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Nine-Year Microflora Study of an Isolator-Maintained Immunodeficient Child

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A male child, maintained in a controlled environment, was monitored each month for bacteria, yeasts, and filamentous fungi recovered from the mouth, nasal passages, feces, and nine body surface sites. Three natural microbial categories became apparent. (i) Incident microorganisms were recovered from within the isolator but did not establish permanent residence. Of the 53 incident types isolated, 20 were filamentous fungi and 4 were yeasts. Some genera, such as *Fusobacterium*, *Lactobacillus*, *Neisseria*, and *Rothia*, which were commonly found in the reference group, did not become permanent inhabitants. (ii) Transient microorganisms were repeatedly recovered but could not be demonstrated within the isolated environment at the end of the study. The loss of only a few of the 19 transient species could be associated with antimicrobial therapy. (iii) Permanent microorganisms consisted of *Pencillium citrinum* and 17 bacterial types, of which alpha-hemolytic streptococci, *Staphylococcus edpidermidis* subgroups II and V, *Micrococcus* groups 1 and 2, *Clostridium bifermentans*, and *Propionibacterium acnes* were recovered throughout the entire 9 years of the study. The number of CFUs recovered from each sample type was generally not unlike that from the reference group of healthy male adults. Also, the number of different aerobic species recovered from the feces was within the normal range of that of the reference group. In contrast, the number of different species recovered from all other samples was less than that commonly found in the reference group.

Children with severe combined immune deficiency (SCID) rarely survive past 1 year of age because of their greatly impaired ability to resist infection. We had the opportunity to monitor the microbial load of an otherwise normal male human child with SCID from years 3 through 12 of his life. The efforts taken to restrict microbial contamination were sufficient to prevent life-threatening infection, although numerous species and strains of microorganisms were introduced into the isolator environment. This phenomenon of extensive, but sublethal, contamination provided a rare opportunity to study the long-term relationships between different microbial species in association with a human subject under controlled conditions in the absence of infection.

MATERIALS AND METHODS

Subject history. This study incorporates a 9-year period from age 3 to age 12 in the life of a male child with congenital SCID. Up to age 12 the subject was retained in a series of isolated enviroments, and he has been the subject of a variety of other investigations (3, 5, 7, 14, 19, 20, 22, 26, 28, 35, 37). Because a deceased antecedent brother of the patient was shown to have SCID which contributed to his death by pneumocystic pneumonia in his first year (35) and because several members of the subject's maternal lineage may have had immune deficiencies (18), the subject was delivered by caesarean section and maintained in isolation until appropriate therapy could be applied to correct the deficiency (29). The caesarean section, performed 21 September 1971, was unusual only in that extraordinary precautions were taken to eliminate air contamination in the delivery room (15).

The diagnosis of severe combined immune deficiency was confirmed by quantitation of the child's immunoglobulins, challenge of his immune system in vivo with standard and experimental antigens and skin grafting, and in vitro testing of his lymphoid cells for reactivity to phytohemagglutinin and pokeweed mitogen. In addition, the child had a diminished number of lymphocytes, very low levels of circulating immunoglobulins G and M, and no immunoglobulin A detectable by quantitative precipitation techniques (29).

As time progressed, protocols for gnotobiotic care and infectious disease prevention were modified so that a disease-free, rather than a germfree, state became the maintenance goal (37). During the very early part of the subject's life, microbiological sampling was conducted by various laboratories as outlined by Wilson et al. (37). *Alcaligenes faecalis* appeared on the second day of life. Subsequent treatment with colistin methate, followed by gentamicin, resulted in repeated negative samples by 14 days. Because of a change in maintenance philosophy and the complexities of preserving strict isolation, the subject did not remain germfree. During the first 28 months of life, 18 different species were reported to have been recovered from the feces or several skin sites or both (37). Of these, the following nine microorganisms were reported to have occurred more than once: *Staphylococcus epidermidis*, *Enterobacter agglomerans*, *Bacillus pulvifaciens*, *Propionibacterium acnes*, *Candida tropicalis*, *Clostridium hastiforme*, *Clostridium perenne*, *Bacteroides oralis*, and *Lactobacillus catenaforme*. At age 6 months, procaine penicillin G therapy resulted in the disappearance (albeit followed by subsequent reappearance) of all fecal contaminants (37).

At age 38 months, we initiated a major program of microbiological screening to determine the nature and variability of microbial contamination. This monitoring was part of a program to provide and test a mobile biological isolation system similar to that developed for immunosuppressed cancer patients (23) which would allow the patient more freedom of movement while maintaining a controlled environment. We have previously reported the results of this

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expanded regimen in which 54 different species or strains of microbial contaminants were shown to have entered the isolator environment during a 20-month period commencing at age 38 months. The presence of these microorganisms (22 were aerobic bacteria, 10 were anaerobic bacteria, 1 was a yeast, and 21 were filamentous fungi) was tolerated without disease event during the monitoring period (32). Subsequently, we extended our analyses to cover the 9-year period between 38 months (3 years, 2 months) and 146 months (12 years, 2 months). During the test period, the subject remained healthy, and the sampling continued at approximately monthly intervals.

