

Carriage *S. aureus*

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QUANTITATIVE STUDIES OF THE FLORA OF THE NASAL VESTIBULE IN RELATION TO NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*

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SUMMARY. Aerobic and anaerobic bacterial flora and yeast flora of the nasal vestibule were studied quantitatively in eight persons who were persistent, transient or non-carriers of *Staphylococcus aureus*. The nasal flora of all the subjects consisted of lipophilic and non-lipophilic aerobic diphtheroids, propionibacteria and different staphylococcal species. Other micro-organisms, such as *Pityrosporum* and *Candida*, micrococci, streptococci and coliforms were found only occasionally. The presence of *S. aureus* was related to diminished numbers of other staphylococcal strains and of propionibacteria.

INTRODUCTION

Nasal carriage of *Staphylococcus aureus* was extensively studied in the years 1940–1960 (see Williams, 1963) in relation to its significance as a source of the staphylococci that caused hospital-acquired infection, which was common during these two decades (Miller *et al.*, 1962; Williams *et al.*, 1966; Henderson, 1967). In some of these studies the flora of the nasal vestibule was investigated with particular reference to a possible antagonistic action of *S. aureus* against other bacteria (O'Grady and Wittstadt, 1963; Martin and White, 1968; Aly *et al.*, 1970), which, it was suspected, might help this organism to establish itself in the nasal vestibule. Indeed, a direct in-vitro antagonism between *S. aureus* and other members of the nasal flora (Pryjma *et al.*, 1971), and an inverse relationship between the occurrence of *S. aureus* and other organisms (Smith, 1969; Aly *et al.*, 1970; Heczko *et al.*, 1973), were

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demonstrated. However, no quantitative studies of the complex bacterial ecology of this specific region of the human skin have yet been reported.

The aim of the present investigation was to examine the quantitative occurrence of various constituents of the microbial flora of the skin of the nasal vestibule in persistent carriers and non-carriers of *S. aureus*. Because of the reports of Watson *et al.* (1962) and Aly *et al.* (1970) on the presence of anaerobes and yeasts in the nasal vestibule, we extended our study to include these groups of micro-organisms.

MATERIALS AND METHODS

Subjects

The nasal flora of eight members of the laboratory staff in Cologne (aged 20–40 years, four male and four female) was studied. In a preliminary experiment, swabs of their nasal vestibules had been examined for the presence of *S. aureus* on six occasions at weekly intervals.

Cotton swabs moistened with physiological saline were used for sampling, and these were seeded on to plates of Mannitol Salt Agar (Difco), which were incubated for 48 h. Representative colonies thought to be of *S. aureus* were subcultured, tested for coagulase activity, and typed by means of the phages of the International Typing Set (Blair and Williams, 1961).

The subjects were divided, according to the criteria of Leedom *et al.* (1965) into three classes of nasal carriers: persistent carriers (PC; four persons); transient carriers (TC; one person); and non-carriers (NC; three persons).

Quantitative estimation of the flora of the nasal vestibule

Collection of specimens. Each subject was examined on three occasions at weekly intervals. The area to be sampled was demarcated by means of an Ayer's chalazion forceps with a round window 8 mm in diameter. The device was fixed by a screw to the interior surface of the lateral side of the ala nasi in a horizontal position, so that the window exposed a 50-mm² area of skin, leaving a margin from the orifice of width 2 mm. The area was then sampled with a small sterile cotton swab by gentle scrubbing for 1 min. The swab had previously been moistened in 0.075M phosphate buffer, pH 7.9, containing Triton X100 0.1% (v/v) (Williamson and Kligman, 1965; Evans, 1975). The procedure was repeated with another swab in the opposite nostril.

