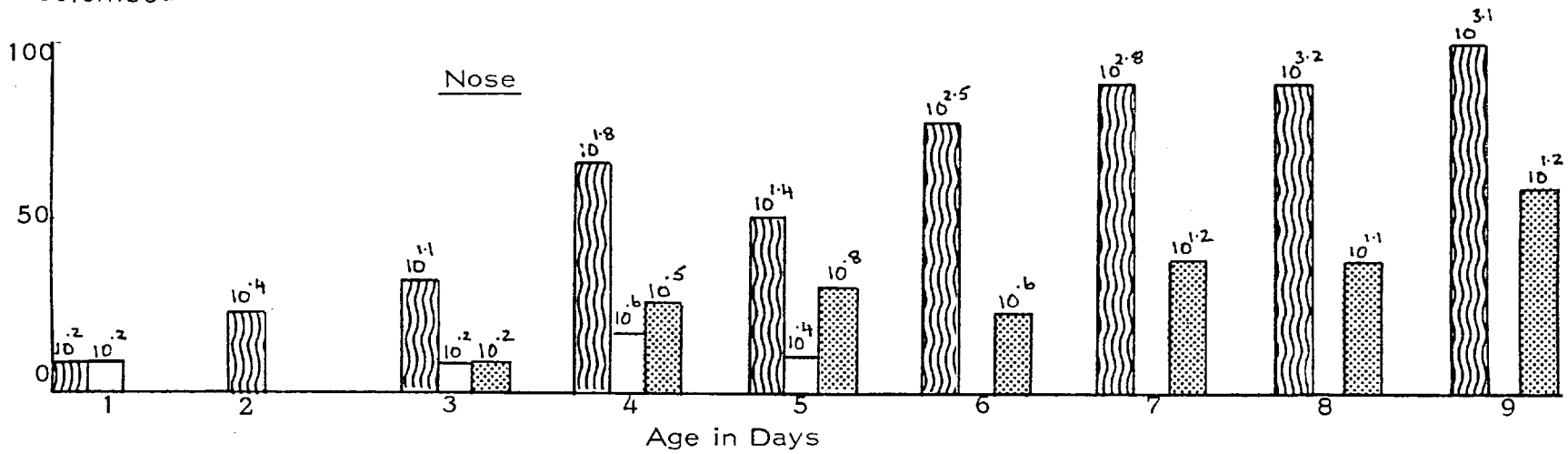
The data on the colonisation of the mouth is given in Figure 6. The predominant organisms both in incidence and numbers were the coagulase-negative staphylococci and by day 8 all infants were

Figure 6. Upper Respiratory Tract Flora of Group 2 Infants

Percentage of infants colonised



Percentage of infants colonised

Key -
See Figure 3

colonised with a geometric mean count of $10^{4.9}$. Gram-negative bacilli were isolated with increasing frequency over the first six days of life and the geometric mean count rose to over 10^3 organisms. Streptococci were less frequently isolated than either staphylococci or Gram-negative bacilli and the isolation rate reached 57% by day 9 with a geometric mean count of $10^{2.7}$ organisms.

The predominant organisms isolated from the nose are also shown in Figure 6. Coagulase-negative staphylococci were the predominant organisms throughout the period of study and by day 9 all infants were colonised and the geometric mean count was $10^{3.1}$ organisms. Gram-negative bacilli were also isolated and the frequency rose to 58% by day 9 with a geometric mean count of $10^{1.2}$ organisms. Streptococci were isolated during the first five days of life only and did not become an established part of the nose flora.

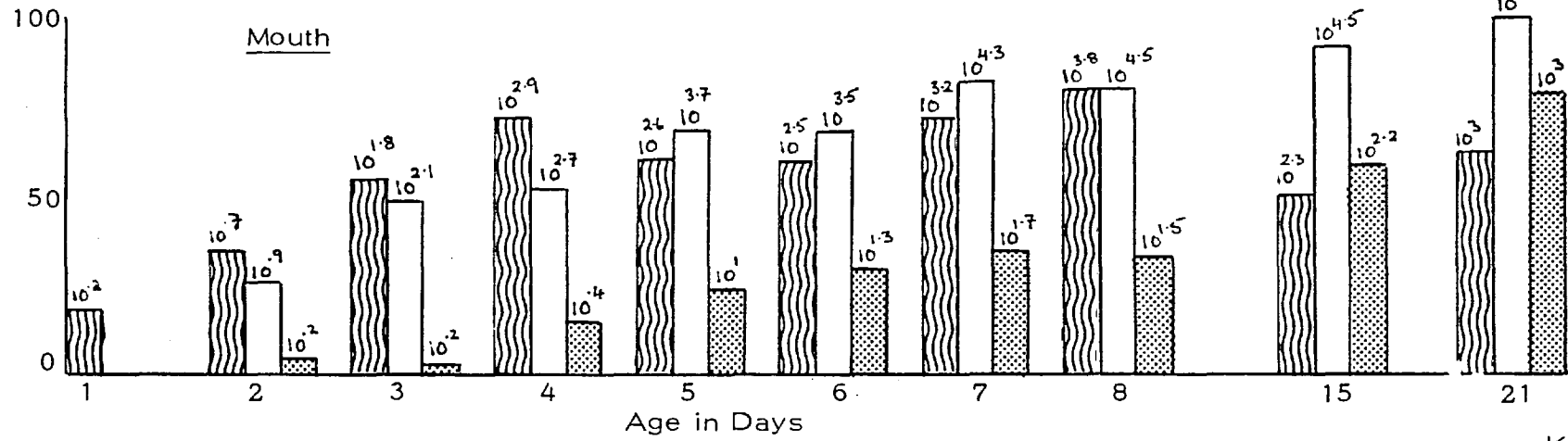
The Gram-negative bacilli, E.coli, Klebsiella and Ps.aeruginosa, were isolated with similar frequencies of 9%, 13.3% and 10.1% respectively.

(iii) Group 3 Infants

The mouth flora of these infants is given in Figure 7. Streptococci were the predominant organisms in the mouth although they did not become established until day 5, but by day 21 all infants were colonised and the geometric mean count reached $10^{5.1}$ organisms. Coagulase-negative staphylococci were initially the predominant isolates but by day 21 only 63% of infants were colonised and the geometric mean count was 10^3 organisms. Gram-negative bacilli were rarely isolated during the first few days of life but the frequency increased and by the end of the third week of life 79% of infants were colonised; however the geometric mean count was the same as that found for the coagulase-

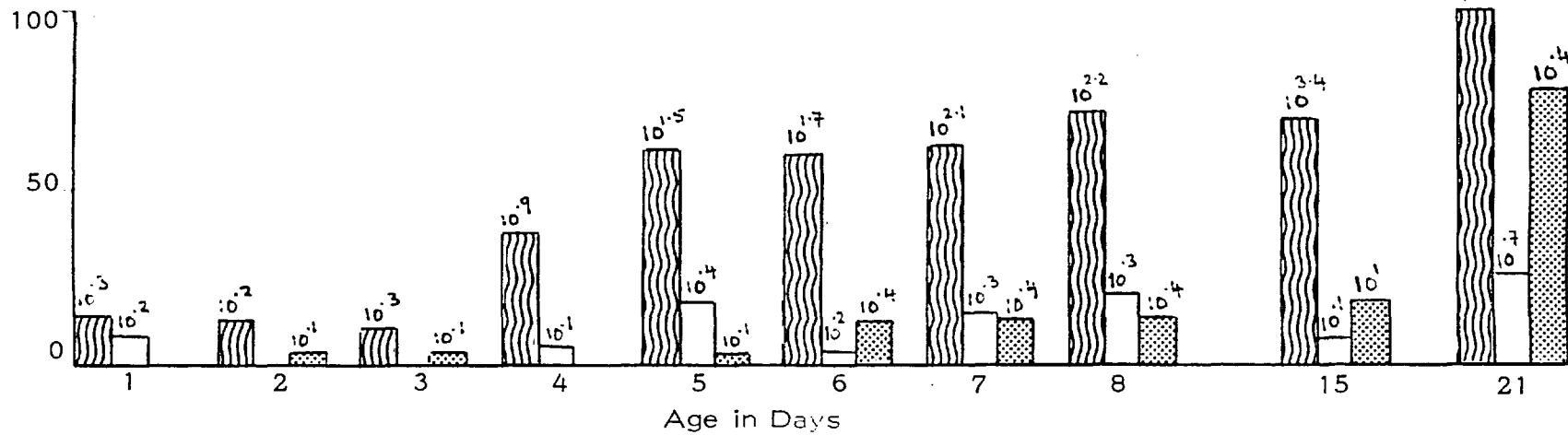
Figure 7. Upper Respiratory Tract Flora of Group 3 Infants

Percentage of infants colonised



Key - See Figure 3

Percentage of infants colonised



negative staphylococci, 10^3 organisms per swab.

The organisms isolated from the nose of these infants is also shown in Figure 7. Coagulase-negative staphylococci remained the predominant organisms throughout the period of study and by day 21 all infants were colonised with a geometric mean count of $10^{3.2}$ organisms. Streptococci were rarely isolated and the maximum isolation rate was only 25%. Gram-negative bacilli were also rarely isolated over the first two weeks of life but by the end of the third week 77% of infants were colonised. Although the percentage isolation rate rose rapidly over this period, the geometric mean count remained low and reached $10^{.4}$ organisms by day 21.

E. coli was the most common Gram-negative bacillus and was isolated from 10.8% of all upper respiratory tract swabs taken in the first nine days. Klebsiella species were not isolated during this period and were infrequently isolated in the remainder of the period of study. Ps aeruginosa was not isolated from these infants.

iv) Comparison of the three groups of ill/low birthweight infants and Normal Infants

a) Mouth

Streptococci were isolated with a lower frequency in Group 1 infants than found in Group 2 infants ($p < 0.05$) and in both these groups of infants the isolation rate was lower than that of normal infants ($p = 0.013$). The isolation rate found in Group 3 infants was higher than that of Group 1 and Group 2 infants, but still lower than that of normal infants; these differences were not significant.

Coagulase-negative staphylococci were less frequent in Group 1 infants than in normal infants. The isolation rate was significantly

higher in Group 2 infants than found in Group 1 infants ($p < 0.01$ on day 4, $p < 0.05$ on day 5) and slightly higher than that found in normal infants. The isolation rate was similar in Group 3 infants and normal infants.

The most significant difference was found in the isolation rate of the Gram-negative bacilli. The isolation rate was similar in Group 1 and Group 2 infants and this was significantly higher than that of normal infants ($p < 0.05$ on days 4 and 5, $p < 0.01$ on day 8). The frequency of isolation was also higher in Group 3 infants than in normal infants but this difference was not significant.

b) Nose

Coagulase-negative staphylococci, the predominant organisms in normal infants, were also isolated from the noses of infants in the P.B.U. The incidence was significantly lower in Group 1 infants than in Group 3 infants ($p < 0.02$ on day 6) but in both groups of infants this was lower than that of normal infants. The isolation rate was similar in Group 3 infants and normal infants.

Gram-negative bacilli were isolated more frequently in Group 1 infants ($p < 0.001$ on day 5) and Group 3 infants ($p < 0.01$ on day 5) than in normal infants. The frequency was also higher in Group 3 infants but this was not a significant difference.

v) Isolation of Gram-Negative Bacilli

E. coli was the most common Gram-negative bacillus isolated from the upper respiratory tract of normal and Group 3 infants. In Group 2 infants E. coli, Klebsiella species and Ps aeruginosa were isolated with similar frequency but in Group 1 infants Ps aeruginosa was the most common Gram-negative bacillus isolated. The isolation of this organism is shown in Figure 8. Pyocine studies were carried

out and are reported in a later part of this thesis (page 116).

vi) Isolation of Staph. aureus

This organism was isolated from the upper respiratory tract of five (7.8%) ill/low birthweight infants but only one (1.6%) infant was colonised on more than one occasion and two of the infants acquired this organism while the 'carrier' infant was in the unit. The isolation rate was also low in normal infants; 4 (10.6%) infants acquired this organism but in only two infants was it isolated on more than one occasion.

DISCUSSION

No organisms were isolated from 86.5% of mouth swabs and 92.1% of nose swabs taken immediately after birth. These figures are in agreement with those reported in earlier works (Bloomfield, 1922; Torrey and Reese, 1945; Smith and Bloomfield, 1950; Evans *et al.*, 1970; and Ehrenkranz, 1970). The mouth quickly became colonised and streptococci were the predominant organisms, coagulase-negative staphylococci were also present. The nose was colonised at a slightly slower rate and this agrees with the results of Evans *et al.*, (1970). Coagulase-negative staphylococci were the predominant organisms and streptococci were isolated in low incidence and numbers.

The isolation rate of Gram-negative bacilli was 17.4%; this was higher than the incidence of 8% reported by Davies *et al.*, (1970) in an earlier study carried out in this hospital, but both these results were lower than the 40% reported by Light *et al.*, (1968), who found that the high incidence of Gram-negative bacilli was related to a reduction in the incidence of Staph. aureus to 5.5% when hexa-

chlorophene was introduced into the ward regime. Hexachlorophene was also used in the wards and the incidence of Staph. aureus was low throughout the study (5.3% of infants were colonised on more than one occasion). Therefore this lower isolation rate of the Gram-negative bacilli was not related to a higher incidence of Staph. aureus and may be related to improved techniques in the nursing of normal infants.

Infants nursed in the P.B.U. acquired a very different pattern of bacterial colonisation to that of normal infants. Initially the proportion of infants colonised was slightly higher, 21.7% in the mouth and 14% in the nose compared to 13.5% and 7.9% respectively in normal infants. This difference between the two groups of infants is due to two factors; treatment of the infant at birth before the swabs were taken and the later time of sampling in the first twenty-four hours of life. The figures obtained were similar to those found by Torrey and Reese (1944), Evans et al., (1970) and Davies et al. (1970).

Gram-negative bacilli were more frequently isolated from the upper respiratory tract of ill/low birthweight infants than normal infants in agreement with the results of Farmer (1967). A previous report (Davies et al., 1970) stated that 58% of infants in the P.B.U. were colonised in the throat with Gram-negative organisms by day 7, a figure slightly higher than the 46% found in this study.

Subdivision of the ill/low birthweight infants into the three groups on the basis of treatment received in the P.B.U. indicated that these infants were not a homogenous group and considerable variation in the flora of the upper respiratory tract was observed.

Infants receiving antibiotic therapy had a slower colonisation

rate of the upper respiratory tract than the other infants and Gram-negative bacilli especially Ps. aeruginosa were present in considerably high numbers and incidence. There seemed to be some variation in the mouth flora with particular antibiotic regime used but this could not be investigated further.

Infants nursed in incubators acquired an upper respiratory tract flora in which the coagulase-negative staphylococci predominated and Gram-negative bacilli were commonly isolated. Unlike Group 1 infants, E. coli, Klebsiella species and Ps. aeruginosa were isolated with a similar frequency over the first nine days of life. These infants represent an intermediate position between Group 1 infants and Group 3 infants.

Infants nursed in open cots (Group 3) acquired a mouth flora in which streptococci predominated, as found in normal infants, but Gram-negative bacilli and coagulase-negative staphylococci were also isolated in high frequency and numbers, as found in Groups 2 and 1. The predominance of the coagulase-negative staphylococci in the nose flora was again similar to that of normal infants but the isolation of Gram-negative bacilli was similar to that of Group 1 and Group 2 infants. The upper respiratory tract flora of these infants represents an intermediate position between Group 2 infants and normal infants.

Staph aureus was isolated from only one ill/low birthweight infant on more than one occasion. This lower incidence of this organism together with the higher incidence of Gram-negative bacilli can be correlated with the intensive hexachlorophene regime used in the P.B.U. and supports the findings of Light et al., (1968) and Davies et al. (1970)

In work carried out in this hospital, Davies et al (1970) reported that the treatment received by the infant may affect the carriage rate of Gram-negative bacilli. This present study indicates that not only is the overall incidence affected but also the relative numbers and types of Gram-negative bacilli are affected by the treatment received by infants in the P.B.U.

SUMMARY

1. Mouth swabs were taken from 37 normal infants immediately after birth and organisms were grown from 5 (13.5%) swabs. All infants were colonised by day 5 and the geometric count became stable at 10^5 organisms. Streptococci were the predominant organisms throughout. Nose swabs were also taken from all 37 normal infants and 3 (7.9%) were positive on the first day of life. By day 8, 17 (73.9%) infants were colonised and the geometric count reached 10^2 organisms. Coagulase-negative staphylococci were the predominant organisms. Gram-negative bacilli were isolated with low frequency from the mouth and rarely from the nose.
2. Coagulase-negative staphylococci were more common in the mouth of breast fed infants than bottle fed infants.
3. Colonisation of the mouth of ill/low birthweight infants occurred at a slower rate than in normal infants and 100% positive was not achieved until day 13. Initially coagulase-negative staphylococci were predominant but by the end of the fourth week of life, streptococci and Gram-negative bacilli were isolated with the same frequency as these organisms.
4. The percentage colonisation of the nose was slightly higher in

ill/low birthweight infants than in normal infants, but in both these groups of infants coagulase-negative staphylococci were the predominant organisms.

5. The incidence of Gram-negative bacilli was higher in infants in the P.B.U. and this incidence increased over the first four weeks of life.

6. Investigation of the three groups of infants nursed in the P.B.U. indicated that differences in the types of Gram-negative bacilli found in the upper respiratory tract were related to the treatment of the infants. Infants treated with antibiotics had the highest incidence of Gram-negative bacilli both in the mouth and nose and Ps . aeruginosa was the most common member of this group of organisms. The incidence of Gram-negative bacilli was lower in Group 2 infants and E. coli, Klebsiella species and Ps. aeruginosa were isolated with similar frequency. Infants nursed in open cots had the lowest incidence of Gram-negative bacilli of the three groups of infants in the P.B.U. and E. coli was the most common isolate.

7. The incidence of Staph. aureus was low, the organism was isolated from 5 (7.8%) of ill/low birthweight infants but only 1 (1.6%) infant could be termed a carrier. Four (10.6%) normal infants acquired this organism but only 2 (5.3%) infants were positive on more than one occasion.

3. SKIN

a) Normal Infants

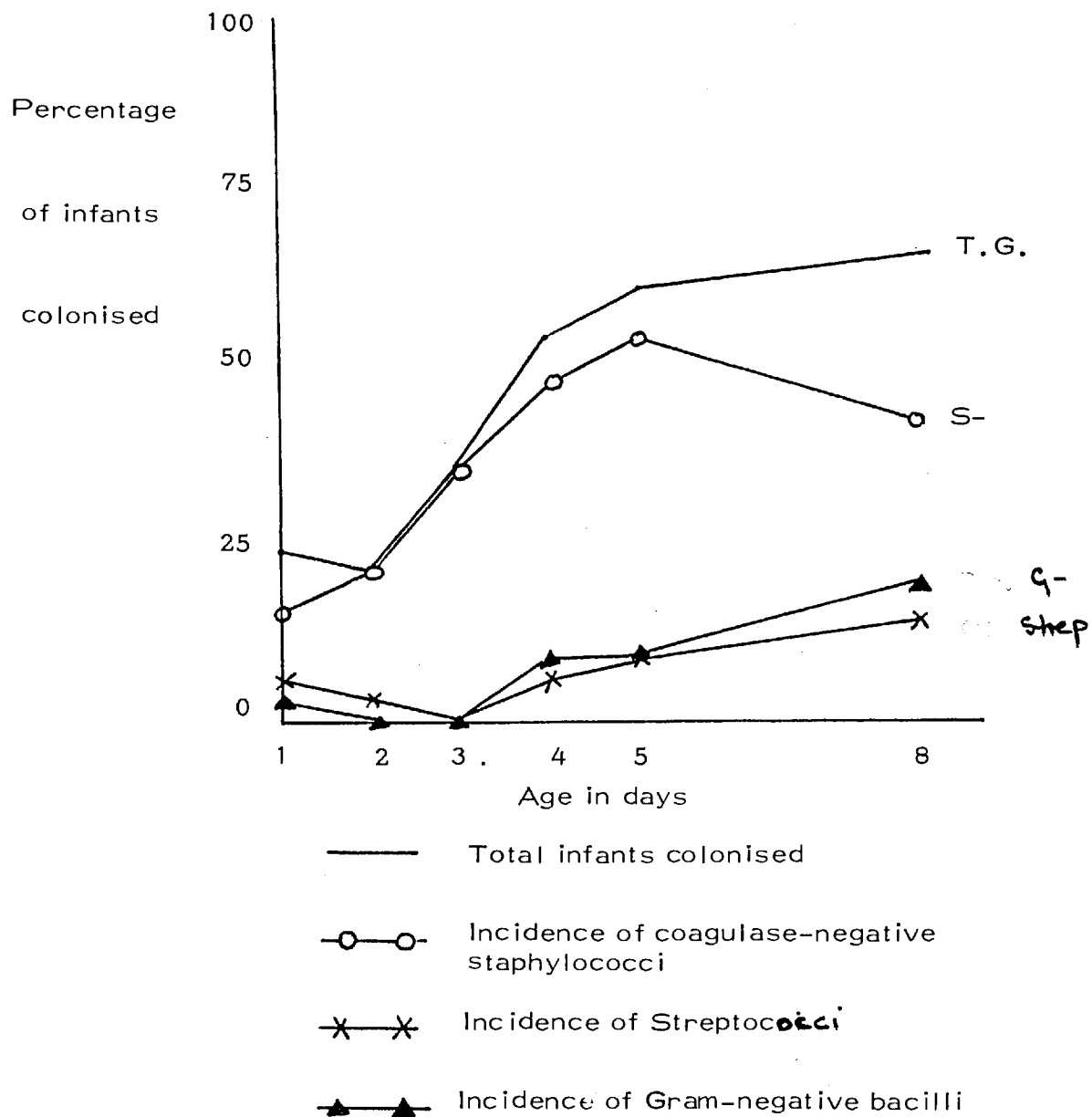
Hand swabs were taken from 33 infants immediately after birth. All six infants born by Caesarian section and 19 (70.4%) infants born by vaginal delivery had sterile swabs. Eight hand swabs were positive; coagulase-negative staphylococci were isolated from five swabs, gram-positive bacilli/micrococci from two and streptococci from one.

The subsequent colonisation of the skin is shown in Figure 9. On day 2 there was a slight fall in the number of positive cultures but the rate quickly increased and by day 8, 66% of infants were colonised. Coagulase-negative staphylococci remained the predominant organisms throughout the eight days although the isolation rate of other Gram-positive organisms increased after an initial fall. No difference was noted between breast fed and bottle fed infants.

Staph. aureus was isolated from three infants (9.1%) and no skin lesions were noted. Swabs from one infant were positive on successive days so this infant could be termed a carrier of this organism, the upper respiratory tract and rectum were also colonised. Swabs taken from the mother were also positive in the mouth and nose throughout her stay in hospital and she was probably the source of this organism. The other two infants were positive on day 8 only and no further tests were possible.

b) III/Low Birthweight Infants

Hand swabs were taken from 47 infants within the first twenty-four hours of life. Eight swabs were positive (17.4%), five (10.9%) grew coagulase-negative staphylococci, and three (6.5%) gram-positive bacilli/micrococci.

Figure 9. Skin Flora of Normal Infants

The rate of colonisation rose steadily during the first four days and stabilised at 50% positive. Coagulase-negative staphylococci were the predominant organisms throughout, the incidence of the other Gram-positive organisms never rose above 20%.

The colonisation of the three groups of infants is shown in Figure 10. The percentage colonisation was lowest in Group 1 infants but wide variations in all three groups were observed. The incidence of Gram-negative bacilli was low; *Klebsiella* species were isolated from Group 1 and Group 2 infants on two occasions, *Ps. aeruginosa* was isolated once from a Group 1 infant, *E. coli* was isolated with low frequency from Group 3 infants (maximum 6%) and once from a Group 2 infant.