Biological isolation. The isolator systems in which the subject lived while at home and while at the hospital have been reported previously by Wilson et al. (36, 37) and are briefly reviewed below. The mobile biological isolation garment, which was supplied by the National Aeronautics and Space Administration, has not been previously reported and is more completely described here.

The hospital isolator consisted of a rigid-walled playroom constructed by Ecodyne Systems, Inc., Houston, Tex., based on a modification of a standard patient isolator developed by Linear Flow Systems, Grand Rapids, Mich. To this main living unit was affixed an intermediate play isolator built by Frank Lucas, Co., Houston, Tex. Flexible film supply and transfer units were built by the Standard Safety Equipment Company, Palatine, Ill., and could temporarily be mated to the other units by special O-ring joints. The exact configuration of the total accessible area changed over the years. In general, the initial home isolator consisted of a playroom (7 by 3.5 by 2 ft [approximately 213 by 107 by 61 cm]), an intermediate unit (5 by 3.5 by 2 ft [approximately 152 by 107 by 61 cm]), and a distal unit (5 by 2 by 2 ft [approximately 152 by 61 by 61 cm]) connected in series. The hospital unit had a main room 10 ft, 7 in (approximately 323 cm) long; 6 ft, 8 in (approximately 203 cm) wide; and 8 ft (approximately 244 cm) high.

HEPA filtered air was blown into the chambers to provide ventilation and to provide a positive outward air flow in the event of containment faults. Heavy duty rubber gloves and access ports were fitted into the walls. When unoccupied, the isolators were sterilized through a 45-min contact with 2% peracetic acid. Supplies were sterilized either with steam or ethylene oxide and introduced into the isolator through an air lock system.

Near the end of the subject's second year it became apparent that he would not soon be removed from the closed system and that major changes in the closed environment would be required. In January 1974, when the subject was in his third year of isolation, the National Aeronautics and Space Administration received a request to develop and provide a mobile biological isolation garment which would allow considerable freedom of movement while maintaining the gnotobiotic isolation. On 19 May 1977, the device was delivered to Texas Children's Hospital where it was used for the first excursion out of the isolation unit on 28 July 1977.

The biological isolation garment consisted of a small unit similar to a miniature space suit with a push cart transporter which carried a life-support system. The suit and pressure retention assembly consisted of a neoprene-coated, nonporous bladder to which was permanently attached a soft, transparent plastic helmet, rubber gloves, boots, and a special tunnel and adapter ring which attached directly to the isolator ports. A lightweight overgarment of porous nylon was worn over the suit to provide additional protection against abrasion, puncture, or fire. Ambient air, filtered to 0.3 μm, was blown into the helmet by one of three redundant ventilator fans and over the body for cooling. Restricted air exit at the ankles maintained a positive pressure within the suit. A lawnmower frame, wheels, handles, and breaks served as the transporter frame. A seat was provided along with special bumpers and a swing arm to allow the subject to ride on the transporter or to walk alongside within the 10-foot (approximately 305-cm) radius provided by the umbilical line. The electrical power system included two blowers, two motors, two 12-V batteries, and the circuitry required to utilize power from a standard household electrical outlet or a motor vehicle cigarette lighter.

Sample collection. A sterile kit containing sterile snap-cap tubes, swabs, and 0.03 mM phosphate buffer was passed into the isolator before each sample collection. Samples were collected at approximately 4-week intervals from the mouth, nasal passage, feces, and body surface areas. Sample sites of the body included the forehead, ear canals, back of neck, hands (palm and between fingers), umbilicus, face (nasal folds), axilla, groin, and toe webs. On the morning of sample collection, a fecal sample was obtained, removed from the isolator as soon as possible, and placed in a reduced-oxygen atmosphere. All of the body surface areas were sampled with sterile swabs wetted in phosphate buffer. The mouth and nasal passages were sampled with dry swabs. After use, the swabs were placed in individual, labeled snap-cap tubes and removed from the isolator in such a way as to avoid back contamination. Five milliliters of sterile Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) was added to each tube. The tubes were packed in ice and transported to the Johnson Space Center Laboratories for processing, where the contents were analyzed quantitatively for aerobic and anaerobic bacteria and qualitatively for yeasts and filamentous fungi. All samples were obtained with the written consent of the subject or his parents, using forms approved by the Institutional Review Boards for Human Research of the Baylor College of Medicine and Texas Children's Hospital.

Quantitation methods. All samples were held from 1 to 2 h at 4°C until processed. After serial dilution in sterile Trypticase soy broth, 0.1-ml portions were transferred to quantitation plates and spread over the surface with an alcohol-flamed glass rod. Media used for quantitation of aerobic bacteria in the various samples were blood agar (Trypticase soy agar with 5% sheep blood; BBL) for all samples, chocolate bacitracin agar (Casmen agar [BBL] with 5% horse blood and 300 μg of bacitracin per ml) for samples from mouth and nasal passages, mitis salivarius agar (Difco Laboratories, Detroit, Mich.) for samples from mouth and feces, and MacConkey agar (BBL) for samples from the feces. The inoculated aerobic media were incubated at 35°C for 48 h.

