Volume 4 Número 3 Jul.-Set. 1973

Revista de Microbiologia

Órgão Oficial da Sociedade Brasileira de Microbiologia
São Paulo – Brasil

Jarras GasPak

nova dimensão no sistema

BBL

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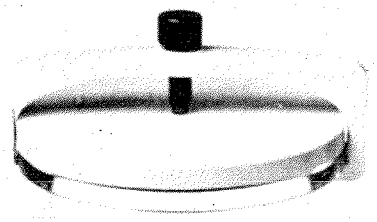

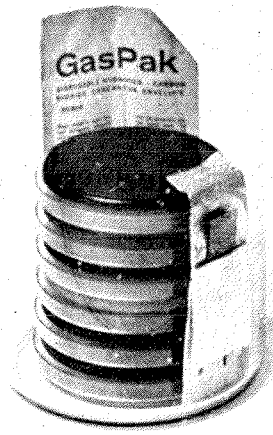

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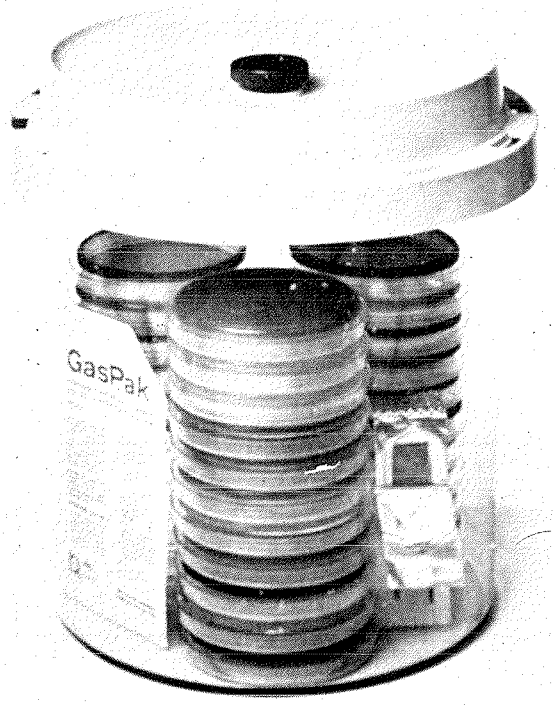

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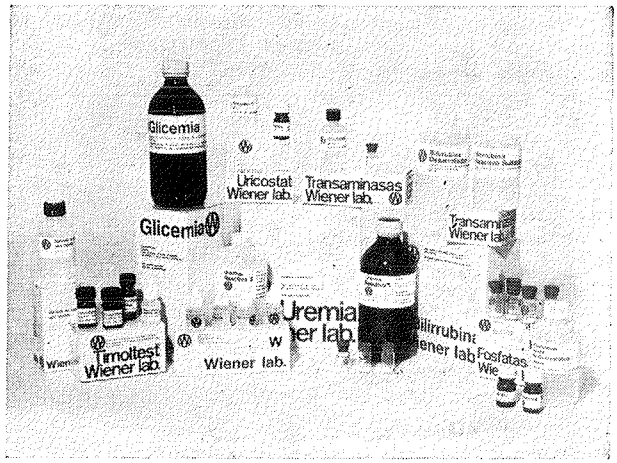

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Aspectos bacteriológicos de paciente com estreitamento uretral crônico*

Milton Barros, Moema M. G. Oliveira & Heonir Rocha

Resumo

Para determinar o método mais adequado de coleta de urina, para urocultura de doentes com estreitamento uretral inflamatório crônico, 25 pacientes nesta condição foram sucessivamente submetidos a punção supra-púbica, coleta do jato urinário médio e cateterismo vesical. O melhor método foi o da punção supra-púbica, sendo o índice de contaminação dos dois outros procedimentos relativamente elevado (12%). Maior número de bactérias foi geralmente observado nas amostras do jato urinário médio e cateterismo vesical, mesmo nos casos infectados. Os contaminantes mais freqüentes foram *Streptococcus faecalis* e *Staphylococcus* sp. Hemoculturas feitas antes, durante e após cateterismo dilatador, comprovaram bacteremia durante ou após as manobras em sete pacientes, cinco dos quais apresentavam, no sangue, a mesma bactéria presente na urina infectada. Não houve relação entre o isolamento de bactérias no sangue e a elevação térmica, mesmo quando a bactéria invasora era gram-negativa, possuidora de endotoxina. A tolerância à endotoxina, aliada à invasão de pequeno número de microrganismos, explica a falta de resposta febril, nestes casos.

Summary

Bacteriological aspects of patients with chronic urethral stenosis

To determine the most appropriate collecting method for quantitative urine culture from patients with inflammatory urethral stenosis, urine was obtained successively by bladder puncture, urethral catheterization and mid stream voiding from 25 patients. The most adequate method was the bladder puncture; the rate of contamination with the other techniques was 12%. A tendency for higher bacterial counts was observed in samples obtained from natural voiding or catheterization, even in infected patients. In a same group of patients, blood cultures obtained either before, during or after urethral dilatation (used as a therapeutic measure) revealed blood stream invasion in seven cases, five of which showing the same bacteria from the urinary tract. No correlation was found between a positive blood culture and the temperature rise. Even when gram-negative, enterotoxin producing bacteria were present, fever was not a constant finding. This discrepancy was probably related to patients toxin tolerance, as the result of a longstanding urinary tract infection by gram-negative rods, and, perhaps, to a low grade bacteremia resulting from urethral dilatation.

Introdução

A obstrução do trato urinário, em qualquer nível, predispõe ao aparecimento de infecção nesta área do organismo[9, 11], especialmente quando, em decorrência de uma atitude propedêutica ou terapêutica, o trato urinário é manipulado com freqüência, como é o caso dos estreitados de uretra. Assim, a uretra de um paciente com estreitamento inflamatório, de longa duração, se apresenta bem mais contaminada, podendo ser fonte permanente de penetração de bactérias em ductos prostáticos e bexiga, favorecendo a contaminação com urina de micção espontânea.

Por outro lado, a passagem de um cateter por área assim poluída transporta bactérias até a bexiga, sendo inevitável a contaminação da amostra de urina colhida. O tratamento destes doentes é feito, na maioria dos casos, por repetido cateterismo uretral dilatador. Esse procedimento não só contamina a bexiga como pode ser fonte de bactérias para o sangue do paciente, com grande possibilidade de complicações[4, 15].

O presente estudo visou: comparar diferentes métodos de coleta de urina, numa tentativa de se determinar o mais adequado para uroculturas quantitativas; verificar com que freqüência os cateterismos dilatadores resultam em invasão da corrente circula-

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Realizado com ajuda do Conselho Nacional de Pesquisas, TC-14898.

tória; e correlacionar a flora da infecção urinária com a flora da bacteremia.

Material e Métodos

Foram estudados 25 pacientes do sexo masculino portadores de estreitamento inflamatório de uretra, documentado clínica e radiologicamente, internados no Hospital Prof. Edgard Santos, Salvador, BA. Além do estudo clínico habitual, todos os pacientes foram submetidos a uroculturas quantitativas, com amostras obtidas por três métodos diferentes, de modo a permitir uma comparação entre a amostra colhida por punção supra-púbica, jato médio e cateterismo vesical. Além disso, foi estudada a influência do cateterismo dilatador na indução de bacteremia.

Os métodos de estudos foram os seguintes: medida da temperatura axilar dos pacientes; antissepsia da face anterior da dobra do cotovelo com álcool iodado e coleta de 16mℓ de sangue, sendo 8mℓ colocados em balão de fundo chato contendo "tryptocase say broth" TSB (BBL), e o restante em outro balão contendo caldo biliado (Difco), ambos incubados a 37°C.

A seguir, o paciente era colocado em decúbito dorsal, na mesa de exame, e realizada ampla antissepsia, com álcool iodado, na região entre a cicatriz umbilical e o terço superior das coxas, incluindo-se genitais externos. De maneira asséptica, foi feita punção supra-púbica da bexiga e aspiração de 10 a 15mℓ de urina, que era transferida para tubo estéril. Logo após, o paciente urinava espontaneamente, sendo colhida, em outro tubo estéril, a porção média do jato urinário. Repetindo-se a antissepsia da região, passou-se um cateter dilatador tipo Phillips, de calibre adequado para o caso, colhendo-se urina em outro tubo de ensaio estéril.

