J. Hyg., Camb. (1982), **88**, 487
Printed in Great Britain

Dispersal of non-sporeforming anaerobic bacteria from the skin

BY EVA BENEDIKTSDÓTTIR AND ANNA HAMBRAEUS
Institute of Clinical Bacteriology, University of Uppsala, Uppsala, Sweden

(*Received* 3 *August* 1981; *accepted* 25 *November* 1981)

SUMMARY

Dispersal of non-sporeforming anaerobic bacteria was studied. Skin samples were taken from the subjects, and dispersal from different parts of the body was examined.

The number of anaerobic bacteria dispersed was not correlated to their density on the surface of skin area exposed. The highest density of anaerobic bacteria on the skin was found in the face and upper trunk, but the highest yield of anaerobic bacteria dispersed came from the lower trunk.

The dominant anaerobic bacteria dispersed were *Propionibacterium acnes*, but *Propionibacterium avidum*, *Propionibacterium granulosum* and Gram-positive cocci were also isolated from the dispersal samples. *Peptococcus magnus* was the most common coccus isolated. For the less frequently isolated bacteria, the best correlation was found between the perineal flora and airborne bacteria.

A comparison was also made of bacterial dispersal by naked and dressed subjects. The dispersal of both aerobic and anaerobic bacteria was higher when the subjects were dressed in conventional operating theatre cotton clothing than when they were naked.

The increased dispersal of anaerobic bacteria when the subjects were dressed was mainly due to increased dispersal of *Propionibacterium* sp.

INTRODUCTION

There have been a number of studies on the dispersal of aerobic bacteria, but little attention has been paid to the nature of the dispersal of anaerobic bacteria. In an earlier paper we showed that strict anaerobic non-sporeforming bacteria can be isolated from the air of occupied rooms, and that they are carried on particles of the same size as aerobic bacteria (Hambraeus & Benediktsdóttir, 1980).

In the present study the dispersal of anaerobic bacteria from different individuals and from different parts of the body has been examined. The study includes the dispersal of anaerobic bacteria in relation to the anaerobic skin flora, as well as a comparison of the dispersal of anaerobic and aerobic bacteria by naked and dressed subjects. Both qualitative and quantitative results of this investigation are presented.

MATERIAL AND METHODS

Air sampling

A Casella slit sampler MK II, maximum capacity 700 l/min, and a Sartorius filter sampler MD, maximum capacity 45 l/min, were used for air sampling. In the filter sampler, gelatin filters, type SM 12652, from Sartorius Membranfilter were used (Hambraeus & Benediktsdóttir, 1980).

Bacteriological methods

The medium used for anaerobic cultivation was Brain Heart Infusion (BHI) agar, containing Bacto agar, 18 g/l; BHI, 33·3 g/l; yeast extract, 4·5 g/l; sheep blood, 50 ml/l; vitamin K, 1·7 mg/l and haemin, 16·2 mg/l.

For aerobic incubation blood agar was used, containing Bacto Beef extract, 3 g/l; Bacto peptone, 5 g/l; Bacto agar, 15 g/l; NaCl, 5 g/l; lactose, 1 g/l and sheep blood, 50 ml/l.

The nutrient agar used to subculture stains of coryneforms contained the same ingredients as the blood agar except the sheep blood.

End products were tested with gas chromatography after two days' cultivation in peptone yeast glucose (PYG), containing peptone, 10 g/l; yeast extract, 10 g/l; glucose, 10 g/l; L-cystein-HCl, 0·5 g/l; 0·01 % Tween 80 and 40 ml of a salt solution consisting of $CaCl_2$, 0·2 g/l; $MgSO_4$, 0·2 g/l; K_2HPO_4, 1 g/l; KH_2PO_4, 1 g/l; $NaHCO_3$, 10 g/l and NaCl, 2 g/l.

The Gas Peak System (Oxoid) was used for anaerobic incubation. The samples were incubated 6 or 7 days at 37 °C. Different types of colonies were then counted and representatives subcultured on both BHI blood agar for anaerobic and blood agar for aerobic incubation. Bacteria that did not grow on blood agar aerobically after 2 days were included for further typing as follows. A total of 50 strains of suspected *Propionibacterium acnes* isolated from 12 different subjects were typed according to the VPI manual (Holdeman *et al.* 1977) with gas chromatography and biochemical patterns, using the BBL Minitek anaerobic test system for indole, nitrate reductase, catalase, sucrose, trehalose, sorbitol, esculin, maltose, mannitol, lactose and adonitol (Stargel *et al.* 1976). All *P. acnes* strains from 12 subjects were serotyped according to Cummins (1975). Remaining strains of *P. acnes* were diagnosed with typical colony form and cell morphology only. All suspected *P. avidum* and *P. granulosum* were typed with gas chromatography and the Minitek system, using the same tests as for *P. acnes*.