Media used for quantitation of anaerobic bacteria were anaerobic blood agar (blood agar with vitamin K, hemin, and yeast extract) for all samples; paromomycin-vancomycin-menadione agar for samples from mouth, nasal passages, and feces; and Rogosa agar (Difco) for samples from the mouth and feces. The inoculated anaerobic media were incubated in GasPak jars (BBL) using GasPak H_2-CO_2 generators for the gaseous phase, at 35°C for 4 days.

Portions of the Trypticase soy broth cultures were streaked onto Sabouraud dextrose agar with antibiotics, corn meal malt extract-yeast extract agar with antibiotics, and Czapek-Dox agar for isolation of yeasts and filamentous fungi. After incubation at 25°C for 4 to 7 days, yeasts were inoculated into Dalmau plates and appropriate biochemical

media; filamentous fungi were transferred to Czapek-Dox agar, malt extract agar, and half-strength corn meal agar. Species were identified as outlined previously (31, 33).

After 48 h of incubation, each morphologically different aerobic bacterial isolate was quantitated and picked to Trypticase soy broth, and a Gram stain was prepared. After 24 h of incubation at 35°C, all cultures were streaked onto blood agar to ensure purity before biochemicals were inoculated.

After 96 h of incubation, each morphologically different anaerobic bacterial isolate was quantitated, Gram stained, picked to a tube of prereduced chopped meat (Virginia Polytechnic Institute, Blacksburg, Va.), and streaked onto a blood agar plate. After 48 h of aerobic incubation at 35°C, the blood agar plates were examined for growth. Biochemicals were inoculated using only those isolates that were strict anaerobes. Unless noted, Virginia Polytechnic Institute anaerobic procedures were followed (11).

The reference group consisted of healthy male adults who were sampled and evaluated by the same methods (2, 4, 10, 30, 34). These subjects cannot be considered to be a control group because of the differences in age and life style. However, their use as a reference group does allow a useful comparative perspective.

RESULTS AND DISCUSSION

During the 9-year sampling period, 92 different species or strains of microorganisms were recovered from the controlled environment. Of these, 36 were aerobic bacteria, 26 were anaerobic bacteria, 25 were filamentous fungi, and 5 were yeasts. All of these microorganisms logically fell into one of three distinct categories. (i) Incident: Those species or strains which were shown to have been within the controlled environment but did not establish a permanent residence. (ii) Transient: Those forms which were repeatedly recovered, thus indicating an established residence, but which were lost from the controlled environment before the end of the study. (iii) Permanent: Those microorganisms which were frequently isolated and which remained in the controlled environment at the end of the monitoring period.

Incident microorganisms. Fifty-three species or strains of microorganisms were recovered from the feces, mouth, nasal cavity, or at least one of the nine skin sites, but they apparently did not establish residency as they were only recovered a few times (Table 1). The 17 transient aerobic bacterial species were mostly bacilli, corynebacteria, or streptococci, whereas half of the 12 anaerobic bacteria were *Bacteroides* or *Clostridium* species. In addition, 4 species of yeasts and 20 species or strains of filamentous forms were isolated but not established. It is not surprising that some of these microorganisms were found to be transient, as they also did not generally frequent the subjects in the reference group (2, 4, 10, 30, 34). Notable exceptions to this expectation were the genera *Lactobacillus*, *Neisseria*, *Rothia*, and *Fusobacterium*, which were nearly ubiquitous in the oral cavities of the reference population. Although members of these genera were recovered from the subject at least once, colonization did not occur.

Transient microorganisms. Transient microorganisms consisted of eight species of aerobic bacteria, six species of anaerobes, and five species of yeasts and filamentous forms that were recovered frequently enough to be considered established. However, for reasons not always well understood, their presence in the controlled environment was interrupted by the end of the monitoring period (Table 2). At

TABLE 1. Incident microorganisms[a]