As amostras de urina, obtidas sucessivamente pelos três diferentes métodos, foram submetidas a urocultura quantitativa. Além de inoculação em placa de agar sangue e desoxicolato agar (Difco) com alça calibrada (0,001mℓ), a urina foi diluída sucessivamente (10^{-1}, 10^{-3}, 10^{-5}) e cultivada em placa com agar simples, com 1mℓ de cada diluição, incubada a 37°C.

As manobras de dilatação foram feitas após a coleta de urina, recolhendo-se uma amostra de sangue para hemocultura. Retirado o cateter, era feita nova hemocultura. A temperatura axilar foi medida cada

Tabela 1

Comparação de resultados de uroculturas quantitativas, obtidas de portadores de estreitamento uretral crônico, por três métodos diferentes

Nº	Iniciais	Punção supra-púbica	Jato médio	Cateterismo
01	EBS	*E. coli* 10^6	*E. coli* 10^6	*E. coli* 10^6
02	JMS	Negativa	Negativa	Negativa
03	MAA	*E. coli* 10^6	*E. coli* 10^6	*E. coli* 10^6
04	AS	*Staph.* $2,4 \times 10^4$	*Staph.* $9,1 \times 10^4$	*Staph.* $5,1 \times 10^4$
05	AFS	*Strep. faecalis* $1,6 \times 10^4$	*Strep. faecalis* $1,6 \times 10^4$	*Strep. faecalis* $1,3 \times 10^5$
06	AFS	*E. coli* 5×10^5	*E. coli* 10^6	*E. coli* 10^6
07	FM	Negativa	Negativa	*Strep. faecalis* 3×10^4
08	BBB	*Alk. faecalis* 5×10^5	*Alk. faecalis* 10^6	*Alk. faecalis* 10^6
09	EFC	Negativa	Negativa	*Strep. faecalis* $9,1 \times 10^5$
10	HC	Negativa	Negativa	Negativa
11	MFA	Negativa	Negativa	Negativa
12	BVA	Negativa	Negativa	Negativa
13	EAS	*Klebsiella-Enterobacter* $3,8 \times 10^6$	*Klebsiella-Enterobacter* $1,7 \times 10^7$	*Klebsiella-Enterobacter* $1,6 \times 10^7$
14	MMT	Negativa	Negativa	Negativa
15	HBC	*Staph.* 5×10^5	*Staph.* $8,0 \times 10^6$	*Staph.* 8×10^5
16	FA	*Proteus** 10^6	*Proteus** 10^6	*Proteus** 10^6
17	MS	*Klebsiella-Enterobacter* $1,5 \times 10^7$	*Klebsiella-Enterobacter* $2,1 \times 10^7$	*Klebsiella-Enterobacter* $2,1 \times 10^7$
18	EMS	*Staph.* $1,5 \times 10^5$	*Staph.* $1,3 \times 10^6$	*Staph.* 3×10^5
19	AFM	Negativa	Negativa	Negativa
20	BRL	*Klebsiella-Enterobacter* $3,8 \times 10^7$	*Klebsiella-Enterobacter* $5,0 \times 10^7$	*Klebsiella-Enterobacter* $6,6 \times 10^6$
21	EPA	Negativa	Negativa	Negativa
22	AA	*Pseudomonas* sp. 1×10^5	*Pseudomonas* sp. 6×10^3	*Pseudomonas* sp. 6×10^3
23	JCM	*Klebsiella-Enterobacter* $5,0 \times 10^7$	*Klebsiella-Enterobacter* $5,0 \times 10^7$	*Klebsiella-Enterobacter* $5,0 \times 10^7$
24	EF	Negativa	*Staph.* $6,9 \times 10^4$	*Staph.* $1,1 \times 10^6$
25	OFS	Negativa	Negativa	Negativa

* Indol negativo

vez que se fazia uma hemocultura, e uma e duas horas após as manobras de dilatação.

Os balões contendo sangue, incubados a 37°C, foram observados durante até 14 dias. Quando havia suspeita de crescimento bacteriano, fazia-se repicagem para agar sangue e procedia-se a identificação da bactéria, que porventura crescesse, por métodos bacteriológicos já consagrados, procedendo-se o antibiograma pelo método do disco.

Resultados

1. *Comparação de métodos de coleta de urina para urocultura em pacientes com estreitamento uretral* — A comparação dos resultados de uroculturas quantitativas revelou que 11 pacientes apresentaram cultura de urina negativa por punção supra-púbica. Cateterismo vesical e jato médio se equivaleram em relação ao número de bactérias isoladas da urina. Vale ressaltar que, em três (27, 2%) dos 11 pacientes com urocultura negativa, quando a coleta foi realizada por punção supra-púbica, houve número considerável de bactérias na urina obtida através de cateterismo vesical (Tabela 1).

2. *Flora bacteriana das infecções urinárias em estreitados* — Neste caso, a flora infectante mostrou predominância de bacilos gram-negativos, fermentadores de lactose. Vale ressaltar, entretanto, o isolamento relativamente freqüente de bactérias do gênero *Staphylococcus* e assinalar que a cultura de urina, obtida por punção supra-púbica em 11 pacientes, foi negativa (Tabela 2).

Tabela 2

Flora infectante do trato urinário, em pacientes com estreitamento uretral crônico. Resultados por punção supra-púbica

Uroculturas positivas	Nº de casos 14	% 56,0
Flora infectante		
Klebsiella-Enterobacter	4	28,6
E. coli	3	21,4
Staphylococcus sp.	3	21,4
Strep. faecalis	1	7,1
Pseudomonas sp.	1	7,1
Proteus sp. indol negativo	1	7,1
Alk. faecalis	1	7,1

3. *Conseqüências bacteriológicas das manobras de dilatação uretral* — Quando submetidos às manobras de dilatação, 76,0% dos pacientes tiveram discreto aumento de temperatura axilar, variando de 0,1 a 1,0°C, com diferença média de 0,3°C, logo após a retirada do cateter dilatador.

Nas tomadas de temperatura axilar, uma e duas horas após o término da dilatação, a tendência foi haver manutenção dos níveis térmicos imediatamente anteriores, com pequenas variações (Fig. 1).

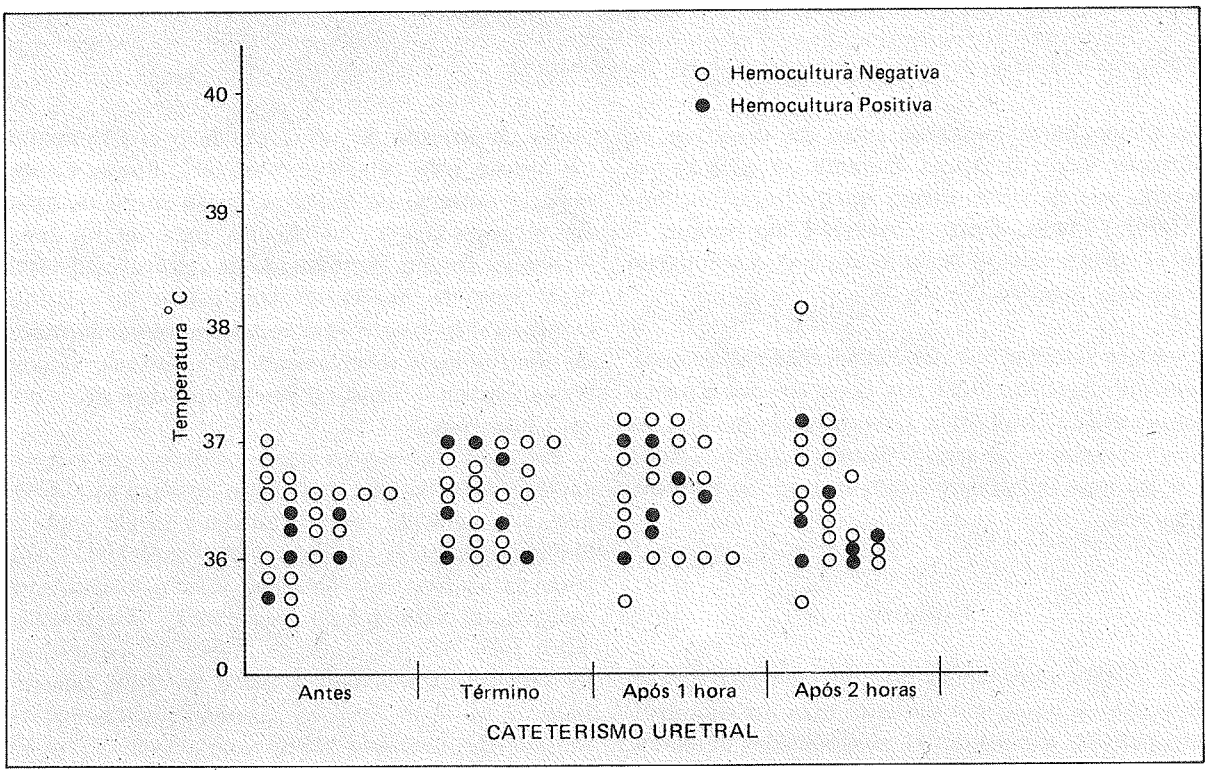

Fig. 1 — Influência do cateterismo uretral dilatador na curva térmica de pacientes com estreitamento inflamatório da uretra masculina.