Only 2 (3.1%) infants became colonised with *Staph. aureus* and in neither case were lesions observed. One infant was colonised in the upper respiratory tract over 21 days but the skin was positive on days 2 and 4 only, which suggests a transient autocolonisation. The other infant was colonised at this site on one day which again suggests a transient colonisation.

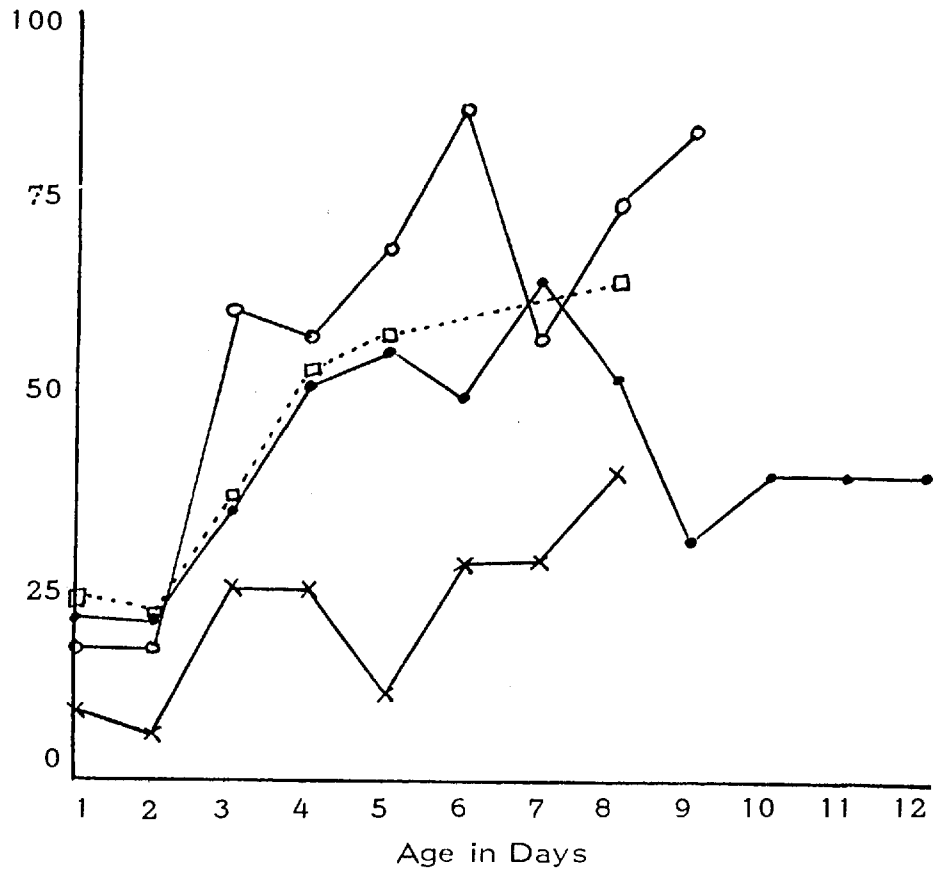
DISCUSSION

Although scrub techniques and hand in glove sampling methods might give more comprehensive results (Shaw et al., 1970; Michaud, McGrath and Goss, 1972) these methods are impractical when dealing with very small infants and a swab technique was considered the best method available.

The skin was sterile in the majority of infants at birth and the rate of colonisation increased over the first four days of life. The predominance of the coagulase-negative staphylococci is in

Fig. 10 Skin Colonisation of III/Low Birthweight
and Normal Infants

Percentage
of infants
colonised



—x—x— Group 1 Infants

- - □ - - - □ - - - Normal infants

—○—○— Group 2 Infants

—●—●— Group 3 Infants

agreement with Sarkany and Gaylarde (1967a).

The lower percentage colonisation of Group 1 infants compared to the other infants nursed in incubators (Group 2) must be related to the antibiotic regime. It was shown earlier (page 58) that the colonisation of the upper respiratory tract was delayed when the infants received antibiotics which may also affect the colonisation of the hands.

The low level of colonisation of infants with Staph. aureus and the absence of skin lesions is in agreement with the findings of Hardyment et al. (1960); Pluëckhahn and Banks (1968) and Forfar et al (1968) and was to be expected with the ward regime used. Gram-negative bacilli were not isolated from the skin of normal infants and were infrequently isolated from ill/low birthweight infants. E. coli was isolated from the hands of infants nursed in open cots and was also the most common Gram-negative bacillus isolated from the upper respiratory tract of these infants. Klebsiella species were isolated from infants in Groups 1 and 2, Ps. aeruginosa was isolated from an infant in Group 1. This again corresponds with the incidence of these organisms in the upper respiratory tract.

SUMMARY

Hand swabs were taken from 33 normal infants immediately after birth. All 6 infants born by Caesarian section had sterile skin swabs but organisms were isolated from 8 (25.8%) infants born by vaginal delivery. Colonisation of the skin occurred and by day 8, 66% of infants had positive skin swabs. Coagulase-negative staphylococci were the predominant organisms throughout.

Forty-seven ill/low birthweight infants were swabbed within the first twenty-four hours of life and organisms were isolated from 8 (17.4%) infants. Coagulase-negative staphylococci were the predominant organisms, the incidence of Gram-negative bacilli was low in all infants nursed in the P.B.U.

Staph. aureus was isolated from 3 (9.1%) normal infants and 2 (3.1%) ill/low birthweight infants. No skin lesions were noted in any of these infants.

UMBILICUS

a) Normal Infants

The rate of colonisation is shown in Table 14.

Table 14

Colonisation of the Umbilicus of Healthy Infants

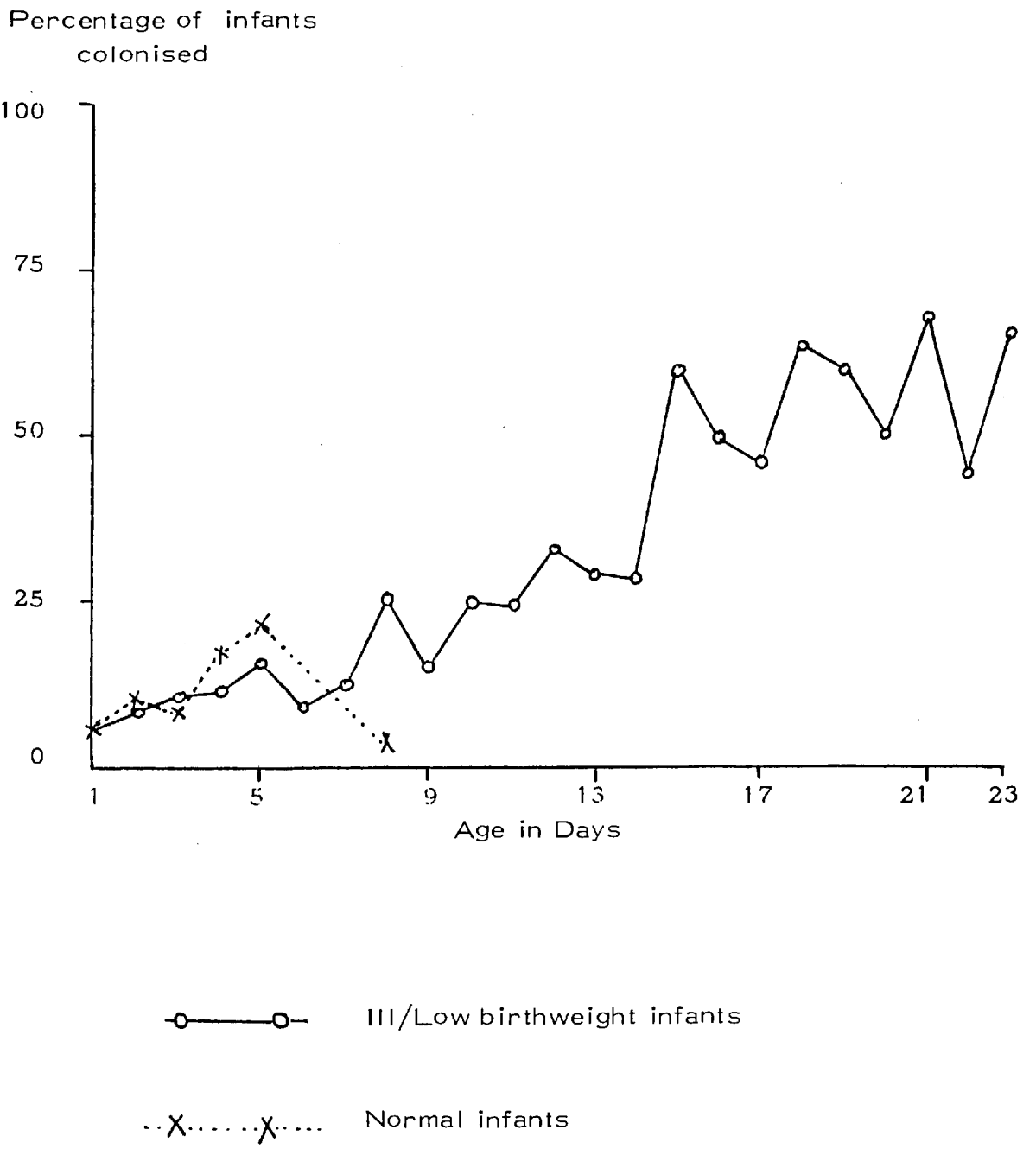
Day	1	2	3	4	5	8
Swabs	36	37	37	35	35	23
No. positive	2	4	3	6	7	1
% positive	5.6	10.8	8.1	17.1	21.2	4.35
% Coagulase negative Staphylococci	2.8	5.4	5.4	14.3	12.1	4.4
% Streptococci	2.8	0	2.7	0	3.3	0
% Klebsiella Species	0	0	0	0	3.0	0

The level of colonisation remained low throughout. In most infants the umbilical stump had separated by the eighth day and this might account for the sudden reduction in the level of colonisation. Coagulase-negative staphylococci were the predominant organisms isolated, sporadic isolates of streptococci were obtained and may have been a transient colonisation due to soiled napkins. The incidence of Gram-negative bacilli was very low, only one isolate of Klebsiella was obtained from the 201 swabs taken.

b) III/Low Birthweight Infants

The rate of colonisation is shown in Figure 11. The percentage colonised increased with time and Gram-positive organisms were more common than Gram-negative organisms throughout. Coagulase-negative staphylococci were the most common isolates and the incidence increased from 2.2% on day 1 to 50% by day 29. The incidence of

Figure 11. Colonisation of the Umbilicus



Gram-negative bacilli increased with time after an initial lag period but the incidence of the various genera was different, E. coli was isolated from 41.3% of swabs on day 29, Klebsiella 12.5%, Proteus species 28%, and Ps. aeruginosa 10%. The overall level of isolation is given in Table 15.

Table 15

Incidence of Organisms Colonising the Umbilicus

Total number of isolates

Total No. of Swabs	No. Sterile	Coagulase Negative Staphylococci	<u>E. Coli</u>	Klebsiella	Proteus Species	<u>Ps. Aeruginosa</u>
790	597	128	37	18	25	2
	(75.6%)	(17.7%)	(4.7%)	(2.3%)	(3.2%)	(0.25%)

Staph. aureus was isolated twice; one isolate was from an infant who was known to carry this organism in the upper respiratory tract over a period of 26 days before it was isolated from the umbilicus, the other isolate was from an infant who was positive for this organism two days earlier but no further isolates were made and this may be considered a transient colonisation.

Analysis of the data showed that there was no significant difference between the three groups of ill/low birthweight infants.

DISCUSSION

Ninety-four per cent of normal infants and 93% of ill/low birthweight infants had a sterile umbilicus on day 1; this figure is slightly higher than the 85% reported by Fairchild et al (1958), and considerably higher than the 19% for healthy infants and the 51% for premature infants reported by Evans et al (1970). All initial swabs were taken after the cord had been clamped and sprayed with polybactrim; some transfer of polybactrim from the sprayed cord to the swab might have

occurred which would affect the number of positive cultures.

The rate of colonisation of the umbilicus was similar in normal and ill/low birthweight infants to that found by Davies et al (1970) in an earlier study in this hospital but these results showed a larger number of sterile swabs than reported by Evans et al (1970) in which only 20% of normal infants and 21.9% of premature infants had a sterile umbilicus by day 5; in this study figures of 78.8% and 83.3% respectively were recorded. In all three reports coagulase-negative staphylococci were the predominant organisms.

Staph. aureus was not isolated from normal infants and only twice from ill/low birthweight infants whereas Laursen (1963) reported an incidence of 29.8% in normal infants and Evans et al (1970) 1.6% - 9.6% in normal infants and 2.4% - 13% in premature infants.

The incidence of Gram-negative bacilli was lower than previously reported. Laursen (1963) isolated coliform bacilli from 10.3% of normal infants and Evans et al (1970) 14.6%; in this study a single isolate was made from normal infants. The incidence in ill/low birthweight infants increased with time but over the first six days was lower than the 14% reported by Evans et al (1970).

The colonisation of the umbilical stump with pathogenic organisms is no longer an inevitable occurrence. The introduction of hexachlorophene baths and the use of topical antibiotics have reduced the overall colonisation and especially colonisation with Staph. aureus and Gram-negative bacilli. The low incidence of these organisms in this study suggests that they may be considered as transient colonisers of the umbilicus of infants in this hospital.

SUMMARY

The colonisation of the umbilicus remained low in both normal and ill/low birthweight infants during the first week of life. A rise in the rate of colonisation from 27% to 60% after the second week of life was found in ill/low birthweight infants. In all cases coagulase-negative staphylococci were the predominant organisms. The use of hexachlorophene baths and topical antibiotics to reduce the level of colonisation with potential pathogens was discussed.

5. RECTAL FLORA

a) Normal Infants

Sixteen infants were solely breast fed and 21 infants received a prepacked artificial milk. A higher percentage of breast fed infants (25%) were colonised at birth than bottle fed infants (19%) but as swabs were taken immediately after birth this difference must be related to the conditions of birth. Once feeding had been established this difference was not maintained and by day 4 all infants were colonised and the geometric mean count stabilised at $10^{7.5}$ organisms by day 5.

The colonisation of both groups of infants is shown in Figure 12. Initially the coagulase-negative staphylococci were predominant but by day 5 the Gram-negative bacilli had become established in the breast fed infants. This transition to a predominantly Gram-negative flora did not occur until day 8 in bottle fed infants but the frequency of Gram-negative bacilli both in numbers and incidence was higher than in breast fed infants.

E. coli was the predominant Gram-negative bacillus and was isolated from 30% of swabs from breast fed infants and 35.2% of swabs from bottle fed infants; *Klebsiella* species were isolated from 11.6% and 7.15% respectively, Ps. aeruginosa was not isolated.

b) III/Low Birthweight Infants

The rate of colonisation is shown in Figure 13. Positive cultures were obtained from most infants by day 4 and the mean count became stable at about 10^8 organisms per swab. Gram-negative bacilli predominated and the incidence was initially higher than found in normal infants fed on the same artificial milk ($p < 0.025$

Figure 12. Rectal Flora of Breast and Bottle Fed Infants

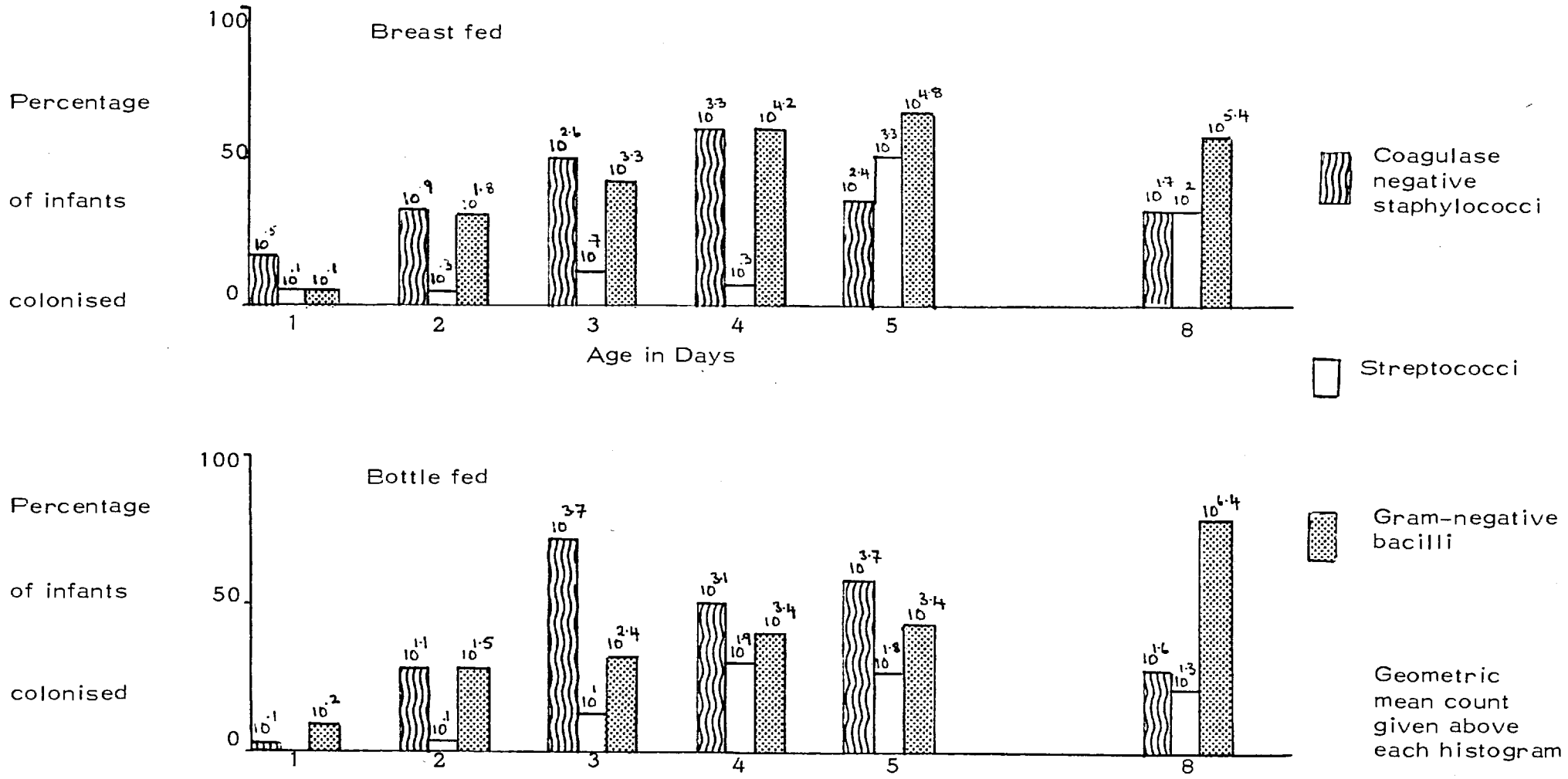
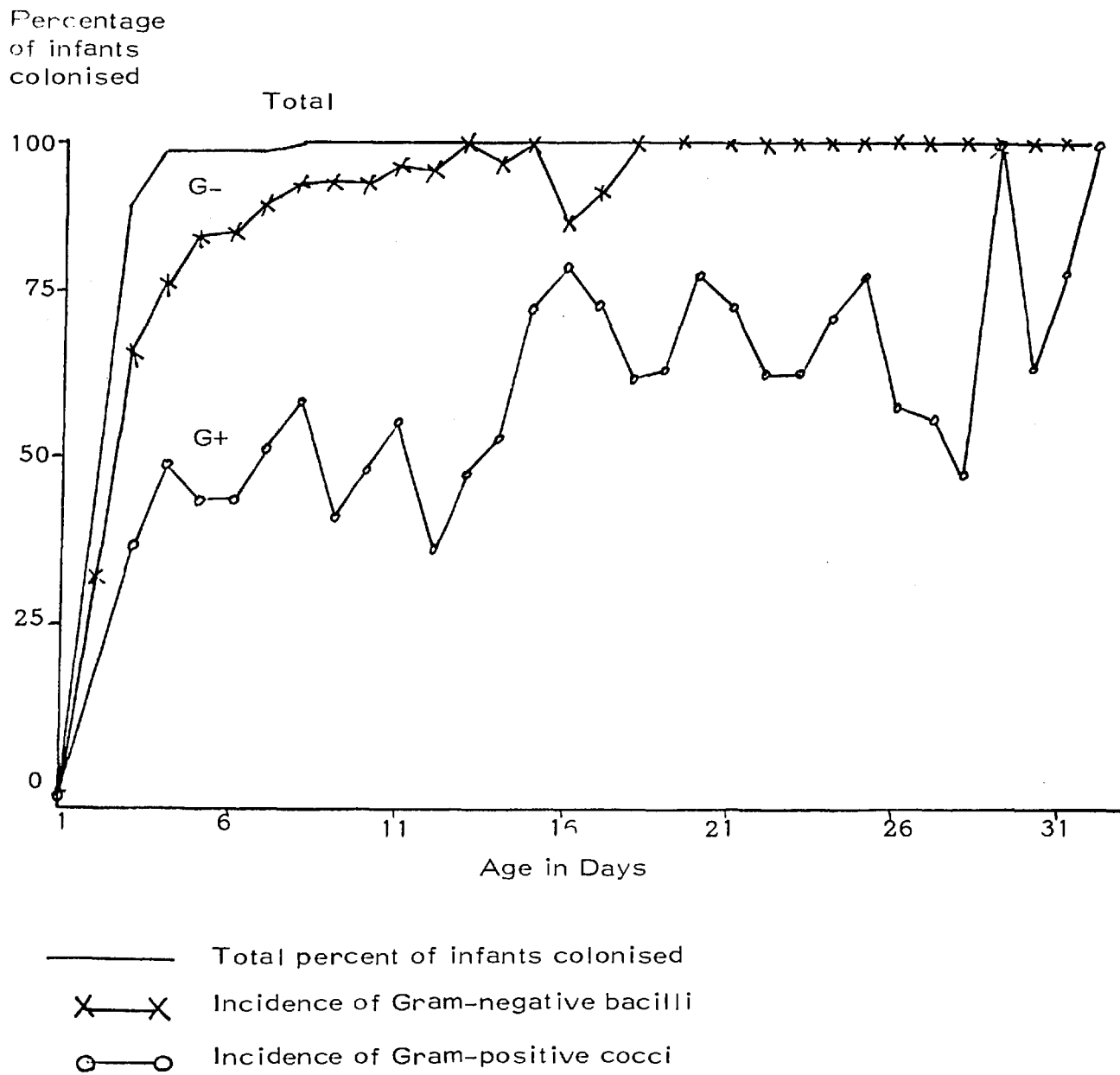


Figure 3. Rectal Flora of Ill/Low Birthweight Infants



on day 3, $p < 0.005$ on day 4 and $p < 0.001$ on day 5) but by day 8 the incidence had increased in normal infants and was similar to that in ill/low birthweight infants.