Group and microorganism	No. of occurrences	Site from which microorganisms were isolated:			
		Feces	Mouth	Nasal	Skin
Aerobic bacteria					
Bacillus brevis	3	X			
Bacillus cereus	1				X
Bacillus megaterium	2	X			X
Corynebacterium A	1	X			
Corynebacterium B	1				X
Corynebacterium C	2				X
Corynebacterium G	3			X	X
Flavobacterium sp.	1	X		X	X
Lactobacillus brevis	1		X		
Micrococcus luteus	1	X			
Neisseria sicca	1				X
Rothia dentocariosa	1	X			
Staphylococcus epidermidis VI	2		X		X
Streptococcus agalactiae	1				X
Streptococcus salivarius	2		X	X	
Streptococcus sp. gamma	4	X			X
Unidentified gram-negative rod	1				X
Anaerobic bacteria					
Bacteroides coagulans	2				X
Bacteroides sp.	5	X			
Clostridium cochlearium	4	X			
Clostridium malenominatum	6	X			
Clostridium tertium	1	X			
Clostridium putrefaciens	2	X			
Corynebacterium pyogenes	1	X			
Fusobacterium naviforme	1		X	X	X
Propionibacterium jensenii	5	X			
Streptococcus intermedius	2	X			
Unidentified gram-positive rod	1	X	X		
Veillonella parvula	4	X			X
Yeasts					
Cryptococcus albidus	1				X
Cryptococcus laurentii	2				X
Rhodotorula rubra	3				X
Torulopsis magnoliae	1				X
Filamentous forms					
Alternaria alternata	1				X
Alternaria radicina	1				X
Aspergillus flavus	3				X
Aspergillus fumigatus	1				X
Aspergillus niger	6	X			X
Aspergillus restrictus	1				X
Aspergillus ustus	2				X
Chaetomium globosum	1				X
Curvularia lunata	1				X
Drechslera coicis	1				X
Microascus intermedium	1				X
Nigrospora oryzae	1				X
Penicillium chrysogenum	5				X
Penicillium granulatum	1			X	
Penicillium janthinellum	1				X
Penicillium lanosum	1				X
Penicillium notatum	8	X		X	X
Penicillium steckii	1	X			
Pithomyces sp.	1				X
Streptomyces sp.	1				X

[a] Those isolated from the subject without becoming established in the isolator environment.

TABLE 2. Transient microorganisms[a]

Microorganism	Length of stay (mo)	Isolation frequency during stay (%)	Site from which microorganisms were isolated							
			Feces		Mouth		Nasal		Skin	
			O[b]	Q[c]	O[b]	Q[c]	O[b]	Q[c]	O[b]	Q[c]
Aerobes										
Enterobacter agglomerans	65	74	37	6.6	35	3.8	9	2.3	34	2.4
Pseudomonas aeruginosa	46	89	35	6.4	37	4.5	0		6	2.1
Bacillus species	42	72	28	7.5	0		0		9	2.2
Bacillus circulans	40	78	27	7.4	0		2	2.0	6	2.3
Bacillus sphaericus	38	74	17	6.8	0		2	2.0	13	2.3
Staphylococcus epidermidis IV	36	80	1	5.0	6	5.5	5	2.3	22	4.5
Micrococcus subgroup 3	22	73	0		1	7.2	3	2.8	14	4.2
Micrococcus subgroup 5	17	87	0		0		3	1.7	13	3.5
Anaerobes										
Clostridium subterminale	46	53	22	7.3	0		0		3	2.4
Bacteroides clostridiiformis	38	100	34	7.9	0		0		0	
Clostridium sporogenes	30	73	19	7.5	0		0		0	
Clostridium tyrobutyricum	19	57	8	7.6	0		0		0	
Clostridium acetobutylicum	15	85	12	8.5	0		0		0	
Clostridium sphenoides	8	71	5	8.0	0		0		0	
Yeasts and filamentous forms										
Candida parapsilosis	64	75	28		2		5		35	
Aspergillus sydowi	43	51	1		0		5		20	
Aspergillus versicolor	48	27	4		1		2		11	
Cladosporium sphaerospermum	75	22	2		1		1		12	
Penicillin decumbens	50	20	1		0		0		11	

[a] Those apparently established within the controlled environment but lost before completion of the monitoring period.
[b] O, Number of occurrences.
[c] Q, Mean quantitation during stay (in $\log_{10}$ CFU per gram of feces or $\log_{10}$ CFU per square centimeter of surface area).

age 63 months, *Micrococcus* subgroups 3 and 5 were lost from the skin in a general autoflora change which also involved colonization by two strains of *Pseudomonas aeruginosa*. Although the impetus for this change is unknown, we can associate the next major autoflora modification with the prophylactic administration of dicloxacillin at age 76 months. This therapy was followed by the loss of *Clostridium sphenoides*, *Clostridium acetobutylicum*, *Clostridium subterminale*, *Bacteroides clostridiiformis*, *Bacillus* species, *Bacillus sphaericus*, *Bacillus circulans*, and *Aspergillus*

TABLE 3. Permanent microorganisms[a]

Microorganism	Length of stay (mo)	Isolation frequency during stay (%)	Site from which microorganism was isolated							
			Feces		Mouth		Nasal		Skin	
			O[b]	Q[c]	O[b]	Q[c]	O[b]	Q[c]	O[b]	Q[c]
Aerobes										
Staphylococcus epidermidis II	108	100	63	7.0	100	5.8	103	4.7	107	6.1
Micrococcus subgroup 1	108	99	6	5.8	58	4.9	49	3.0	107	6.0
Micrococcus subgroup 2	108	81	2	7.5	12	5.2	8	3.5	87	4.4
Staphylococcus epidermidis V	108	68	8	5.8	34	5.5	11	2.4	65	3.0
Streptococcus species (alpha)	108	14	6	7.0	1	7.2	1	4.0	9	3.3
Pseudomonas aeruginosa	84	99	68	6.5	76	4.6	6	2.5	22	2.0
Streptococcus lactis	67	95	38	8.1	55	4.5	29	2.1	59	2.5
Corynebacterium species VIII	63	90	2	8.0	0		0		37	6.3
Klebsiella pneumoniae	42	97	24	7.7	36	6.0	21	2.1	33	2.1
Anaerobes										
Propionibacterium acnes	108	22	3	8.3	0		3	1.8	22	2.0
Clostridium bifermentans	108	52	55	6.2	0		0		2	2.3
Clostridium species	108	23	25	7.1	0		0		1	0.7
Eubacterium contortum	70	60	41	9.1	0		0		0	
Clostridium ramosum	65	31	16	7.0	3	5.4	0		0	
Clostridium paraputrificum	69	37	25	8.4	0		0		0	
Clostridium perfringens	59	86	30	7.0	0		0		0	
Clostridium clostridiiforme	58	30	16	8.8	0		0		0	
Fungi										
Penicillium citrinum	108	33	1		2		6		31	