Method of culture. The swabs were placed in tubes containing 1 ml of the buffer and shaken for 1 min at room temperature. Ten consecutive tenfold dilutions were made in the buffer diluted 1 in 2 with sterile distilled water. Then a 0.02-ml drop of each dilution was placed on a plate of the following media: Mannitol Salt Agar (Difco), Tryptic Soy Agar (Difco) with 5% (v/v) of defibrinated sheep blood (TSAB), selective Furoxone-Tween 80-Oil Red O (FTO) medium (Smith, 1969), A-agar for the cultivation of propionibacteria (Pulverer and Ko, 1973), Endo Agar (Difco), and maltose-extract agar (ME) for *Pityrosporum* (Randjandiche, 1975).

The samples were spread on the plates with glass rods. All plates except for those of A-agar and ME agar were incubated aerobically at 37°C for 48 h. Plates of A-agar were incubated for 5 days at 37°C in an anaerobic incubator (type VT/N2, Heraeus) in an atmosphere of 95% N₂ and 5% CO₂ at normal pressure. Plates with ME agar were incubated at 35°C for 7 days in a moist atmosphere.

Bacterial and yeast colonies with the same morphological appearances were counted on several plates containing different dilutions of the samples and their mean numbers per 1 cm² of skin were calculated. Ten representative colonies of each morphological type on one plate of a given medium were picked and transferred on to appropriate media for further characterisation. Single colonies occurring on the same plate, which did not represent populations over 10² organisms, were omitted. Staphylococcal strains were classified into species according to Kloos and Schleifer (1975), micrococci were identified as described by Baird-Parker (1963) and aerobic

diphtheroids were distinguished according to Somerville (1972) by means of lipid-free and Tween 80-supplemented Tryptic Soy Broth (Difco). Bacteria of the genus *Propionibacterium* were examined by the Minitec Anaerobic System (BBL) and classified according to the key given by Moore and Holdeman (1974). Results were confirmed by serological typing as described by Höffler, Ko and Pulverer (1977). Streptococci and gram-negative rods were identified by standard methods (Lenette, Spaulding and Truant, 1974). All *S. aureus* isolates were phage-typed as described above.

Presentation of quantitative results. Geometric means of six counts per subject (left and right; on three occasions) for different organisms per 1 cm² of vestibular skin were calculated, together with the appropriate log₁₀ standard deviation values (Kligman, Leyden and McGinley, 1976). Mean values for all persons of the same carriage class, with standard error values, were also determined.

RESULTS

Representatives of eight of the ten coagulase-negative staphylococcal species recognised by Kloos and Schleifer (1975) were found (table I). Staphylococci belonging to the species *S. epidermidis*, *S. xylosum*, and *S. hominis* occurred most frequently while the others were found only occasionally; some isolates remained unclassified. Also, two micrococcal strains were isolated from one person (not included in table I). *S. aureus* strains isolated from three persons differed in their phage-typing patterns while two persons harboured *S. aureus* strains with identical phage patterns. No changes in phage pattern were seen in *S. aureus* strains consecutively isolated from the same subject. Several other bacterial strains were found in two of the subjects; most of these were identified as *Escherichia coli* or streptococci of serological group G. *Pityrosporum* spp. were isolated from four persons, two of whom also yielded *Candida albicans*.

TABLE I
Classification of staphylococci isolated from nasal vestibules

Species of <i>Staphylococcus</i> *	Number of strains†	Percentage
<i>aureus</i> ‡	18	11.6
<i>capitis</i>	2	1.3
<i>cohnii</i>	4	2.6
<i>epidermidis</i>	48	30.9
<i>haemolyticus</i>	0	0
<i>hominis</i>	19	12.3
<i>saprophyticus</i>	0	0
<i>simulans</i>	2	1.3
<i>warneri</i>	0	0
<i>xylosum</i>	45	29.0
Unclassified	17	11.0
Total	155	100.0

* According to Kloos and Schleifer (1975).

† All isolates of the same species obtained from one nostril of a given person at the same time were regarded as one strain.

‡ *S. aureus* = coagulase positive; other species = coagulase negative.

