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Temporal Study of the Staphylococci and Micrococci of Normal Infant Skin†

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Staphylococcus and *Micrococcus* populations were collected from the healthy skin of 10 infant subjects. Infants were sampled from 1 day to 32 weeks of age. Species were characterized by approximately 30 different morphological, physiological, and biochemical characters. Staphylococci were the predominant inhabitants of normal skin, whereas micrococci were found only occasionally in this environment. *Staphylococcus epidermidis, S. haemolyticus,* and *S. hominis* were the predominant and persistent staphylococci. These species constituted a high percentage of the total aerobic bacterial flora of infant skin. *Micrococcus luteus* and *M. kristinae* were the prevalent micrococci found on infant skin. Only limited correlation between *Staphylococcus* and *Micrococcus* populations and infant age or body area sampled was indicated by this study.

The majority of previous investigations of the aerobic flora of infant skin have been concerned with the study of *Staphylococcus aureus.* The source (9, 15, 17, 27) and prevention (1, 2, 6, 7, 22-24) of infant colonization by *S. aureus* has been of particular medical interest. Coagulase-negative staphylococci have understandably produced fewer problems for infants and those responsible for their care. As a consequence, study of the presence of these staphylococci on infant skin has been somewhat limited.

Investigation of the flora of the umbilical stump reported by Fairchild (5) showed the occurrence of *Staphylococcus albus* and/or *Staphylococcus citreus* on 13% of 6-day-old infants and on 87% of 30-day-old infants. Sarkany and Gaylarde (19) reported the predominance of coagulase-negative staphylococci on infant skin. Their study included five skin sites of infants from 1 to 6 days of age. The following year these researchers reported the presence of *S. albus* at birth on virtually all infants (20). Somerville (26) reported the isolation of *Micrococcaceae* (coagulase-negative staphylococci and micrococci) from all infants studied, except when the umbilicus was the skin site sampled. Sixty-eight percent of infants had *Micrococcaceae* on the umbilicus. In 1970, Evans et al. (3) reported the isolation of *Staphylococcus epidermidis* from 40% of full-term newborn infants when umbilical sites were sampled. Fifty percent of full-term newborn infants had *S. epidermidis* in the nares. Evans et al. (4) also found that 71% of infants 21 days of age had *S. epidermidis* in the nares and 82% of 21-day-old infants had *S. epidermidis* on umbilical sites. Montes et al. (16) reported the occurrence of coagulase-negative staphylococci on 44% of 25 infants free of diaper dermatitis.

This study was undertaken primarily to determine which species of coagulase-negative staphylococci occur normally on infant skin as well as the population sizes of these species in this particular environment. Recent advances in the identification of staphylococci to species (11, 12, 21) facilitated the classification of the coagulase-negative staphylococci.

The occurrence of micrococci on infant skin has likewise received limited interest (16, 25, 26). Recent identification of this genus to species (13) facilitated its incorporation in this investigation.

A few of the previously used taxonomic schemes for separating the species of *Staphylococcus* and *Micrococcus* have resulted in incorrect classification of many of these organisms. This study clarifies the classification and resolution of these genera and their respective species as they are found on normal infant skin.

MATERIALS AND METHODS

Bacterial strains. Staphylococci and micrococci strains were collected from the healthy skin of 10 infants. Nine infants were residents of Raleigh, N.C.; one was a resident of Knightdale, N.C. The race, sex, family economic condition, sibling placement, skin care, and any medications of each child were not considered in choosing infants for this study. Eight Caucasians, one Negro, and one child of Asiatic heri-

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tage were included. The sex ratio was seven females to three males. All infant subjects were volunteered for the study by their parents.

Bacterial collections were taken from all infants at 1, 6 to 8, 10 to 12, 16 to 20, and 28 to 32 weeks of age. Collections were taken from five infants also at 1 and 3 days of age.

Samples of the skin flora of each infant were collected from 12 body sites comprising eight major body areas. The sites included were forehead, right or left naris, neck, upper chest, peri-umbilicus, right axilla, left axilla, right arm, left arm, right leg, left leg, and right upper foot. The upper foot was included with legs in considering major body areas.

Procedure for collecting bacterial strains. Cotton swabs saturated with sterile physiological saline (approximately 0.5 ml/swab) were used to collect the cutaneous flora of each body site. A swab was rubbed vigorously, with rotation, for roughly 5 s over an approximate 8-cm^2 area of each body site. Each swab was then used to inoculate the entire surface of a 100-mm-diameter agar plate.