Tabela 3

Relação entre bactéria isolada da urina (por punção supra-púbica) e do sangue, em pacientes com estreitamento uretral crônico, submetidos a cateterismo dilatador

Nº caso	Iniciais	Urocultura	HEMOCULTURA RELACIONADA AO CATETERISMO DILATADOR		
			Antes	Durante	Após
01	EBS	*E. coli*	Neg.	Neg.	Neg.
02	JMS	Neg.	Neg.	*E. coli*	*E. coli*
03	MAA	*E. coli*	Neg.	Neg.	Neg.
04	AS	*Staph.*	Neg.	*Staph.*	Neg.
05	AFS	*Strept. faecalis*	Neg.	Neg.	Neg.
06	AFS	*E. coli*	Neg.	Neg.	Neg.
07	FM	Neg.*	Neg.	*Strept. faecalis*	*Strept. faecalis*
08	BBB	*Alk. faecalis*	Neg.	Neg.	Neg.
09	EFC	Neg.*	Neg.	Neg.	Neg.
10	AC	Neg.	Neg.	Neg.	Neg.
11	MFA	Neg.	Neg.	Neg.	*Strept. faecalis*
12	BVA	Neg.	Neg.	Neg.	Neg.
13	EAS	*Klebsiella-Enterobacter*	Neg.	Neg.	Neg.
14	MMF	Neg.	Neg.	Neg.	Neg.
15	HBC	*Staph.*	Neg.	Neg.	*Staph.*
16	FA	*Proteus* sp.	Neg.	Neg.	Neg.
17	MS	*Klebsiella-Enterobacter*	Neg.	Neg.	Neg.
18	EMS	*Staph.*	Neg.	Neg.	Neg.
19	AFM	Neg.	Neg	Neg.	Neg.
20	BRL	*Klebsiella-Enterobacter*	Neg	Neg.	Neg.
21	EPA	Neg.	Neg.	Neg.	Neg.
22	AA	*Pseudomonas* sp.	Neg.	Neg.	*Pseudomonas* sp.
23	JCM	*Klebsiella-Enterobacter*	Neg.	*Klebsiella-Enterobacter*	*Klebsiella-Enterobacter*
24	EF	Neg.	Neg.	Neg.	Neg.
25	OFS	Neg.	Neg.	Neg.	Neg.

* Urocultura, realizada por cateterismo, revelou $> 10^4$ *Strept. faecalis*

As hemoculturas realizadas antes das manobras de dilatação foram negativas. Durante ou após as manobras, sete pacientes deram hemoculturas positivas (28,0%). Destes, cinco mostraram, no sangue, o mesmo germe isolado do trato urinário, e dois deram urocultura negativa (Tabela 3).

É curioso ressaltar que não houve, sistematicamente, aumento de temperatura nos casos em que a hemocultura se tornou positiva. Em quatro casos, bactérias foram isoladas do sangue, enquanto o paciente estava e se manteve apirético, mesmo tratando-se de gram-negativos.

Discussão

A punção supra-púbica foi o método mais preciso de coleta de urina em paciente com estreitamento inflamatório, eliminando as possibilidades de contaminação. Isso, aliás, foi o esperado, levando-se em conta a poluição da uretra e a inevitável contaminação da amostra de urina obtida pelo jato médio ou mesmo pelo cateterismo vesical[7]. Apesar de ser método especial, de difícil aplicação rotineira, deve ser o procedimento de escolha para dirimir uma dúvida decorrente de resultado de valor questionável neste tipo de doente. O jato médio se mostrou comparável ao cateterismo vesical, ambos com índice de contaminação relativamente alto. Isso não surpreendeu, porque a urina ou cateter têm que atravessar zona infectada.

Por maior que tivesse sido o cuidado para evitar a contaminação durante a dilatação uretral e para diminuir o trauma da uretra, usando a sonda gomada Phillips, precedida de condutores filiformes[3], não pode ser evitado que bactérias fossem lançadas na corrente circulatória. Esta possibilidade, já conhecida desde 1780 como a causa da "febre do cateter" só foi melhor documentada a partir de 1929, quando Scott[11], no John Hopkins Hospital, realizou hemoculturas de pacientes febris, encontrando 82 casos com cultura positiva, após teram sido submetidos a manobras instrumentais do trato urinário, inclusive

Tabela 4

Bacteremia após vários procedimentos no trato urinário

AUTORES	ANO	Nº DE DOENTES	PROCEDIMENTO	HEMOCULTURA POSITIVA
Barrington & Wright[1]	1930	23	Operação na uretra ou dilatação uretral	13
Powers[8]	1936	16	Dilatação de estreitamento uretral	3
Birow*	1951	106	Ressecção transuretral de próstata	13
Creewy & Freeney[2]	1954	356	Prostatectomia	157
Slade[13]	1958	50	Cateterismo vesical	10
Mitchell & col.[6]	1962	144	Dilatação de estreitamento uretral	14
Tulloch & col.[14]	1964	14	Dilatação de estreitamento uretral	5
Rodin & Murray[10]	1966	37	Dilatação de estreitamento uretral	1
		39	Massagem prostática	0
		22	Uretroscopia anterior	0
Barros e col.**	1973	25	Dilatação de estreitamento uretral	7

* Citado por Merrit[5]

** Presente estudo

dilatação uretral. Inúmeros autores, posteriormente, documentaram bacteremia em percentagem variável, após vários procedimentos urológicos, incluindo-se intervenções na próstata ou uretra[1,2,5], dilatação uretral[6,14], massagem prostática ou uretroscopia[10]. Como estas áreas do organismo não são estéreis, manobras traumáticas, com ou sem lesão aparente de suas superfícies, podem determinar a inoculação de carga variável de bactérias no sangue. Os resultados observados não diferiram de outros na literatura (Tabela 4). A variabilidade encontrada depende muito do tipo de doente ou condição clínica estudada, da natureza do procedimento urológico, e do cuidado e propriedade da metodologia.

Curioso foi a falta de correlação entre a presença de bactérias no sangue circulante, algumas delas possuidoras de endotoxina, e a elevação da temperatura corporal. A quase totalidade dos pacientes em que foram encontradas bactérias no sangue, através hemoculturas, não exibiu elevação da curva térmica. Este fato sugere que, nestes casos, a penetração de bactérias na corrente circulatória, durante as manobras de dilatação, foi pequena e transitória, com desaparecimento da bacteremia ao fim de alguns minutos, pela ação das defesas do hospedeiro. Isso não quer dizer que a invasão circulatória, por bactérias existentes no trato urinário, não represente perigo. São numerosos os casos documentados de choque séptico, após manipulação do trato urinário inferior e, sem dúvida, de que septicemia por gram-negativos tem o trato urinário[12] como principal fonte infectante. De outra parte, a penetração de enterococos na corrente circulatória pode resultar, se existiram condições predisponentes, em endocardite bacteriana[4,5].

Outra explicação para inexistência de febre, nestes casos, se relaciona ao fenômeno da tolerância ao pirogênio. Os doentes sofrem de infecção do trato urinário por tempo prolongado e continuada, em alguns casos, com possibilidade de surtos de bacteremia. O repetido contato com endotoxina de infectantes gram-negativos poderá resultar em tolerância, propiciando não só a falta de resposta pirogênica, como a retirada, mais pronta, da bactéria invasora da corrente circulatória. É fato seguro que a inexistência de febre não indica, na situação e condição estudadas, inexistência de bacteremia.

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Human dermatophilosis: the first case in Brazil

A.T. Londero*, Cecy D. Ramos* & Lenita P. Souza**

Summary

The first human case of dermatophilosis in Brazil is reported. Characteristic branched hyphae and coccoid elements of *Dermatophilus congolensis* were disclosed both in the lesions of the hand and in pure cultures.

Resumo

Dermatofilose humana: o primeiro caso no Brasil

É relatado o primeiro caso de dermatofilose humana ocorrido no Brasil. As hifas ramificadas e os elementos cocóides, característicos de *Dermatophilus congolensis,* foram verificados tanto nas lesões, como nas culturas isoladas.