TABLE II
Aerobic flora of the skin of the anterior nares in relation to nasal carriage of *S. aureus*

Subject	Carrier class*	Geometric mean count/cm ² (and log SD), for the stated subject, of									
		<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. xylosum</i>	<i>S. hominis</i>	other staphylococci	<i>Micrococcus</i> spp.	lipophilic diphtheroids	non-lipophilic diphtheroids	yeasts†	others‡
1	PC	1.3 × 10 ³ (0.81) 4,1	2.6 × 10 ³ (1.93) 2,4	4.4 × 10 ³ (2.17) 5,6	-	1.1 × 10 ⁴ (2.35)	-	9.4 × 10 ³ (1.76)	1.6 × 10 ⁷ (2.35)	-	-
2	PC	8.8 × 10 ³ (1.61) 3,4	4.6 × 10 ⁵ (1.84) 5,†	1.4 × 10 ⁴ (1.68) 4,1	2.4 × 10 ⁴ (1.43) 4,3	-	-	5.9 × 10 ⁵ (0.99)	1.3 × 10 ⁷ (1.66)	1.2 × 10 ³ (1.89)	-
3	PC	1.0 × 10 ⁴ (1.12) 4	3.2 × 10 ³ (2.27) 3,5	3.1 × 10 ¹ (0.91) 7	5.6 × 10 ¹ (0.61) 1,†	-	1.7 × 10 ² (1.63)	1.6 × 10 ⁴ (1.38)	5.3 × 10 ⁶ (2.54)	-	-
4	PC	1.8 × 10 ⁴ (1.43) 4,3	1.9 × 10 ² (1.02) 2,3	-	-	-	-	7.2 × 10 ⁴ (1.72)	1.4 × 10 ⁶ (1.85)	3.9 × 10 ³ (1.13)	-
5	TC	1.6 × 10 ⁴ (1.14) 4,2	3.9 × 10 ³ (1.45) 3,6	7.5 × 10 ³ (0.98)	2.8 × 10 ⁴ (0.33)	-	-	2.4 × 10 ⁴ (1.25)	2.0 × 10 ⁵ (2.16)	1.9 × 10 ³ (1.36)	7.9 × 10 ³ (2.51)
6	NC	-	4.8 × 10 ⁴ (0.66) 4,†	6.6 × 10 ³ (0.75) 3,8	2.4 × 10 ⁴ (0.78)	-	-	3.9 × 10 ⁵ (1.36)	6.7 × 10 ⁶ (1.88)	1.3 × 10 ³ (1.83)	7.2 × 10 ² (1.57)
7	NC	-	1.2 × 10 ⁴ (0.81)	1.0 × 10 ⁴ (0.90)	4.1 × 10 ⁴ (1.14)	-	-	7.6 × 10 ⁴ (0.96)	4.7 × 10 ⁵ (1.13)	-	-
8	NC	-	3.8 × 10 ⁴ (1.12)	7.9 × 10 ⁴ (0.72)	7.0 × 10 ⁴ (0.83)	-	-	3.3 × 10 ⁵ (0.89)	3.5 × 10 ⁷ (2.16)	-	-

- = Less than 10² organisms/cm².

* According to Leedom *et al.* (1965): PC = persistent carrier; TC = transient carrier; NC = non-carrier.

† *Pityrosporum* spp. and *Candida albicans* (presented jointly).

‡ Streptococci and gram-negative rods (presented jointly).

Total
counts

4, 3
5, 7
~~3, 7~~
3, 7
- 2, 3
4, 6
4, 9
4, 5
5, 3

54764
54765
54766
54767
54768
54769
54770
54771
54772
54773
54774
54775
54776
54777
54778
54779
54780