The flora collected from each body area with high microbial density was diluted by rinsing the appropriate swab in 2.0 ml of sterile saline. This swab was then used to inoculate an agar plate. A 0.01 dilution of the saline rinse was also cultured. The collected flora from naris, neck, peri-umbilicus, right axilla, and left axilla were so diluted for better separation and resolution of collected strains.

Culture conditions. Cultures were incubated at 34°C and maintained at 4°C. Inocula for tests were prepared from 18- to 24-h-old cultures.

Culture medium. The medium used for the collection, isolation, propagation, and maintenance of all strains was P agar (18). This is a nonselective medium of the following composition: peptone (Difco), 10 g; yeast extract (Difco), 5 g; sodium chloride, 5 g; glucose, 1 g; agar (Difco), 15 g; and distilled water, 1,000 ml.

Procedure for selection of bacterial strains for analysis. Bacterial colonies on original collection plates were counted to determine what percentage of this value (the total number of aerobic bacteria collected) was either staphylococci or micrococci. Yeasts and molds were not included in this count. Small lipophilic diphtheroids were counted. Rare isolates of certain fastidious bacteria would not be expected to grow on the nonselective isolation medium, but they are not considered part of the normal flora.

Only strains thought to be micrococci or staphylococci were isolated. Determination of several characters of isolated cultures made it possible to recognize what were assumed to be identical strains of staphylococci or of micrococci. Multiple cultures ascertained to be identical strains were discarded except for a single representative culture. The representative culture of each potentially unique strain was characterized.

Procedure for identification of genus and species *Staphylococcus* and *Micrococcus*. Preliminary identification of *Staphylococcus* and *Micrococcus* strains was made from colony morphology, pigmentation, and growth rate (11, 13, 14, 21) observed on original collection plates. This identification was further substantiated by the same characters seen in pure cultures of these strains.

Final identification of *Staphylococcus* strains was according to the scheme of Kloos and Schleifer (11, 21). Identification of *Micrococcus* strains was according to the scheme of Kloos et al. (13). Approximately 30 different morphological, physiological, and biochemical characters were determined for each strain.

RESULTS

***Staphylococcus* populations of normal infant skin.** *Staphylococcus* was the most widely distributed, most predominant, and most persistent aerobic genus found on infant skin (Fig. 1). A summary of the 480 samples of bacterial skin flora collected from all infants (Fig. 2) illustrates the predominance of staphylococci over micrococci. Of the total 480 samples, less than 4% failed to contain staphylococci. Of the areas tested, the axilla produced the greatest number of samples, with 75% or more of their aerobic flora being staphylococci.

The occurrence of *Staphylococcus* species on normal infant skin is shown in Fig. 3. *S. epidermidis* was most prevalent. Of the total 480 samples, 83% contained this species. Strains of *S. haemolyticus* were isolated from 62%, *S. hominis* from 49%, *S. xylosus* from 14%, *S. aureus* from 10%, *S. warneri* from 9%, and *S. capitis, S. cohnii, S. simulans,* and *S. saprophyticus* from 5% of these samples. Body area appeared to have little effect upon the distribution of *Staphylococcus* species on normal infant skin.

Effect of infant age upon the occurrence of staphylococci. Negligible correlation existed between infant age and the occurrence or relative percentage of staphylococci on infant skin (Fig. 1 and 3; Tables 1 and 2). However, the occurrence of *S. xylosus* appeared to increase appreciably as infants became older (Fig. 3; Table 2).

***Micrococcus* populations of normal infant skin.** *Micrococcus* was infrequently isolated from normal infant skin (Fig. 1 and 2). Of the total samples taken, only 30% contained members of this genus. Micrococci occurred less frequently and in lower relative percentages on several body areas of high microbial density, such as the nares and the umbilicus. Micrococci were slightly more prevalent on the arms and legs. No one body area was appreciably more colonized by micrococci than were other areas.