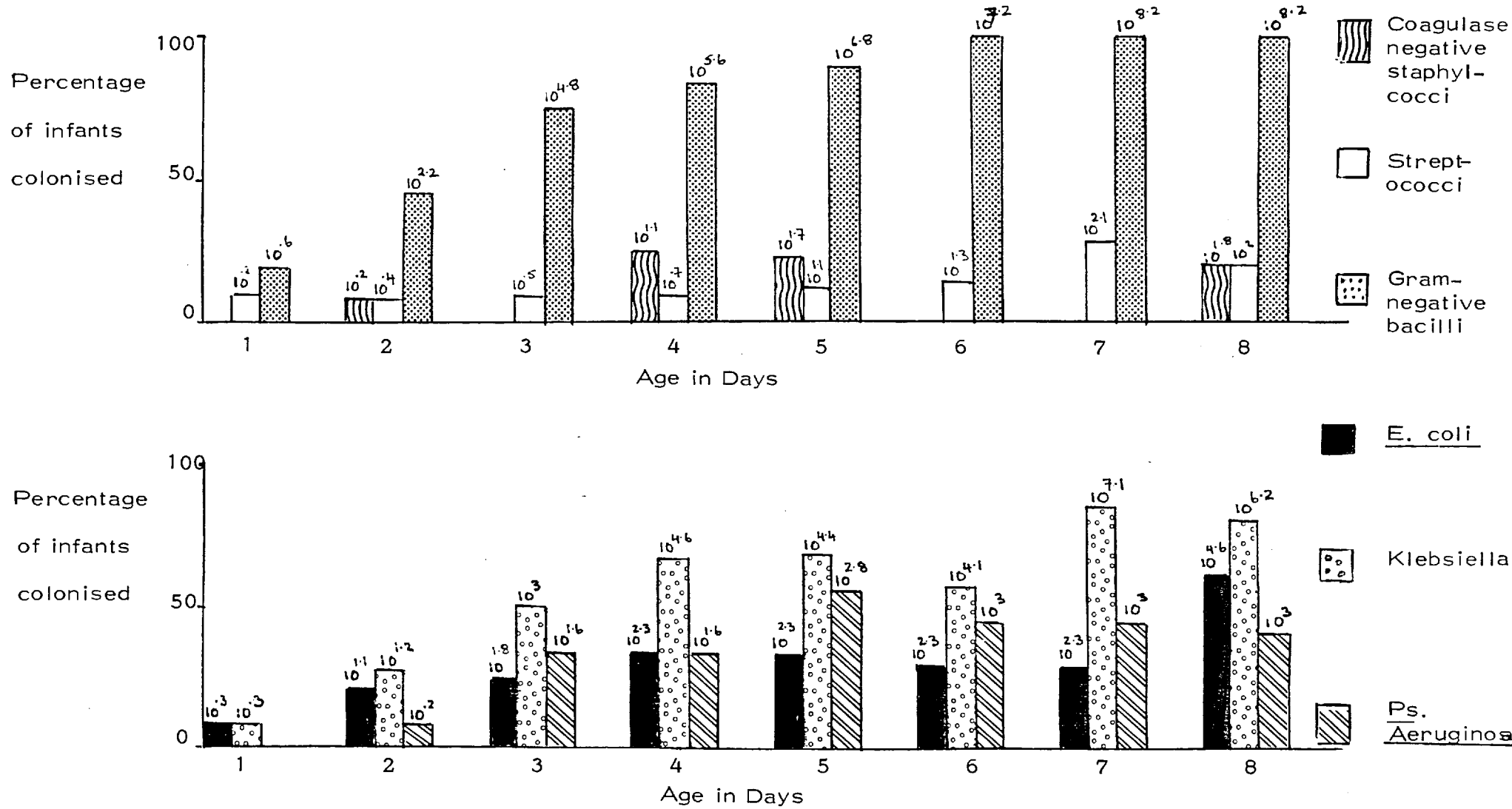
E. coli was the most common Gram-negative bacillus and was isolated from 44.7% of swabs over the first 8 days. Klebsiella species were isolated from 30.1% of swabs and this was significantly higher than in normal infants either breast fed or bottle fed ($p < 0.001$). Ps. aeruginosa was isolated from 8.9% of swabs.

The incidence of coagulase-negative staphylococci in ill/low birthweight infants was significantly lower in the first few days of life than found in normal infants ($p < 0.005$ on day 3).

1) Group 1 Infants

The infants receiving antibiotic therapy nursed in incubators were colonised at a slightly slower rate than other infants and 100% positive was not recorded until day 6. Figure 14 shows the colonisation of these infants.

Gram-positive cocci were isolated in low incidence and numbers during the first 8 days and the flora consisted mainly of Gram-negative bacilli. Klebsiella species were the predominant isolates and by day 8 85.7% of infants were colonised. Ps. aeruginosa was also common in these infants and 55% of infants were colonised by day 5; this figure slowly declined and by day 8 reached 40%. The incidence of E. coli varied, rising to 60% by day 8, but it was never predominant. The total isolation rate over the first 8 days of Ps. aeruginosa and E. coli were similar, 28.6% and 27.3% respectively; Klebsiella species predominated with an isolation rate of 50.7%.

Figure 14. Rectal Flora of Group 1 Infants

2) Group 2 Infants

Infants nursed in incubators were colonised at a similar rate to that of normal infants and 100% positive was reached by day 5. The colonisation is shown in Figure 15.

Initially all swabs were sterile, by day 2 coagulase-negative staphylococci were dominant but by day 3 Gram-negative bacilli had become established. E. coli and Klebsiella species were isolated with a similar overall isolation rate of 31.8% but daily variations in incidence were observed. Ps. aeruginosa was isolated with increasing frequency reaching 29% by day 9 with an overall isolation rate of 13.6%, this organism was the least common of the three groups of Gram-negative bacilli.

3) Group 3 Infants

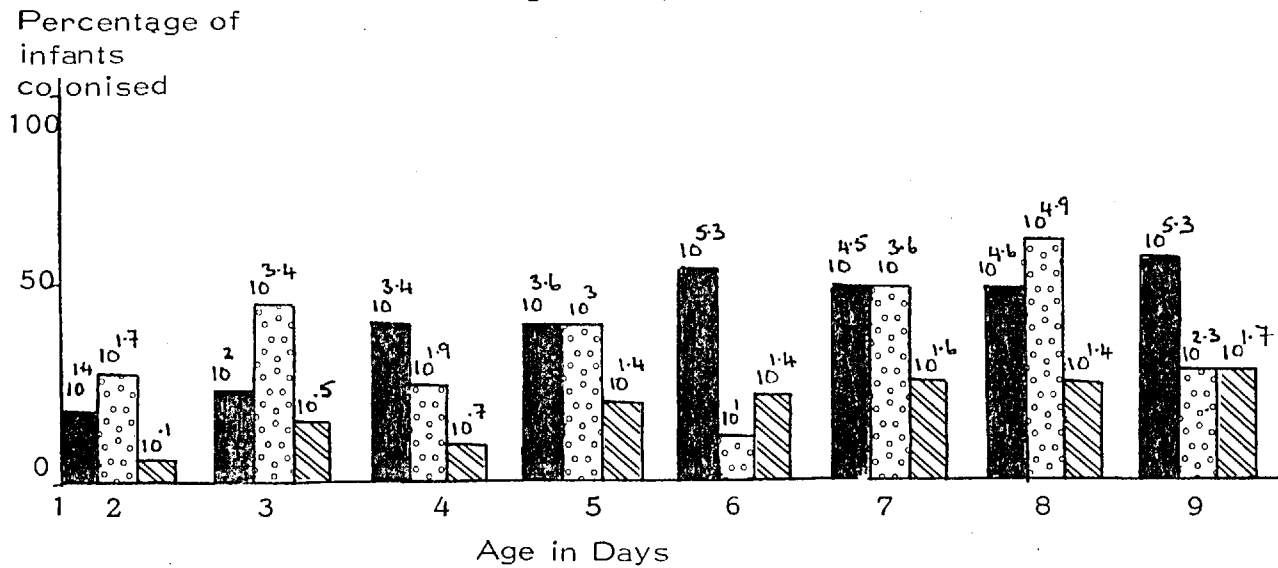
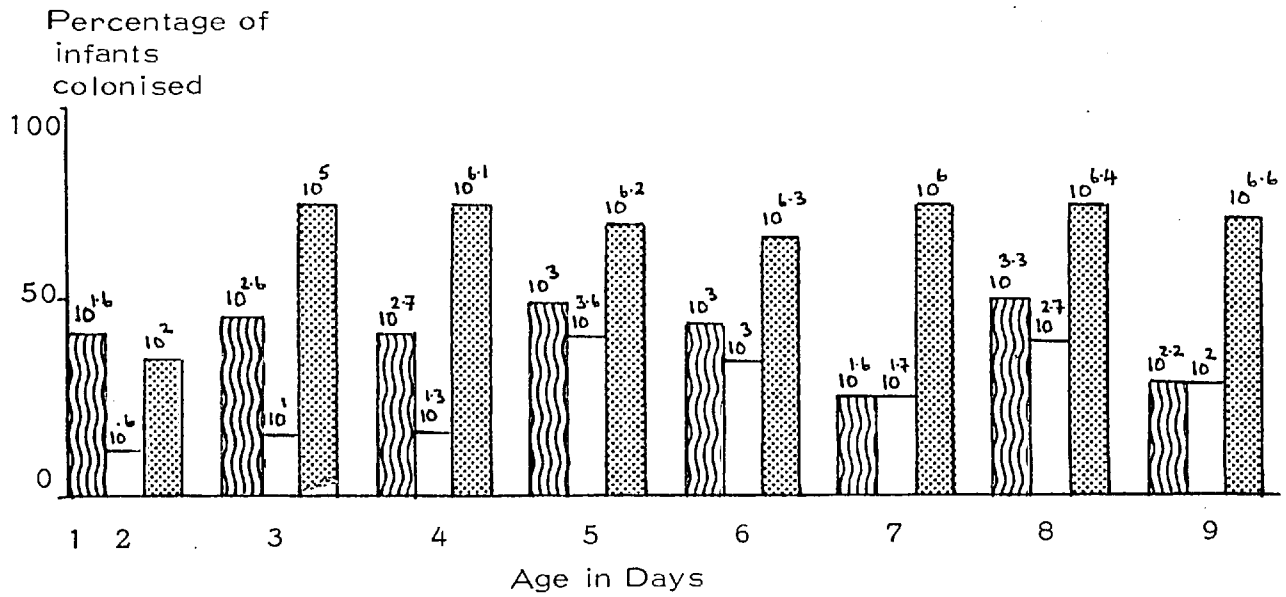
Infants nursed in cots were quickly colonised and 100% positive was found on day 4. The data on the first 8 days of life is shown in Figure 16; after this time the flora remained stable.

The rectal flora was dominated by Gram-negative bacilli throughout with lower but increasing incidence of streptococci and a low incidence of coagulase-negative staphylococci. E. coli was the dominant isolate and by day 8 85% of infants were colonised and the overall isolation rate was 52.3%. Klebsiella species were isolated from 23% of infants and this figure remained stable over days 3 - 8; the overall isolation rate was 23%.

4) Comparison of Infants

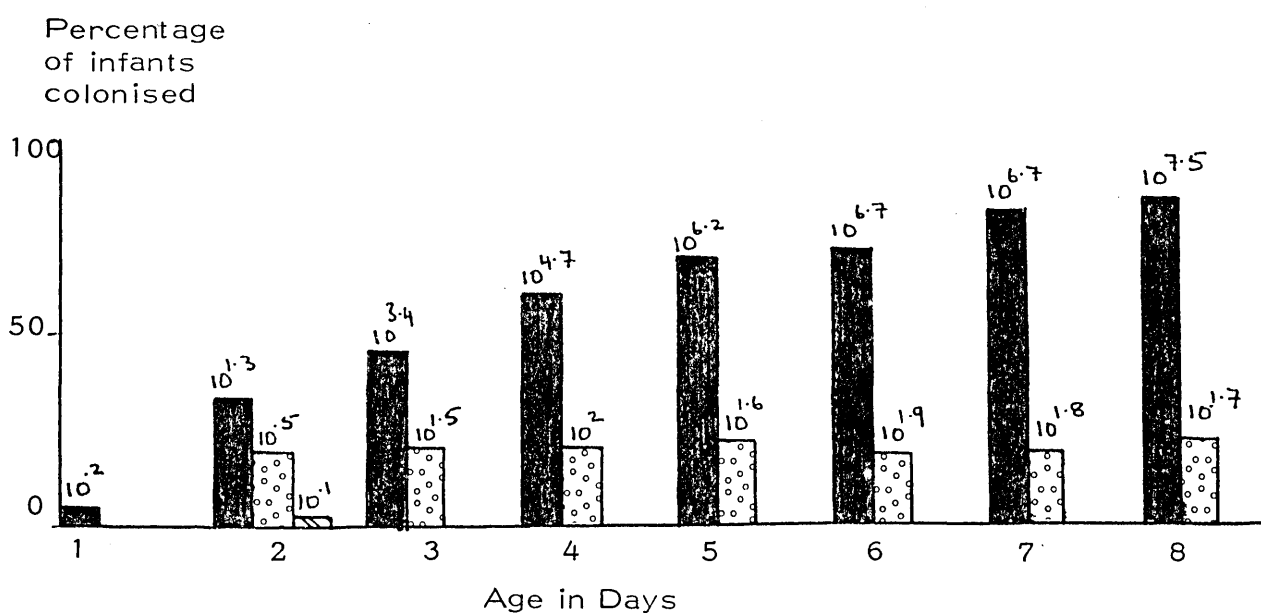
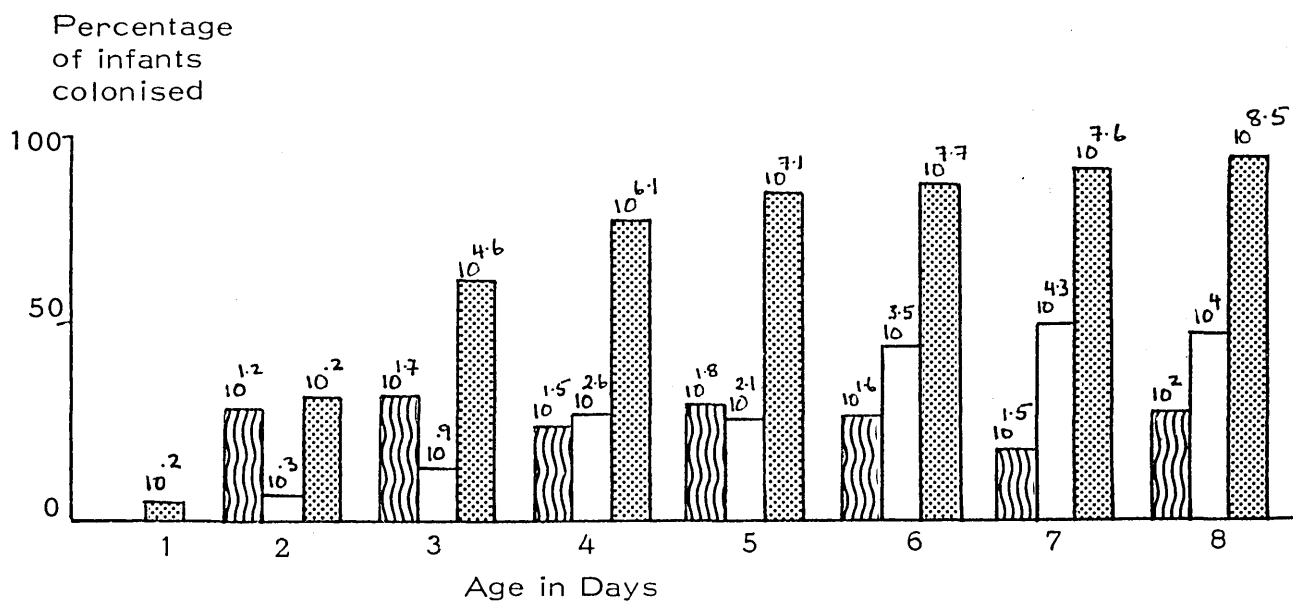
The incidence of the coagulase-negative staphylococci was significantly lower in Group 1 infants by day 3 than in Group 2

Figure 15. Rectal Flora of Group 2 Infants



Key - See Figure 14

Figure 16. Rectal Flora of Group 3 Infants



Key - See Figure 14

infants ($p < 0.05$), Group 3 infants ($p < 0.005$) or normal infants ($p < 0.025$).

In all three groups of infants in the P.B.U. Gram-negative bacilli were significantly more common than in normal infants fed on the same artificial milk ($p < 0.05$ on day 3 - $p < 0.025$ on day 5) but by day 8 the difference was not significant.

The incidence of E. coli was similar in normal bottle fed infants, Group 1 infants and Group 2 infants but was significantly higher in Group 3 infants ($p < 0.001$). Klebsiella species were most common in Group 1 infants and least common in normal infants and significant differences were found between all groups of infants; the incidence in Group 1 was higher than in Group 2 ($p < 0.025$), Group 2 higher than Group 3 ($p < 0.01$) and Group 3 higher than normal infants ($p < 0.05$). Ps. aeruginosa was not isolated from normal infants and only once from a Group 3 infant. The incidence in Group 1 infants was significantly higher than Group 2 infants ($p < 0.05$) and in both these groups of infants was higher than Group 3 infants ($p < 0.001$), as shown in Figure 8.

DISCUSSION

The comparison of the faecal flora of infants studied can be considered in two parts.

In the first part of the study, normal infants fed on breast milk or an artificial milk were compared. In both groups of infants there was an initial high incidence of Gram-positive cocci especially the coagulase-negative staphylococci. This flora was only transient and a Gram-negative flora became established. Although this

transition occurred earlier in breast fed infants, by day 8 the incidence, both in frequency and numbers of Gram-negative bacilli was higher in infants fed on the artificial milk. In all normal infants E. coli was the predominant Gram-negative bacillus and Klebsiella species were isolated with low frequency.

In the second part, infants fed on the same artificial milk but nursed in different environments and receiving different treatments were compared. The infants nursed in the P.B.U. had a significantly higher colonisation with Gram-negative bacilli than bottle fed normal infants and variations within the groups of ill/low birthweight infants were also apparent.

Infants in group 1 had a delayed colonisation, a situation also recorded in the upper respiratory tract. Klebsiella species were the predominant Gram-negative bacilli, Ps. aeruginosa and E. coli were isolated with similar frequency which was considerably lower than that of Klebsiella species. Infants in Group 2 had a different pattern of colonisation with Gram-negative bacilli; Klebsiella species and E. coli were the predominant organisms and were isolated with a similar frequency, Ps. aeruginosa was isolated but with a lower frequency. Infants in Group 3 more closely resembled normal infants in that E. coli was the predominant Gram-negative bacillus and Ps. aeruginosa was rarely isolated. Although the relative incidence of these organisms was similar to normal infants, the total isolation rate was significantly higher and in agreement with the isolation rate found in the other two groups of infants in the P.B.U.

Differences in the isolation rate of Gram-positive cocci were

also observed between the groups of ill/low birthweight infants. Group 1 infants had the lowest isolation rate, infants in Groups 2 and 3 had a similar incidence but in Group 2 infants the incidence of coagulase-negative staphylococci was initially high then fell to the same level as the streptococci whereas in Group 3 infants streptococci slowly rose in incidence to dominate the coagulase-negative staphylococci.

These results agree with Farmer (1967) and Davies et al. (1969) who found that Gram-negative bacilli were more common in ill/low birthweight infants. However the differences in the relative incidence of the types of Gram-negative bacilli indicated that infants can be divided into groups on the basis of such factors as antibiotic therapy and nursing in open cots or incubators as well as the accepted division of breast or bottle fed infants. The types of Gram-negative bacilli were also consistent with the colonisation of the upper respiratory tract, i. e. the higher incidence of *Klebsiella* species and *Ps. aeruginosa* in Group 1 infants, the intermediate position of Group 2 infants, and the prevalence of *E. coli* in Group 3 infants.

Gram-negative bacilli were isolated from the rectum before isolation from the upper respiratory tract and two possibilities can be considered. Firstly colonisation occurred via the anus at birth and followed by auto- or cross-colonisation of the upper respiratory tract, or secondly the numbers of gram-negative bacilli in the upper respiratory tract were so small that they were not detected initially. The next section of this thesis investigated whether strains of bacteria colonising one site of an infant were the same as those isolated from other sites of the same infant and from other infants.

SUMMARY

The rectal flora of normal infants, after passing through a transition period in which the Gram-positive cocci dominated, became predominantly Gram-negative by the eighth day of life. Although this transition was shorter in breast fed infants, bottle fed infants had a higher incidence, both in numbers and frequency, of Gram-negative bacilli by day 8. In both groups of normal infants E. coli was the predominant Gram-negative bacillus isolated with a low incidence of Klebsiella species. Ps. aeruginosa was not isolated from normal infants.

Ill/low birthweight infants in the P.B.U. had a significantly higher incidence of Gram-negative bacilli than either breast or bottle fed normal infants and differences in the relative incidence of the different types of organisms occurred within the three groups of ill/low birthweight infants. Infants treated with antibiotics acquired Klebsiella species as the predominant Gram-negative bacilli with Ps. aeruginosa and E. coli isolated with a similar but lower frequency. Infants nursed in incubators had a high incidence of Klebsiella species and E. coli and a low incidence of Ps. aeruginosa. In infants nursed in open cots E. coli was the predominant Gram-negative bacillus, Klebsiella species were isolated in low frequency and Ps. aeruginosa was rarely isolated. This pattern of colonisation with Gram-negative bacilli in the three groups of ill/low birthweight infants was similar to that found in the upper respiratory tract.

SECTION 5

FURTHER IDENTIFICATION OF ISOLATES

COAGULASE-NEGATIVE STAPHYLOCOCCI

a) Review of the Literature

In the late 1960s there was an upsurge of interest in the coagulase-negative staphylococci when it was realised that these organisms caused endocarditis and bacteraemia associated with Spitz-Holter valves. In order to carry out any epidemiological studies it was necessary to develop a typing system to distinguish strains of this organism. A bacteriophage typing system had been developed for Staph. aureus by Williams and Rippon (1952), but even in the early 1960s it was thought that lysogenicity in other staphylococci was rare and classification was made on biochemical criteria (Baird-Parker, 1963). However, bacteriophages were isolated and Verhoef, Van Eoven and Winkler (1972) reported the development of a bacteriophage typing system.

Strains may be lysed by a number of bacteriophages and the occurrence of long lysis patterns gives rise to many difficulties when assessing the relationship of strains. Williams and Rippon (1952) investigated the variation in lysis patterns in Staph. aureus and found that similarity could not be excluded when strains differed by less than two strong reactions. This rule has been accepted for the coagulase-negative staphylococci.