All Gram-positive rods, not having the typical colony morphology of *P. acnes*, were tested for end products from PYG with gas chromatography. Those which only showed acetic acid and not propionic acid in ether extract were grouped as coryneforms. A limited number of coryneform strains were tested for prolonged aerobic incubation on blood agar and for 2 days' incubation on nutrient agar with and without 1 % Tween 80. Most of the strains tested grew to visible colonies on blood agar when incubated 4–7 days, and on nutrient agar when incubated 2 days, and often the size of the colonies was increased when the nutrient agar was supplemented with Tween 80. Some of these strains lost their capability to grow anaerobically when subcultured two or three times.

Gram-positive cocci were typed according to the VPI manual by using the BBL Minitek anaerobic test system for indole, nitrate reductase, catalase, sucrose, xylose, lactose, maltose, glucose, aesculin, cellobiose and mannose, and with gas chromatography. They were also tested for sensitivity to metronidazole Watt & Jack, 1977). All the typable Gram-positive cocci were sensitive to metronidazole.

Serotyping of P. acnes

Typing sera were prepared by injecting rabbits intravenously with purified cell wall preparations in saline, prepared according to Cummins (1975). Injections were made on three successive days, and three weeks later a booster was given on three successive days. Three or four days later the animals were bled out. The strains used were *P. acnes* NCTC 737 (serotype 1) and *P. acnes* ATCC 11828 (serotype 2) obtained from Statens Bakteriologiska Laboratorium in Stockholm.

Typing was made using immunodiffusion tests against bacterial extracts prepared according to Cummins (1975).

Skin sampling

Samples for qualitative studies were taken from the hair by contact plate technique, and from the axilla, groins and perineum by swab technique. From other skin areas, quantitative sampling of anaerobic skin bacteria was performed according to Williamson & Kligman (1965). A sterile ring, 2·6 cm in diameter and 2 cm deep, was placed on the sampling site. One ml of sterile phosphate buffer containing 0·1 % Triton X-100, pH 7·9, was placed in the ring and rubbed over the skin with a glass rod for half a minute. The fluid was removed and the procedure repeated once. The two samples were pooled. After that ten-fold dilutions were made in saline and viable counts made on 0·1 ml of the dilutions.

Efficiency of the quantitative skin sampling technique

To get an estimate of the removal of anaerobic bacteria by the scrub method, five serial ½ min washes were made on the same site on the forehead and forearm (hairy part) on seven subjects. A mathematical model, in which the amount of bacteria removed per wash was assumed to be directly proportional to the amount of bacteria remaining on the skin (Aly, Maibach & Bloom, 1978), was used to calculate the fraction of the total population removed per wash. Only anaerobic bacteria were counted.

The test chamber

The dispersal experiments were performed in a chamber 2·1 m high × 1·2 × 1·27 m. This was made of hardboard plates covered with vinyl sheets and with a vinyl floor. The connexions between the ceiling and walls were sealed with silicon gum. At 1·2 m height there was a hole, in which a 22 × 22 × 40 cm aluminium pipe was inserted. Air was extracted at the bottom of the pipe, through one or two holes that fitted the filter holder used.

Air was blown into the chamber through the ceiling after passing a prefilter and a high-efficiency filter, giving a uniform downflow. The flow could be varied, the

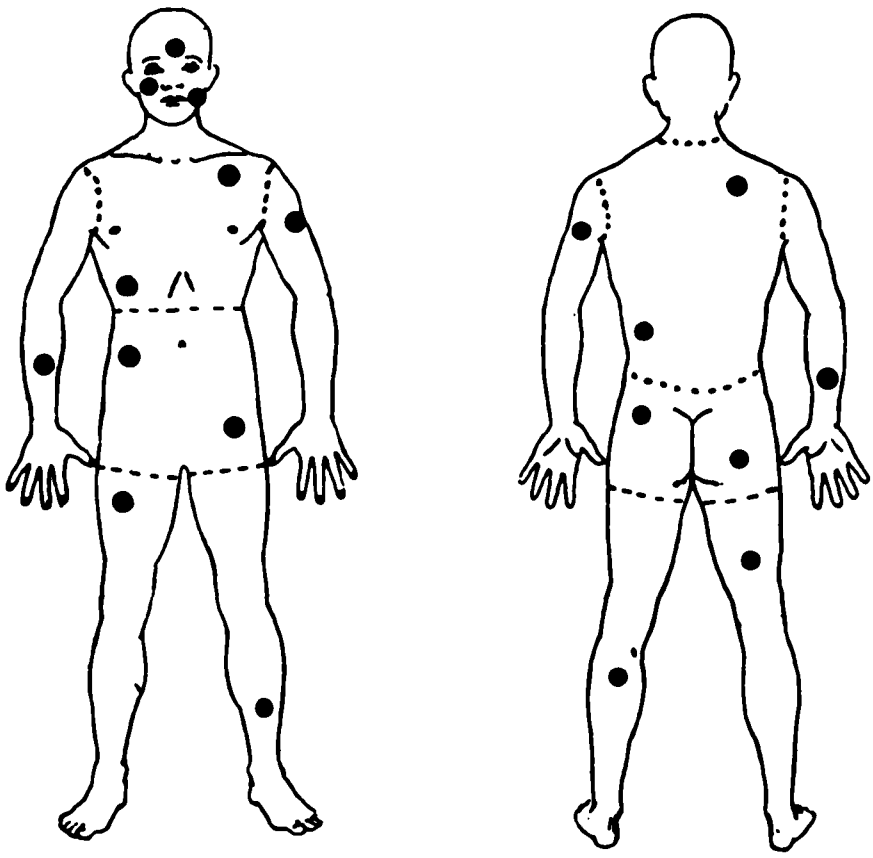

Fig. 1. The sites (●) from which skin flora was sampled with the scrub method.

maximum capacity was 1900 m^3/h. During the dispersal experiments a slight overpressure was kept in the chamber.