[a] Those remaining at the end of the monitoring period.
[b] O, Number of occurrences.
[c] Q, Mean quantitation during stay (in $\log_{10}$ CFU per gram of feces or $\log_{10}$ CFU per square centimeter of surface area).

versicolor from the feces, with the subsequent introduction of *Eubacterium contortum* and *Clostridium paraputrificum*. Conversely, such an autoflora change cannot be demonstrated following additional prophylactic therapy with dicloxacillin at age 89 months.

Permanent microorganisms. Nine species of aerobic bacteria, eight species of anaerobes, and one filamentous fungus were repeatedly isolated from the subject samples through the completion of this study. Of these, the alpha-hemolytic streptococci, *Staphylococcus epidermidis* subgroups II and V, micrococci groups 1 and 2, *Penicillium citrinum*, *Clostridium bifermentans*, and *Propionibacterium acnes* were recovered throughout the entire 9 years of the study. It is most logical to consider those microorganisms listed in Table 3 which were frequently isolated (for example, 50% or greater isolation frequency) as comprising the true autoflora of the subject.

Fecal autoflora. Table 4 lists the genera isolated at least one-third of the time from the samples of either the subject or the reference group. The species isolated from the subject differ from those of the reference group in several ways. The common genera *Streptococcus*, *Escherichia*, *Bifidobacterium*, and *Fusobacterium* were always absent from the patient's fecal samples. *Bacteroides clostridiiformis*, *Clostridium subterminale*, *Clostridium bifermentans*, *Staphylococcus epidermidis*, *Bacillus circulans*, and a variety of other bacilli were routinely recovered. In addition, *Enterobacter agglomerans*, infrequently found in the reference group, was generally present in the patient. The yeast *Candida parapsilosis* was isolated from the patient 45.5% of the time.

Although recovery of *Bacteroides* species is not uncommon in normal subjects (6, 8), it should be noted, in light of the subject's immunodeficient state, that *Bacteroides clostridiiformis* has been implicated in tissue and intestinal abscesses. *Candida parapsilosis* has been associated with nail infections, endocarditis, and ulcerations. Although *Enterobacter agglomerans* (*Erwinia erbicola*) has been isolated from human infections (24), its presence did not cause an infection in this severely immunodeficient patient.

The distribution of fecal anaerobes and fecal aerobes throughout the 9-year test period are illustrated in Fig. 1. Within the reference group, there were generally more than nine different anaerobic species in the feces for a combined quantitation in excess of a billion CFU per gram of wet sample. Although the subject's viable anaerobe load approached that of the reference group norm at times during the study, the number of different species was always much lower. It is not surprising that the fecal anaerobe autoflora of an isolated individual would be less complex than those of the free reference subjects. However, it is interesting that the total viable cell load grew to approach that of the reference norm, apparently independent of the number of species present. In contrast, the subject's fecal aerobe autoflora, when viewed in terms of species number and viable cell number, was virtually indistinguishable from that of the reference group (Fig. 1).

Autoflora of the oral cavity. The genera isolated at least one-third of the time from the mouth of either the patient or the reference group are listed in Table 4. Although very few cohesive data exist on the composition of the oral flora during childhood (9), it is likely that by the time the primary dentation has formed, autoflora components are essentially the same as those in adults. Of course, there is considerable variability, depending on diet, hygiene regimen, presence of caries, etc. In one study, it has been shown that by 12 months of age, all children tested had the genera *Streptococcus*, *Staphylococcus*, *Veillonella*, and *Neisseria* in the oral flora (17). At least half of those tested had the genera *Actinomyces*, *Lactobacillus*, *Rothia* (*Nocardia*), and *Fusobacterium*. This observation was borne out by the reference group used in this study (Table 4), except that the genera *Corynebacterium*, *Haemophilus*, *Micrococcus*, and *Bacteroides* also were found frequently. This difference can be explained largely by differences in methodology. This is especially true in the case of *Haemophilus*, which was always isolated from reference individuals but may be easily overlooked if not actively sought out. Of the common aerobic genera, *Corynebacterium*, *Haemophilus*, *Lactobacillus*, *Neisseria*, and *Rothia* were either missing or were only occasionally isolated from the mouth of the immune-deficient patient. Although a variety of bacterial species were recovered at least once from the subject's mouth over