Numbers of *S. aureus* ranged from 10^3 to 10^4 /cm² in the nostrils of PC and TC, but three species of coagulase-negative staphylococci, *S. epidermidis*, *S. xylosus* and *S. hominis*, formed populations of a more variable density (10^2 to 10^5 /cm²; table II). Other staphylococci and *Micrococcus* spp. were each found in only one person. Aerobic diphtheroids formed the most dense populations (10^5 – $>10^7$ /cm²). Lipophilic and non-lipophilic aerobic diphtheroids were present in all persons studied, but the numbers of non-lipophilic organisms always exceeded considerably those of the lipophilic. An inverse effect of sample dilution was observed in all aerobic cultures from PC and TC on TSAB medium: the numbers of the non-lipophilic diphtheroids calculated from higher dilutions exceeded those for lower dilutions of the same samples, probably because of some antagonistic action of *S. aureus* present in these cultures. However, zones of inhibition of the diphtheroids around staphylococcal colonies were not observed. Therefore, the data given in table II refer to calculations based on counting of the diphtheroids growing on FTO medium. Yeasts occurred in four subjects regardless of their carrier class and in numbers that never exceeded 10^3 /cm². Coliform bacilli and streptococci were found together in the nostrils of two persons.

Propionibacteria were present on the nostril skin of seven subjects (table III); *P. acnes* was identified in all of them, either alone (1 person) or together with *P. granulosum* (2 persons), *P. avidum* (2 persons), or both of these species (2 persons). Numbers of *P. acnes* ranged from 10^2 to 10^5 organisms/cm², and

TABLE III

Anaerobic flora of the skin of the anterior nares in relation to nasal carriage of *S. aureus*

Subject no.	Carrier class*	Geometric mean count/cm ² (and log SD), for the stated subject, of <i>Propionibacterium</i>		
		<i>acnes</i>	<i>granulosum</i>	<i>avidum</i>
1	PC	3.2×10^4 (1.27)	3.9×10^2 (1.62)	3.9×10^2 (1.03)
2	PC	3.3×10^5 (2.12)	3.0×10^4 (2.94)	1.9×10^3 (0.95)
3	PC	1.9×10^2 (1.43)	–	–
4	PC	–	–	–
5	TC	1.7×10^5 (0.85)	1.1×10^4 (2.94)	–
6	NC	3.7×10^5 (0.72)	–	8.6×10^3 (0.92)
7	NC	8.3×10^5 (0.88)	–	1.9×10^4 (1.68)
8	NC	6.2×10^5 (1.08)	7.9×10^4 (2.52)	–

* See footnotes to table II.