This technique has been developed recently and there are few reports in the literature. Verhoef et al (1972) reported that the flora of the nose varied widely, some people carried a strain for several months but others carried one or more strains for short periods of time. Dean et al (1973) investigating carriage by

members of a laboratory staff in the nose and on the skin showed that people can carry a large number of strains on the body at one time and may carry the same strain for periods of up to 16 weeks.

b) Method

Over a period of 18 months, 30 strains were sent each week to Dr. M. T. Parker, Cross Infection Reference Laboratory, Central Public Health Laboratory, Colindale, who very kindly bacteriophage typed them. Whenever possible, all the strains from one patient were sent in one batch.

c) Results

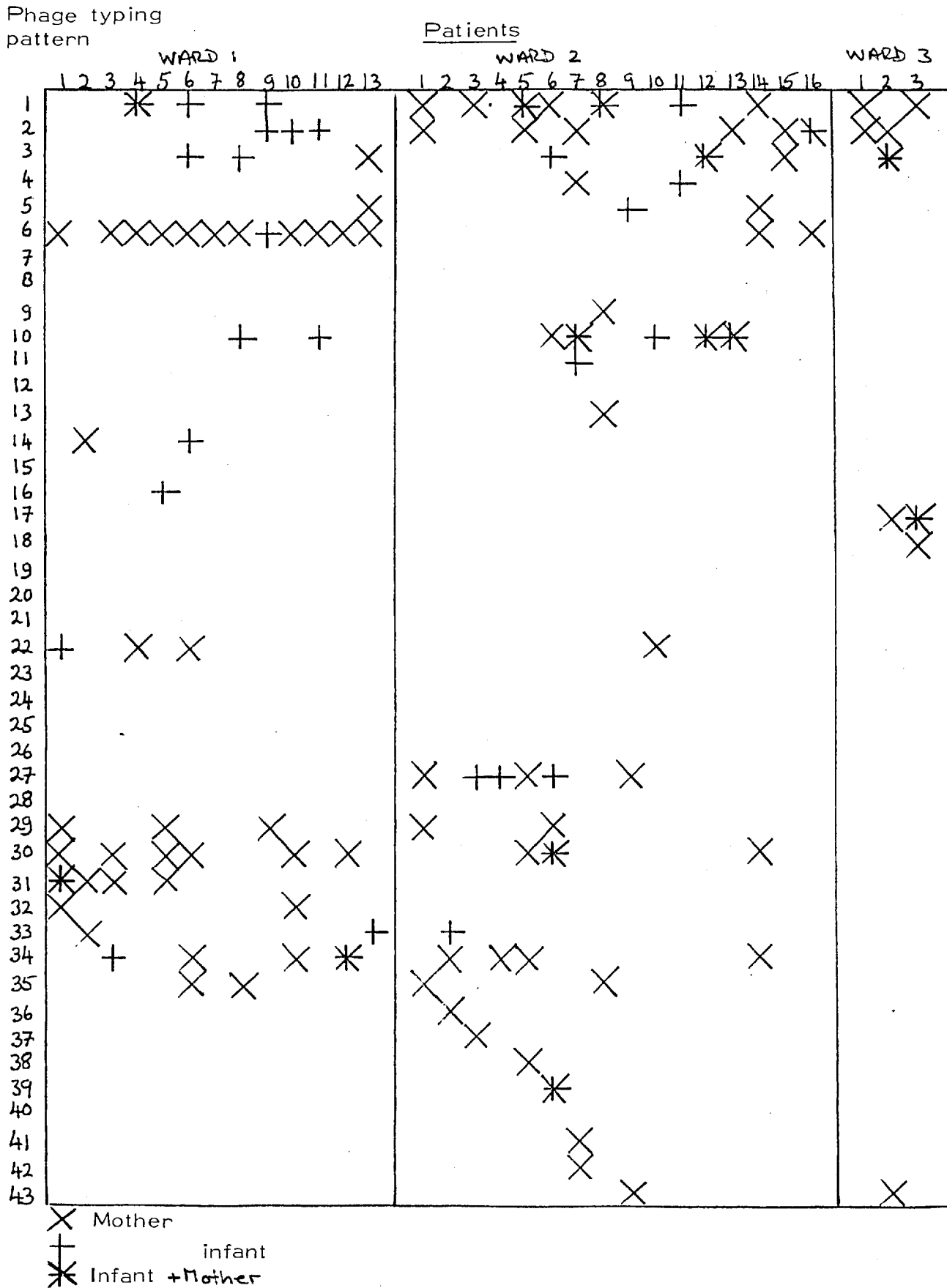
Two thousand four hundred and ten strains were typed using bacteriophages 1-19 and E1. Lysis patterns were allocated using the rules of Williams and Rippon (1952) and the 43 patterns identified are given in Table 1 in the Appendix.

1) Normal infants and their mothers

One thousand and fifty strains isolated from 34 normal infants and their mothers and the attendant staff were typed, 355 (33.5%) were typeable with the bacteriophages stated and 695 (66.5%) were non-typeable.

More typeable strains were isolated from mothers than from their infants and this is shown in Figure 17. Pattern 6 was the most common pattern and was isolated from 13 mothers. This pattern was particularly common in one ward where it was isolated from 11 (84.6%) mothers but only from one (15.4%) infant. Pattern 1 was the next most common and was isolated from nine mothers and six infants, this type was isolated from three infants on a

Figure 17. Phage typing patterns of coagulase-negative staphylococci isolated from normal infants and their mothers



number of occasions and three infants only once.

Seventeen infants (50%) had strains indistinguishable from those of their mothers but no correlation was found with the mode of feeding; nine infants were fed on an artificial milk and eight infants on breast milk. Thirteen of the 17 infants who acquired strains carried by their mothers acquired only one strain whereas 4 infants acquired two strains.

Strains isolated from the attendant staff were also identified. One nurse had a strain, pattern 6, which was common, one nurse had pattern 18 which was not isolated from the infant and mother investigated at that time, one nurse had a strain, pattern 22, which was isolated from the infant in the ward at that time and one nurse had pattern 31, which was isolated from a mother.

2) III/low birthweight infants

One thousand three hundred and sixty strains from 55 infants were phage typed, 471 (34.6%) were typeable and 889 (55.4%) could not be typed with the bacteriophages used. The results are shown in Figure 18.

Pattern 3 was the most common and was isolated from 18 (32.7%) infants over a period of 18 months. The next two common types were patterns 2 (21.8% of infants) and 1 (18.2% of infants). Pattern 6, the most common isolate in the normal infants and their mothers, was isolated from 8 (14.5%) infants.

Coagulase-negative staphylococci were isolated from 5 members of the medical staff; one isolate was non-typeable, two were pattern 9, two pattern 6 and one pattern 19. Both patterns 9 and 6 were relatively common in these infants but 19 was only isolated from one infant over the total period of this investigation.

DISCUSSION

This study was begun in November 1971 before the report by Dean et al (1973) that individuals carry numerous strains of staphylococci. Only one isolate per swab was phage typed unless there were marked colonial differences; however the repeated isolation of a particular type from the mother suggested the predominance of this type and therefore the greater possibility of acquisition by the infant. The closer proximity of breast fed infants to their mothers compared to bottle fed infants did not affect the colonisation of the infant with the types isolated from the mother.

The isolation of certain types common to the lying-in wards or the P.B.U. indicated that there were a number of hospital types which remained constant despite changes in personnel. The isolation of similar types from staff and patients would suggest a common source of these organisms but the low percent of strains which were phage typed does not allow further discussion of this possibility.

SUMMARY

Only one third of the 2410 strains of coagulase-negative staphylococci could be further identified by bacteriophage typing. The identification of certain recurrent types both in the lying-in wards and the P.B.U. suggested a continuing presence of hospital types. Common types were also isolated from hospital staff. Seventeen normal infants had types indistinguishable from those isolated from their mothers but no correlation could be made between the acquisition of these organisms and the feeding regime.

SEROTYPING OF E. COLI

a) Review of the Literature

Since the first isolation from faeces by Escherich in 1855, E. coli has been regarded as one of the more important organisms in the bacterial flora of man. The elucidation of the antigenic structure and the subsequent development of a serotyping system proved to be a major development in epidemiological investigations of this organism.

The acquisition of E. coli by newborn infants can be considered as occurring from two sources; the mother during birth and the environment, staff and other infants after birth. These two are not mutually exclusive. Gareau et al (1959) found that 5 of 20 infants acquired a serotype which had been isolated from the mother and in two cases were isolated at birth, a further indication of a maternal source. The isolation of E. coli after birth could be indicative of an external course but the absence of a common serotype did not support this hypothesis. Bettelheim, Faiers and Shooter (1974) investigated mucous extracts from the mouths of infants immediately after birth and rectal swabs taken from infants and their mothers; in 22 of 33 mothers and infants, the infant acquired a serotype which had been isolated from the mother and in 12 cases, a serotype was isolated from the mucous extract and subsequent stool cultures. An earlier paper (Bettelheim, Faiers and Shooter, 1972) showed that it was not possible to identify all the serotypes in a stool specimen and therefore not possible to state that a serotype was not present only not identified.

The investigations of the acquisition of E. coli from other

sources has been associated with outbreaks of infantile gastroenteritis where epidemiological investigations have been important. E. coli was first suggested as a causative agent of infantile gastroenteritis by Bray in 1945 and later studies have identified certain serotypes as associated with this disease (Taylor 1961).

The epidemiology of an outbreak of E. coli gastroenteritis was reported by Rogers (1951); when an infant with gastroenteritis was admitted into a cubicled ward, not only did the cubicle become heavily contaminated but also the whole ward via the use of communal articles. In this manner other infants in the ward became colonised and a single source caused an epidemic. Rogers also showed that the organism could remain viable in dust for 27 days, an important factor in the control of outbreaks. Admission of infants from other hospitals with outbreaks of gastroenteritis could be correlated with outbreaks in the admitting hospital (Rogers and Keegler, 1951) and in this manner spread between hospitals. The spread of certain serotypes among infants in wards or nurseries has been reported by many workers (Taylor and Charter, 1952; McDonald and Charter, 1952; Anderson, Crockah and Rose, 1954; Mushin and Ashburner, 1964; Farmer and Hassall, 1974).

The above studies all indicate that enteropathogenic E. coli can spread through a neonatal ward but few studies have investigated the spread of other serotypes in newborn infants. Laurell (1952) found that E. coli serotypes in the nose and throat were identical and 06 and 018 were the common serotypes. Serotype 06 was found to be common in the throat and rectum (Mushin and Ashburner, 1964). Orskov (1956) found that 02, 08, 075, 06, 01, 04 and 021

were the common serotypes in the stools of healthy infants. In infants with diarrhoea these serotypes and 057 were isolated but the distribution within these serotypes was different. In a study of a provincial children's home Orskov, Orskov and Paerregaard (1956) 0118, 021 and 068 were the predominant serotypes, cross infection occurred but there was a constant change of serotypes and most infants acquired more than one serotype.

Investigation of E. coli in two institutions (Stuart and Van Stratum, 1945) showed that there was a high percentage of infants in the same institution who carried E. coli with the same antigenic structure but there was little correlation between the two units. This result was also obtained by Orskov et al (1956) who investigated infants in two rooms in a children's hospital. Although certain serotypes were common in both sets of infants, further identification of strains using the H antigens and biochemical reactions indicated that the strains isolated in the two rooms were not identical. Serotypes were isolated from infants over short periods and no serotypes were consistently isolated.

The distribution of common serotypes has been reported by many workers. Turck et al (1969) found that 04, 06 and 075 were more common in adult hospital patients than in the general community and patients acquired these serotypes in relation to the duration of their stay in hospital. Geographical factors also affect the distribution of serotypes (Gruneberg, Leigh and Brumfitt, 1968), 01, 02 and 04 were the common serotypes cited in European papers and 075, 01, 02, 018 and 06 in the American papers. Vahlne (1945) found that 09, 02, 018, 025 and 01 were the common

serotypes and that 35 out of 87 patients in hospital carried 018 which was evidence for a hospital-acquired serotype.

The mode of spread of E. coli has proved very difficult to determine. Cooke et al (1970) isolated it from food and this has been thought to be one mode of spread in adult patients (Vahlne, 1945). This organism has also been isolated from hands after washing with Betadine (Brunn and Solberg, 1973), this was the handwash preparation in use in the P.B.U. during this study. Ironside et al (1970) in their investigation of E. coli gastro-enteritis concluded that even with the best barrier nursing techniques it was not possible to prevent cross-infection.

The high incidence of E. coli in infants in the P.B.U. has already been noted. The object of this part of the study was to determine if serotypes isolated from the upper respiratory tract were similar to those isolated from the rectum and if there was a predominance of certain serotypes throughout the period of study.

b) Method

Two hundred and twenty-four isolates from 82 sites of normal infants and 321 isolates from 158 sites of 34 ill/lowbirthweight infants were serotyped by Dr. B. Rowe of the Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale.

Two thousand two hundred and eighty-three isolates from 837 sites of 52 ill/low birthweight infants were serotyped, 0 antigen only, using a limited range of sera 01, 02, 06, 07, 08, 011, 015, 021 and 075 by Dr. Joan Taylor using the following method. Over-

night cultures grown in nutrient broth were steamed for 30 minutes then tested against two polyvalent sera 1) 01, 02, 06, 015 and 075; 2) 07, 011, and 021. If more than one isolate from a site was positive with a pooled serum, only one isolate was tested with the individual sera to determine the O group. Negative strains were tested with a monovalent serum, 08, and any negative strains were called non-typeable. No identification of K or H antigens was carried out.

c) Results

1) Normal Infants

E. coli isolated from 9 (32%) of the 28 rectal swabs taken from mothers immediately after birth and from 14 infants during their stay in hospital were serotyped. The results are given in Table 18.

Table 18

E. Coli Isolated from Mothers and Infants

Mother and Infant	Mother	Infant
02 K? H5		
02 K? H6		
075 K? H5		
01 K51 H7	076 K? H14	+06 K53 H1
018ac K? H-		02 K? H6, 075 K? H5
0R H4	0? H39	0? H6, 021 H?, 0? H7
0? H-, 0? H4		0? H7
027 K? H7	075 K? H-, 0? H-	086 K? H51, 0? H8, 0? H10, 0? H1
	0148 K? H8	064 K? H4
		075
		*07
		*0? H6
		02 K7 H-, 04 K? H5, 075 K? H5
		01 K7 H-
		020 K7 H12
		07 H10, 0? H6
		078 K? H9

+ Isolated immediately after birth from mouth, ear and rectum, probably maternal origin

* Caesarian section

In eight cases, a serotype isolated from the mother was also isolated from her infant; in six of these cases identification was made on the O and H antigens but in two cases the O antigen could not be determined and the H antigen was the basis of similarity.

Fourteen infants acquired serotypes not isolated from the mother. In one of these infants E. coli 06 K53 H1 was isolated from the ear, mouth and rectum immediately after birth, and although this serotype was not isolated from the mother, was suggestive of a maternal origin; the membranes had been ruptured for over 20 hours which would increase the possibility of colonisation of the infant by the mother. In 4 cases the infant and mother had one serotype in common, in one case the serotype isolated from the mother and infant was different. In eight cases no E. coli was isolated from the mother, in two of these cases birth was by Caesarian section which indicated that colonisation occurred from sources other than the mother.

A total of 13 different O serotypes and 5 serotypes in which the O antigen could not be identified but the H antigen was were isolated from infants and 7 O serotypes from the mothers. However no two infants were in the same ward at the same time and therefore no inference about the presence or absence of a hospital flora in the wards can be made.

2) III/Low Birthweight Infants

Three hundred and twenty-one Isolates were serotyped by Dr B. Rowe, 99 (62%) were fully identified and 122 (38%) were either rough or could not be serotyped.

Two thousand two hundred and eighty-three isolates were

investigated by Dr Joan Taylor, 1681 (73.9%) were identified with the polyvalent sera and of these strains 848 (37.1%) were identified. At least one isolate from each swab was identified whenever possible.

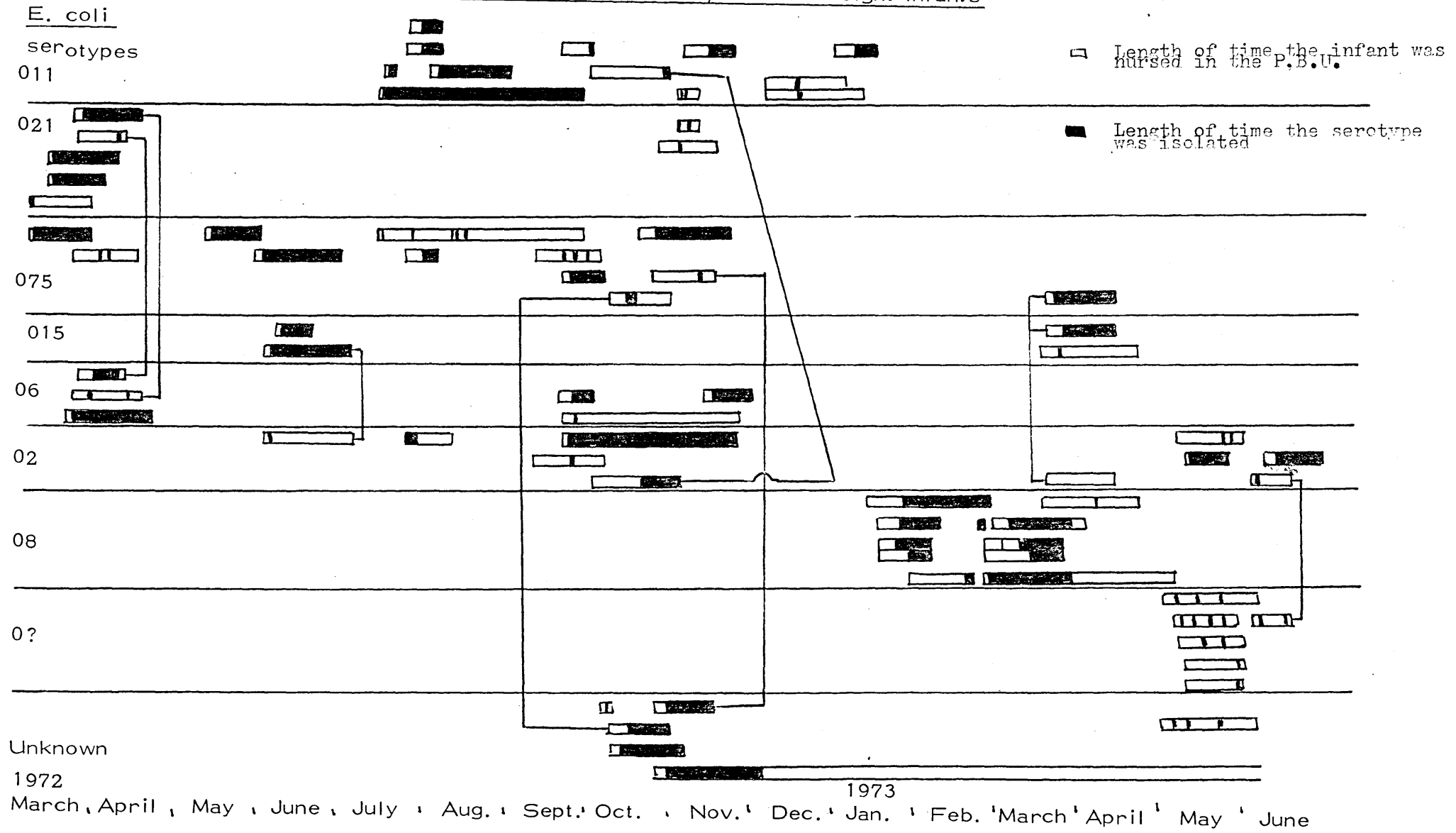
The results are shown in Figure 19. More than one serotype was isolated from some infants who are therefore represented twice in the diagram, a line connects the two or more representations of these infants.

Six serotypes, 02, 06, 08, 011, 021 and 075 were the common serotypes isolated throughout the investigation. Clusters are evident in the diagram when a serotype was isolated from more than one infant at that point in time; this was particularly evident in February-March 1973 when 08 was isolated from 10 infants.

Initially 021 was a common isolate and consistently isolated from 3 infants. Another infant initially acquired this serotype but all subsequent isolates were 075, a serotype isolated at regular intervals over the first nine months of this investigation. A change of serotype was also noted in one other infant who was born by Caesarian section; 02 was isolated on days 2 and 3 but from day 4 onwards 015 was isolated. This serotype was subsequently isolated from another infant during this period.

In September 1972, 011 formed a cluster of isolates; one infant carried this serotype for $2\frac{1}{2}$ months and 075 was isolated at infrequent intervals from this infant: 011 and 075 were isolated from infants over the next few months during which time three infants acquired 02 and five acquired E. coli which could not be identified using the range of sera: 083 was isolated twice from one infant at a four day interval but it was not possible to

Figure 19. E. coli serotypes isolated from ill/low birthweight infants



determine if this serotype could be isolated throughout the infant's stay in the unit.

After the cluster of isolations of 08 in early 1973, there was a small cluster of infants carrying a strain identified by Dr. Rowe as 0 unidentifiable H10. As six infants in the unit over a short period of time all acquired E. coli with this structure, it is probable that it was a single strain and is represented in Figure 19 as such.

Investigation of the serotypes isolated from each infant indicated that in most cases one serotype was consistently isolated during the stay in the unit. Only in one infant were two serotypes regularly isolated. Identification of serotypes from the upper respiratory tract and the rectum showed that the same serotype was present in both sites.

Treatment received by the infant in the unit did not influence the serotype of E. coli acquired by the infant.

DISCUSSION

The isolation of an E. coli serotype common to the mother and her infant indicated that the infant acquired some of his bacterial flora from the mother during the birth process. This has been reported by Gareau et al (1959) and Bettelheim et al (1974). In one case a serotype was isolated from three sites of an infant immediately after birth and although this serotype was not isolated from the mother it was probably of maternal origin. Thirteen other infants acquired serotypes not isolated from the mother but three factors must be taken into consideration. Firstly

all mothers were given a soap and water enema during the early part of labour. Secondly, the nursing staff changed from time to time, so that the rectal swabs taken by them could not follow the standard procedures used by me. Thirdly, only three isolates per swab were identified and, as Bettelheim et al (1972) showed, it is not possible to identify all the serotypes present in a sample which contains large numbers of E. coli. However the wide range of serotypes isolated from normal infants suggested that the mother is a more important factor in the acquisition of E. coli by a normal infant than the hospital environment. Two infants were born by Caesarian section and, as organisms would not be acquired during birth, their bacterial flora originated from sources other than the mothers' vaginal flora. These two infants acquired E. coli with different serotypes but as they were born two months apart, this small number does not allow any inference about the presence or absence of hospital serotypes.

There was a high incidence of E. coli in the ill/low birthweight infants and the number of strains identified indicated the common serotype(s) of each infant. Infants were investigated over a period of seventeen months and although it was not possible to identify all patterns of cross-colonisation, clusters of serotypes were isolated. The occurrence of hospital serotypes was detected by Turck et al (1968) and in that situation 04, 06, and 075 were the common serotypes. Gruneberg et al (1968) found that E. coli serotypes had a geographical variation not only between America and Europe but also within areas of the same city. In their study of domiciliary practice, 01, 02, 04, 06, 07, 011, 018, 039 and

075 constituted 51.5% of the normal faecal flora in adults. In this study 02, 06, 08, 011, 015, 021 and 075 were the common serotypes in the P.B.U. and were isolated from 85% of the infants colonised with E. coli.

Cooke, Ewins and Shooter (1969) investigated the change in serotypes at weekly intervals in patients in an adult female ward. They found that the faecal population was constantly changing and small clusters of patients carrying the same serotype were noted. In this study, the majority of infants retained the same serotype throughout their stay but in one case a change of serotype occurred in the first few days and in another infant two serotypes were regularly isolated.

E. coli from the upper respiratory tract was investigated by Laurell (1952) who found that these strains could be serotyped. In this study the serotype isolated from the upper respiratory tract and the rectum were similar. Colonisation of the infant could have occurred by one of two routes. The first possibility is that auto-colonisation of the infant occurred in which case the serotypes from the various sites of the infant would be similar. The second possibility is that the upper respiratory tract was colonised by E. coli from another infant in the unit, as more than one infant carried the same serotype at the same time it is possible that cross-colonisation with the serotype already carried by that infant could have occurred. However the presence of more than one serotype in the unit at any one time would mean that cross-colonisation of the infant with a different serotype could also occur; this was not found in this study. Some cross-colonisation of infants must occur because clusters of a serotype were clearly evident.

Ironside et al (1971) stated that even with an experienced nursing staff using conventional barrier nursing techniques it was not possible to prevent cross-infection in infantile gastroenteritis. This result was also found in the P.E.U. where hand-washing technique was rigorously enforced, and the nursing staff were fully aware of aseptic technique. This cross-colonisation of infants also occurred with non-enteropathogenic serotypes, for example ten infants acquired E. coli 08 at a time when the unit was particularly full. The infants would be closer together than at other times and this factor increased the possibility of cross-colonisation of infants. Not all infants in the unit at any one time were investigated and therefore the full extent of the cross-colonisation was not determined. However the lack of distinction in E. coli serotypes from infants born by Caesarian section and infants born by vaginal delivery indicated the predominance of the environmental serotypes in the determination of the infants' flora. These serotypes were isolated over a period of seventeen months despite changes in staff and this constant isolation of serotypes agrees with the findings of Kennedy, Florde and Petersdorf (1965). The results of Kennedy et al (1965) and Gruneberg et al (1968) suggest that a hospital flora is specific for that situation and variation between hospitals occurs. Investigation of this variation might indicate the selective pressures which produce a hospital flora and this would be of interest in the control of hospital infection.