Introduction

Dermatophilosis or cutaneous streptothricosis is a cosmopolitan infectious disease caused by an actinomycete, *Dermatophilus congolensis.* It affects many species of domestic and wild animals, in which the disease appears as an exudative dermatitis[5], except in cat[1]. Human infection by *D. congolensis* has been diagnosed very rarely. It was reported by Dean & col.[3], in the United States, and by Kaminski[6], in Austrália. Recently, Rubel[7] pointed *D. congolensis* as the agent of pitted keratolysis of man.

Although animal infection by *D. congolensis* has been found frequently in Brazil[2] specially in cattle and sheep, human infection by this actinomycete is now reported by the first time in this country.

Case Report

Clinical history: a 38 years-old white agriculture worker complained of lesions of one year duration. The lesions began as non-painfull pustules on the palmar surface of the left hand, at the basis of the middle, ring and little fingers. Few days later the pustules ruptured, scared and healed. From time to time, the lesions reappeared at the same site. Later on, the patient noticed a lesion on the web between his left middle and ring fingers. An exfoliated area was seen on the palmar surface, as result of the rupture of three pustular lesions; a white macerated epidermis was also seen on the finger web (Fig. 1). The patient

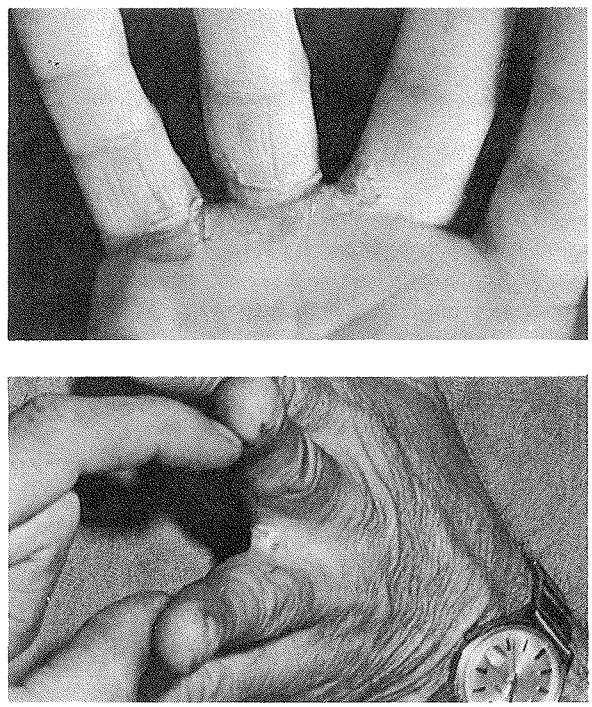

Fig. 1 — Human dermatophilosis: palmar (a) and intertriginous (b) lesions.

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was examined twice (every 15 days) during a month and specimens were collected for laboratory procedures. The lesions remained unchanged, and the patient was treated with intramuscular dihydrostreptomycin and topical applications of timerosal.

Laboratory findings: scrapings were taken from the borders of the lesions and skin scales fixed in methyl alcohol (1 minute) and stained with Giemsa (5 minutes) for microscopic examination. Some scales were cultured on slants of Mycosel agar (BBL) and Sabouraud dextrose agar plus cloramphenicol-cycloheximide and incubated at 24°C; other scales were processed according to Haalstra's technique[4], and cultured on Brain Heart Infusion agar (BBL) enriched with blood and incubated at 37°C, under anaerobic condition.

Microscopical preparations revealed branched hyphae 1 to 6 μm wide, divided both transversal and longitudinally (Fig. 2). Either coccoid bundles in sarcinae-like arrangement at the extremity of the wider hyphae, or masses of coccoid elements not associated with the filaments were seen (Fig. 3). Both types resulted from dissociation of the wider hyphae.

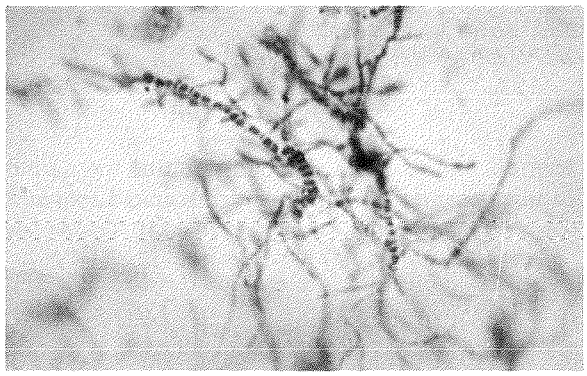


Fig. 2 – Branched filaments of *D. congolensis* in clinical material, stained by Giemsa, × 870.

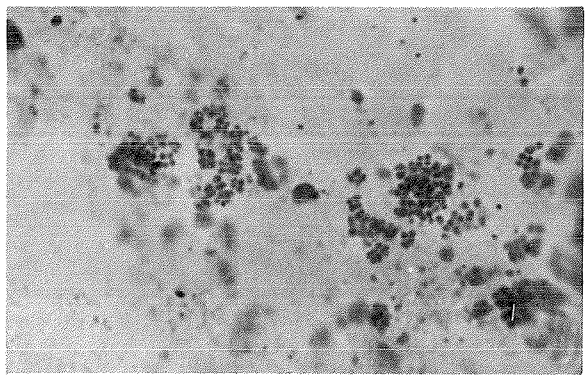
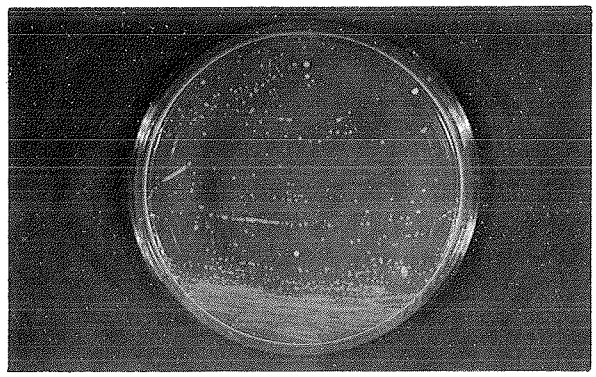


Fig. 3 – Coccoid elements in skin scraping, stained by Giemsa, × 870.

Cultures on Mycosel and Sabouraud's media yielded no pathogenic fungi. On Brain Heart Infusion Agar, grayish, rough, raised colonies markedly depressing the agar surface were obtained (Fig. 4a). The microscopic morphology of the organism in culture was similar to that from infected skin (Fig. 4 b).



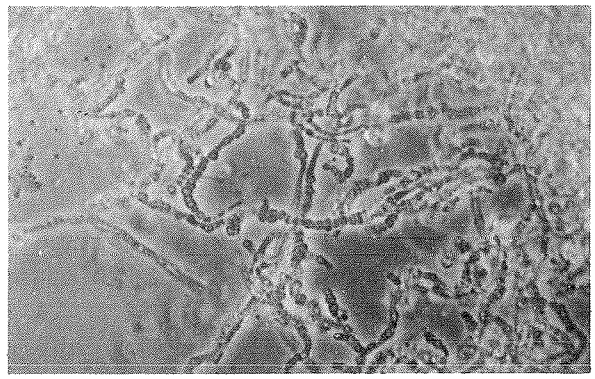


Fig. 4 – Seven-days subculture of *D. congolensis* on BHI agar at 37°C (a); and structure of the actinomycete in culture, × 870 (b).

Epidemiological data: no domestic animals in the patient's little farm and neighborhood presented cutaneous dermatophilosis.

Discussion

The typical aspects of *D. congolensis* seen from direct examination and the absence of other pathogenic fungi in culture isolations suggested that this actinomycete was the agent of the skin lesions observed. The source of the infection could not be traced but it is probable that the disease was acquired by a contact with an infected animal.

Lesions of human dermatophilosis have been described as an acute self-limited infection appearing as furuncles, foliculitis or pustular lesions on the upper limbs[3,6]. Two new clinical forms may be added to the spectrum of the disease: pitted keratolysis[7] and a recurrent pustular eruption plus

intertriginous lesion.

Although *D. congolensis* presents a minimal pathogenicity to man, dermatophilosis is becoming an important zoonosis from the public health standpoint. The rarity of this disease in man, specially in areas where sheeps are raized, seems related to the simple fact that the infection has not been sufficiently recognized.