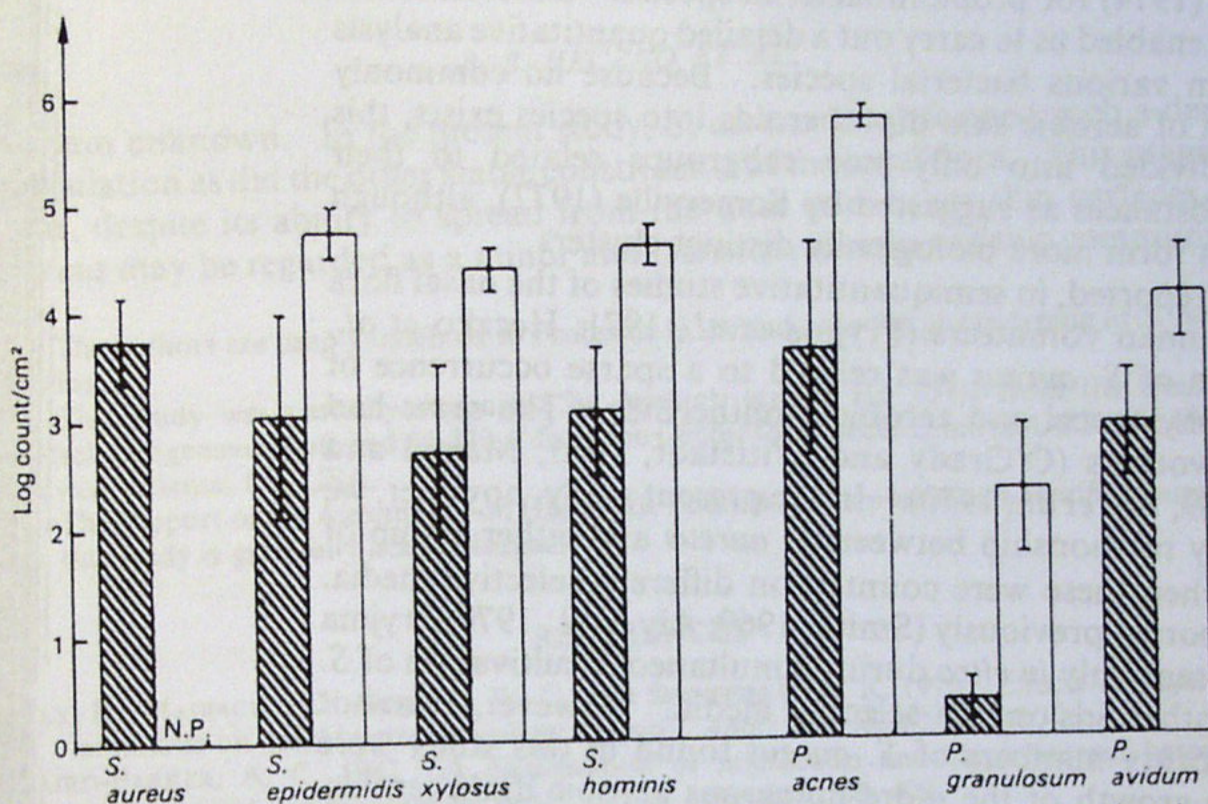


FIG.—Numbers of staphylococci and propionibacteria per cm² of the skin of the anterior nares of persistent carriers \blacksquare and non-carriers \square of *S. aureus*. N.P. = not present.

were always higher than those of members of the remaining propionibacterial species.

A comparison of cumulative mean numbers of selected species of *Staphylococcus* and *Propionibacterium* calculated from results of testing of four PC persons versus three NC persons (the figure) showed significant differences in the quantitative composition of the bacterial flora of the nasal vestibule depending on carrier class. These were related to the presence of decreased numbers of three staphylococcal species: *S. epidermidis*, *S. xylosus* and *S. hominis* in the PC group in comparison with their numbers in the NC group. There were even more evident differences in the numbers of each of the species of *Propionibacterium*: the densities of these bacteria in the NC group exceeded those found in the PC group by about 100 times for *P. acnes* and *P. granulosum* and about 10 times for *P. avidum*.

No significant differences were found between the PC and the NC groups in the numbers of other micro-organisms: the remaining staphylococcal species, lipophilic and non-lipophilic aerobic diphtheroids, micrococci, yeasts and others.

DISCUSSION

Subsequent biochemical examination of morphologically distinct colonies confirmed our ability to recognise different species of staphylococci and propionibacteria visually. This observation is in keeping with the findings of Kloos, Musselwhite and Zimmerman (1976) for staphylococcal and of

Marples and McGinley (1974) for propionibacterial species. Differentiation by colonial morphology enabled us to carry out a detailed quantitative analysis of relationships between various bacterial species. Because no commonly recognised classification of aerobic skin diphtheroids into species exists, this bacterial group was divided into only two subgroups related to their dependence on fatty substances as suggested by Somerville (1972), although obviously these bacteria form more biologically distinct clusters.

We have previously reported, in semiquantitative studies of the nasal flora of some hundreds of human volunteers (Pryjma *et al.*, 1971; Heczko *et al.*, 1973), that the presence of *S. aureus* was related to a sparse occurrence of coagulase-negative staphylococci and aerobic diphtheroids. The same had been found by other workers (O'Grady and Wittstadt, 1963; Martin and White, 1968; Smith, 1969; Aly *et al.*, 1970). In the present study, however, we are unable to find any relationship between *S. aureus* and either group of aerobic diphtheroids when these were counted on different selective media. Therefore, the effect reported previously (Smith, 1969; Aly *et al.*, 1970; Pryjma *et al.*, 1971) may be present only *in vitro* during simultaneous cultivation of *S. aureus* and aerobic diphtheroids on non-selective media. However, it cannot be excluded that the scanty numbers of *S. aureus* found in this study were unable to suppress the growth of the more numerous aerobic diphtheroids. Bibel, Greenberg and Cook (1977) recently reported that *S. aureus* in densities lower than 10^4 cells/cm² on the skin of patients with atopic dermatitis did not alter the quantitative composition of the aerobic flora while *S. aureus* in higher densities, up to 10^7 cells/cm², eliminated other aerobic bacteria.