Between the experiments the walls of the chamber were sprayed with 70% ethanol in water and then flushed with filtered air at maximum flow for several minutes. Before each experiment, a sample was taken from the empty chamber to give the background value, and counts less than 3 c.f.u. over this value were not regarded as significant.

The dispersal tests

The tests persons were all young, healthy technicians or students. During the dispersal experiments they performed walking movements.

Those parts of the body that were not to be exposed were covered with plastic. The face was always exposed. All connexions between the plastic and the skin were sealed with a sticky tape to make them tight.

Three different series of investigations were made.

(A) *Dispersal in relation to the skin flora.* In this series dispersal and skin flora were studied, and each subject was investigated on two successive days. Dispersal was studied from eight different parts of the body: (1) the whole body, (2) hair and arms, (3) hair, (4) face only, (5) thighs and knees, (6) below the knees, (7) upper trunk including the axilla, and (8) lower trunk.

On the first day, 1–6 were exposed, and on the following day, 1, 7 and 8. At each experiment two 2 min samples, 40 l/min, were taken with the Sartorius filter sampler.

https://doi.org/10.1017/S0022172400070340 Published online by Cambridge University Press

Before the dispersal experiments each day, skin samples were taken from the sites that were to be exposed that day. The skin sites from which the scrub samples were taken are shown in Fig. 1. In all, 19 samples were taken from the skin of each subject. Six females and two males took part in this series.

(B) *Expanded dispersal experiments.* Based on the results from the first series, a simplified experimental layout was used in these experiments. Dispersal was studied from four different parts of the body: (1) The whole body, (2) above the waist, (3) lower trunk, and (4) legs (thighs, feet). Both ½ min and 2 min samples were taken with the Sartorius filter sampler, capacity 40 l/min. One ½ min sample was made with the Casella slit sampler, capacity 700 l/min, for a qualitative study. Only swab skin samples from the axilla, groins and perineum were made. Two females and six males took part in this series.

(C) *Dispersal in relation to clothing.* This series was carried out to study the effect of clothing on dispersal of anaerobic bacteria. The clothes used were sterile conventional operating theatre cotton clothing, trousers and a shirt.

The volunteers put on the clothes and walked around for 30 min before entering the chamber. In order to approach steady state in the chamber they stayed 3 min performing walking movements before sampling and ventilation was begun. Then the ventilation of 700 l/min was put on. Two ½ min samples and two 2 min samples were taken with the Sartorius filter sampler. Afterwards, dispersal was studied in the same way from the naked subjects.

Five females and five males took part in these series. Two of the males were the same as in series B (C1 = B2 and C5 = B3).

RESULTS

Efficiency of the skin sampling technique

In five subjects no decrease in the number of bacteria could be shown in samples from the forehead with repeated washes. In two subjects the decrease corresponded to a removal of 5 and 9 %, respectively, of the resident anaerobic flora at the first wash, according to the model of Aly, Maibach & Bloom (1978). On the forearm, decrease was noted in all the seven subjects. The average decrease from the forearm corresponded to a removal of 37 % of the anaerobes at the first wash and 60 % with two serial scrubbings.

Quantitative analysis of skin sampling and dispersal experiments

In Table 1, the results of the quantitative skin sampling of the coryneforms and the anaerobic bacteria are presented. As can be seen, the highest density was found in the face, $7{\cdot}4 \times 10^3$ to $1{\cdot}4 \times 10^6$ c.f.u./cm^2, and the upper trunk $6{\cdot}6 \times 10^2$ to $1{\cdot}9 \times 10^5$ c.f.u./cm^2. In six subjects the face gave the highest yield, but in two females, A7 and A8, the highest yield came from the upper trunk. The arms and the lower trunk had counts 10–2000 times lower than the face, and the lowest yield was generally from the thighs and legs.