TABLE 4. Microorganisms commonly recovered from immune-deficient patient or reference group[a]

Genus	Site	Mean quantitation (log_{10} CFU)[b]		Isolation frequency (%)	
		Patient	Reference group	Patient	Reference group
Bacillus	Skin	1.9	2.1	24.1[c]	79.5
	Feces	7.8[d]	5.0	77.3[c]	23.7
Bacteroides	Mouth	0[d]	5.1	0[d]	100.0
	Feces	7.9[d]	10.1	75.0	100.0
Bifidobacterium	Feces	0[d]	8.9	0[d]	100.0
Candida	Feces	2.7	2.8	45.5	12.8
Clostridium	Feces	8.2[d]	5.6	97.7	85.7
Corynebacterium	Nasal	4.6	4.5	1.3[d]	84.6
	Mouth	6.6	5.6	0.1[d]	86.7
	Skin	3.2	5.5	25.3	100.0
Enterobacter	Nasal	2.5	2.0	11.0	33.3
	Mouth	3.7	2.9	40.2	35.7
	Skin	2.0	1.8	40.2	23.2
	Feces	6.6[c]	4.6	79.5[d]	7.9
Escherichia	Feces	0	6.8	0[d]	94.7
Eubacterium	Feces	8.9	9.7	18.2	71.4
Fusobacterium	Mouth	1.6	4.5	0.1[d]	87.2
	Feces	0[d]	8.6	0[c]	71.4
Haemophilus	Mouth	0[d]	6.2	0[d]	100.0
Lactobacillus	Mouth	3.0	3.4	0.1[d]	93.3
Leptotrichia	Mouth	0[d]	3.2	0[c]	53.9
Micrococcus	Nasal	3.2	3.4	56.3[c]	20.5
	Mouth	4.7	5.3	58.6	63.3
	Skin	5.7[c]	4.6	100.0	100.0
Neisseria	Mouth	0[d]	6.0	0[d]	93.3
Peptococcus	Skin	0[d]	3.4	0[d]	53.8
Propionibacterium	Nasal	1.7[d]	3.6	1.3[d]	51.3
	Skin	1.3[d]	3.2	11.0[d]	100.0
Pseudomonas	Mouth	4.3	3.1	41.4	9.0
	Feces	7.2	5.6	52.3[d]	3.2
Rothia	Mouth	7.0	5.3	0.1[d]	90.0
Staphylococcus	Nasal	4.7	4.3	96.5	89.7
	Mouth	5.7[c]	3.2	97.7	76.7
	Skin	5.7[c]	4.2	100.0	100.0
	Feces	7.2[d]	3.2	88.6[d]	5.3
Streptococcus	Nasal	2.4[d]	3.4	26.4	46.2
	Mouth	4.3[c]	7.0	41.4	100.0
	Skin	2.3	1.9	48.3	53.8
	Feces	8.5[c]	6.0	15.9[d]	94.7
Veillonella	Mouth	0[d]	5.4	0[d]	94.9

[a] Only genera isolated at least one-third of the time are presented.
[b] Quantitations given as log_{10} CFU per square centimeter of surface area (nasal, mouth, and skin) or per gram of wet feces.
[c] $P < 0.05$ compared with reference group.
[d] $P < 0.01$ compared with reference group.