The quantitative composition of the bacterial flora of the skin of the nasal vestibule differs from that of the normal human skin of dry regions, such as the extremities and the lower trunk (Kligman *et al.*, 1976). It resembles, however, that of skin regions with increased humidity, such as the axilla or interdigital spaces, where the numbers of aerobic diphtheroids are much higher than those of coagulase-negative staphylococci (Kligman *et al.*, 1976). On the other hand, densities of propionibacteria found in the nasal vestibule were nearly as high as those of these bacteria in sebum-rich areas of the skin (Leyden *et al.*, 1975). The mean numbers of propionibacteria in the nasal vestibules of our PC group were comparable with those reported by McGinley, Webster and Leyden (1978), but the numbers in our NC group were greater than these. However, we made observations only on eight persons who lived in a different geographical region from the subjects studied by McGinley *et al.* (1978). These authors classified the inner surface of the anterior nares as a mucosa. In our previous studies we had demonstrated, however, that the microbial flora multiplies only on a narrow margin of true skin covering the nasal vestibule (Heczko *et al.*, 1973).

Thus, the human nasal vestibule forms a specialised ecological niche rather similar to the other wet areas of the skin, but it supports the growth of *S. aureus* only in some persons. In spite of extensive studies (O'Grady and Wittstadt, 1963; Martin and White, 1968; Aly *et al.*, 1970), the factors responsible for the multiplication of this organism in these persons, or for preventing it in others,

remain unknown. In the present study, *S. aureus* never formed such a dense population as did the other stable constituents of the nasal flora. This suggests that, despite its ability to spread from the nose over other skin surfaces, *S. aureus* may be regarded as a minor and transient member of this community.

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REFERENCES

- ALY, R., MAIBACH, H. I., STRAUSS, W. G. AND SHINEFIELD, H. R. 1970. Effects of a systemic antibiotic on nasal bacterial ecology in man. *Appl. Microbiol.*, **20**, 240.
- BAIRD-PARKER, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. *J. gen. Microbiol.*, **30**, 409.
- BIBEL, D. J., GREENBERG, J. H. AND COOK, J. L. 1977. *Staphylococcus aureus* and the microbial ecology of atopic dermatitis. *Can. J. Microbiol.*, **23**, 1062.
- BLAIR, J. E. AND WILLIAMS, R. E. O. 1961. Phage typing of staphylococci. *Bull. Wld Hlth Org.*, **24**, 771.
- EVANS, C. A. 1975. Persistent individual differences in the bacterial flora of the skin of the forehead: numbers of propionibacteria. *J. invest. Derm.*, **64**, 42.
- HECZKO, P. B., PRYJMA, J., KASPROWICZ, A. AND KRAWIEC, H. 1973. Influence of host and parasite factors on the nasal carriage of staphylococci. In *Staphylococci and staphylococcal infections: recent progress*, edited by J. Jeljaszewicz, Karger, Basel, p. 581.
- HENDERSON, R. J. 1967. Staphylococcal infection of surgical wounds: the source of infection. *Br. J. Surg.*, **54**, 756.
- HÖFFLER, U., KO, H. L. AND PULVERER, G. 1977. Serotyping of *Propionibacterium acnes* and related microbial species. *F.E.M.S. Microbiol. Lett.*, **2**, 5.
- KLIGMAN, A. M., LEYDEN, J. J. AND MCGINLEY, K. J. 1976. Bacteriology. *J. invest. Derm.*, **67**, 160.
- KLOOS, W. E., MUSSELWHITE, M. S. AND ZIMMERMAN, R. J. 1976. A comparison of the distribution of *Staphylococcus* species on human and animal skin. In *Staphylococci and staphylococcal diseases*, edited by J. Jeljaszewicz, Fischer-Verlag, Stuttgart, p. 967.
- KLOOS, W. E. AND SCHLEIFER, K. H. 1975. Simplified scheme for routine identification of human *Staphylococcus* species. *J. clin. Microbiol.*, **1**, 82.
- LEEDOM, J. M., KENNEDY, R. P., LEPPER, M. H., JACKSON, G. G. AND DOWLING, H. F. 1965. Observations of the staphylococcal nasal carrier state. *Ann. N.Y. Acad. Sci.*, **128**, 381.
- LENNETTE, E. H., SPAULDING, E. H. AND TRUANT, J. P. 1974. *Manual of clinical microbiology*, 2nd ed., American Society for Microbiology, Washington.
- LEYDEN, J. J., MCGINLEY, K. J., MILLS, O. H. AND KLIGMAN, A. M. 1975. Propionibacterium levels in patients with and without acne vulgaris. *J. invest. Derm.*, **65**, 382.
- MCGINLEY, K. J., WEBSTER, G. F. AND LEYDEN, J. J. 1978. Regional variations of cutaneous propionibacteria. *Appl. envir. Microbiol.*, **35**, 62.
- MARPLES, R. R. AND MCGINLEY, K. J. 1974. *Corynebacterium acnes* and other anaerobic diphtheroids from human skin. *J. med. Microbiol.*, **7**, 349.
- MARTIN, R. R. AND WHITE, A. 1968. The reacquisition of staphylococci by treated carriers: a demonstration of bacterial interference. *J. Lab. clin. Med.*, **71**, 791.
- MILLER, D. L., McDONALD, J. C., JEVONS, M. P. AND WILLIAMS, R. E. O. 1962. Staphylococcal disease and nasal carriage in the Royal Air Force. *J. Hyg., Camb.*, **60**, 451.
- MOORE, W. E. C. AND HOLDEMAN, L. V. 1974. Genus *Propionibacterium* Orla-Jensen. In