SUMMARY

E. coli from 9 mothers and 17 normal infants were serotyped, in 8 instances the same serotype was isolated from the mother and

infant, in 14 cases a serotype was isolated from the infant which was not isolated from the mother although in one infant it was isolated immediately after birth and was probably of maternal origin.

E. coli from 52 ill/low birthweight infants were also serotyped. Seven serotypes, 02, 06, 08, 011, 015, 021 and 075 were the common serotypes and the isolation of these serotypes occurred in clusters rather than a uniform distribution. E. coli isolated from the upper respiratory tract and the rectum were of the same serotype which suggested an intra-infant colonisation although the presence of other infants in the unit carrying that serotype would not preclude inter-infant colonisation. The E.coli flora of these infants tended to remain constant over the period of investigation.

PSEUDOMONAS AERUGINOSA

a) Review of the Literature

Over the last fourteen years, Gram-negative bacilli especially Ps. aeruginosa have been found to be important causes of infection. This organism is known to be a frequent contaminant of hospital equipment and is highly resistant to many antibiotics and disinfectants (Heckman, Babcock and Rose, 1972).

In order to carry out any epidemiological studies, it was necessary to develop an effective and practical means of typing this organism. In 1960, Holloway suggested that pyocine production by Ps. aeruginosa might form the basis of a typing system. Wahba (1963) investigated the factors affecting pyocine production and in 1964, Darrell and Wahba reported the results of an investigation of 1,000 strains of Ps. aeruginosa using 12 pyocine indicators, Gillies and Govan (1966) proposed a pyocine typing system using a modified medium and 8 indicator strains which gave 35 patterns of inhibition.

Investigations indicated that patients could carry more than one pyocine type and the predominant types varied from one hospital to another. Heckman et al (1972), using this method and indicator strains, found that although the most common pyocine type was the same as found by Gillies and Govan, pyocine type 1, there was a large variation in the distribution of the other pyocine types.

In 1969, Govan and Gillies subdivided pyocine type 1 into eight subtypes and found inter-hospital variation in the predominance of the subtypes although 1b and 1h were the most common. Mushin and Ziv (1973) investigated numerous strains of Ps. aeruginosa and

found that 1h was the most common subtype.

Carriage of Ps. aeruginosa by newborn infants was reported by Cole, Thom and Watrasiewicz (1971) who found that 16 of 20 infants were colonised with this organism; only 2 infants showed clinical symptoms and both responded to treatment. This indicated that infants could carry this organism without clinical symptoms.

Morehead and Houck (1972) investigated two outbreaks of Ps. aeruginosa infection in an intensive care unit involving 14 infants over a period of 6 months. In the second outbreak, 3 of 4 infants were infected with the same pyocine type. Fourteen cultures of the environment yielded only two isolates; a suction bottle of a colonised infant and a metal jar containing used suction catheters found in the utility room. Thirty-three swabs from personnel before and after handling infants and after washing gave only two positive cultures, both of which were after handling a colonised infant and prior to washing. This study emphasised the great difficulty in the elucidation of the mode of spread of this organism.

Previous work on pyocine types found in the F.B.U. was reported by Davies et al (1970) who isolated a non-typeable strain over a period of 6 months indicating that colonisation of infants must occur from one infant to another. In 1971, 27 strains of this organism isolated from infants in the unit were typed (D. Adams unpublished data); ten isolates belonged to type 10, six to type 1 of which three were 1b, one was 1c and two were not subtyped; two belonged to type 22 and nine were non-typeable. This work identified the pyocine types present in the unit before this study was started.

b) Method

Two hundred and eight strains of Ps. aeruginosa were isolated from 11 ill/low birthweight infants and 9 strains from the environment. Each strain was defined as Ps. aeruginosa using the criteria stated previously (page 32).

The indicator strains were kindly supplied by Dr. Gillies.

1) Culture Media

Nutrient broth No. 2 (Oxoid) was used to grow the test strains and the indicator strains. Tryptone soya agar was prepared according to the manufacturer's instructions and 5% of horse blood was added.

2) Technique

The method of Gillies and Govan (1966) was used. The apparatus devised by Wahba and Lidwell (1963) was used to apply the indicator strains at right angles to the original line of inoculum. Any strains belonging to pyocine type 1 were subtyped using the method of Govan and Gillies (1969).

c) Results

1) Normal Infants

Ps. aeruginosa was not isolated from the 37 normal infants born at Hammersmith Hospital and nursed in the maternity wards.

2) Ill/low birthweight infants

Ps. aeruginosa was isolated from 11 of the 64 infants studied. Seven infants, once colonised, remained carriers for short periods, three were positive on one occasion only and one was positive for three days.

The clinical data obtained for these 11 infants is shown in Table 19.

Table 19

Patients Colonised with Ps. aeruginosa

<u>Patient No.</u>	<u>No. of isolates</u>	<u>Site</u>	<u>Age in days</u>	<u>Place of birth</u>	<u>Relevant Clinical Details</u>		
24	1	R	10	H.H.			
26*	16	M.N.R.	3-32	H.H.	Intubated for 25 minutes.	I.P.P.V.	
87**	6	M.R.	12-14	H.H.			
89*	17	M.N.R.	3- 8	H.H.	Ventilator, C.P.A.P.	1 - 5 days	
94*	44	M.E.N.C.R.H.	2-31	H.H.	Intubated for 35 minutes		
95	1	R	2	H.H.			
98*	42	M.N.R.	2-32	W.M.H.	Ventilator for 6½ hours.		
101**	40	M.E.N.R.	2-31	P			
105**	39	M.N.R.	2-31	H.H.	Intubated		
116*	28	M.N.R.	1-28	P	Intubated. Ps. aeruginosa isolated from endotracheal tube on admission.		
M	Mouth	C	Umbilicus	H.H.	Hammersmith Hospital	C.P.A.P.	Constant
E	Ear	R	Rectum	W.M.H.	West Middlesex Hospital		Positive Airway
N	Nose	H	Hands	P	Perivale Hospital	P.P.P.V.	Pressure
*	Group 1 Infants						Intermittent
**	Group 2 Infants						Positive Pressure
							Ventilation

A total of 235 strains of Ps. aeruginosa were isolated; 34 cultures (14.5%) failed to grow on subculture after storage and 201 (85.5%) strains were typeable. The results are shown in Table 20 and depicted on a time scale in Figure 20 together with the results of the routine weekly mouth and rectal swabs taken from all infants in the P.B.U.

When infant 24 was admitted to the unit, another infant was known to be a carrier of Ps. aeruginosa and this infant was still in the unit when infant 26 was admitted. The initial swabs from infant 26 were sterile and colonisation with Ps. aeruginosa was first detected on the third day of life, the 22nd March 1972. The same pyocine type, type 10, was isolated from infant 24 on the 23rd March.

Infant 89 was admitted to the P.B.U. when there was one carrier of Ps. aeruginosa, unknown pyocine type, already in the unit. Infant 89 became colonised with type 10 on the third day of life and remained a carrier for the duration of his investigation. At this time, infant 87 was in the unit and he acquired Ps. aeruginosa type 10 relatively late in life, day 12. The only other infant in the unit colonised with Ps. aeruginosa was infant 89 who carried type 10 also.

Infants 95 and 98 became colonised with Ps. aeruginosa type 1b at about the same time when the unit had two infants carrying Ps. aeruginosa, infants 89 and 94, but neither carried type 1b. Infant 98 was admitted from another hospital but became colonised with a type known to be in the unit; he was the only infant studied who showed a permanent change of pyocine type. Initially this

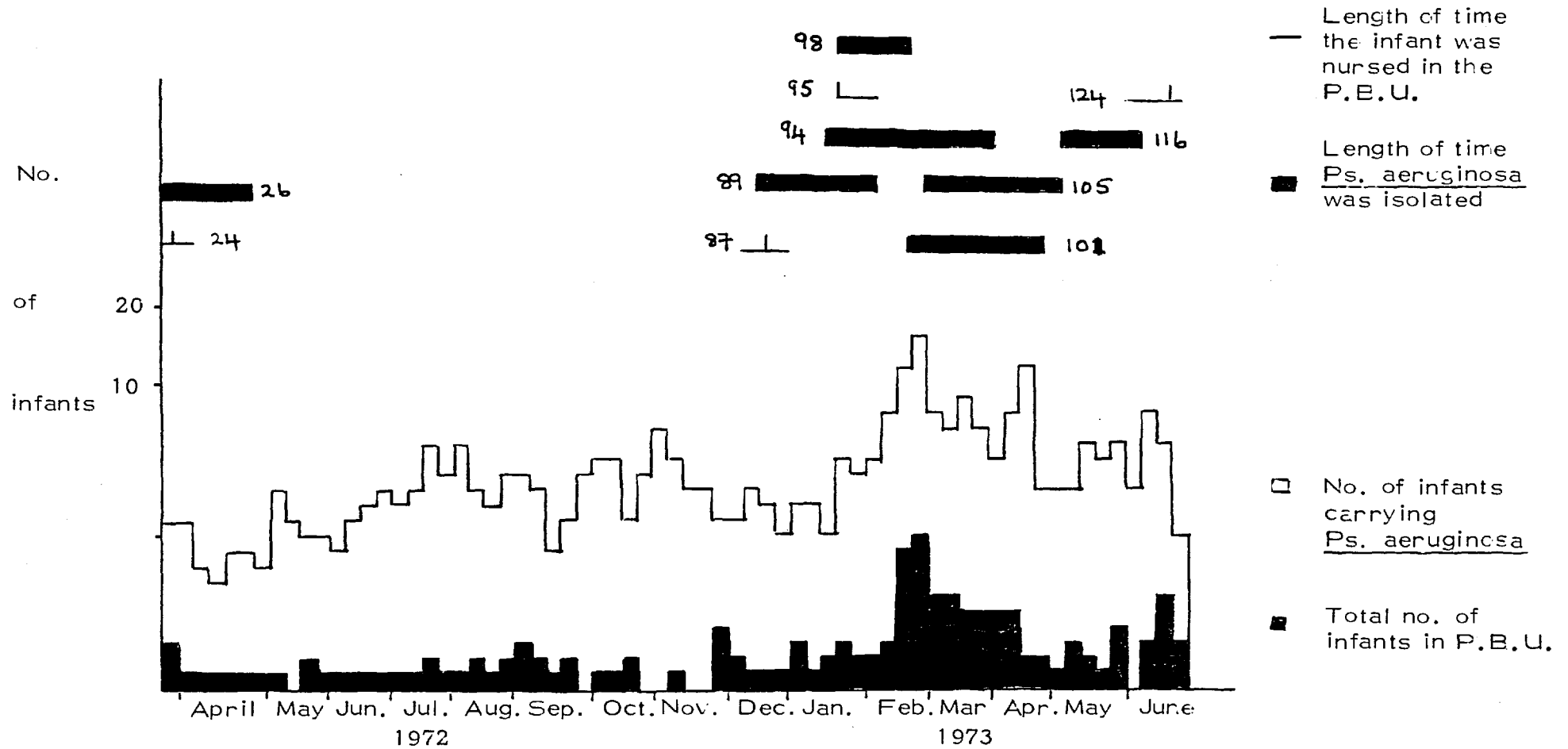
Table 20

Pyocine Types isolated from infants
in the PEU

<u>Patient</u> <u>No.</u>	<u>No. of</u> <u>isolates</u>	<u>No. pyocine</u> <u>typed</u>	<u>Date of</u> <u>isolation</u>	<u>Site</u>	<u>Pyocine</u> <u>Type</u>
24	1	1	23. 3. 72	R	10
26	16	16	22. 3. 72-20. 4. 72	M, N, R.	10
87	6	6	18. 12. 72-20. 12. 72	M, R.	10
89	17	17	15. 12. 72-20. 12. 72	M, N, R.	10
94	44	42	15. 1. 73-13. 2. 73 17. 1. 73	M, E, N, H, R. R	3 10
95	1	1	20. 1. 73	R	1b
98	42	34	21. 1. 73-25. 1. 73 26. 1. 73 26. 1. 73 28. 1. 73 28. 1. 73 29. 1. 73-20. 2. 73	M, R. M. R M R M, N, R.	1b 1b 3 3 1b 3
101	40	29	28. 2. 73-29. 3. 73	M, E, N, R.	1b
105	39	35	28. 2. 73-27. 3. 73	M, N, R.	1b
116	28	19	8. 5. 73- 1. 6. 73	M, N, R.	10
124	1	1	19. 6. 73	R	10

E = Ear
M = Mouth
N = Nose
R = Rectum
H = Hands

Figure 20. Ps. aeruginosa in ill/low birthweight infants



infant was nursed in one section of the unit and acquired type 1b but on the 24th January 1973 was moved to the isolation room which was occupied by infant 94. Within two days infant 98 acquired the same pyocine type as carried by infant 94, type 3. Both infants were cared for by the same nursing staff and were also in a confined area.

When infants 101 and 105 were admitted to the unit, infants 94 and 98 were in the isolation room. Both infants became carriers of the same pyocine type initially acquired by infant 98, type 1b. At this time ten infants in the unit were carriers of Ps. aeruginosa and there was a high probability that cross-colonisation would occur.

Infant 116 was admitted from another hospital already colonised with Ps. aeruginosa type 10. This infant, like infants 26, 89, 94, 101 and 105 retained the same pyocine type throughout the period of investigation.

In June 1973, when the number of infants colonised with Ps. aeruginosa was high, an infant (infant 124) became colonised with this organism pyocine type 10, a type also isolated from infants 24, 26, 87, 89 and 116.

3) Environment of the P.E.U.

Swabs taken from the sinks and equipment were tested for the presence of Ps. aeruginosa and the pyocine type determined. Sinks in the P.E.U. were negative except for one sink at the entrance to the unit which was the only one to have a S-bend waste pipe. Ps. aeruginosa was isolated from sinks in the laboratory adjacent to the unit and these sinks all had S-bend waste pipes. This organism was also isolated from the blood-gas machine which was also in the laboratory.

The pyocine types are shown in Table 21 .

Table 21.

<u>Pyocine Types Isolated from the Environment</u>		
<u>Site</u>	<u>Date</u>	<u>Pyocine Type</u>
Sink at entrance to P.B.U.	24. 4. 1973	3
	15. 5.	Not typeable
Sinks in laboratory		
Sink 1	25. 4. 1973	1h
2	10. 5.	1b
3	25. 4.	10
	10. 5.	1b
Blood gas machine	25. 4. 1973	10
	2. 5.	10
Sink 3	5. 1. 1974	3

In May 1973, the laboratory was thorough cleaned and no further isolates were made from the equipment. However it proved impossible to eradicate Ps. aeruginosa from the sinks with S-bends and a later investigation in January 1974 yielded pyocine type 3.

DISCUSSION

Factors which are thought to affect the carrier rate of Ps. aeruginosa in premature infants include lack of antibodies, use of antibiotics which favours the selection of resistant organisms such as Ps. aeruginosa, high humidity and better treatment leading to the survival of more premature infants (Jellard and Churcher, 1967).

Antibiotics were not used prophylactically in the unit; five of the infants permanently colonised were treated with antibiotics to which the organism was sensitive in vitro but this failed to eradicate the organism although the counts were reduced.

All seven infants permanently colonised with Ps. aeruginosa

were nursed in incubators which possessed humidifying units and the high humidity obtained in incubators favours the colonisation of infants with this organism (Hoffman and Finberg, 1955; Evans et al, 1970). Ventilation therapy is also thought to be a factor favouring the colonisation of infants (Bassett et al, 1965; Rubbo, Gardner and Franklin, 1966); seven infants in this study had prolonged ventilation therapy, three died and two of the surviving four infants acquired Ps. aeruginosa.

If the carrier rate in the unit is high, there is an increased chance that any infant entering the unit may become colonised. This high carrier rate at certain times may account for the transient colonisation of the four infants and be a factor in favour of the permanent colonisation of the other seven infants.

None of the infants colonised with Ps. aeruginosa showed symptoms of infection which agrees with the findings of Cole et al (1971) that response to Ps. aeruginosa is unpredictable and individualistic and that most infants will not show clinical symptoms.

The change in pyocine type that occurred in infant 98 indicated that despite the stringent washing techniques followed in the unit, cross-colonisation of infants took place. The two infants were close together in the isolation room which played an important role in this occurrence.

The environment contained all the types isolated from infants in the unit. Jellard and Churcher (1967) considered that the environment was not the main source of Ps. aeruginosa infection but the fact that the laboratory opened on to the unit and had many sources of this organism must present a potentially hazardous situation. Infants who had ventilation therapy and the ill infants nursed in incubators were handled

more than infants nursed in cots, they also had more tests, e. g. blood gas measurements, than the well infants. Medical staff were not required to change their gowns on returning to the unit from the laboratory and, unlike the nursing staff, did not put on a separate overgown for each infant. These two factors increased the chance of indirect cross-colonisation especially when the unit had a large number of ill infants and crowding would act as a third factor. It is suggested that whenever possible gowns should be changed when re-entering the unit from the laboratory and after handling any infant known to be a carrier of Ps. aeruginosa.

Jellard and Church (1967) regarded faeces as the main reservoir of this organism and suggested that great care should be taken when changing napkins which must be placed in a bag or impervious container and removed from the wards. Infants admitted from other hospitals should, if possible, be placed in an isolation room until the results of the initial swabs are known.

Observation of these precautions may result in a reduction in the carriage rates of this organism within the unit. Antibiotic therapy will not eradicate Ps. aeruginosa and prevention of colonisation must be the desired objective.

SUMMARY

Ps. aeruginosa was not isolated from normal infants but was isolated from eleven ill/low birthweight infants. Seven infants became carriers and six of these cases retained the same pyocine type throughout their stay in the unit; one infant had a change of pyocine type when placed in an isolation room with an infant

colonised with a different pyocine type. Six infants received ventilation therapy and five of them were treated with antibiotics to which the organism was sensitive, but this did not eradicate the organism. Four infants became transiently colonised, in three cases with a pyocine type known to be carried by other infants in the unit at that time. The same pyocine types as those isolated from the infants were also isolated from the laboratory separated from the unit by a door which was usually open

SECTION 6

ANTIBIOTIC RESISTANCE OF E. COLI

ANTIBIOTIC RESISTANCE OF *E. COLI*

a) Review of the Literature

Antibiotic resistance has become an increasing problem in recent years. Smith and Hall (1966) reported that 15 out of 24 healthy adults carried resistant *E. coli* and Datta (1969) found a carriage rate of 52% in patients in hospital. Moorhouse (1969) reported a rate of 71% in Dublin children under 2 years of age, this level was similar to that reported by Linton *et al* (1972) who found that 67% of children and 46% of adults carried resistant *E. coli*. The higher carriage rate found in children might be due to the higher rate of illness and resultant use of antibiotics.

Shallard and Williams (1965) investigated neonates who had received antibiotic therapy and found that these infants had a higher rate of colonisation with resistant organisms. In 1966, they reported that increased lengths of hospitalisation could be correlated with increased colonisation with these organisms.

The antibiotics used in the neonatal period vary with hospital policy but ampicillin and kanomycin have been widely used in America and Great Britain. In 1971, Starkey and Gregory in an investigation in a general hospital, reported a rise in ampicillin and kanomycin resistance reaching 36% and 33% respectively in 1969. Franco, Eitzman and Baer (1973) followed the change in kanamycin resistance in a premature baby unit over a period of $3\frac{1}{4}$ years. Initially this was the first drug of choice and the carriage rate of resistant organisms was 10%. After the drug was withdrawn the carriage rate fell to 0.9%, this rose again when the drug was reintroduced and then fell when the antibiotic was withdrawn again. Gentamicin

replaced kanamycin and after 25 months a resistance rate of 2.5% was reported. Infants colonised with resistant organisms tended to remain carriers for the duration of their stay in hospital.

Baker (1974) investigated 150 well and 68 ill neonates and found a significant difference in the levels of kanamycin resistance; 4% of well neonates acquired kanamycin resistant E. coli compared with 10% of the ill neonates. The resistance to ampicillin was also significantly different; 19% in the well group and 35% in the ill group. No significant difference in gentamicin resistance was found.

Farmer (1967) compared the carriage rate in normal infants and infants nursed in a P.B.U. Premature infants had a high carriage rate of Gram-negative bacilli in the upper respiratory tract; if these E. coli organisms were acquired at birth they tended to be sensitive to the commonly used antibiotics but 50% of strains acquired in the P.B.U. were resistant to four or more antibiotics. This pattern was also found in isolates of Klebsiella species.

b) Method

Antibiotic sensitivities were determined for all isolates of E. coli using impregnated paper discs and the Stokes (1968) method which was simple, quick and overcame many of the disadvantages based on individual plate variation. The control organism used was the Oxford Hi strain of Staphylococcus aureus (NCTC 6571). Eight antibiotic discs were used: ampicillin 10 ug, kanamycin 30 ug, gentamicin 10 ug, carbenicillin 100 ug, sulphafurazole 500 ug, trimethoprim 1.25 ug, cephaloridine 5 ug and streptomycin 10 ug.

Lysed blood plates were inoculated and incubated overnight at 37°C,

zones of inhibition were measured using a millimeter ruler. The following definitions were used:

Sensitive	zone radius of test organism = \pm 2 mm. ^{control} not organism
Partially resistant	zone radius of test organism more than 2 mm. smaller than that of control organism
Resistant	test organism has no zone of inhibition

c) Results

Twenty-five resistance patterns were identified and are listed in Table 22. Patterns 1 and 2 were resistant to five of the eight antibiotics tested, the number of resistances decreased and patterns 14 - 25 were not resistant to any of the antibiotics.

Many infants carried E. coli with more than one resistance pattern although in most cases one or two patterns were consistently isolated from infants and the remainder were sporadic isolates.

The distribution of the different resistance patterns is shown in Figure 21. Forty (62.5%) ill/low birthweight infants acquired resistant E. coli compared with 5 (13.5%) normal infants; this difference was highly significant ($p < 0.001$). Six infants (42.8%) in the P.B.U. receiving antibiotic therapy acquired resistant E. coli compared with 34 (68%) infants who did not receive antibiotics but acquired resistant E. coli, this difference was not significant.

Figure 21 indicated that multiple resistance was not uncommon in ill/low birthweight infants and the incidence of this is shown in Table 23.