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Bacterial adherence in infection and immunity*

Ronald J. Gibbons**

Summary

Studies of streptococci indigenous to the mouth have revealed that bacterial species exhibit surprising differences in their ability to attach to teeth and various mucosal surfaces. In all instances studied to date, the relative ability of a species to attach to an oral surface has been found to correlate with the proportions of that species found indigenously. It appears that in environments which contain surfaces exposed to a fluid flow, such as the oral cavity, the nasopharyngeal area, and portions of the intestinal canal, bacteria must become firmly attached to the surface to colonize. Otherwise they are washed away. These ecological principles are applicable to pathogenic bacteria. In the case of *Strep. pyogenes*, a correlation was observed between the ability of strains to attach to human epithelial cells and their virulence. The type-specific M protein surface antigen of *Strep. pyogenes* was found to be associated with its ability to adhere. There have also been reports of correlations between the adherence and the virulence of strains of *E. coli, Shigella,* and *Mycoplasma* species.

Electron photomicrographs have revealed that cells of indigenous and pathogenic streptococci adhere to epithelial cells by means of thin surface fibrils which form a "fuzzy coating" on the organisms. Tryptic removal of this fuzzy coating impairs the ability of these streptococci to attach. Immunoglobulins, including IgA antibodies contained in secretions, have been found to bind to bacterial surface antigenic components, and impair their adherence. Since colonization of a mucosal surface requires continuous bacterial reattachment to new surfaces resulting from desquamation, the ability of antibodies in secretions to specifically inhibit bacterial attachment can provide a basis of protective immunity on mucosal surfaces. The immunological selection pressure thereby imposed upon bacteria colonizing mucosal surfaces can provide an explanation for the apparent ecological aggressiveness of pathogenic organisms, and the basis of the carrier state.

Resumo

Aderência bacteriana em infecção e imunidade

Estudos de estreptococos orais indígenos têm revelado que estas bactérias exibem diferenças surpreendentes em sua capacidade de aderência aos dentes e várias superfícies mucosas. Em todos os casos até agora estudados, a habilidade relativa de uma espécie se fixar a uma superfície oral tem sido correlacionada com as proporções das espécies encontradas indigenamente. Parece que, em ambientes nos quais são encontradas superfícies expostas a correntes fluidas, tais como a cavidade oral, área nasofaringeana, e porções do canal intestinal, as bactérias necessitam se fixar firmemente às superfícies para colonizá-las. De outra maneira, serão removidas. Estes princípios ecológicos são aplicáveis a bactérias patogênicas. No caso de *Streptococcus pyogenes,* foi observada a correlação entre a habilidade de fixação de linhagens a células epiteliais humanas e a virulência. A proteína M, antígeno espécie-específico da superfície de *S. pyogenes*, foi observada em associação com a habilidade de aderência da bactéria. Há também relatos sobre a correlação entre aderência e virulência de linhagens de *E. coli* e espécies de *Shigella* e *Mycoplasma*. Fotomicrografias eletrônicas têm mostrado que células de estreptococos indígenos e patogênicos aderem-se a células epiteliais por meio de fibrilas superficiais finas que formam uma cobertura "fuzzy" sobre o organismo. Remoção dessa cobertura por tripsina prejudica a habilidade desses estreptococos se aderirem. Imunoglobulinas, inclusive anticorpos IgA contidos nas secreções, ligam-se a componentes antigênicos da superfície bacteriana e impedem a aderência. Uma vez que a colonização da superfície mucosa requer a contínua readerência de bactérias a novas superfícies resultantes da descamação, a habilidade dos anticorpos na inibição específica da aderência bacteriana pode fornecer a base para a imunidade protetiva às superfícies mucosas. A pressão da seleção imunológica imposta sobre as bactérias colonizadoras das superfícies mucosas pode fornecer a explicação para a agressividade ecológica aparente dos organismos patogênicos e a base do estágio transportador.

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To be pathogenic, an organism must first colonize a host, and secondly cause tissue damage. Most pathogenic bacteria initially colonize a mucosal surface[62,66]. There the organism may replicate and remain localized, or it may subsequently disseminate throughout the body. Depending upon the circumstances, immune phenomena could interfere with colonization of a pathogen locally on a mucosal surface or systemically when the organism invades the tissues of the host. Antibodies may also neutralize bacterial toxins, though this does not necessarily affect either the local or systemic colonization of the pathogen. In the past, factors considered most important for bacterial colonization were those which related to microbial growth or death[38,66]. Attempts to induce immunity to infectious bacterial agents have therefore been aimed at stimulating immunoglobulins which were capable of exerting bactericidal effects, or which could assist phagocytes in engulfing and killing bacteria. Immunoglobulins possessing bactericidal and opsonizing properties are present in highest titres in serum, and thereby provide the basis of systemic immunity. However serum antibodies are less likely to exert significant effects upon the initial colonization of pathogenic bacteria on mucosal surfaces. Rather, the secretory immune system has greater opportunity to act on such surfaces. However, immunoglobulin A, the predominant antibody species in secretions, is not generally considered to possess bactericidal or opsonizing properties, and consequently the role of the secretory immune system in affording immunity to bacterial infections has not been clearly delineated[77,78,79].

Over the past few years, our laboratories have studied the ecology of streptococci indigenous to the mouth. These investigations have revealed a parameter which is required for bacterial colonization on mucosal surfaces which is independent of those effecting bacterial growth or death. It has been shown that bacteria must adhere to a mucosal surface if they are to colonize successfully[32,35,50,51,80,81,82]. Although this seems obvious after a moments reflection, it has been given little attention in the past. Yet bacterial adherence appears to be of fundamental importance for understanding the natural ecology of infectious bacteria, and it can help to explain how secretory antibodies may exert a protective effect. Consequently, I would like to discuss some aspects of the role which bacterial adherence may play in the colonization of indigenous and pathogenic bacteria.

Role of bacterial adherence in the ecology of indigenous oral bacteria. The role of bacterial adherence as an ecological determinant influencing the colonization of bacteria on mucosal surfaces was first suggested from studies of *Streptococcus mutans*. This organism is associated with dental caries activity in both experimental animals and in man[32,44]. *S. mutans* has been found to possess unusual caries-inducing activity when tested in a variety of experimental animals. There are compelling data which indicate that the organism's cariogenic potential is associated with its ability to accumulate on the surfaces of teeth. The bacterial deposits which form on teeth are referred to collectively as dental plaque. Plaque formation by *S. mutans* has been found to be related to the organisms ability to synthesize extracellular polysaccharides specifically from sucrose. For example, *S. mutans* forms adherent microbial deposits on the walls of culture vessels when grown in sucrose broth, and it initiates large bacterial plaques in experimental animals fed diets containing sucrose. The organism has been found to synthesize extracellular glucans and fructans specifically from sucrose, but not from other common sugars. Thus both polysaccharide synthesis and plaque formation by this organism are specifically dependent upon this sugar[32,44].

Not only is sucrose required by *S. mutans* to form plaque, but several studies have shown that this sugar greatly fosters colonization of the organism in the mouths of experimental animals and in humans. Since *S. mutans* can grow on, and metabolize other sugars such as glucose as readily as sucrose, it's specific dependence on sucrose for colonization is attibutable to something other than growth. This is likely to be related to the organism's ability to adhere to the surfaces of teeth[32]. Thus *S. mutans* appeared to provide an example of an organism whose ability to colonize the oral cavity was strongly influenced by its ability to attach to an oral surface.

The apparent importance of adherence in the ecology of *S. mutans* raised the possibility that colonization of other indigenous bacteria might also depend upon their ability to adhere to oral surfaces. It has been recognized for several years that different bacterial species preferentially colonize different sites in the mouth (Table 1). For example, *S. sanguis* and *S. mitis* generally comprise high percentages of the streptococcal populations found on teeth; yet *S. salivarius* does not[13,51,81]. Rather, *S. salivarius* preferentially colonizes the tongue[34,46]. *S. mutans* also selectively colonizes teeth, but it is rarely found in significant proportions on oral mucosal surfaces[13,32,79]. The reasons for the selective colonization of streptococci and other bacteria within various niches of the mouth have never been clear. The elective localization has been vaguely attributed to differences in nutrient availability postulated to exist between sites. However recent studies of the nutritional requirements of oral *Streptococcus* species have indicated that these organisms possess an overall nutritional similarity, and the data do not substantiate nutritional parameters as being primarily responsible

for their intra-oral localization[14, 59].

Recent studies in our laboratories have demonstrated that these streptococci, and other oral species, differ widely in their abilities to adhere to the various surfaces of the mouth[32, 35, 50, 51, 80, 81, 82]. In all cases studied, using both *in vitro* and *in vivo* techniques, the ability of an organism to adhere to an oral surface has been found to correlate directly with the proportions of the organism found indigenously (Table 1).