- Bergey's manual of determinative bacteriology, edited by R. E. Buchanan, and N. E. Gibbons, 8th ed., Williams & Wilkins, Baltimore, p. 633.
- O'GRADY, F. AND WITTSTADT, F. B. 1963. Nasal carriage of *Staphylococcus pyogenes*. II. Bacterial ecology of the nose. *Am. J. Hyg.*, **77**, 187.
- PRYJMA, J., BOBR, J., HECZKO, P. B., KASPROWICZ, A. AND KRAWIEC, H. 1971. Studies on carriers of *Staphylococcus aureus*. I. Antagonistic properties of the bacterial flora of the nasal vestibule. *Epidem. Rev.*, **25**, 190.
- PULVERER, G. AND KO, H. L. 1973. Fermentative and serological studies on *Propionibacterium acnes*. *Appl. Microbiol.*, **25**, 222.
- RANDJANDICHE, M. 1975. Fréquence de *Pityrosporum ovale* dans l'oreille humaine. *Dermatologica*, **151**, 100.
- SMITH, R. F. 1969. A medium for the study of the ecology of human cutaneous diphtheroids. *J. gen. Microbiol.*, **57**, 411.
- SOMERVILLE, D. A. 1972. The microbiology of the cutaneous diphtheroids. *Br. J. Derm.*, **86**, suppl. 8, 16.
- WATSON, E. D., HOFFMAN, N. J., SIMMERS, R. W. AND ROSEBURY, T. 1962. Aerobic and anaerobic bacterial counts of nasal washings: presence of organisms resembling *Corynebacterium acnes*. *J. Bact.*, **83**, 144.
- WILLIAMS, R. E. O. 1963. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bact. Rev.*, **27**, 56.
- WILLIAMS, R. E. O., BLOWERS, R., GARROD, L. P. AND SHOOTER, R. A. 1966. Hospital infection: causes and prevention, 2nd ed., Lloyd-Luke, London.
- WILLIAMSON, P. AND KLIGMAN, A. M. 1965. A new method for the quantitative investigation of cutaneous bacteria. *J. invest. Derm.*, **45**, 498.