The percentage of normal and ill/low birthweight infants acquiring fully sensitive E. coli was similar, the incidence of a single resistant E. coli was higher in infants in the P.B.U. than normal infants but this difference was not significant. E. coli

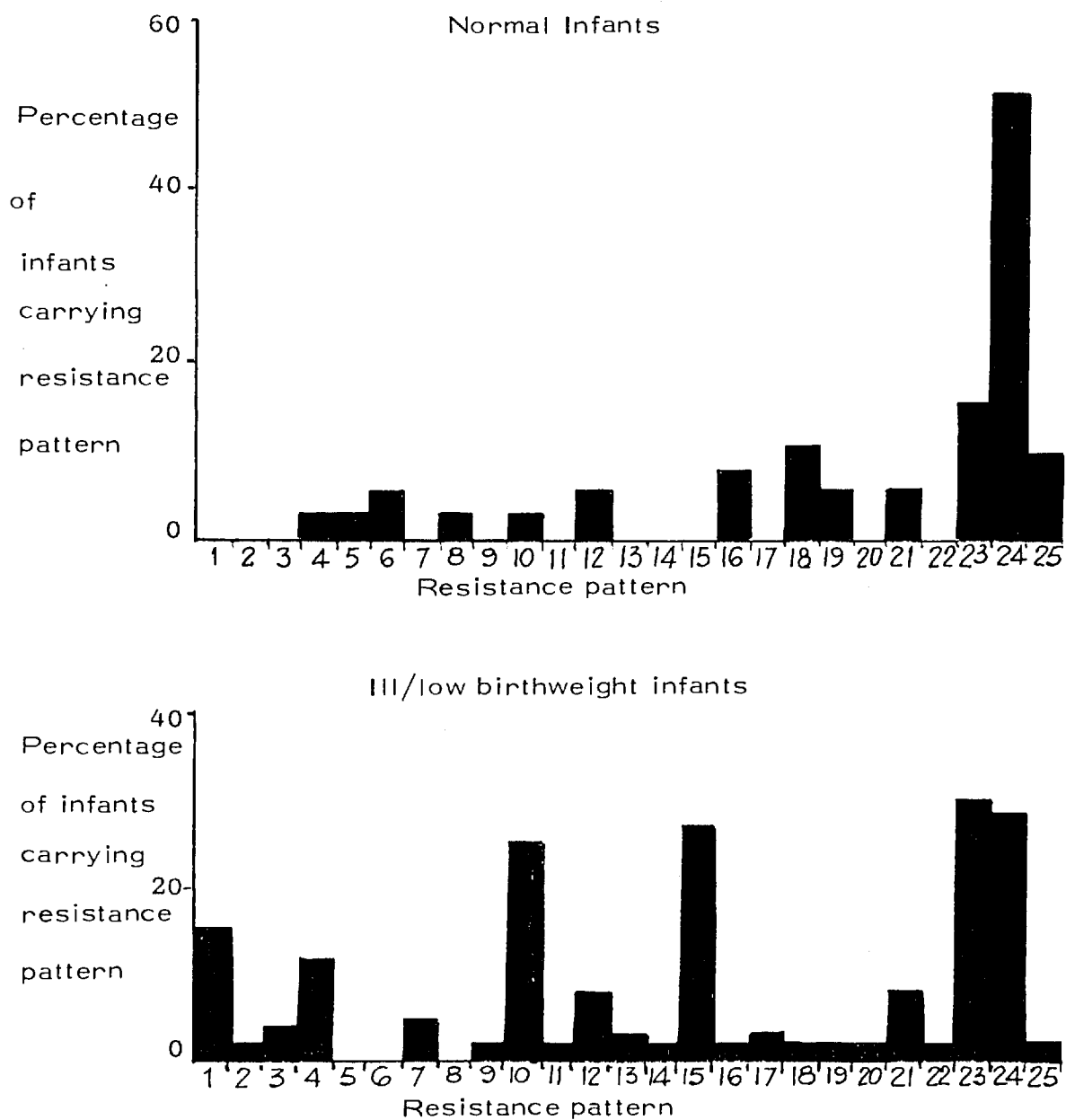
Table 22

Antibiotic Resistance Patterns of E. coli

Pattern No.	No Resistance	AMP	KAN	GEN	SUL	TRI	CEPH	CARE	STR
1	5	R	R	PR	R	S	PR	R	R
2		R	PR	S	R	R	R	PR	R
3	4	R	PR	PR	R	S	PR	R	R
4		R	R	S	R	S	PR	R	PR
5	3	R	S	S	R	S	PR	R	PR
6		R	PR	PR	PR	S	R	PR	R
7		R	S	S	PR	S	PR	R	S
8	2	R	S	S	S	S	R	PR	S
9		PR	S	S	R	S	R	PR	S
10		PR	S	S	R	S	PR	PR	R
11		R	S	S	PR	S	PR	PR	S
12	1	PR	S	S	R	S	PR	PR	S
13		PR	S	S	PR	S	R	PR	S
14		PR	PR	PR	PR	S	PR	PR	PR
15	0	PR	PR	PR	PR	S	PR	PR	S
16		PR	PR	S	PR	S	PR	PR	PR
17		PR	PR	PR	S	S	PR	PR	PR
18		PR	PR	PR	S	S	PR	PR	S
19		PR	S	PR	PR	S	PR	PR	S
20		PR	S	S	PR	S	PR	PR	PR
21		PR	PR	S	PR	S	PR	PR	S
22		PR	PR	S	S	S	PR	PR	S
23		PR	S	S	PR	S	PR	PR	S
24		PR	S	S	S	S	PR	PR	S
25		S	S	S	S	S	S	S	S

R = Resistant
PR = Partially Resistant
S = Sensitive

Figure 21 Antibiotic Resistance of E. coli isolated from Normal and III/low birthweight infants



resistant to 2 of 8 antibiotics tested was significantly more common ($p < 0.01$) in ill/low birthweight infants, this was also found for resistance to 4 of 8 ($p < 0.002$) and 5 of 8 ($p < 0.001$) antibiotics. Resistance to 3 of 8 antibiotics was found in three normal infants but was not found in ill/low birthweight infants, this was in contrast to the general trend of increased resistance in *E. coli* isolated from infants in the P.B.U.

Table 2 .
Incidence of Multiple Resistant *E. coli*

Infants	0	1	2	3	4	5
Ill/low birthweight	43	7	20	0	10	11
%	67.2	10.9	31.1	0	15.6	17.2
Normal	23	2	2	3	1	0
%	62.1	5.4	5.4	8.1	2.7	

Antibiotics were not used prophylactically in the unit. In the first part of the study, penicillin and kanamycin were the antibiotics of choice changing to penicillin and gentamicin or carbenicillin and gentamicin when *Ps. aeruginosa* was isolated. In the latter part of the study, kanamycin was withdrawn due to an increase in the number of resistant strains and penicillin and gentamicin were the chosen antibiotics.

Kanamycin resistance was found in 17 (26.6%) infants in the P.B.U. but in only 1 (2.7%) normal infant, a significant difference ($p < 0.005$). Carbenicillin resistance was found in 21 (37.8%) ill/low birthweight infants and 2 (5.4%) normal infants, this also was significant ($p < 0.01$). There was a significant difference in the

incidence of ampicillin resistance in the two groups of infants; 23 (35.9%) ill/low birthweight and 4 (10.8%) normal infants acquired this resistant E. coli ($p < 0.025$). There was no significant difference in ampicillin, kanamycin and carbenicillin resistance between infants in the P.E.U. receiving antibiotics and those not receiving antibiotics.

No gentamicin resistance was found during the study which was carried out from November 1972 to June 1973.

DISCUSSION

Previous work carried out in this hospital (Davies et al, 1970) reported that the majority of isolates of E. coli from infants in the P.B.U. were fully sensitive. These results show that there has been a sharp increase in the incidence of resistant E. coli as over half the infants in the unit acquired these organisms. This is in agreement with the results of Farmer (1968) who found a higher incidence in premature infants compared to normal infants and also increased multiple resistance in the premature infants.

Ampicillin resistance 35%, was found to be similar to that noted by Starkey (1971). This antibiotic was used widely throughout the hospital and might account for the level of ampicillin resistance found in normal infants. Carbenicillin was used less widely in the hospital and the level of resistance found in normal infants was lower than that found for ampicillin resistance.

Kanamycin was used solely in the paediatric department and the incidence of resistance was less than that of ampicillin or carbenicillin in normal infants. Kanomycin was withdrawn as a first line antibiotic and no further isolates of kanomycin resistant E. coli were identified.

This suggested that the appearance of E. coli with a specific resistance can be controlled by the selection pressure and that by removing this the resistance might be lost. After a suitable length of time, it might be possible to reintroduce the antibiotic into the therapy schedule.

No significant increase in antibiotic resistance was found in infants receiving antibiotic therapy, however 4 out of the 14 infants in this group died and the numbers were very small. The course of treatment was normally five days and if resistant E. coli were isolated after cessation of antibiotic therapy it was not possible to state if this was due to the antibiotics or cross-infection from infants in the unit carrying resistant E. coli.

Many infants carried multiple resistant E. coli and in some cases was the predominant resistance pattern isolated. This could not be correlated with increased hospitalization as no change of antibiotic resistance occurred over the first month of life in infants nursed in the unit.

These results indicate that there was a high level of antibiotic resistance in infants in the P.B.U. and this was not related to the use of antibiotics.

SUMMARY

Infants in the P.B.U. acquired resistant E. coli more readily than normal infants and multiple resistance, up to 5 of 8 antibiotics tested, was not uncommon in these infants. Ampicillin, carbenicillin and kanamycin resistance were significantly more common in ill/low birthweight infants than in normal infants. No difference in incidence of antibiotic resistance was noted between infants treated and infants not treated with antibiotics

PART 2

AEROBIC AND ANAEROBIC FLORA

INTRODUCTION

In the review of the literature on the establishment of the faecal flora in infants, it became apparent that there was a significant difference between that of breast fed infants and artificially fed infants. In both groups of infants the first organisms to colonise the gastro-intestinal tract were the gram-positive cocci, followed by a transition period in which gram-negative bacilli and gram-positive bacilli appeared. Breast fed infants then developed a faecal flora in which the bifidobacteria predominated whereas artificially fed infants retained a mixed faecal flora comprised of E. coli, bifidobacteria, Lactobacillus acidophilus, streptococcus, and sarcina (Olsen 1949).

The results of the first part of this thesis confirmed this pattern of colonisation with aerobic organisms in normal infants and determined the aerobic flora of three groups of ill/low birthweight infants which were predominantly gram-negative. The object of this part of the study was to investigate the anaerobic flora of breast fed and bottle fed normal infants nursed in this hospital and to compare the results with previous work. The anaerobic flora of the ill/low birthweight infants could then be compared with that of normal infants fed on the same artificial milk. Although the aerobic flora, especially the gram-negative flora of premature infants, had been investigated by other workers (Farmer, 1968; Davies et al, 1970) the anaerobic flora of these infants has not been determined and this was the object of this section of this work.

The isolation of Clostridium perfringens and bacteroides from the faeces of normal infants remains a controversial subject (Smith

and Crabb, 1961; Eullen and Willis, 1971). In this study the isolation rates in normal infants was determined and compared with the isolation rates in ill/low birthweight infants.

ANAEROBE STUDY

1. Materials and Methods

a) Media

Media described in the first part of this thesis were used throughout this work. In addition the following media were used for the isolation of anaerobic bacteria:

Neomycin Blood Agar	Blood Agar + 25 ugm neomycin/ml
Tomato Juice Agar	Oxoid + lactic acid (pH 5.1)
Thioglycollate Broth	Difco Bacto NIH Thioglycollate Broth
Nagler Plate	Nutrient Agar (Oxoid) 5% Fildes medium 5% egg yolk Neomycin 100 ugm/ml

All media were prepared in accordance with the manufacturers' instructions.

b) Anaerobic Jars

Stainless steel milking machine pails with vacuum tight lids (Fullwood and Bland Limited, 35 Bevenden Street, N1) were fitted with vacuum taps and cold catalysts. These churns were used for the incubation of the anaerobic media (Schaedler, Dubos and Costello 1965). If the number of plates to be incubated anaerobically did not exceed ten, Baird and Tatlock jars were used.

c) Anaerobic Conditions

Each jar or pail was evacuated to 650 mm Hg and the air replaced with oxygen-free nitrogen. The jar or pail was then re-

evacuated to 650 mm Hg and nitrogen containing 10% carbon dioxide was introduced. The nitrogen was left attached to the container for a few minutes to allow the gasses to equilibrate.

A Lucas methylene blue indicator tube (Stokes 1968) was included in each container.

All media used for anaerobic work was pre-anaerobised under these conditions for 24 hours before use.

d) Collection and treatment of specimens

A swab was taken of the mouth area and immediately placed in 10 mls. of sterile thioglycollate broth. The bottle was quickly shaken by hand to disperse the organisms in the fluid medium. A fresh sample of faeces was placed in a preweighed universal containing 1 ml. thioglycollate broth. The specimens were taken immediately to the laboratory. The universal container was reweighed and thioglycollate broth added to make a 1 in 10 dilution of the faeces. The two suspensions were then thoroughly mixed, using a whirlmixer.

Serial 1 in 100 dilutions were made using thioglycollate broth and 0.1 ml of the dilution was spread on the media listed in Table 24. Plating out was performed on the bench top as quickly as possible, the plates put into the pails which were immediately evacuated as stated.

Table 24
Media and Incubation Conditions used in the Study

<u>Specimen</u>	<u>Dilution</u>	<u>Media</u>	<u>Incubation</u>	<u>Time</u>
Mouth swab	10^{-1}	Cetrimide	Air	24 hours at 37°C
	$10^{-1} + 10^{-3}$	Blood Agar MacConkey Agar	Air	24 hours at 37°C
		Blood Agar Neomycin Blood Agar Tomato Juice Agar	AnO ₂	72 hours at 37°C
Faeces	10^{-1}	Cetrimide	Air	24 hours at 37°C
	$10^{-1}, 10^{-3}, 10^{-5}$ or $10^{-3}, 10^{-5}, 10^{-7}$	Blood Agar MacConkey Agar	Air	24 hours at 37°C.
		Blood Agar Neomycin Blood Agar Tomato Juice Agar	AnO ₂	72 hours at 37°C

e) Viable Count

Plates incubated aerobically were examined after 24 hours. The total number of colonies was counted and the number of each colonial type noted. One colony of each colonial type was streaked on to half a blood agar plate which was incubated at 37°C for 24 hours.

After 72 hours incubation the plates incubated anaerobically were examined. The viable count of each colonial type was recorded and one colony of each colonial type was plated on to a quarter of a preanaerobised blood agar plate and a slide was also prepared. The plates were then incubated at 37°C for 48 hours.

f) Identification of Isolates

(i) Aerobic organisms Each isolate was identified using the methods stated in the first part of this thesis except that no distinction

was made between coagulase-negative staphylococci and micrococci.

Each isolate was identified as belonging to one of the following groups:

Gram Positive Bacteria

Staph. aureus
Coagulase-negative staphylococci/
Micrococci
Streptococci
Gram-positive bacilli

Gram-Negative Bacteria

E. coli
Klebsiella-Enterobacter-Serratia (KES)
Proteus species
Ps. aeruginosa
Other gram-negative bacilli
Gram-negative cocci

(ii) Anaerobic Organisms When each colony was plated on to a blood agar plate for anaerobic culture, a slide preparation was made which was gram stained and examined. After anaerobic incubation, each isolate was subcultured on to blood agar, and incubated in air for 24 hours at 37^o C. Identification of isolates was made on the basis of the gram stain, colonial morphology, growth on selective media and ability to grow in air. The identification scheme used is given in Table 25.

Table 25
Identification of Anaerobic Bacteria

<u>Gram Stain</u>	<u>Grown on</u>		<u>Growth in Air</u>	<u>Identification</u>
	<u>NBA</u>	<u>TJA</u>		
Positive Bacillus	+		+/-	Clostridium Species
Negative Bacillus	+		-	Bacteroides Species
Positive Bacillus		+	+/-	Lactobacillus Species/ Bifidobacteria
Negative Coccus	+		-	Veillonella
Positive Coccus	+		-	Anaerobic Streptococci

NEA - Neomycin blood agar

TJA - Tomato Juice agar

Each isolate of Clostridium species was further subdivided into Cl. perfringens or Clostridium species (non-perfringens) on the basis of the Nagler Reaction.

Nagler Reaction

Five drops of Cl. perfringens antitoxin (Wellcome - Mixed Gas Gangrene Antitoxin) were spread over half a Nagler Plate. Each of six test organisms plus a known culture of Cl. perfringens were spotted on to each half of the plate. The plate was then incubated anaerobically at 37°C overnight. Any test organism which produced a cloudy halo on the untreated half of the plate and no halo on the half treated with antitoxin was identified as Cl. perfringens.

2. Clinical Information

a) Normal Infants

One hundred and seventy infants were included in this study; all were born at term, 101 (59.4%) were male and 69 (40.6%) were female; 77 (45.3%) were breast fed and 93 (54.7%) were fed using a prepacked sterile cows milk preparation as described in the first part of this thesis.

Whenever possible, samples were taken on days 1, 3, 5 and 7 but in many cases either a fresh specimen could not be obtained or the infant had left hospital; no infant was investigated on all sample days.

The number of breast and artificially fed infants investigated on each days is shown in Table 26.

Table 26

<u>Day</u>	<u>Normal Infants Sampled</u>		JUNE 1973	JUNE 1974
	1	3	5	7
<u>Normal Infants</u>				
<u>Breast fed</u>	36	35	30	27
Male	22	20	17	15
Female	14	15	13	12
<u>Bottle Fed</u>	40	38	46	27
Male	23	25	28	16
Female	17	13	18	11
Total	76	73	76	54

Total number of swabs taken - breast fed - 128

bottled fed - 151

An attempt was made to keep the number of breast fed and bottle fed infants investigated on any one day as equal as possible but it was not possible to investigate equal numbers of male and female infants.

b) III/low birthweight infants

Forty-nine infants nursed in the P.B.U. were admitted to this study. Seven (14%) infants were born at other hospitals and transferred to the P.B.U. within the first day of life. Forty two (86%) infants were born at Hammersmith Hospital and admitted to the P.B.U. immediately after birth.

One (2%) infant was post-term, 6 (12%) infants were term and 42 (86%) infants were pre-term. The birth weights ranged from 1180 grams to 3940 grams with a mean of 2300 grams.

No infant received antibiotic therapy before or during his inclusion in this study

All infants included in this study were fed on a cows milk preparation which was prepared as already described. A few infants had received expressed breast milk but all infants received cows milk feeds before and during the time of investigation.

For the reasons stated for normal infants, specimens were not obtained from all infants on all days and the number investigated each day is given in Table 27.

Table 27

<u>Day</u>	<u>Number of Ill/low Birthweight Infants Investigated on</u>										
	<u>each day</u>					<u>JUNE 1973</u>					
	1	3	5	7	9	11	14	18	21	25	28
<u>Infants</u>											
Male	11	7	13	14	10	11	11	9	7	5	2
Female	7	12	16	13	12	16	18	15	11	7	8
Total	18	19	29	27	22	27	29	24	18	12	10

Twenty (40.8%) were male infants and 29 (59.2%) were female infants but the ratio of male to female varied on each day

3) Results

a) Mouth Flora

(i) Normal infants The mouth flora of breast fed and bottle fed infants is compared in Table 28.

Table 28

Mouth Flora of Breast Fed and Bottle Fed Infants

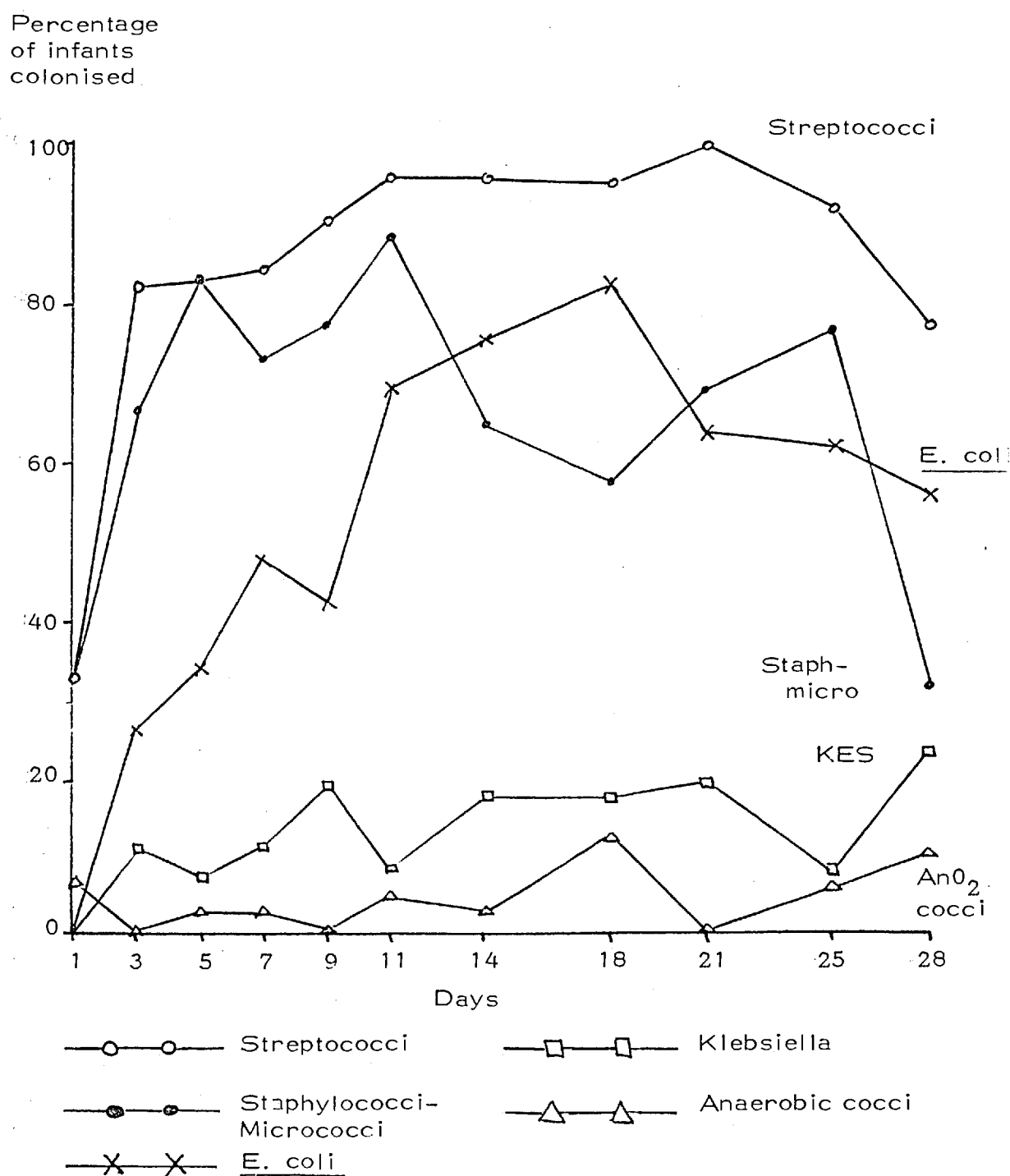
Days	<u>Breast Fed</u>				<u>Bottle Fed</u>			
	1	3	5	7	1	3	5	7
Percentage Incidence								
<u>Aerobic Organisms</u>	54.1	100	100	100	52.5	100	100	100
Gram positive	54.1	100	100	100	52.5	100	100	100
Staph-Micro	44.6	85.7	90	100	40	73.7	86.9	85.2
Streptococci	41.8	85.7	90	88.8	32.5	100	100	100
Gram negative	2.8	25.7	16.6	37	7.5	21.1	19.6	33.3
Bacilli	2.8	8.6	3.3	11.1	5.9	7.9	7.7	22.2
Cocci	0	17.1	16.6	29.6	2.5	13.2	10.9	18.5
<u>Anaerobic cocci</u>	0	2.9	0	0	2.5	5.3	2.2	3.7

The incidence of aerobic organisms was similar to that found in the first part of this thesis.

Anaerobic organisms were infrequently isolated, anaerobic streptococci were isolated more from bottle fed infants than breast fed infants but in both cases these organisms were never predominant. Anaerobic bacilli were not isolated.

(ii) III/low birthweight infants The numbers and types of organisms which form the mouth flora of III/low birthweight infants is shown in Figure 22. The colonisation with aerobic organisms was similar to that found in the first part of this thesis.