In series A, no significant dispersal was found from the face of any subject. Significant dispersal from the upper trunk and legs below the knees was only found

https://doi.org/10.1017/S0022172400070340 Published online by Cambridge University Press

Table 1. *Results of quantitative skin samples from six different parts of the body (series A)*

		Geometrical mean of anaerobic bacteria and coryneforms/cm²					
Subject	Sex	Face (3)	Arms (4)	Upper trunk (4)	Lower trunk (4)	Thighs (2)	Legs (2)
A2	M	$6{\cdot}6 \times 10^5$	$2{\cdot}5 \times 10^{3}$*	$4{\cdot}1 \times 10^3$	$3{\cdot}5 \times 10^2$	ND†	ND
A5	M	$1{\cdot}3 \times 10^4$	$1{\cdot}0 \times 10^2$	$2{\cdot}3 \times 10^2$	$1{\cdot}3 \times 10^2$	$1{\cdot}1 \times 10^2$	$1{\cdot}2 \times 10^2$
A1	F	$5{\cdot}5 \times 10^5$	$4{\cdot}9 \times 10^{3}$*	$1{\cdot}4 \times 10^4$	$3{\cdot}8 \times 10^2$	ND	ND
A3	F	$7{\cdot}4 \times 10^3$	$6{\cdot}7 \times 10^1$	$6{\cdot}6 \times 10^2$	$1{\cdot}3 \times 10^2$	—‡	—‡
A4	F	$1{\cdot}4 \times 10^6$	$7{\cdot}9 \times 10^3$	$1{\cdot}9 \times 10^5$	$2{\cdot}4 \times 10^4$	$6{\cdot}0 \times 10^2$	$3{\cdot}2 \times 10^2$
A6	F	$4{\cdot}6 \times 10^4$	$3{\cdot}2 \times 10^1$	$3{\cdot}8 \times 10^3$	$4{\cdot}0 \times 10^1$	$3{\cdot}0 \times 10^1$	6·2
A7	F	$2{\cdot}1 \times 10^4$	$2{\cdot}7 \times 10^2$	$1{\cdot}7 \times 10^5$	$1{\cdot}2 \times 10^2$	$4{\cdot}7 \times 10^1$	$1{\cdot}8 \times 10^1$
A8	F	$7{\cdot}9 \times 10^4$	$1{\cdot}5 \times 10^3$	$1{\cdot}4 \times 10^5$	$4{\cdot}1 \times 10^2$	$1{\cdot}5 \times 10^2$	$5{\cdot}2 \times 10^1$

In parentheses are the number of sites sampled on each part (see Fig. 1).
* Mean calculated from eight sites sampled.
† ND = Not done.
‡ One or more plates had no anaerobic bacteria, mean was not calculated.

Table 2. *Dispersal of anaerobic bacteria and coryneforms from different parts of the body (series A + B)*

		Geometrical mean of c.f.u./80 l			
Subject	Sex	Naked	Lower trunk	Above waist	Legs
A2	M	134·8	29·6	NS*	ND†
A5	M	>‡	>‡	1·4	4·5
A1	F	53·9	27·7	NS	ND
A3	F	20·3	4·2	NS	2·8
A4	F	22·4	2·8	16·0	NS
A6	F	10·8	6·3	NS	NS
A7	F	11·0	9·2	3·5	NS
A8	F	13·4	1·7	NS	3·4
B2	M	213·8	25·0	12·8	1·7
B3	M	152·0	18·0	NS	NS
B5	M	152·0	30·4	10·9	4·2
B6	M	17·7	NS	NS	NS
B1	F	7·7	6·8	1·4	NS
B4	F	20·4	6·5§	NS	

* NS = Plate count not significant (see text) or one or more plates had zero value.
† ND = Not done.
‡ Total plate count was more than 3000 c.f.u., not possible to distinguish between anaerobes and facultatives.
§ Lower trunk and legs exposed together.

in one person, from the hair and arms in two persons and from the thighs in three persons. In Table 2, the dispersal of anaerobic bacteria and the coryneforms from both the first series (subjects A1–A8) and the simplified series (subjects B1–B6) is shown. For the former the dispersal from 'hair and arms' is added to the dispersal from the 'upper trunk' and presented as 'above waist', also 'legs' represent added counts from the 'thighs' and 'below knees'.

https://doi.org/10.1017/S0022172400070340 Published online by Cambridge University Press

Table 3. *Coryneforms and types of anaerobic bacteria isolated from the skin samples in series A and B*

	Number of subjects with positive findings								
	Axilla (12)	Groins (12)	Perin. (11)	Face (8)	Arms (8)	U. tr. (8)	L. tr. (8)	Legs (6)	Hair (4)
Rods									
Coryneforms	6	10	9	0	5	3	2	1	1
P. acnes	7	6	3	8	8	8	8	6	4
P. avidum	2	2	4	0	0	0	0	0	0
P. granulosum	1	0	1	0	0	0	0	0	0
Cocci									
Pc. magnus	0	0	5	0	0	0	0	0	0
'Gaffkya anaerobica'	0	0	2	0	0	0	0	0	0
Pc. saccharolyticus	1	0	1	0	0	0	1	0	0
Pc. asaccharolyticus	0	0	2	0	0	0	1	0	0
Pc. prevotii	0	1	1	0	0	1	0	0	1
Ps. anaerobius	1	0	0	0	0	0	1	0	0

In parentheses are the total number of subjects sampled for respective site.