The study of *Micrococcus* species isolated from infant skin is shown in Fig. 4. *M. luteus* was the most prevalent species, occurring in 21% of the total 480 samples taken. *M. kristinae* was isolated from 9% of the samples, *M. varians* and *M. lylae* from 3%, and *M. nishinomiyaensis* from

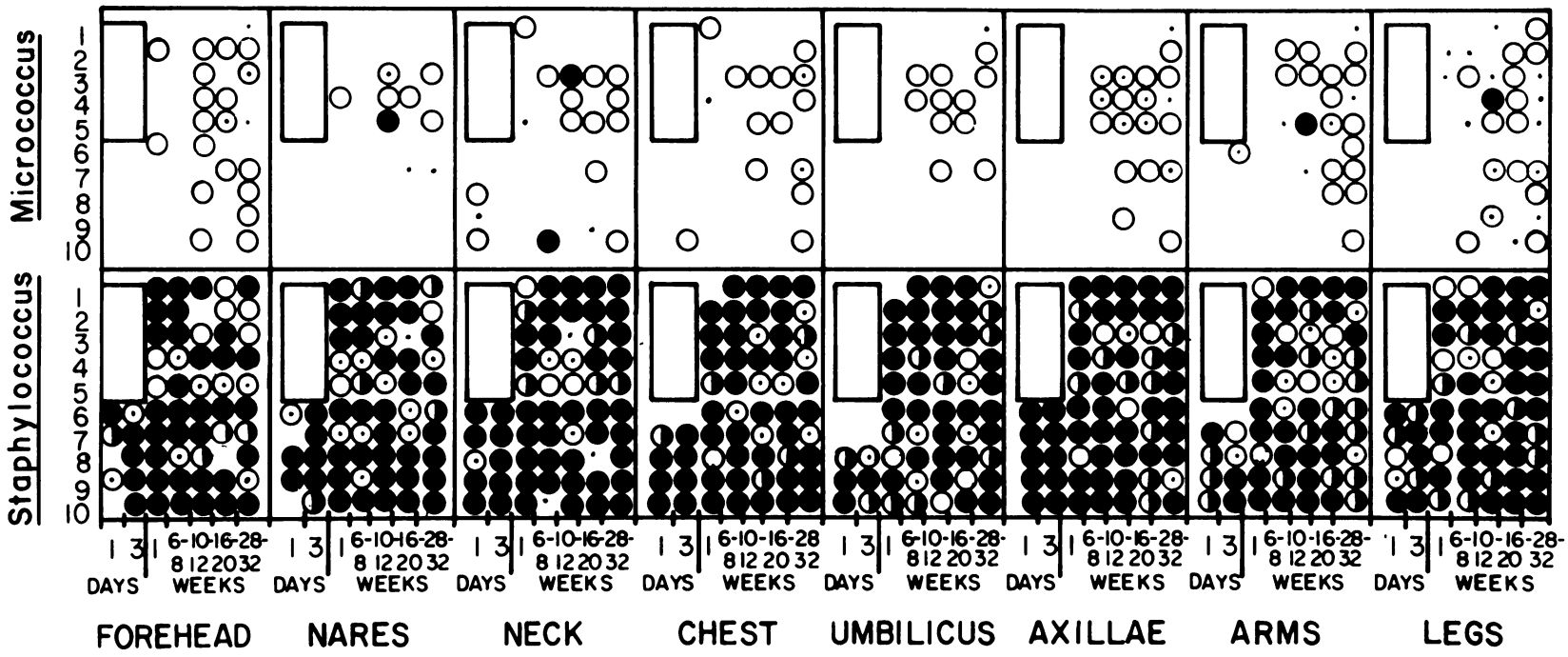

FIG. 1. *Temporal study of staphylococci and micrococci isolated from infant skin. Each infant is represented by a single number along the ordinate. Collection times are represented for each body area along the abscissa. The percentage of total collected aerobic bacteria represented by each genus (referred to in the text as the relative percentage of the genus) is designated according to the following symbols: , <1; ○, 1 to 24; ⊙, 25 to 49; ◐, 50 to 74; ●, 75 to 100; no symbol, 0. Outlined areas are indicative of no collection.*

2%. *M. sedentarius* and *M. roseus* were isolated only once throughout the study.

Effect of infant age upon the occurrence of micrococci. The data presented in Fig. 1 and 4 and in Tables 1 and 3 suggest an effect of infant age upon the occurrence of micrococci. According to these data, the occurrence of micrococci increased with increasing age. This increase in occurrence of micrococci is best demonstrated with the predominant species *M. luteus* and *M. kristinae*. It is noted that micrococci were rarely isolated from infants less than 1 week in age.