The incidence and numbers of anaerobic organisms isolated remained

Figure 22. Mouth Flora of III/low Birthweight Infants

low throughout and did not increase over the period of the study. Anaerobic streptococci were isolated from 11 swabs (5.2%); anaerobic bacilli were isolated infrequently, clostridia were isolated four times (1.8%) and lactobacilli bifidobacter twice (0.9%).

b) Faecal Flora

(i) Normal Infants The rate of colonisation of these infants is given in Table 29

Table 29
Percentage Colonisation of Breast Fed and Bottle Fed
Normal Infants

	Day 1		Day 3		Day 5		Day 7	
	Breast	Bottle	Breast	Bottle	Breast	Bottle	Breast	Bottle
No. infants	32	39	36	38	31	45	28	27
No. colonised	20	28	36	38	31	45	28	27
% colonised	63%	72%	100%	100%	100%	100%	100%	100%
<u>Aerobes</u>	59%	72%	100%	95%	100%	100%	100%	100%
<u>Gram positive organisms</u>	53%	54%	83%	82%	89%	93%	96%	89%
Staph-Micro	38%	51%	67%	58%	71%	64%	86%	67%
Streptococci	28%	15%	55%	60%	55% ⁺	82%	68%	78%
<u>Gram negative organisms</u>	28%	31%	69%	63%	64%	64%	68%	78%
<u>E. coli</u>	16%	20%	47%	47%	42%	44%	28% ⁺	67%
Klebsiella	9%	10%	25%	16%	23%	16%	36%	15%
<u>Anaerobes</u>	22%	36%	100%	97%	100%	98%	97%	100%
Clostridia	16%	20%	67%	74%	77%	80%	71%	89%
Bacteroides	6%	18%	53%	68%	19%*	44%	39%*	67%
Bifidobacteria/ Lactobacilli	9%	20%	58%	55%	77%	55%	82%	63%

+ $p < 0.025$

* $p < 0.05$

Infants were up to twenty-four hours old when investigated on day 1 and some had acquired a faecal flora. The percentage of infants colonised on day 1 was higher than reported in the first part of this study when infants were swabbed immediately after birth.

Breast Fed Normal Infants

The pattern of colonisation with aerobic organisms was similar to that found in the first part of this study and the data is shown in Table 29.

The overall colonisation of these infants with anaerobic organisms was equal to that of aerobic organisms and by day 3 all infants were colonised with both anaerobic and aerobic organisms. Initially the clostridia were the most common anaerobic isolates and were found in 22% of infants on day 1, 67% on day 3, and 77% on day 5 with a geometric mean count which rose to 10^7 by this time. During this period, bifidobacteria/lactobacilli became established and the rate of isolation increased from 9% on day 1 to 82% on day 7 with a geometric mean count of $10^{7.5}$, these organisms were the predominant anaerobes at this time. The incidence of bacteroides remained low throughout the first week of life and by day 7, only 39% of infants were colonised with these organisms.

Bottle Fed Normal Infants

Table 29 indicated that the faecal flora of infants fed on a cows milk preparation was not significantly different to that of breast fed infants over the first five days of life. However by day 7, the higher colonisation of bottle fed infants with gram negative organisms reported in the first part of this study was reaffirmed.

Although both breast fed and bottle fed infants became rapidly colonised with anaerobic bacteria there was a difference in the

relative importance of the three main groups of these organisms. Clostridia remained the predominant anaerobes throughout the seven days and the rise in incidence and numbers of the lactobacilli/bifidobacteria found in breast fed infants did not occur. The geometric mean count of clostridia was 10^7 and was a hundred-fold higher than that of either lactobacilli/bifidobacteria or bacteroides. The incidence of bacteroides was however higher than found in breast fed infants, 67% compared to 39% on day 7; this incidence was slightly higher than that of the lactobacilli/bifidobacteria which was isolated from 63% of infants on day 7. Table 30 lists the numbers of organisms isolated from both breast fed and bottle fed infants.

Table 30

<u>Geometric Mean Count of Organisms Commonly Isolated from the Faeces of Normal Infants</u>				
	10^7	10^6	10^5	$> 10^5$
Breast fed	Lactobacilli Bifidobacteria	Staph Micrococcus Streptococci	Clostridia	Bacteroides Klebsiella <u>E. coli</u>
Bottle fed	Clostridia Streptococci		<u>E. coli</u> Bacteroides Stap. Micrococcus Lactobacilli/ Bifidobacteria	Klebsiella

In bottle fed infants, clostridia were the predominant organisms both in incidence and numbers, streptococci were the next most common and both these groups of organisms were present in numbers ten-fold higher than the other organisms. In breast fed infants the lactobacilli/bifidobacteria dominated the faecal flora.

(ii) III/low Birthweight Infants Samples of meconium were obtained from 18 infants within the first twenty-four hours of life. Seven specimens

were sterile (38.9), aerobic organisms were isolated from 11 (61.1%) specimens and anaerobic organisms were isolated from 4 (22.2%). Gram positive cocci were predominant and isolated from 8 (44.4%) infants; the incidence of gram-negative organisms was lower, 5 infants (27.8%) were colonised. All four infants who had acquired anaerobic organisms acquired clostridia, in addition one infant carried bacteroides and another lactobacilli/bifidobacteria.

The faecal flora quickly became established and the percentage colonisation with the principal organisms is shown in Figure 23.

The incidence of aerobic organisms was similar to that found in the first part of this study and the predominance of gram-negative bacilli was reaffirmed. The incidence of anaerobic organisms increased rapidly over the first week of life. On day 1, clostridia were isolated from 22% of infants, but by day 5 this had increased to 83% and for the remainder of the first four weeks of life fluctuated between 75% and 95% with a geometric mean count between 10^6 and 10^8 organisms/gm. of faeces. These organisms remained the predominant anaerobic bacilli throughout but the incidence of lactobacilli/bifidobacteria increased in the first eleven days and then both the number and incidence of organisms resembled that of the clostridia. The incidence of bacteroides increased on day 3 but then decreased in frequency of isolation and then never rose above 40% isolation rate.

A comparison of the faecal flora of ill/low birthweight infants and breast fed and bottle fed normal infants is given in Table 31.

Figure 23. Faecal Flora of Ill/low Birthweight Infants

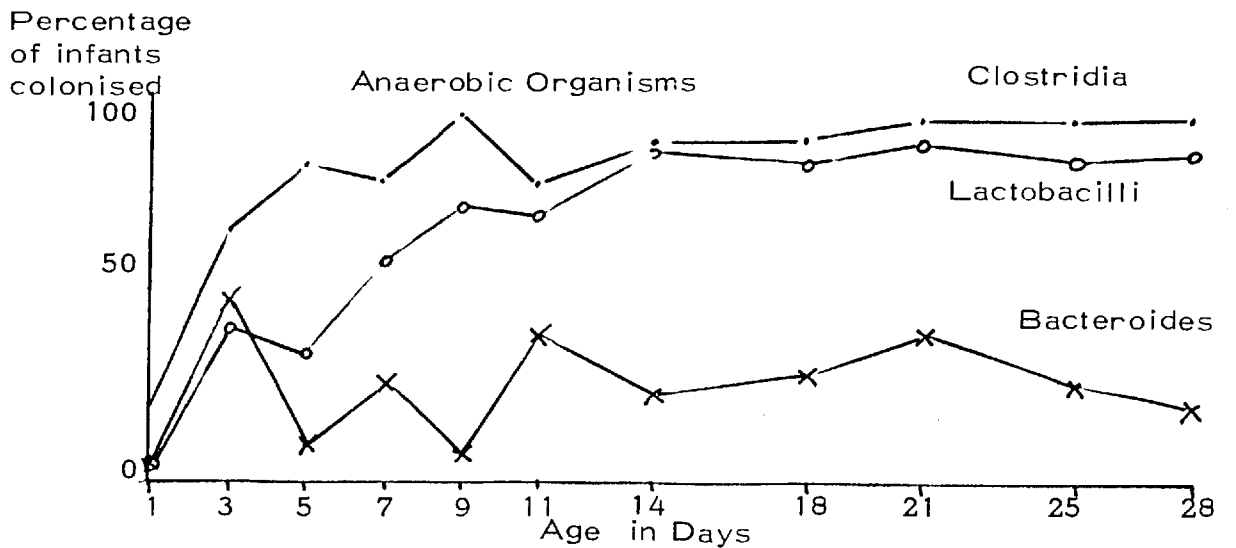
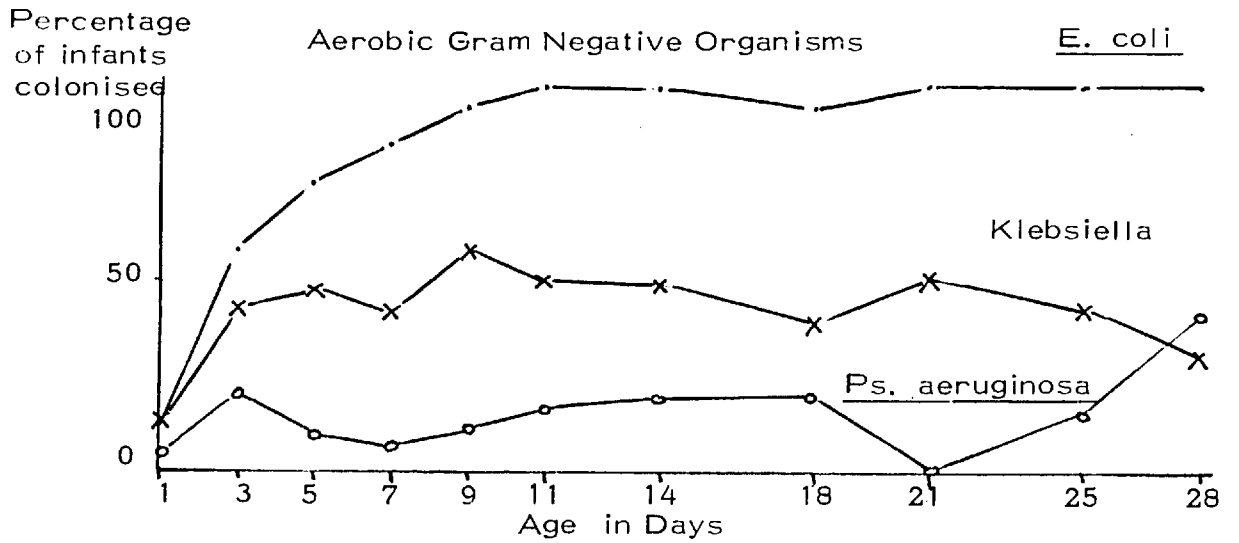
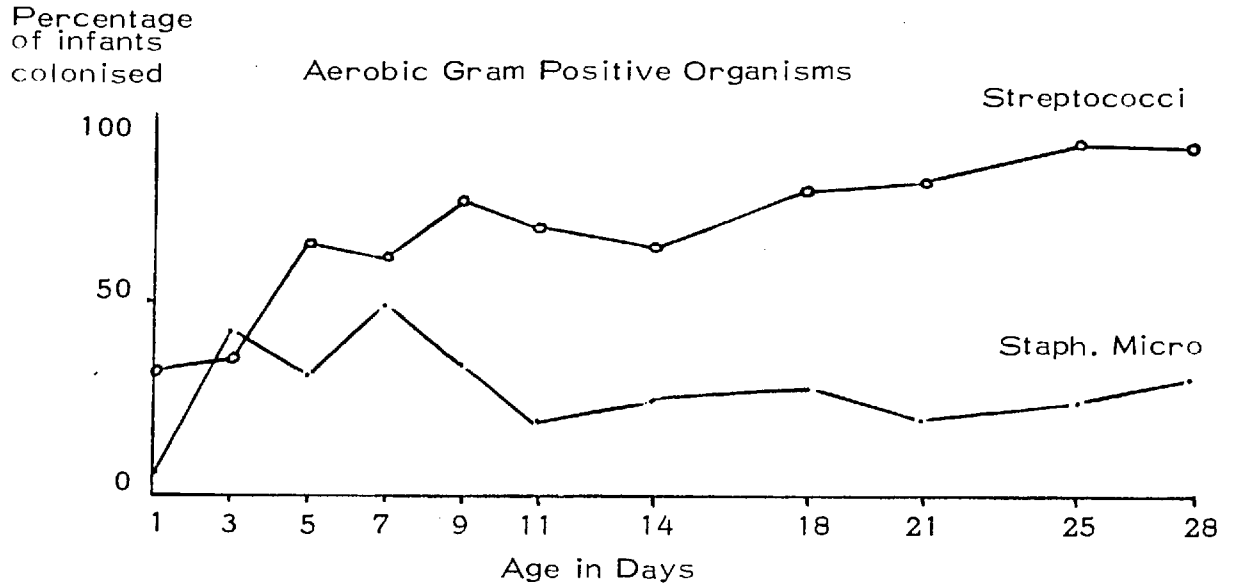


Table 31.

The Faecal Flora of Normal and Ill/Low Birthweight
Infants on Day 7

	<u>% Colonised</u>			<u>Geometric Mean Count</u>		
	<u>Normal</u>		<u>Ill/low</u>	<u>Normal</u>		<u>Ill/low</u>
	Breast	Bottle	Birthweight	Breast	Bottle	Birthweight
Staph-Micro	86 ⁺	67	52 ⁺	10 ^{6.8}	10 ^{5.3}	10 ^{2.7}
Streptococci	68	78	63	10 ^{6.6}	10 ⁷	10 ^{5.8}
<u>E. coli</u>	28 ^{*+}	67 ⁺	85*	10 ^{2.5}	10 ^{5.9}	10 ^{8.2}
Klebsiella	36	15	41	10 ^{3.1}	10 ^{1.3}	10 ^{3.5}
<u>Ps. aeruginosa</u>	0	0	77	0	0	10 ^{0.2}
Clostridium	71	89	78	10 ^{5.5}	10 ^{7.6}	10 ^{6.6}
Bacteroides	39	67 ^o	26 ^o	10 ^{3.2}	10 ^{5.5}	10 ²
Lactobacilli- Bifidobacteria	82	63	59	10 ^{7.5}	10 ^{5.5}	10 ^{5.2}

^o p < 0.01 + p < 0.025 * p < 0.001

The faecal flora of breast fed and ill/low birthweight infants was significantly different and this might be expected being related to a difference in feed. However, if bottle fed normal and ill/low birthweight infants are compared there are also differences in the faecal flora, although both these two groups of infants were fed on the same artificial milk. The incidence of gram-positive cocci was lower in the ill/low birthweight infants and that of gram-negative aerobic bacilli higher than in normal bottle fed infants. The total count of aerobic organisms was identical, 10^{9.7} per gram of faeces, in both groups of infants. There was a difference in the incidence of anaerobic organisms in these infants, by day 7 the total count of anaerobic organisms was ten-fold lower in the ill/low birthweight infants reaching only 10^{8.2} organisms

per gram of faeces compared to $10^{9.3}$ in bottle fed normal infants.

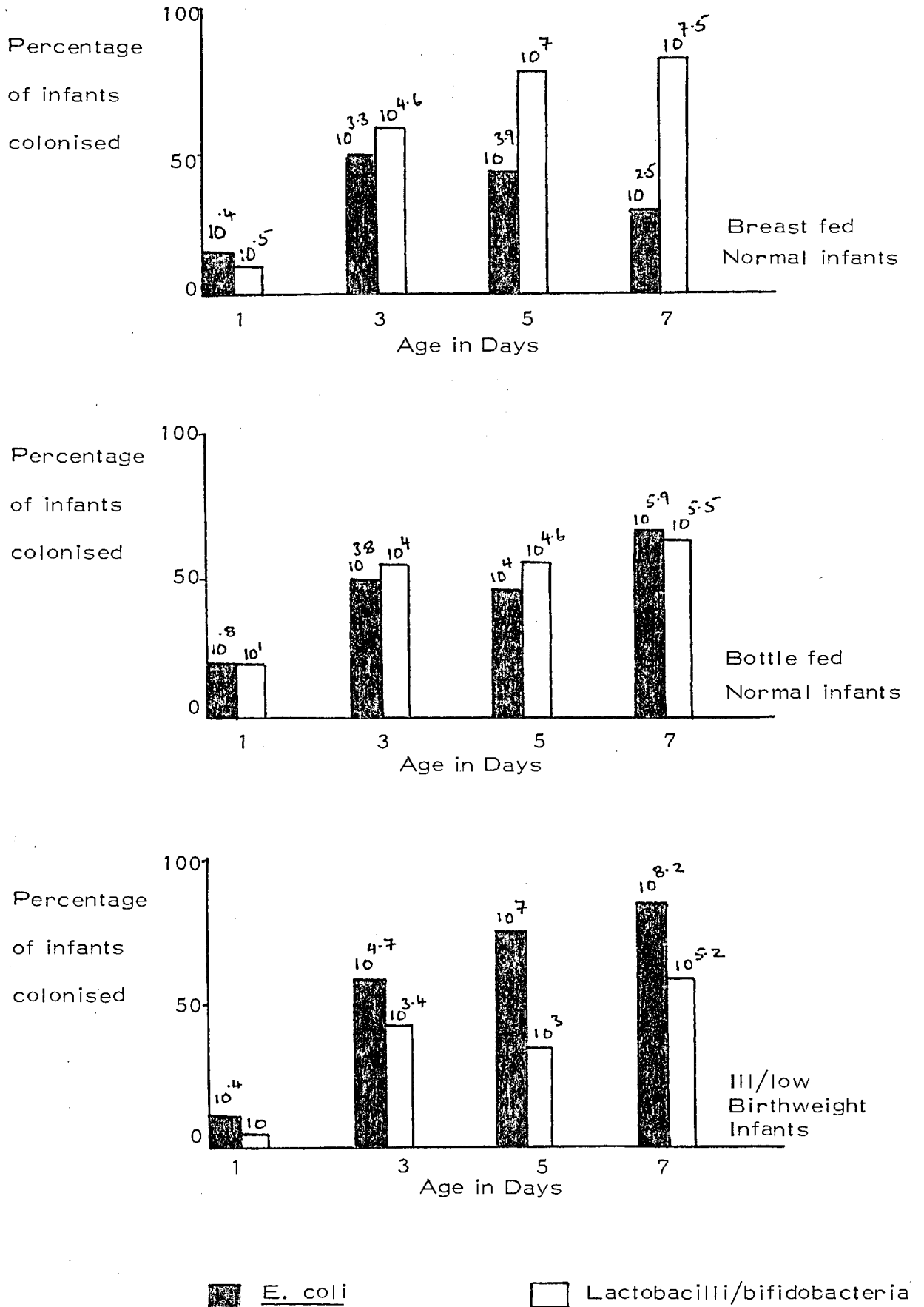
Clostridia were the predominant anaerobes in both groups of infants but the incidence and numbers in the normal bottle fed infants was higher than in the ill/low birthweight infants. The incidence of lactobacilli/bifidobacteria was similar in both groups of infants; also the geometric mean count was about 10^5 . There was a significant difference in the isolation rate of bacteroides; by day 7, 67% of normal bottle fed infants had acquired these organisms compared to 26% of ill/low birthweight infants ($p = 0.01$). This low incidence of bacteroides was lower than found in normal breast fed infants who had an incidence rate of 39% at this time.

c) Relationship of E. Coli and Lactobacilli/Bifidobacteria

(i) Introduction Bullen and Willis (1971) proposed a mechanism based on the redox potential in the intestine which would account for the difference in the faecal flora of breast fed and bottle fed normal infants. In breast fed infants the establishment of the lactobacilli/bifidobacteria due to the low Eh and pH was related to a decrease in incidence of E. coli, in bottle fed infants the high buffering capacity of artificial milk would not allow the pH to fall and the lactobacilli/bifidobacteria would not become established. In the first part of this study the ill/low birthweight infants were found to have a high incidence of gram-negative bacilli; this second part of the study was to determine the relation of the incidence of E. coli and the lactobacilli/bifidobacteria in normal infants and in the ill/low birthweight infants.

(ii) Results Figure 24 represents the percentage of infants colonised with E. coli and lactobacilli/bifidobacteria, the geometric mean count is given above each percentage.

Figure 24. Lactobacillus-bifidobacteria and E. coli in the faeces of normal and ill/low birthweight infants



In breast fed infants, the gram-positive bacilli predominated both in numbers and incidence by day 3 and this became more pronounced with time. By day 7, E. coli was isolated from only 28.6% of infants and lactobacilli/bifidobacteria from 82%. The difference in the mean count of these two organisms was 10^5 .

Bottle fed normal infants acquired a mixed flora and neither E. coli or lactobacilli/bifidobacteria predominated in numbers or frequency. The difference in isolation rate did not rise above 10% and the geometric mean counts did not differ by more than $10^{0.6}$.

The incidence of lactobacilli/bifidobacteria in low birthweight infants was similar to that found in normal bottle fed infants. The incidence of E. coli was higher in ill/low birthweight infants than that found in normal bottle fed infants but this difference was not significant. However, the ratio of E. coli to the lactobacilli/bifidobacteria in these infants was very different. In the ill/low birthweight infants the difference in incidence of these organisms was 25% with a mean count of E. coli 10^3 times higher than that of the lactobacilli/bifidobacteria. This high count of E. coli in relation to that of lactobacilli/bifidobacteria continued throughout the four weeks of study of the infants and a comparison of the geometric mean count and incidence of these organisms is given in Table 32.

By day 11 all infants were colonised with E. coli but the incidence of lactobacilli/bifidobacteria was still increasing slowly by day 28. The ratio of the geometric mean counts remained in excess of 10^1 throughout the four weeks and on most days exceeded 10^2 indicating the great excess of the gram-negative aerobic bacilli.

During the first week of life this ratio reached 10^3 and was in contrast to the results obtained for normal bottle fed and breast fed infants.

Table 32

Comparison of the Incidence and Numbers of E. Coli
and Lactobacilli-Bifidobacteria in ill/low birthweight
Infants

Day	% Incidence			Geometric Mean Count <u>E. coli</u>		
	E. coli	L-B	Difference	E. coli	L-B	L-E
1	11.11	55.6	53.6 44.5	$10^{0.4}$	$10^{0.2}$	$10^{0.2}$
3	57.9	42.1	15.8	$10^{4.6}$	$10^{3.5}$	$10^{1.1}$
5	75.9	34.5	41.4	10^7	10^3	10^4
7	85.2	59.3	25.9	$10^{8.2}$	$10^{5.2}$	10^3
9	95.5	72.7	22.8	$10^{8.7}$	$10^{6.7}$	10^2
11	100	70.4	29.6	$10^{9.7}$	$10^{6.3}$	$10^{3.4}$
14	100	86.2	13.8	$10^{9.9}$	$10^{7.8}$	$10^{2.1}$
18	95.8	83.3	12.5	$10^{9.5}$	$10^{7.6}$	$10^{1.9}$
21	100	88.9	11.1	$10^{9.7}$	$10^{7.8}$	$10^{1.9}$
25	100	83.3	16.7	$10^{9.5}$	$10^{7.3}$	$10^{2.2}$
28	100	90	10	$10^{9.4}$	$10^{8.3}$	$10^{1.1}$

The distribution of the geometric mean counts of lactobacilli/bifidobacteria in all three groups of infants were compared and no significant difference was found. However, the geometric mean count of E. coli was considerably higher in the ill/low birthweight infants and the distribution of the counts is given in Table 33.

Statistical analysis indicated that the counts were significantly higher ($p < 0.05$) in the ill/low birthweight infants than in normal bottle fed infants ($p < 0.05$) or breast fed infants ($p < 0.001$). These results are in agreement with the first part of this thesis.