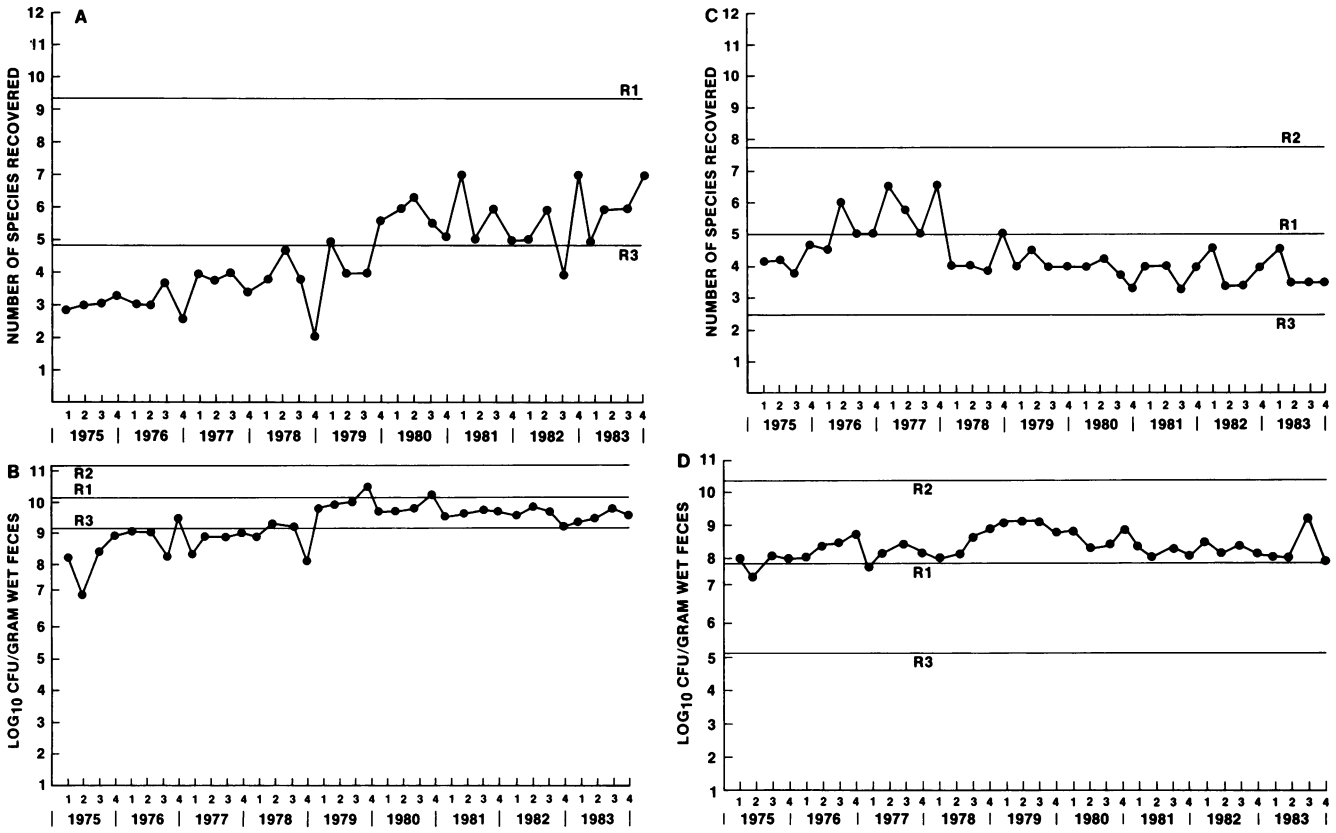

FIG. 1. Nine-year history of anaerobic bacterial load (A and B) and aerobic bacterial load (C and D) recovered from the feces of an isolator-maintained human subject. Line R1, Mean values for the reference group; line R2, 95% upper limit for the reference group; line R3, 95% lower limit for the reference group. Numbers above the years at the bottom of the panels indicate the quarter during which the sample was collected.

the 9-year test period, only the genera *Enterobacter*, *Micrococcus*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus* were recovered frequently enough to be considered part of the autoflora (Table 4). These genera may have colonized in high numbers because of the absence of competition from other common oral microorganisms which either were never introduced into the isolator or were present but failed to colonize in the mouth.

Although the complexity of the patient's oral autoflora was reduced when compared with that of the reference group (Fig. 2), the presence of even one species should be considered medically important because of his immunodeficiency. The fact that there were no oral infections throughout the test period indicates the importance of other factors in controlling infection. Probably the most important is the fact that he remained in good general health and was not subject to frank pathogens. Likewise, species such as *Enterobacter agglomerans* and *Staphylococcus epidermidis* dominated the autoflora. This may signify the benign nature of these species. Other affecting factors could include the antagonism between the established species and newcomers (21), salivary lysozyme, and production of hydrogen peroxide (12) and bacteriocin (13) by species present, as well as diet and hygiene.

Autoflora of the nasal cavity. As with the oral cavity autoflora and the fecal anaerobes, the population within the nasal cavity was less complex, albeit as numerous as that in the reference group (Fig. 2). Fewer corynebacteria and proprionibacteria were recovered from the subject (Table 4), whereas the quantitation of staphylococci and micrococci were higher when compared with that of the reference group.

Autoflora of the skin. The data shown in Fig. 2 demonstrate the usual population pattern in which the autoflora is simplified, but viable cells are as numerous as in the reference population. The species isolated at least one-third of the time from the patient's skin samples are shown in Table 4. As might be expected (16), strains of members of the family *Micrococcaceae* predominate in the integumentary autoflora; three strains of *Micrococcus* and three of *Staphylococcus*, as identified by the Baird-Parker procedure (1), were routinely recovered. The recovery of these microorganisms from the patient's skin compared well with those from the reference group, as well as with other studies in which *Staphylococcus epidermidis* II and *Micrococcus* groups 2 and 3 have been found to predominate in the skin autoflora of normal adults (27). In addition *Candida parapsilosis* and *Enterobacter agglomerans*, infrequently recovered from the normal male adult control group, were often recovered from the patient's skin. It is likely that those two species are inhabitants of the lower digestive tract and occur on the skin only as secondary contaminants.