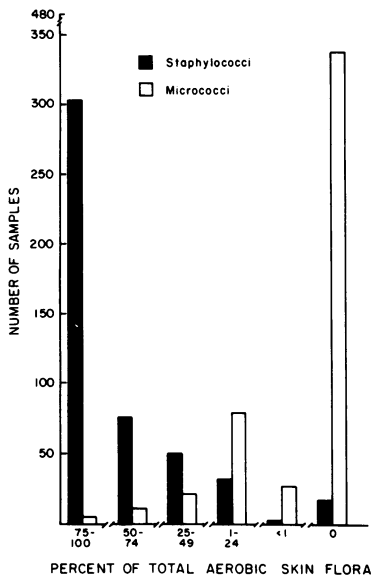

FIG. 2. *Comparison of the relative percentages of staphylococci and micrococci isolated from infant skin.*

DISCUSSION

A comparison of the occurrence and relative percentage of staphylococci and micrococci on infant skin has illustrated the overwhelming dominance of staphylococci. A major source of infant colonization has been shown by some researchers to be those adults to which the infant is exposed (1, 7, 8, 15, 27). The predominance of staphylococci over micrococci on the infants in this study could be a reflection of the composition of the skin flora of the adults caring for these infants. Alternatively, infant skin may favor the colonization of staphylococci over micrococci.

A study of the species of staphylococci and micrococci of normal infant skin has demonstrated that a few species are especially prevalent. These species are those generally most prevalent on adult skin (10). The occurrence of these particular species on infant skin lends support to the first hypothesis stated above. However, infant skin did, in general, contain slightly elevated populations of *S. haemolyticus* as compared with adult skin (10). *S. capitis* was less frequently isolated from infant than from adult subjects (10).

When compared with adult skin flora (10),

TABLE 1. *Percentage of samples containing micrococci and staphylococci*

Organism	Infant age						
	1 day	3 days	1 week	6–8 weeks	10–12 weeks	16–20 weeks	28–32 weeks
Micrococci	5.0	5.0	11.3	20.0	43.8	35.0	62.5
Staphylococci	80.0	90.0	97.5	100.0	98.8	97.5	100.0

TABLE 2. *Percentage of samples containing Staphylococcus species*

Staphylococcus species	Infant age						
	1 day	3 days	1 week	6–8 weeks	10–12 weeks	16–20 weeks	28–32 weeks
S. aureus	7.5	10.0	10.0	8.8	3.8	7.5	18.8
S. simulans	–[a]	2.5	8.8	1.3	3.8	–	12.5
S. xylosus	–	5.0	3.8	8.8	12.5	20.0	35.0
S. cohnii	2.5	2.5	10.0	6.3	1.3	5.0	3.8
S. saprophyticus	–	–	–	2.5	11.3	7.5	7.5
S. haemolyticus	32.5	50.0	50.0	67.5	57.5	68.8	72.5
S. warneri	3.8	3.8	3.8	15.0	13.8	10.0	5.0
S. hominis	20.0	50.0	55.0	37.5	48.8	53.8	63.8
S. epidermidis	60.0	67.5	87.5	86.3	82.5	86.3	90.0
S. capitis	–	–	5.0	10.0	7.5	2.5	6.3

[a] –, Absence of species.