Table 1

Proportion of bacteria found indigenously on oral surfaces, and their relative adherence as determined experimentally

Bacteria	Proportions Found Indigenously			Experimentally Demonstrable Adherence		
	% of Streptococcal Flora*			Relative Adherence*		
	Tooth	Tongue	Cheek	Tooth	Tongue	Cheek
Streptococcus mutans	0-50	< 1	< 1	low to high**	low	low
Streptococcus sanguis	30-40	5-15	10-20	high	moderate	moderate
Streptococcus mitis (S. miteor)	30-40	10-20	70-90	high	moderate	high
Streptococcus salivarius	< 1	40-60	10-20	low	high	moderate
	% of Total Cultivable Flora					
Lactobacilli	0-0.05	0.01-0.1	0.01-0.2	low	low	low
Veillonella	1-3	10-15	< 1	low	high	low
Neisseria	1-2	1-2	1-2	low	low	low

* Estimates derived from data of several studies [32, 35, 50, 51, 80, 81, 82]

** High when mediated by dextran

For example, *S. sanguis* and *S. mitis* were found to adhere to teeth far better than either *S. salivarius* or *Veillonella* species, thereby reflecting the distribution of these species found naturally on teeth (Table 1)[35, 50, 51, 80, 81]. In marked contrast, the latter species have been found to adhere well to the dorsal surface of the tongue, which agrees with their observed intra-oral localization.

Bacteria in many natural environments are recognized to have a predilection for growing on surfaces. Soil bacteria are generally found adsorbed to the surfaces of colloidal clay particles, while the accumulation of microbial and other forms on objects immersed in the sea is well known. Similarly, the vast majority of bacteria found in the oral cavity[32, 34, 39] and in the intestinal canal[62] of man are firmly attached to the surfaces present. Studies revealing the selectivity of the attachment of indigenous bacteria to surfaces of the mouth have evolved a simple but generally unappreciated ecological determinant[32]. In environments which contain surfaces exposed to a fluid flow, bacteria must either adhere to a surface, or else grow at a rate which exceeds the dilution rate resulting from the fluid flow, if they are to colonize successfully. Otherwise they are simply washed away. A flowing stream, the oral cavity, the nasopharyngyeal area and portions of the gastrointestinal canal represent examples of such environments. The rate of growth of bacteria in the oral cavity[31] and in the intestinal canal appears to be slow[33]. Indeed it is likely much lower than the dilution rate affected by the flow of secretions. Consequently the ability of an organism to adhere to a freely exposed surface or to become mechanically entrapped in a protected niche appears to be a prerequisite for colonization. Since epithelial surfaces desquamate continuously, bacteria may not proliferate for extended periods of time on any given epithelial cell. The relatively slow rate of bacterial growth occurring in such environments also tends to minimize microbial population shifts due to differences in the rates of growth of various species. Organisms which become dislodged, or their progeny, must continuously reattach to these ever-renewing surfaces for colonization to continue. Hence, the extent to which an organism may colonize a mucosal surface would seem to be largely dependent upon the number of cells available for attachment, and the organism's innate capacity to adhere[32, 35].

Adherence in the ecology of overtly pathogenic bacteria. The importance of bacterial adherence in regulating the ecology and colonization of indigenous oral bacteria implies that it should also be significant in the primary colonization of overt pathogens on mucous surfaces. Moreover, if colonization of a pathogen is dependent upon its ability to adhere to a mucosal surface, then it follows that the extent to which the pathogen adheres should influence the extent to which it may colonize. Hence, adherence should be a parameter which relates to the virulence of infectious organisms.

The literature, in fact, contains several reports of correlations which have been observed between the ability of pathogenic bacteria to adhere to mucosal surfaces and their virulence. For example, pathogenic strains of *Mycoplasma* species have been found to adhere to tracheal epithelial cells, while nonpathogenic strains generally do not[62]. Similarly, several investigators have reported direct correlations between the ability of strains of *Shigella,* and enteropathogenic *Escherichia coli* to adhere to the lining of the small intestine and the virulence of the organisms[2, 6, 7, 19, 43, 48, 67, 69]. In many instances, these correlations were interpreted to mean that the more pathogenic strains could more readily damage the underlying tissues with toxic products. In the case of *Shigella* species, the greater adherence of virulent strains appeared to be related to "invasiveness". Other pathogens which have been observed to adhere to mucosal epithelium include gonococci[86], *Clostridium perfringens*[3], *Salmonella*[73], *Vibrio cholerae*[28, 30], and *S. pyogenes*[21]. Frequently, the simple, yet fundamental role of adherence as a prerequisite for colonization of these organisms was not considered.

Our laboratories have recently attempted to apply the principles of bacterial adherence in studies of the ecology of *Streptococcus pyogenes*[21]. *S. pyogenes*

possesses an array of antigens, but the most significant of these is the M antigen, a surface protein which serves as the type specific antigen for this species[49]. It has been known for many years that the presence of M protein correlates with the virulence of *S. pyogenes*, and that naturally acquired immunity to infection is directed against this antigen. Yet M protein is nontoxic to healthy subjects, and only previously recognized function in the pathogenesis of group A streptococcal infections has been its ability to impede phagocytosis[49, 52, 88]. We have found that virulent strains of *S. pyogenes* possessing M protein adhere well to human oral and pharyngeal epithelial cells, whereas an avirulent strain lacking M protein adhered poorly[21]. Tryptic removal of M protein from virulent strains markedly impaired their ability to adhere. When mixtures of virulent and avirulent strains of *S. pyogenes* were introduced into the mouths of mice, the avirulent strain lacking M protein was cleared rapidly, while the greater adherence of the M positive virulent strain increased the organism's retention on mucosal surfaces thereby fostering its colonization. These findings, plus the observation that type specific anti-M serum inhibited the adherence of virulent strains to epithelial surfaces[21], indicate that the M antigen of *S. pyogenes* participates in its adherence to mucosal surfaces. Thus a primary function of M protein in the pathogenesis of group A streptococcal infections is related to the initial colonization of the organism. Since phagocytic cells are not commonly found on non-inflamed mucosal surfaces, the action of M protein in impeding phagocytosis would occur secondarily. It would assume importance only when colonization had progressed to a point where inflammatory reactions were evoked, or when the infection had become systemic.

Subsequent to these studies, an analogous situation has been found to exist for enteropathogenic strains of *E. coli* associated with acute diarrhea in piglets. Some enteropathogenic strains of *E. coli* possess a surface protein, designated the K88 antigen. Pathogenic strains possessing K88 antigen have been observed to adhere better to segments of small intestine *in vitro* than avirulent strains lacking this antigen[43]. Upon oral administration, K88 strains were found to become associated with the surface of the small intestine, whereas avirulent strains tended to remain free in the lumen. Adherence of the virulent organism was also found to be inhibited by antiserum to the K88 antigen. Thus the greater adhesion of strains possessing K88 antigen would help prevent their removal by peristaltic or villous motility, thereby facilitating colonization[43]. Other enteropathogenic strains of *E. coli* probably possess other antigenic components which are involved in adherence.

It is interesting to speculate that the organ and tissue predilection of some infectious bacteria may be related to their ability to adhere to the surfaces of the affected organ, in a manner analogous to the way that adherence influences the selective colonization of bacteria in various niches in the mouth. It is conceivable that one of the reasons why Gram positive bacteria are most commonly associated with infections of the heart is that this reflects not only their opportunity but also their ability to attach to and hence colonize this organ. Preliminary findings from our laboratory are consistent with this view, for virulent strains of *S. pyogenes* have been found to adhere far better to human buccal and pharyngeal epithelial cells than an enteropathogenic strain of *E. coli*[22]. Similar observations have been made *in vivo* in experimental animals. Hence the ability of these respective pathogens to adhere to a tissue surface seems to correlate with the predilection of that tissue for infection.

Bacterial surface components involved in adherence. The surprising differences in adherence among various bacterial species to a given surface indicates that they must possess widely different surface constituents. Electron microscopic observations of oral streptococci have indicated that *S. salivarius, S. mitis, S. sanguis* and *S. mutans* each possess a distinctive surface morphology[63]. Cells of *S. salivarius* possess a dense fibrillar fuzzy surface coating (Fig. 1) when grown under a variety of conditions, including in a defined culture medium[36, 63]. These surface components seem to differ from bacterial pili on a morphological basis. Cells of *S. mitis* also possess surface fibers (Fig. 2), though they differ morphologically from *S. salivarius*[51, 63]. *S. sanguis* and *S. mutans*, the two species which preferentially colonize teeth, possess less developed surface structures (Fig. 3).