The dispersal of these groups of bacteria from naked persons varied between 7·7 and 213·8 c.f.u./80 l. Dispersal from the lower trunk showed significant counts from all subjects except one, but the dispersal was lower than from the whole body, between zero and 29·6 c.f.u./80 l. One high degree disperser in series A dispersed more than 3000 c.f.u./80 l in total count from anaerobic incubation from both the lower trunk and the whole body.

Three of the six subjects in series B dispersed significant numbers of bacteria from 'above waist', and two of them also from the legs, but the counts were lower than from the lower trunk.

Qualitative analysis of skin sampling and dispersal experiments

The different types of anaerobic bacteria and the coryneforms found in the different skin samples are presented in Table 3.

Anaerobic Gram-positive rods. P. acnes was the predominant bacterium in the scrub samples from all persons. Strains from five persons were serotyped. More than one type was found in all of them (serotype 1, serotype 2 or not typable *P. acnes*).

From the intertriginous areas *P. acnes* was isolated from the groins in six of twelve, from the perineum in three of eleven and from the axilla in seven of twelve subjects. *P. avidum* was isolated from the intertriginous areas of one female and three males and *P. granulosum* from one female and one male. Neither of these two types were found in the scrub samples.

Coryneforms were isolated from the scrub samples from six persons. In five of these they were only found in one to five sampling sites, but in one male they were the dominant type in samples from the lower trunk and legs.

In the intertriginous areas the coryneforms were found more often than *P. acnes*: in the groins in ten of twelve, in the perineum in nine of eleven and in the axilla in six of twelve persons.

Anaerobic Gram-positive cocci. Anaerobic Gram-positive cocci were most often found in the perineal area. Samples from 11 persons were taken from this area. *Peptococcus magnus* were isolated in five, '*Gaffkya anaerobica*' and *Pc. asaccharolyticus* in two, *Pc. saccharolyticus* and *Pc. prevotii* in one of these. One female carried *Pc. prevotii* on the upper trunk as well as in the hair and the groins. *Pc. asaccharolyticus* and *Peptostreptococcus anaerobius* were found on the lower trunk of the same person. The remaining positive sites, two with *Pc saccharolyticus* and one with *Ps. anaerobius*, represent three different persons.

Untypable anaerobic Gram-positive cocci were divided into metronidazole-resistant and metronidazole-sensitive groups (Watt & Jack, 1977). Metronidazole-resistant cocci were found in scrub samples from four persons. In two of these samples they constituted $> 50\%$ of the skin flora. Metronidazole-sensitive cocci were isolated from the perineum in three persons and from the axilla in three persons. They were only isolated in scrub samples from one person.

In Table 4 the different types of anaerobic bacteria as well as the coryneforms found in the air during dispersal experiments A and B are presented.

Anaerobic Gram-positive rods. P. acnes and coryneforms were dispersed from all 14 volunteers when naked and from 14 and 12, respectively, when the lower trunk

Table 4. *Coryneforms and types of anaerobic bacteria isolated from the air samples in series A and B*

	Number of subjects with positive findings			
	Whole body (14)	Lower trunk (14)	Above waist (14)	Legs (12)
Rods				
Coryneforms	14	12	5	2
P. acnes	14	14	9	5
P. avidum	6	3	0	0
P. granulosum	1	0	0	0
Cocci				
Pc. magnus	7	3	1	0
'*Gaffkya anaerobica*'	1	1	1	0
Pc. saccharolyticus	0	1	0	0
Pc. asaccharolyticus	2	1	0	0
Pc. prevotii	0	1	1	0

In parentheses are the total number of subjects sampled for respective area.

was exposed. Either of these bacteria were found in significant numbers in the air in nine cases when 'above waist' and in five cases when the legs were exposed. *P. acnes* from 11 dispersal experiments were serotyped. In all but two cases more than one type was found.

P. avidum was isolated in air samples when the lower trunk and/or the whole body was exposed in seven cases, three females and four males. *P. granulosum* was found in one dispersal experiment only.

Anaerobic Gram-positive cocci. Anaerobic Gram-positive cocci were isolated from the air during dispersal experiments with eight females and three males. From all except two the anaerobic cocci were only found when the whole body or the lower trunk was exposed. *Pc. magnus* was the type most frequently dispersed, by five females and two males. As shown in Table 4, other cocci were found only sporadically. The three types found when 'above waist' was exposed represent one female dispersing '*Gaffkya anaerobica*' and *Pc. prevotii*, and one male dispersing *Pc. magnus*.

Non-typable anaerobic Gram-positive cocci were found in the air in three experiments; Metronidazole-sensitive cocci in two cases when the whole body was exposed and metronidazole-resistant cocci in one experiment when the lower trunk was exposed.