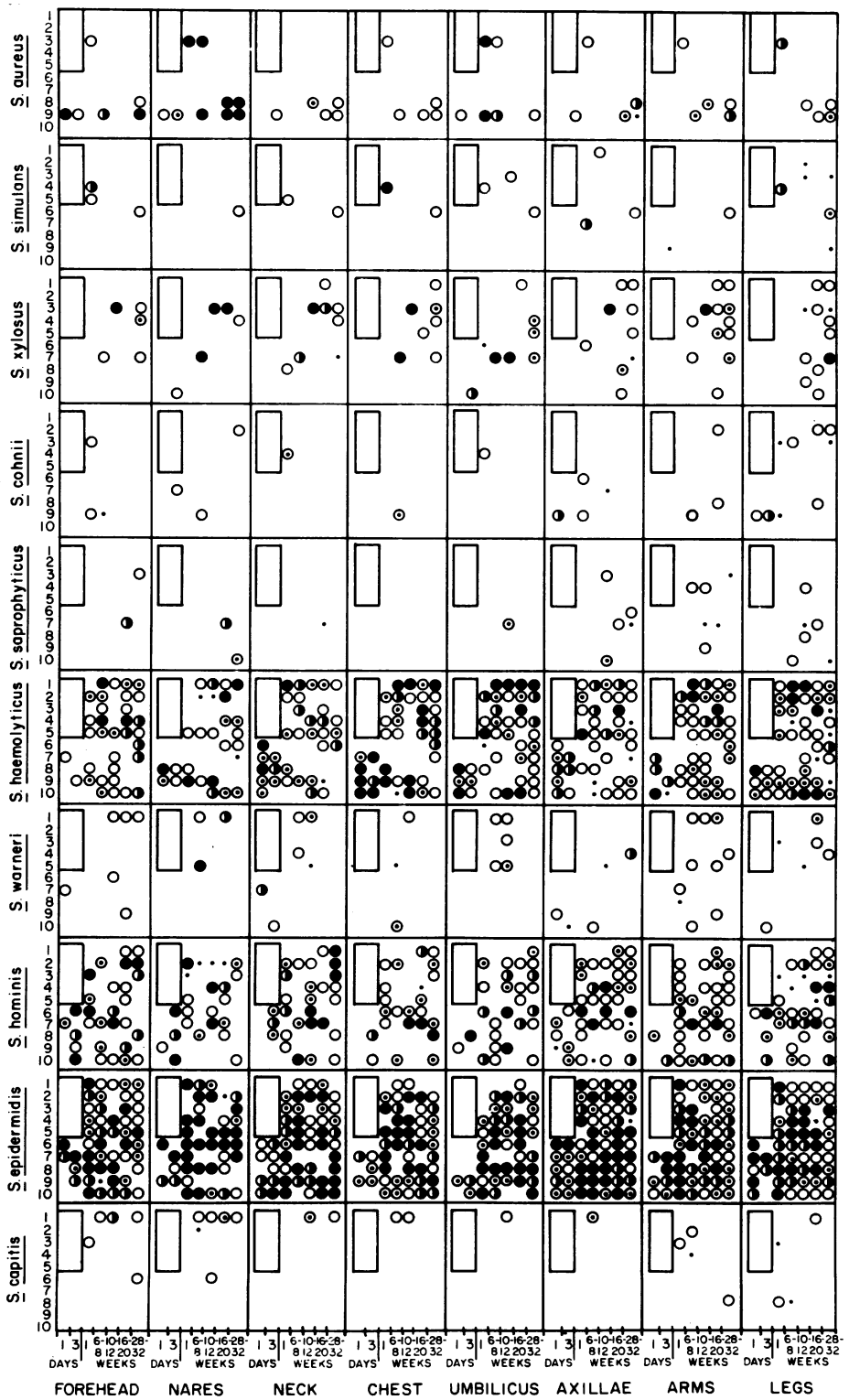

FIG. 3. *Temporal study of the Staphylococcus species isolated from infant skin. Explanation and value of symbols as in Fig. 1. Symbols indicate the percentage of total staphylococci represented by each species (referred to in the text as the relative percentage of the species).*