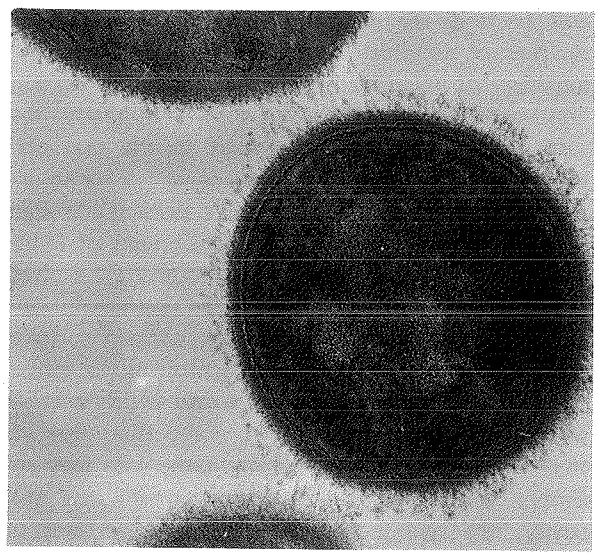

Fig. 1 — A cell of *Streptococcus salivarius* showing fibrillar surface components external to its cell wall[63].

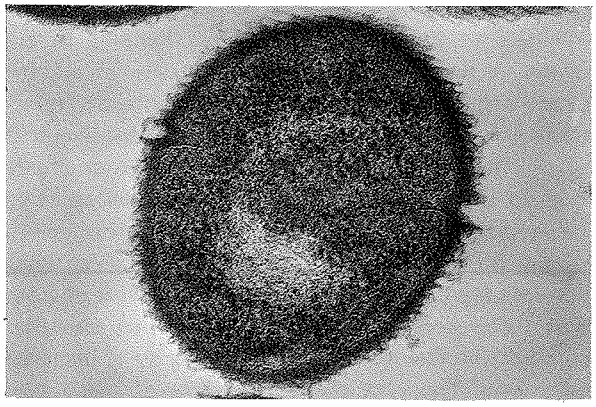

Fig. 2 — Cell of *Streptococcus mitis* (*S. miteor*) showing surface components external to its cell wall[63].

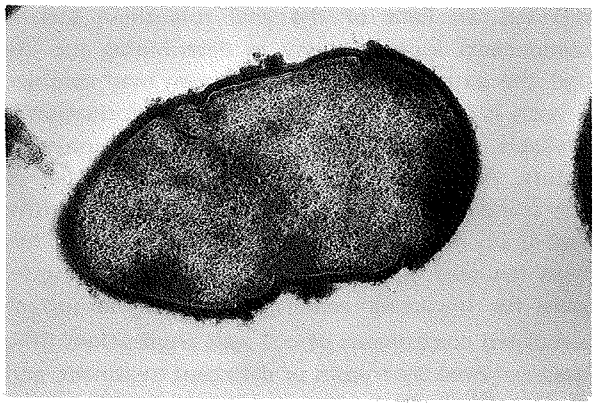

Fig. 3 — Cell of *Streptococcus sanguis.* This organism possesses a less fibrillar surface than *S. salivarius* or *S. mitis*[63].

Electron photomicrographs of cells of *S. salivarius* attached to cheek cells obtained from germfree rats suggest that the organism's adherence is mediated by its fibrillar fuzzy coating, for these surface fibrils seem to bridge the space between the bacterial cell wall and the epithelial cell membrane (Fig. 4)[36].

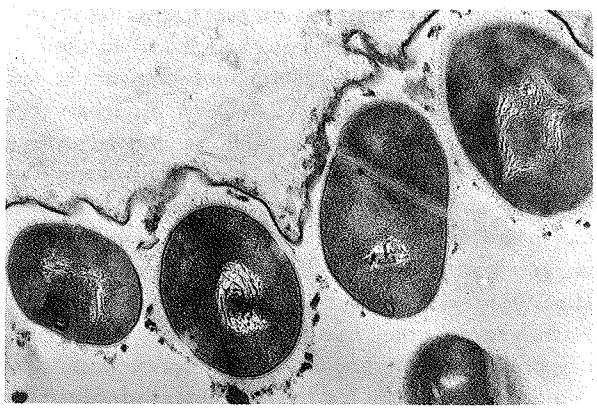

Fig. 4 — Cells of *Streptococcus salivarius* attached to a cheek epithelial cell obtained from a germ-free rat. The bacteria possess fibrillar surface components which appear to bind them to the epithelial cell membrane. Reprinted from the Journal of Dental Research[36].

Similar observations have been made for *S. mitis*[51]. Direct data substantiating the involvement of these surface fibrils in the adherence of bacteria to mucosal surfaces have been obtained. It has been found that trypsin partially or completely removes the surface fibrils from *S. salivarius* without producing other morphologically detectable alterations[36]. In fact, *S. salivarius* grows well in the presence of trypsin. However, trypsintreated cells of the organism, lacking the external fuzzy coat, no longer adhere well to epithelial cells[36]. Wheatgerm and pancreatic lipase also have been found to partially remove the fuzzy surface components of *S. salivarius*, and thus treatment also impairs the adherence of the organism to human oral epithelial cells. These observations further suggest that the organisms fuzzy coating contains proteinacious and lipid constituents[36].

The possession of a fuzzy surface coating is not unique to *S. salivarius* and *S. mitis*. Virtually all of the Gram positive bacteria present indigenously on human cheek or tongue cells have been observed to possess a comparable fuzzy coating which appears to mediate their attachment to the epithelial cell surface[36]. Several pathogenic species have also been found to possess dense surface fibrils. For example, Swanson and co-workers[72] observed that virulent strains of *S. pyogenes* contain surface fibrils which are morphologically similar to those possessed by *S. salivarius.* Treatment of *S. pyogenes* cells with trypsin removed these surface fibrils, and this procedure is known to remove M protein. In addition, these investigators found that an M negative mutant of *S. pyogenes* differed from its virulent parent by not possessing these fuzzy surface components[72]. Ferritin-labeled antibodies against M protein were also observed to localize on the fuzzy coating of virulent strains. On the basis of these observations, Swanson and co-workers[72] concluded that the external fuzzy surface coating of *S. pyogenes* in part represented M protein anatomically. We have further observed that these M protein containing fibrils of *S. pyogenes* appear to mediate the organism's attachment to tongue cells obtained from germfree rats in a manner quite analogous to that observed with *S. salivarius* (Fig. 5)[21]. These observations help to explain how the M protein facilitates the adherence of *S. pyogenes* to mucosal surfaces.

Analogous data are available in the case of other pathogenic species. We have observed that virulent pneumococci are well endowed with fuzzy surface fibrils (Fig. 6). Similarly Hard[37] observed that virulent strains of *Corynebacterium ovis*, an organism associated with lymphadenitis in cattle, possessed a dense surface coating which consisted in part of lipid. An avirulent mutant of *C. ovis* lacked these surface components. Jolly[42] obtained evidence which suggested that the surface lipids of the virulent strain

did not differ in toxicity from those of avirulent strains. It would appear likely that the surface components of the virulent strain mediated its attachment to mucosal surfaces thereby facilitating colonization, while the altered surface of the avirulent mutant rendered it less adherent.

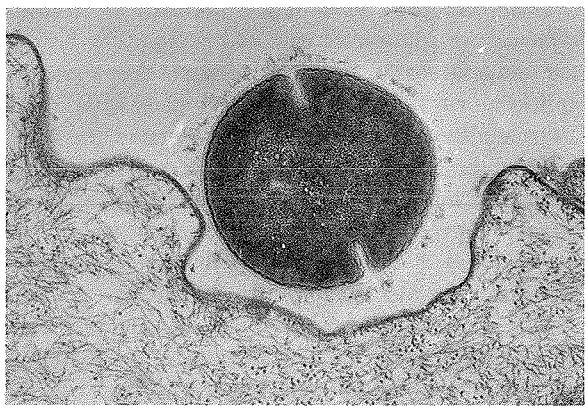

Fig. 5 — Cell of *Streptococcus pyogenes* attached to a tongue epithelial from a germ-free rat. The fibrillar coating of the bacterium, which contains the M antigen, appears to bridge the space between the bacterial cell wall and the epithelial cell membrane. Reprinted from Infection and Immunity[21].