Except for the two dominant groups, *P. acnes* and the coryneforms, the bacterial species found in the air were not always found in the skin samples of the exposed body surface and vice versa. For the less frequently isolated bacteria, *P. avidum*, *P. granulosum* and the Gram-positive cocci, the best correlation was found between perineal flora and airborne bacteria. The bacteria isolated from the perineum were found in the air samples in 11 of 16 cases. Five of these were *Pc. magnus* and four *P. avidum*. Of 13 isolates from other skin sites, the same types were found in the

https://doi.org/10.1017/S0022172400070340 Published online by Cambridge University Press

Table 5(*a*). *Dispersal in relation to clothing*

		Geometrical mean of c.f.u./80 l			
		Aerobes		Anaerobes and coryneforms	
Subject	Sex	Naked	Clothed	Naked	Clothed
C1	M	32·0	82·5	265·3	151·6
C5	M	25·9	120·0	71·9	436·0
C6	M	29·8	172·0	42·3	941·3
C7	M	64·0	25·3	29·7	28·3
C8	M	31·0	85·7	50·0	112·6
C2	F	56·3	160·5	151·8	407·6
C3	F	31·8	58·0	12·1	84·1
C4	F	12·1	103·7	16·1	373·8
C9	F	71·6	79·0	47·3	87·6
C10	F	77·4	41·0	19·6	39·5
Geometrical mean:		37·8	80·6	44·8	156·5

Table 5(*b*). *Dispersal of the coryneforms and the two groups of anaerobes*

		Geometrical mean of c.f.u./80 l					
		Propionibacterium sp.		Coryneforms		Anaerobic cocci	
Subject	Sex	Naked	Clothed	Naked	Clothed	Naked	Clothed
C1	M	41·5	80·6	198·0	44·7	(23·0)	23·1
C5	M	19·6	152·0	49·6	284·0	0	0
C6	M	35·9	935·9	(4·5)	0	0	(6·0)
C7	M	(2·0)	6·9	(22·0)	9·8	(8·0)	6·9
C8	M	0	9·8	43·8	76·3	5·7	17·0
C2	F	23·7	273·1	124·1	132·7	4·0	0
C3	F	(4·0)	81·6	(7·0)	(2·0)	(2·8)	0
C4	F	12·9	347·8	(4·0)	13·9	(1·0)	(14·0)
C9	F	15·0	72·7	24·0	12·6	0	0
C10	F	(6·0)	28·1	(4·0)	8·9	8·0	(0·5)

The numbers in parentheses are arithmetical means, calculated when one or more of the plates had zero value.

dispersal samples in only three cases, and in two of these the source could also have been the perineum.

Bacterial types that had not been isolated from the skin were found in air samples in 13 cases.

Dispersal in relation to clothing

In Table 5(*a*), dispersal of bacteria, from the same persons naked and clothed is shown. The dispersal of aerobes from naked persons ranged from 12·1 to 77·4 c.f.u./80 l. The geometrical mean was 37·8 c.f.u./80 l. When dressed, six of the subjects more than doubled their dispersal of aerobic bacteria, the range being 41 to 172 c.f.u./80 l and geometrical mean 80·6 c.f.u./80 l.

https://doi.org/10.1017/S0022172400070340 Published online by Cambridge University Press

The dispersal of anaerobes and coryneforms varied between 12·1 and 265·3 c.f.u./80 l, geometrical mean 44·8 c.f.u./80 l from naked subjects. When dressed, the dispersal increased and ranged between 28·3 and 941·3 c.f.u./80 l, geometrical mean 156·5 c.f.u./80 l.

In Table 5(*b*) the variation of dispersal between *Propionibacterium* sp., coryneforms and anaerobic cocci from naked and dressed persons is shown. As can be seen from the table, the increased dispersal of anaerobes is mainly due to increase in dispersal of *Propionibacterium* sp. Eight persons dispersed over 3 times more propionibacteria when they were dressed and three of them more than 20 times. The amount dispersed ranged from 0 to 41·5 c.f.u./80 l from naked persons and from 6·9 to 935·9 c.f.u./80 l when dressed. The mean count of the coryneforms was about the same from naked and dressed persons, range 4–198 c.f.u./80 l and 0–284 c.f.u./80 l, respectively. One male increased his dispersal of these bacteria 5·7 times when clothed, another decreased his dispersal 4·4 times. Eight persons dispersed anaerobic cocci. The number sampled was too small to allow for any conclusions concerning differences in dispersal between naked and dressed persons.

DISCUSSION

As a result of normal desquamation, any organism appearing on the skin, either as a resident or transient member of the skin flora, may be dispersed into the air. In this investigation dispersal of anaerobic bacteria from the body surface into the air was studied and an attempt made to correlate the anaerobic skin flora with dispersed bacteria.

Besides strict anaerobic bacteria, we have also studied the dispersal of all skin bacteria growing anaerobically but not aerobically after 2 days' incubation on blood agar. Thus, microaerophilic bacteria such as metronidazole-resistant cocci (Watt & Jack, 1977), *P. avidum*, which on subcultivation often is capable of growing under microaerophilic conditions (own observations) as well as a heterogenous group of coryneforms have been included. These coryneforms consist partially of lipophilic diphtheroids (Smith, 1969) and their slow aerobic growth on blood agar is often caused by an inhibition effect of the blood.