Table 33

<u>Counts of E. coli</u>				
<u>E. coli</u>	10^8	10^{8-9}	10^{9-10}	10^{10}
Breast ^{†*}	22	2	4	0
Bottle ^{o*}	12	3	9	3
Ill/low birthweight ^{o†}	5	4	7	11

[†] p < 0.001 ^o p < 0.05 * p < 0.025

d) Clostridium Perfringens

The results shown in Table 29 indicate that there was a higher incidence of clostridia in bottle fed normal infants than in infants who were breast fed and this difference became more marked with time. These organisms were further investigated using the Nagler test to determine the incidence of Clostridium perfringens. The incidence of Cl. perfringens in normal infants is given in Table 34.

Table 34

Incidence of Cl. Perfringens in Normal Infants

	<u>Cl. perfringens</u>	
	Breast fed	Bottle fed
Day 1	12%	15%
Day 3	16%	34%
Day 5	35% [†]	62%
Day 7	43% [*]	78%

[†] p < 0.05

* p < 0.025

On day 1 Cl. perfringens was the most common isolate of the clostridia in both groups of infants although the overall isolation rate

was low. By day 3 clostridia were isolated from 67% of breast fed infants and 74% of bottle fed infants but other members of the clostridia formed the major part of this genus and Cl. perfringens was only isolated from 25% and 46% respectively of the total specimens containing clostridia and from only 16% and 34% respectively of the total number of breast and bottle fed infants. Bottle fed infants had a higher incidence of Cl. perfringens on day 3 and by day 5 this difference between bottle fed and breast fed infants had become significant ($p < 0.05$) and more significant by day 7 ($p < 0.025$).

The isolation rates of clostridia and Cl. perfringens in ill/low birthweight infants is given in Table 35.

Table 35

Incidence of Cl. Perfringens in Ill/low Birthweight
Infants

Age in Days	1	3	5	7	9	11	14	18	21	24	28
% incidence	31	45	60	84	87	68	89	95	94	89	100

The incidence of Cl. perfringens was higher on days 1 and 3 than found in either bottle fed or breast fed normal infants but by day 5 the isolation rate, 60%, was similar to that obtained in bottle fed normal infants, 62%. This trend was also noted on day 7 when the incidence in ill/low birthweight infants had reached 84% and in bottle fed normal infants 78%. These figures were significantly higher than found in breast fed normal infants ($p < 0.025$).

The isolation of Cl. perfringens from all specimens containing clostridia was 100% on day 1; this figure decreased on day 3 to 56% but rose again on day 5 to 80% and remained between 80% and 100% for the rest of the study. This high incidence of Cl. perfringens in specimens containing clostridia was also found in bottle fed normal

infants who had a maximum incidence of 60% of infants colonised.

e) Antibiotic Sensitivities of E. coli

Antibiotic sensitivities were determined for all isolates of E. coli as described in the first part of this thesis. Fourteen new patterns of antibiotic sensitivity were identified and the full list of antibiotic sensitivities is given in Table 36.

Two new patterns showed antibiotic resistance to five of the eight antibiotics tested, three were resistant to four antibiotics, six to three antibiotics, two to two antibiotics and one to one antibiotic.

No difference in antibiotic resistance patterns was found between breast and bottle fed normal infants. Most normal infants acquired E. coli which were not resistant to the antibiotics tested but infants in the P.B.U. acquired resistant E. coli. The distribution is given in Table 37.

Table 37

Number of Infants Carrying Resistant E. Coli

No. of Antibiotic Resistances	Normal			III/low Birthweight
	Breast	Bottle	Total	
5	2	2	4	6
4	3	4	7	30
3	1	2	3	29
2	3	8	11	10
1	1	4	5	5
0	23	30	53	9

Ill/low birthweight infants carried multiple resistant E. coli and the incidence was significantly higher, $p < 0.001$, than in normal infants. This result was also found in the first part of this thesis and Table 38 compares the incidence of antibiotic resistance found in the two studies in the ill/low birthweight infants.

Table 38

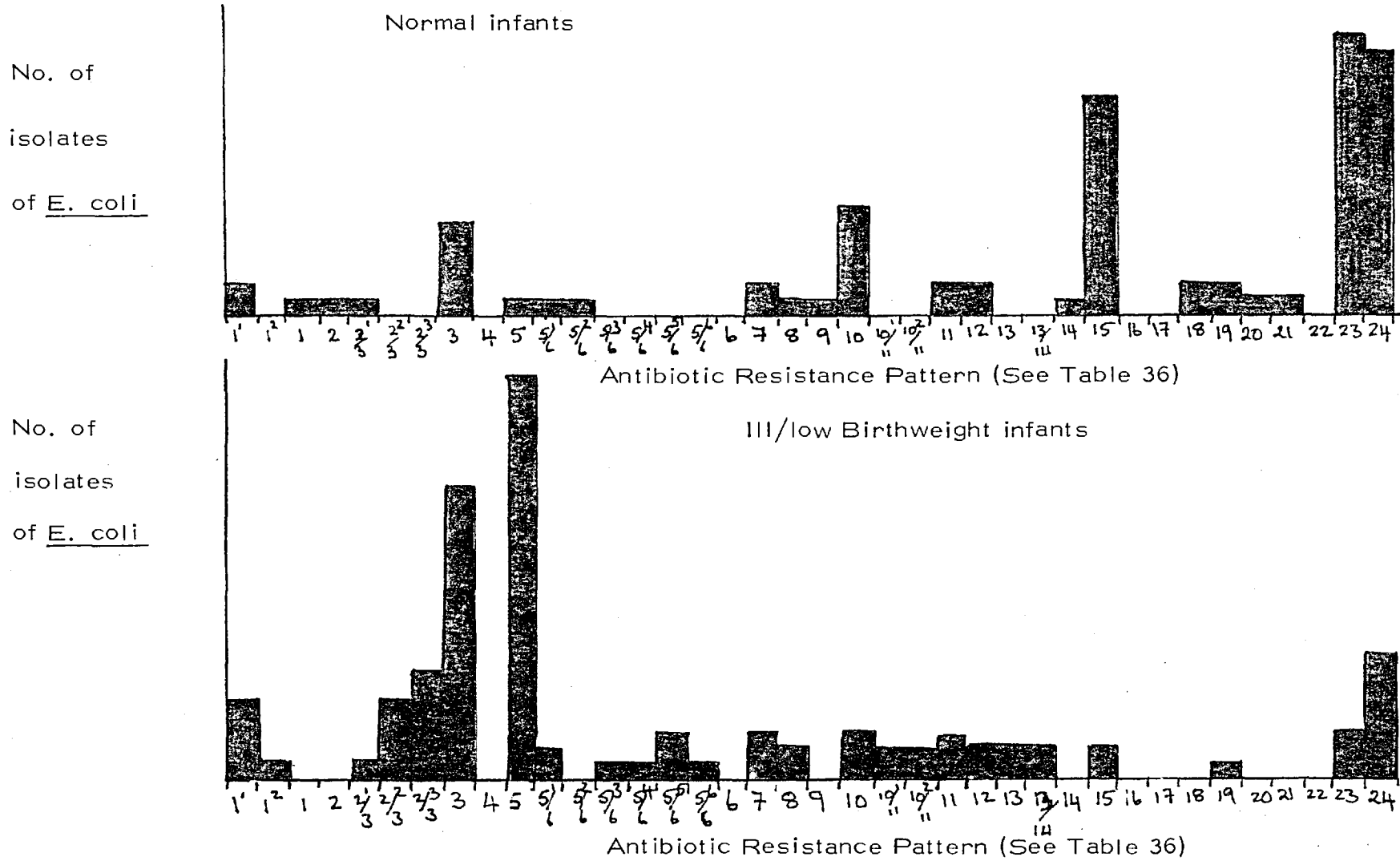
Antibiotic Resistance in Ill/low Birthweight
Infants

	No. of antibiotic resistances out of the 8 antibiotics tested					
	0	1	2	3	4	5
First part of the study - Feb. 1972 to June 1973	67.2	10.9	31.1	0	15.6	17.2
Second part of the study - Feb. 1974 to June 1974	23.7	13.1	26.3	76.3	79	15.7

In the second part of this study there was a significant increase in the number of ill/low birthweight infants colonised with resistant E. coli compared with the first part of this study. This increase was accompanied by a decrease in the number of infants carrying E. coli which was sensitive to the antibiotics tested.

The distribution of the individual resistance patterns in normal and ill/low birthweight infants is shown in Figure 25. A few normal infants became colonised with E. coli resistant to one or more antibiotics; resistance to two antibiotics was the most common and 7 of 11 infants carried pattern No. 10. Pattern No. 3 was the most common resistant to four antibiotics and was isolated from 6 of 7

Figure 25. Antibiotic Resistance Patterns of E. coli isolated from Normal and III/low Birthweight Infants



infants, this pattern was also common in the ill/low birthweight infants.

Kanamycin resistance was found in patterns 1 and 4 and these two patterns were isolated in the first part of this study. Kanamycin was withdrawn from usage during the first part of the study and was not used at all during the second part; kanamycin resistant E. coli was not isolated during the second part of this study.

The high incidence of patterns 5, 23 and 24 found in normal infants in the first part of this study was also found in the second part, but the incidence in ill/low birthweight infants was lower.

There was no change to a more resistant E. coli noted in the ill/low birthweight infants over the 28 days of study, but no infants in this part of the study were treated with antibiotics. Although gentamicin was used in this part of the study as one of the first antibiotics of choice, there were no isolations of a gentamicin resistant E. coli.

DISCUSSION

The isolation of anaerobic organisms from the mouths of infants can be correlated with dentition and these organisms are rarely isolated from newborn infants. Hurst (1954) used an enrichment technique but could only isolate fusiforms from 9% of infants under 10 days old but could isolate these organisms from 94% of infants aged 2 months to 6 months old. In this study anaerobic organisms were rarely isolated from the mouths of infants up to 28 days old.

Most workers investigating the faecal flora of newborn infants have been concerned with the difference between breast fed and artificially fed normal infants (Olsen, 1949; Bullen and Willis, 1971) and the factors in breast milk which contribute to this difference.

This study can be divided into two parts; in the first part the faecal flora of breast fed and bottle fed normal infants was compared. As expected, the faecal flora of breast fed infants, once established, was composed predominantly of lactobacilli/bifidobacteria. The emergence of these organisms as the predominant organisms was coincident with a reduction in the incidence of E. coli as reported by Bullen and Willis (1971).

The isolation of other organisms from the faeces of breast fed infants is still a subject of much controversy. In this study clostridia were isolated from over 70% of infants at one week of life and the incidence of bacteroides remained low throughout, under 40%. These results agree with those of Mata and Urrutia (1971) but differ to those of Bullen and Willis (1971) who reported that clostridia and bacteroides were rarely isolated from the faeces of breast fed normal infants. Smith and Crabb (1961) found that in the one infant studied the isolation rate of Cl. welchii (Cl. perfringens), although initially reaching counts of 10^8 per gram of faeces was not isolated by the end of the first week of life but bacteroides remained the predominant organism throughout the first year of life.

In infants fed on an artificial milk the faecal flora remained a mixed flora and no one organism became established as predominant. The percentage colonisation and geometric counts of lactobacilli/bifidobacteria and E. coli remained similar throughout the first week of life and clostridia, especially Cl. perfringens, was frequently isolated; this isolation of Cl. perfringens had been noted by Bullen and Willis (1971).

In the second part of this study of the anaerobic flora, the

faecal flora of ill/low birthweight infants was determined. Although the incidence of certain aerobic organisms e. g. E. coli (Farmer, 1971) had been investigated in these infants the total faecal flora had not been identified. This study indicated that the anaerobic flora was similar to that found in normal bottle fed infants and therefore different to that of breast fed infants. However, it is not possible to consider one section of the total flora without due reference to the other organisms present. In the ill/low birthweight infants, although the incidence of the lactobacilli/ bifidobacteria was similar to that found in normal bottle fed infants, the faecal flora was dominated by the aerobic gram-negative bacilli, especially E. coli. This result was in contrast to that in normal infants; bottle fed infants retained a mixed faecal flora with both aerobic and anaerobic organisms present in similar frequency and breast fed infants had a predominance of anaerobic bacilli. It was not possible to investigate the faecal flora of ill/low birthweight infants fed solely on expressed breast milk which would complete the investigation of the effect of environment and feed on the faecal flora of infants.

The incidence of bacteroides in ill/low birthweight infants had also not been determined and in this study was found to be much lower than that of bottle fed normal infants or breast fed infants. Reports on the incidence of bacteroides vary widely (Smith and Crabb, 1961; Bullen and Willis, 1971) and the factors which affect the establishment of this organism are not known. The incidence of anaerobic organisms in ill/low birthweight infants was

considerably less than in normal infants; this reduction in anaerobic organisms was not uniform and the bacteroides was the group most affected by this reduction in the anaerobic flora.

In the first part of this study, although multiple resistance in E. coli was not uncommon in the isolates from ill/low birth-weight infants, E. coli which were fully sensitive to the eight antibiotics tested were also frequently isolated. In this second part of the investigation which was carried out nine months later, fully sensitive E. coli were significantly less common and most ill/low birthweight infants acquired E. coli which were resistant to three or four antibiotics out of the eight tested. The absence of kanamycin resistance, 21 months after the use of this antibiotic was stopped, indicated that by the alteration of the selective pressures it was possible to alter the frequency of isolation of that antibiotic resistance pattern and it may be possible to reintroduce the antibiotic at a later date.

The establishment of the lactobacilli/bifidobacteria in the faecal flora of breast fed normal infants and the higher resistance of these infants to gastro-enteritis are now accepted facts. This predominance of the gram-positive bacilli is thought to be promoted by two mechanisms; firstly the physical properties of breast milk enhance the growth of lactobacilli/bifidobacteria resulting in conditions which are unfavourable for the growth of E. coli (Eullen and Willis, 1971); secondly the inhibition of E. coli by the iron binding proteins promotes the establishment of lactobacilli/bifidobacteria. In bottle fed normal infants, the physical properties of the milk and the lack of the iron binding proteins results in a mixed faecal flora without the predominance of any one species. In

these infants the difference in feed can be related to the difference in faecal flora. If this difference in feed was the determining factor in the establishment of the faecal flora it would be expected that the faecal flora of all infants fed on the same artificial milk would be the same, however comparison of the faecal flora of the ill/low birthweight infants and normal bottle fed infants showed significant differences in the faecal flora. The high incidence of aerobic gram negative bacilli in relation to the other organisms isolated from the faeces of ill/low birthweight infants was considered abnormal or dysbiotic by Haenel (1961) and was found in suckling infants in hospital, infants who were thriving poorly or in infants who were otherwise healthy but had slight throat infections. Haenel considered that many factors can easily affect the faecal flora of newborn infants but in adults the flora is more resistant to change. The results of this study define the faecal flora of infants nursed in this hospital and indicate the variation in faecal flora in normal infants; this difference is related to the feeding regime but in ill/low birthweight infants the environment and the physical state of the infants play a role equally important to that of feed in the determination of the faecal flora of the infants. These results also indicate that infants nursed in hospital acquire a faecal flora which has been considered abnormal and the effect of this flora on the development and progress of the infant is not known.

SUMMARY

1. Anaerobic organisms were isolated infrequently from the mouths of both normal and ill/low birthweight infants and these organisms did not form an integral part of the mouth flora.
2. The faecal flora of breast fed normal infants was comprised predominantly of lactobacilli/bifidobacteria by the end of the first week of life. Other organisms both anaerobes and aerobes were isolated but these organisms were less common both in incidence and numbers than the lactobacilli/bifidobacteria.
3. Normal infants fed on an artificial milk retained a mixed faecal flora throughout the first week of life and aerobic and anaerobic organisms were isolated with similar frequency.
4. The faecal flora of ill/low birthweight infants had a predominance of E. coli by the end of the first week of life, and although anaerobic organisms were isolated they did not achieve the importance found in normal infants, either breast or bottle fed.
5. The incidence of clostridia and lactobacilli/bifidobacteria was similar in ill/low birthweight and bottle fed normal infants but the incidence of bacteroides was significantly lower in the ill/low birthweight infants. However, the faecal flora of these ill/low birthweight infants was completely dominated by the high incidence of the gram-negative bacilli as found in the first part of this study. E. coli was isolated in high frequency throughout the study and the isolation of clostridia and lactobacilli/bifidobacteria increased during the first month of life.
6. Cl.perfringens was isolated from normal and ill/low birth-

weight infants, the incidence and numbers were similar irrespective of feeding regime or treatment.

7. Resistant E. coli were significantly more common in the ill/low birthweight infants than in normal infants and was also more common than in the first part of this study.

APPENDIX

APPENDIX 1

IDENTIFICATION OF GRAM-POSITIVE COCCI

Catalase Production

The test organism was inoculated into a tube containing nutrient broth and incubated overnight at 37°C. 0.1 mls. was pipetted into a tube for the coagulase test and catalase reagent (3 volume hydrogen peroxide) was added to the remaining broth. The tube was shaken gently and examined for the production of gas. Any tube which did not produce gas was incubated at 37°C and re-examined after 5 minutes. Organisms which produced gas were denoted catalase positive and tested for the production of coagulase.

Coagulase Production

Any organisms which had the colonial morphology of Staph. aureus were tested for coagulase production using the slide coagulase method, all other catalase positive gram-positive cocci were tested using the tube method.

Slide Coagulase Method

A colony was emulsified in a drop of water on a microscope slide. A straight wire was dipped in plasma and then used to stir the suspension. Any organism which produced clumping within 5 seconds was called coagulase positive.

Tube Coagulase Method

To 0.1 mls. of the overnight broth culture was added 0.5 mls. of a 1 in 10 dilution of plasma in saline. The tube was incubated at 37°C for 6 hours and then at room temperature overnight. A

positive result was indicated by the formation of a definite clot.

Fermentation of Glucose

A straight wire was used to inoculate one tube of O-F medium (Hugh and Leifson). The surface was then sealed to a depth of 1 cm. with sterile liquid paraffin. The tube was incubated for up to 14 days. Any tube which had a change of colour from green to yellow was regarded as positive for the fermentation of glucose.

WARD REGIME

1. Normal Infants

a) Labour Ward

After birth, each infant was swathed and placed in a small nursery in the labour ward. Infants born by normal delivery and with a rectal temperature of over 35°C (97°F) were bathed after one hour. Any infant born by Caesarian section, assisted delivery or was a breech presentation stayed in the nursery for six hours before bathing. After the infant was bathed, he was transferred to the maternity wards.

b) Maternity Wards

The wards consisted of two single rooms, five four-bedded wards and a nursery at the far end of the ward. Staff entering the wards were required to change into clean white coats and put on face masks. Hands were washed with soap and water before and after handling each infant.

Infants were kept in the nursery until feeding time. The nursing staff changed the napkins of all the infants of three days or under in the nursery. After this time the infants were changed

by their mothers in the wards prior to feeding. After feeding, the infants were returned to the nursery.

Infants were fed on breastmilk or a prepacked half cream cows milk preparation.

c) Bathing regime

Infants were bathed in the labour ward, then on the fourth day and on the last day in hospital. Cotton wool was soaked with 2 mls. of a 3% hexachlorophene detergent lotion and used to wipe the infant. Care was taken by the nursing staff to avoid any raw areas, the eyes and the mouth. The infant was then rinsed immediately in warm water and dabbed dry.

d) Treatment of the Umbilical Cord

After birth, the base and the first two centimetres of the cord were sprayed with Polybactrin (polymixin, bacitracin, neomycin) spray. A clip was applied, the cord cut and the cut end was sprayed. The cord was sprayed daily until it separated.

2. III/low Birthweight Infants

a) Transfer to the Premature Baby Unit (P.B.U.)

Infants born at Hammersmith Hospital were treated for any immediate birth problems, swathed in a silver swaddler, placed in a prewarmed incubator and transferred to the P.B.U.

Infants born at other hospitals and transferred to the P.B.U. within 24 hours of birth were admitted to this study. Treatment received by the infant varied with the type of birth problem and the hospital. The infants were collected by the neonatal residents and wherever possible full details of the treatment given to each infant were entered into the infants' notes.

b) Admission to P.B.U.

Infants weighing less than 2,200 gms., less than 35 weeks gestational age or presenting with problems at birth were admitted to the P.B.U. Infants requiring intensive care or unable to maintain body temperature were nursed in incubators, other infants were nursed in open cots.

c) Clothing and Equipment

(i) Staff Nursing staff wore thin cotton dresses in the unit and changed into regulation uniform before leaving. Medical staff, visitors and parents were required to put on cotton overshoes, wash with Betidine (povidone iodine 1% available iodine Berk Pharmaceuticals) and wear cotton gowns before entering the unit. Hands were washed before and after handling an infant.

(ii) Infants Each infant had a locker underneath the incubator or cot containing his own feeding tubes or teats which were kept soaked in Milton, creams, cotton wool and thermometer. By the side of each infant was a stethoscope for use on that infant only and an overgown for the nursing staff. Overgowns were changed twice weekly. All equipment was either sterilized when an infant left the unit or discarded.

d) Bathing

Infants were not bathed on the labour ward before transfer to the unit. Infants nursed in incubators and those in open cots before the cord had separated were 'top and tailed' using 3% hexachlorophene detergent solution. Once the cord had separated, infants nursed in open cots were bathed daily using the procedure carried out in the labour ward and lying-in wards.

e) Treatment of the Umbilical Cord

This was treated as stated for normal infants.

f) Feeds

Feeds were prepared each morning in a milk kitchen which was at one end of the P.B.U. Artificial feeds, full strength, half-cream cows milk preparation was prepared exactly to the manufacturer's instructions. The milk was divided into measured amounts, autoclaved at 212°C for 20 minutes and stored in the fridge until use. Expressed breast milk was measured out, autoclaved and stored as for artificial milk. If there was an excess of expressed breast milk this was boiled and placed in a deep freeze until required.

Ill infants or those fed at intervals of less than three hours were fed by indwelling nasogastric tubes. Larger, well infants were fed by orogastric tube passed at each feed. Infants were fed from a bottle whenever possible but this was rarely at less than 35 weeks gestation.

g) Treatment

Antibiotics were not used prophylactically in the P.B.U. and were prescribed for the following conditions:

1. Infants with respiratory distress who appeared to be deteriorating or developing moist sounds in the lungs.
2. Infants who presented with lethargy, failure to gain weight and weight loss, cyanotic attacks or fever.

The antibiotics used in the P.B.U. were in accordance with the policy of the Paediatric Department (Davies et al, 1972) although the kanamycin was withdrawn during this work and

gentamicin was used.

Infants were nursed in incubators until they were able to maintain body temperature and then, if well enough, were transferred to an open cot.

APPENDIX 3

Bacteriophage Lysis Patterns of Coagulase-Negative Staphylococci

		Phage																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Phage Pattern Number	1																			#
	2		#																	
	3		#	±		#		#	#	#						±		#	#	
	4					#												#		
	5					#														+
	6		#			+	#	+		#	#	+	#	±	#	#		#	#	#
	7						#													±
	8		±				+			#	±	±	±	±			±		+	±
	9				±				#		+									
	10								#	±	±									±
	11																	#	#	
	12					#			#								#			
	13						#													
	14			#								#			#					
	15						±		±				#							
	16			#	#	#														
	17		#		#	#	#			#	#	#	#	#	±	+		#	#	#
	18																			#
	19																			
	20																			
	21		+				+					#								
	22				±		#		#	#	#									±
	23		#				#			#				#		#		#	#	#
	24									±	#									
	25											#	+	±						±
	26					#														
	27						#			#	#		#			#		+	±	±
	28		#	+						#			#					#	#	#
	29						#						#	+						#
	30		#		+	+	#		#	#	#									
	31		#				±			#	+					#				
	32		#		±	#	#			#		#	#			#			#	+
	33		+							+		±					±	±	±	±
	34			#		#														
	35			#		#				#	#	#					#	+	#	
	36																#			
	37		±				±			±	±	±								±
	38								#											#
	39		#				#			#	#					±	±		#	#
	40						±					+						#		

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