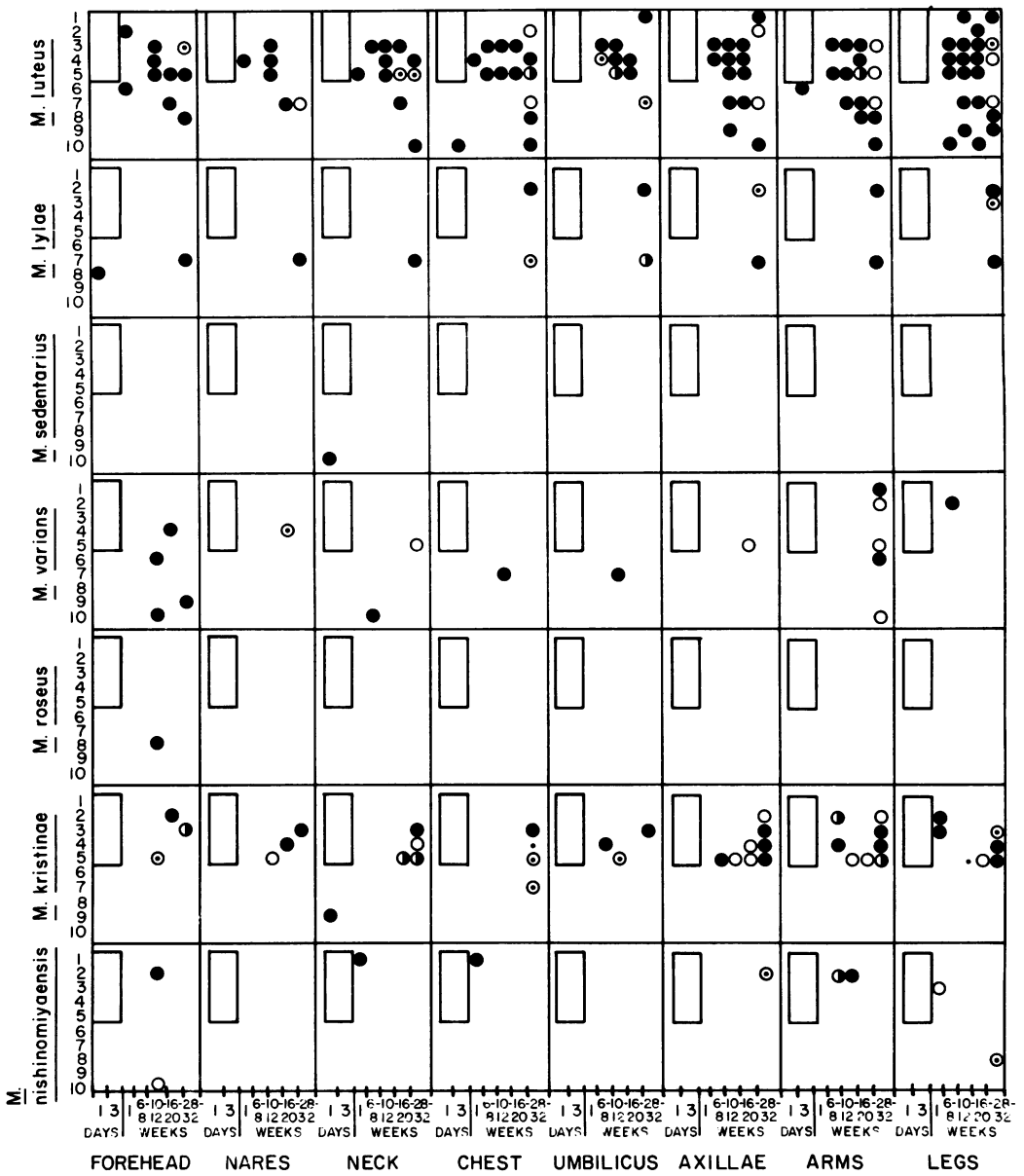

FIG. 4. *Temporal study of the Micrococcus species isolated from infant skin. Explanation and value of symbols as in Fig. 1. Symbols indicate the percentage of total micrococci represented by each species (referred to in the text as simply the relative percentage of the species).*

the staphylococci and micrococci found on infant skin appear to have no specificity for body area. Infant skin lacks some of the niche differentiation that is developed in adult skin. The usual handling by parents and others of infants in our study may have influenced the composition of the skin flora. Likewise, the small body size of infants would tend to make the skin flora more uniform by reducing the spatial separation of any possibly distinct microbial populations.

The bacteria collected from infant skin in this study should be considered representative of the normal flora. A knowledge of any normal situation serves as a means for analyzing the abnormal situation. This is the first temporal study to include a species analysis of the staphylococci and micrococci of infant skin. Such detailed analysis extends and strengthens its use as a

TABLE 3. *Percentage of samples containing Micrococcus species*

Micrococcus species	Infant age						
	1 day	3 days	1 week	6–8 weeks	10–12 weeks	16–20 weeks	28–32 weeks
M. luteus	–[a]	5.0	6.3	16.3	35.0	31.3	37.5
M. lylae	2.5	–	–	–	–	–	17.5
M. sedentarius	2.5	–	–	–	–	–	–
M. varians	–	–	–	2.5	5.0	3.8	8.8
M. roseus	–	–	–	–	1.3	–	–
M. kristinae	2.5	–	2.5	5.0	7.5	8.8	26.3
M. nishino-miyaensis	–	–	3.8	1.3	3.8	–	1.3

[a] –, Absence of species.

reference. As the pathogenic importance of coagulase-negative staphylococci is ascertained, it is hoped this reference will be of particular medical value.

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