The reason for including these bacteria is the lack of information in the literature concerning dispersal of not only anaerobic bacteria but also of microaerophilic bacteria and presumably also the aerobically slowly growing coryneforms.

The skin sampling method of Williamson & Kligman (1965) removes 80 % of the aerobic skin flora from the forearm during one 1-min wash. The method seems to be less effective at removing anaerobic bacteria, it has been reported to remove only 10 % of the anaerobic skin flora from the forehead during one 1-min wash (Aly *et al.* 1978). Our results on sampling efficiency of the anaerobic bacteria from the forehead are consistent with those of Aly *et al.* However, in our investigation removal from the arms was higher than from the forehead, 37 % with one ½ min wash. This variation between different skin sites can probably be explained with difference in the size of the sebaceous follicles where the anaerobic skin bacteria are located (Puhvel *et al.* 1975).

P. acnes was the predominant bacterium in all scrub samples. The highest yield was from the face and the upper trunk and the lowest from the thighs and the legs below the knees. A similar distribution of *P. acnes* on the body surface was found by Somerville & Murphy (1973).

The second most common rods found on the skin were coryneforms. They were found mainly in the intertriginous areas. Anaerobic Gram-positive cocci were most frequently isolated from the perineal area, but were occasionally also found in other areas.

The predominant anaerobic bacteria dispersed into the air were *P. acnes*. *P. acnes* from twelve subjects were studied to see if serotyping of *P. acnes* could be used to help in tracing studies. The results indicate that serotyping alone is not of much help as a marker.

From series A, where dispersal from eight different parts of the body was studied in detail, it was found that the sum of the dispersal from exposed parts of the body was always less than the number dispersed from the whole body. This was probably due to the fact that skin particles adhered by electrostatic forces to the plastic that covered the rest of the body. In this series there was no correlation between the number of skin bacteria on the area exposed and the number of bacteria dispersed. There was for example no significant dispersal when the face was exposed in any experiment, but always when the lower part of the trunk was exposed.

Although Gram-positive anaerobic cocci were found in skin samples from parts of the body other than the perineal area, they were only once found in air samples when other parts of the body were exposed in series A. In this series large areas of the body were covered with plastic in each experiment and only small air volumes, 2×80 l, were sampled. This might have made it impossible to detect dispersal of small numbers of uncommon bacterial species. Based on the experience from series A the experimental layout was altered in series B. In this series larger areas of the skin were exposed and a $\frac{1}{2}$ min sample of 350 l was added to each experiment. However, the findings in series B were similar to those in series A: The highest number of anaerobic bacteria were dispersed from the lower trunk. Dispersal of anaerobic cocci was correlated to their presence in the perineal area. These findings are consistent with those concerning dispersal of aerobic bacteria. The dispersal of aerobic bacteria by naked subjects has been shown to be reduced by about 80% when the perineal area is covered (May & Pomeroy, 1973; Mitchell & Gamble, 1974).

The lack of correlation found in this study between the number of anaerobic bacteria on skin and the dispersal of them into the air may be explained by the fact that high counts of *P. acnes* are found in sites where the sebum contents are high, and a high sebum content may prevent desquamation.

In the last series, series C, a study of the dispersal of anaerobic bacteria and coryneforms from dressed and naked persons was performed. In this series the dispersal of aerobes other than the slowly growing coryneforms was also included.

To make it possible to compare dispersal from a person under different circumstances and between different individuals, measurements must be made as close as possible to steady state, i.e. when the number of bacteria dispersed into

the chamber is the same as the number of bacteria eliminated from the chamber. At this level the concentration in the chamber, C_{st}, is

$$C_{st} = \frac{D}{E},$$

where D stands for the dispersal per unit time and volume, and E stands for the elimination of the particles per unit time, here due to sedimentation and ventilation, according to the formula:

$$E = \frac{\text{sedimentation rate}}{\text{height of chamber}} + \frac{\text{ventilation rate}}{\text{volume of chamber}}$$

As the concentration each time is

$$C_t = \frac{D}{E}(1 - e^{-Et}),$$

where e is the base of the natural logarithm and t is the time, the % concentration at each time can be calculated by division. The settling rate of human generated particles carrying microorganisms is about 0·37 m/min (Noble *et al.* 1963), so in our chamber losses due to sedimentation correspond to 10·5 air changes/h. Thus, when the ventilation is 40 l/min, as in series A and B, 43 % of the steady state level is reached after 3 min and 67 % after 6 min. The bacterial level achieved in 3 min with no ventilation is 92 % of the steady state level approached when the ventilation is 700 l/min, so in series C the steady state level was approached to more than 90 % when the sampling was performed.

These calculations also make it possible to convert the counts found per 80 l into c.f.u./min. D is calculated from C_{st} and E, and the whole dispersal per time unit is $D \times 3{\cdot}2$ m^3 (the volume of the chamber is 3·2 m^3). If the counts from series C, presented in Table 5(*a*), are converted into c.f.u./min, the geometrical mean of aerobic bacteria dispersed is 595 c.f.u./min when the subjects are naked and 1268 c.f.u./min when they are dressed. This compares well with the results of others: Whyte, Vesley & Hodgson (1976) found the median value from 18 tests on three subjects to be 1338 c.f.u./min when the subjects were wearing conventional operating clothes. May & Pomeroy (1973) found the mean total output of aerobic bacteria from 17 naked males to be 4247 c.f.u./min, and the dispersal from 11 females was 810 c.f.u./min when naked.