It has been suggested that in the normal cutaneous environment, the coagulase-negative staphylococci and micrococci dominate the skin surface, by virtue of the bacteriocins and other inhibitors that they produce (25). These factors, which traditionally have been considered minor defense mechanisms in normal individuals, may have

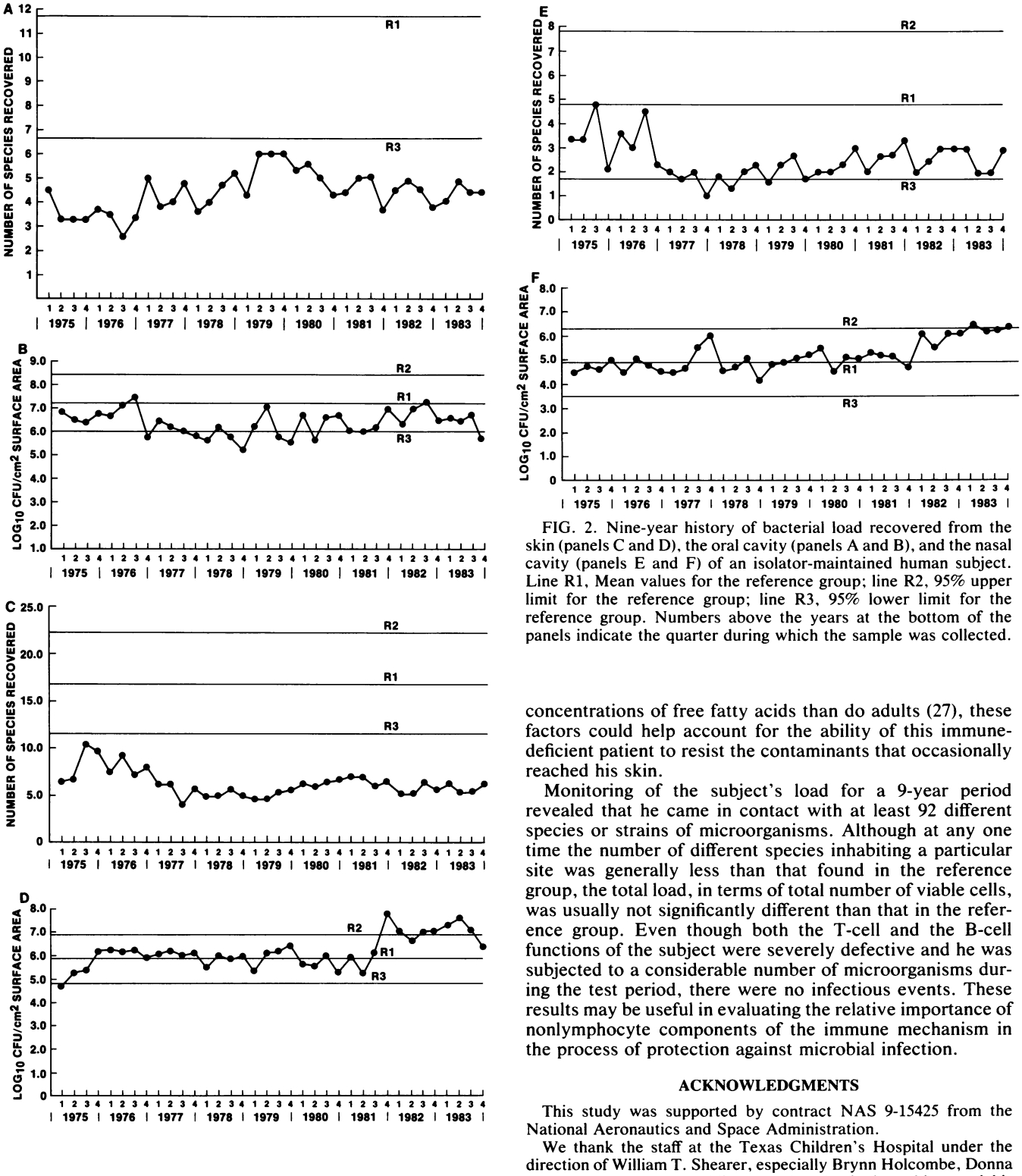

FIG. 2. Nine-year history of bacterial load recovered from the skin (panels C and D), the oral cavity (panels A and B), and the nasal cavity (panels E and F) of an isolator-maintained human subject. Line R1, Mean values for the reference group; line R2, 95% upper limit for the reference group; line R3, 95% lower limit for the reference group. Numbers above the years at the bottom of the panels indicate the quarter during which the sample was collected.

been a more important means of protection to the subject of this report. Because he lived in a controlled environment throughout the test period, the subject was exposed less frequently to new microbial contaminants. Cutaneous lysozyme, free fatty acids, bacteriocins, micrococcines, and epidermins (25) have been shown to selectively inhibit microbial growth. Although children typically have lower concentrations of free fatty acids than do adults (27), these factors could help account for the ability of this immune-deficient patient to resist the contaminants that occasionally reached his skin.

Monitoring of the subject's load for a 9-year period revealed that he came in contact with at least 92 different species or strains of microorganisms. Although at any one time the number of different species inhabiting a particular site was generally less than that found in the reference group, the total load, in terms of total number of viable cells, was usually not significantly different than that in the reference group. Even though both the T-cell and the B-cell functions of the subject were severely defective and he was subjected to a considerable number of microorganisms during the test period, there were no infectious events. These results may be useful in evaluating the relative importance of nonlymphocyte components of the immune mechanism in the process of protection against microbial infection.

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