The dispersal of anaerobes and coryneforms per time unit can be calculated in the same way, as these bacteria have been shown to have the same die-away rate as aerobes (Hambraeus & Benediktsdóttir, 1980). The geometrical mean of anaerobes and coryneforms dispersed is then 704 c.f.u./min when the subjects are naked and 2460 c.f.u./min when they are dressed.

Thus, the real dispersal of bacteria is at least twice that found in investigations where the samples are made on blood agar and incubated aerobically only.

Clothing is one of the factors that may change the pattern of dispersal of skin bacteria. The literature is somewhat confusing concerning the dispersal of aerobic bacteria from naked and dressed persons. Some authors find an increased dispersal when the subjects are dressed (Hill, Howell & Blowers, 1974; Mitchell & Gamble,

1974) and others a decrease (May & Pomeroy, 1973). In the present study, the dispersal of aerobic bacteria was increased in most persons when they were dressed. The dispersal of *Propionibacterium* sp. was increased when the subjects were dressed whereas the change in dispersal of the coryneforms was variable. This may be explained by the different localization of the two bacterial types. *P. acnes* is mostly located in areas other than the intertriginous ones and may therefore be exposed to friction by clothes. This agrees with findings by Mitchell & Gamble (1974), where they showed that dispersal of aerobic bacteria from other sites than the perineum was strikingly increased by wearing clothes. Dispersal of the coryneforms seems to be due to skin to skin friction, as these bacteria are localized to the intertriginous areas. This friction may be so large under any circumstances that it is not strikingly changed by clothing. The diverging results in earlier publications on dispersal of aerobic bacteria by naked-versus-dressed persons could perhaps be explained by the fact that only a total number of aerobic bacteria and not species are presented in the investigations. A separation into intertriginous skin flora and skin flora from other parts of the body might give more clear-cut results.

REFERENCES

ALY, R., MAIBACH, H. I. & BLOOM, E. (1978). Quantification of anaerobic diphtheroids on the skin. *Acta Dermato-Venereologica* (*Stockholm*) **58**, 501–504.

CUMMINS, C. S. (1975). Identification of *Propionibacterium acnes* and related organisms by precipitin tests with trichloroacetic acid extracts. *Journal of Clinical Microbiology* **2**, 104–110.

HAMBRAEUS, A. & BENEDIKTSDÓTTIR, E. (1980). Air-borne nonsporeforming anaerobic bacteria. *Journal of Hygiene* **84**, 181–189.

HILL, J., HOWELL, A. & BLOWERS, R. (1974). Effect of clothing on dispersal of *Staphylococcus aureus* by males and females. *Lancet* ii, 1131–1133.

HOLDEMAN, L. V., CATO, E. P. & MOORE, W. E. C. (Eds.) (1977). *Anaerobe Laboratory Manual*. Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg.

MAY, K. R. & POMEROY, N. P. (1973). Bacterial dispersion from the body surface. In *Airborne Transmission and Airborne Infection*, pp. 426–432. Utrecht: Oosthoek.

MITCHELL, N. J. & GAMBLE, D. R. (1974). Clothing design for operating room personnel. *Lancet* ii, 1133–1136.

NOBLE, W. C., LIDWELL, O. M. & KINGSTON, D. (1963). The size distribution of airborne particles carrying micro-organisms. *Journal of Hygiene* **61**, 385–391.

PUHVEL, S. M., REISNER, R. M. & AMIRIAN, D. A. (1975). Quantification of bacteria in isolated pilosebaceous follicles in normal skin. *Journal of Investigative Dermatology* **65**, 525–531.

SMITH, R. F. (1969). Characterization of human cutaneous lipophilic diphtheroids. *Journal of General Microbiology* **55**, 433–443.

SOMMERVILLE, D. A. & MURPHY, C. T. (1973). Quantitation of *Corynebacterium acnes* on healthy human skin. *Journal of Investigative Dermatology* **60**, 231–233.

STARGEL, M. D., THOMPSON, F. S., PHILLIPS, S. E., LOMBARD, G. L. & DOWELL JR, V. R. (1976). Modification of the Minitek miniaturized differentiation system for characterization of anaerobic bacteria. *Journal of Clinical Microbiology* **3**, 291–301.

WATT, B. & JACK, E. P. (1977). What are anaerobic cocci? *Journal of Medical Microbiology* **10**, 461–468.

WHYTE, W., VESLEY, D. & HODGSON, R. (1976). Bacterial dispersion in relation to operating room clothing. *Journal of Hygiene* **76**, 367–378.

WILLIAMSON, P. & KLIGMAN, A. M. (1965). A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology* **45**, 498–503.