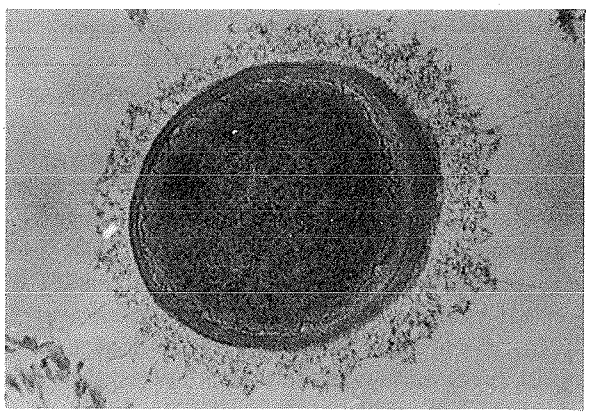

Fig. 6 — A virulent pneumococcus cell showing fibrillar surface components (Liljemark, W. F. & Gibbons, R. J., unpublished).

Other examples of surface components involved in the adherence of infectious agents are available. Trypsin treatment has been found to affect the adherence of *Mycoplasma* species to epithelial cells, and thus these organisms also appear to possess surface proteins which are required for attachment[40, 76]. Similarly, the K88 antigen possessed by some enteropathogenic strains of *E. coli*, which is related to their ability to adhere to the walls of the small intestine[43], has been shown to consist of long thin sparcely distributed filaments[71]. Bacterial pili, which are possessed by many Gram negative organisms, would also appear to function in an analogous manner. Pili have long been recognized to impart adhesive qualities to bacteria which possess them[11]. In addition it has been found that virtually all of the Gram negative bacteria isolated from bladder infections are piliated[11]. However the likely involvement of pili in facilitating the attachment of these organisms to the epithelial lining of the bladder so as to foster their colonization was not generally considered.

Besides the morphological differences which exist between the external coatings of bacteria, it is obvious that differences must also exist in their chemical composition to account for the specificity of bacterial adherence. The type specific antigens of many bacteria are surface components, and many of these are protein in nature. In most instances, these are the antigens to which naturally acquired immunity is directed. In light of the vital role these surface components appear to play in determining the site and the extent to which a pathogen may colonize a host, it is remarkable that so little information is available concerning their chemical and physical nature. Although there have been many studies of the composition of the cell walls of Gram positive and Gram negative bacteria, the major emphasis has been focussed upon the peptidoglycan layer responsible for imparting wall rigidity[61, 75]. The surface associated components of cell wall preparations have been removed by trypsin or detergent treatment, and discarded[61, 75]. Preliminary analyses of the material released by trypsin from cells of *S. salivarius* indicate it contains protein, lipid and carbohydrate. Thus these surface constituents appear to be complex.

There is also little information available about the nature of the receptors present on mucosal and other epithelial cells which influence bacterial adherence. The adsorption of influenza viruses to erythrocytes is well known to entail sialic acid containing receptors, since treating erythrocytes with neuraminidase renders them unsuitable for viral attachment. Similarly, treatment of erythrocytes and tracheal epithelial cells with neuraminidase has been found to destroy the receptors to which *Mycoplasma pneumonia* and *M. gallisepticum* adsorb[68, 75]. However it appears that different receptors are required for the adherence of other *Mycoplasma* species, for the adsorption of *M. orale* and *M. pulmonis* to chicken erythrocytes was not effected by neuraminidase[68]. Similarly, this enzyme had little effect upon the ability of hamster cheek epithelium to adsorb *Streptococcus salivarius*[36]. Rather, pretreating hamster epithelium with lipase preparations, including several phospholipases, rendered it less suitable for the attachment of this streptococcus[36]. This suggests that lipid and perhaps phospholipid moieties serve as receptors for this organism. Treatment of cells of *S. salivarius* with microgram quantities of several phospholipids blocked the attachment of the organism, further suggesting that

the cell receptors involved contain phospholipids[36]. Phospholipids also block the adherence of *S. pyogenes* to human buccal epithelial cells, and consequently these components may be part of the receptors for this pathogen[22].

Although knowledge of the epithelial cell surface components involved in bacterial adherence is scant, it is likely that significant differences exist between cells of various organs, tissues, and species. Certainly marked differences have been observed in the adsorption of oral streptococci to cheek and tongue epithelium (Table 1). Other observations clearly indicate that there are differences in the nature of the surfaces of certain cells between various species. This may be deduced from observations concerning hemagglutination induced by bacteria. Bacteria with pili, and certain *Mycoplasma* species are known to adsorb to erythrocytes of some species of animals and fowl, causing hemagglutination, but not to erythrocytes of other species[20,68]. Thus even cells as basic as erythrocytes possess subtle differences in surface components between species which dramatically influence bacterial adherence.

Nonspecific clearance of bacterial populations on mucosal surfaces. Several studies have shown that when various bacteria are introduced into the mouth, the nasopharyngeal area, or the intestinal canal, the organisms are generally rapidly cleared[8,9,17,18]. Thus the cleansing action existent on mucosal surfaces is apparent. The bathing mucinous secretions flowing across and coating respiratory, oral and intestinal epithelium, of course, play a major role. Their cleansing action may be augmented by the cilial beating of epithelial cells of the trachea, and by the forces imposed by tongue movements and mastication in the oral cavity. Similarly, intestinal motility and peristaltic movements are well known to cleanse the surfaces of the upper intestinal canal of their microbial burden[18]. In fact, to produce infections of the small intestine experimentally in animals, it is frequently necessary to inhibit peristalsis with narcotics to enable introduced pathogens to colonize[26]. Epithelial cell shedding on mucosal surfaces would also appear to be an effective way of limiting bacterial colonization. In this regard, it is interesting to note that the rate of turnover of intestinal epithelial cells is slower in germfree rats compared to conventional animals[1]. This suggests that the rate of epithelial cell shedding is increased when there is need to control bacterial colonization.

Specific inhibition of bacterial adherence by secretory immunoglobulins. The specificity involved in the attachment of bacteria to mucosal surfaces, and its role as a prerequisite for bacterial colonization have provided a novel hypothesis to explain how immunoglobulins may assist bathing secretions in their cleansing action and provide protective immunity[89]. The antibodies present in secretions differ from those in serum in that secretory immunoglobulin A (S-IgA) is the predominant immunoglobulin class in secretions, whereas immunoglobulin G (IgG) predominates in serum[77,78,79]. S-IgA has been demonstrated to serve a protective function against viral infections, but how, or if S-IgA antibodies may affect bacteria is not well understood[77,78,79]. This is because most investigators have found that S-IgA does not mediate complement dependent bacterial lysis, nor does it enhance phagocytosis in a manner comparable to serum IgG[77,78,79]. These are the two activities which have been most extensively studied in experimental efforts to induce and understand immunity to bacterial infections. However, most experimental studies of infectious bacteria use systemic inoculations, and this by-passes the natural ecological events involved in the primary colonization of the pathogen. Hence, factors relating to systemic immunity are not necessarily related to those acting locally on mucous membranes.

Initially, most pathogens colonize mucosal surfaces[62,66], and it is likely that neither complement-mediated bactericidal reactions, nor phagocytosis are important in such an environment. This is because the secretions bathing these surfaces do not contain several components of the complement system, and most secretions possess anti-complementary activity[47,79,83,*]. In addition, few active leukocytes would appear to be present on most mucosal surfaces because of the hypotonic nature of the secretions, and because the surfaces are normally not acutely inflamed.

It is well documented that S-IgA antibodies are capable of specifically binding to antigenic components on the surfaces of bacteria, and of causing agglutination[64,77,79]. In light of the requirement of bacterial adherence for colonization, this property of S-IgA *per se* could provide the basis for protective immunity on mucosal surfaces. This was also suggested by the observations that antisera directed against surface antigens of *S. pyogenes*[21], enteropathogenic strains of *E. coli*[43], or *S. salivarius*[89] do inhibit the adherence of these species as discussed above. We have recently shown that S-IgA isolated from human parotid saliva can, in fact, inhibit the adherence of specific strains of *S. salivarius* and *S. mitis* to human buccal epithelial cells *in vitro*[89]. These antibodies evidently react with bacterial surface antigens and sterically hinder their attachment to epithelial cells. Because continued colonization of a mucosal surface *in vivo* requires continuous bacterial reattachment due to epithelial cell shedding, it is

* See also Genko, R.J. & Taubman, M.A. — Biological properties of secretory IgA antibodies. AAS Symposium on Comparative Immunology of the Oral Cavity, 1973. In press.

evident that the immunoglobulin mediated inhibition of adherence of one serotype of an organism relative to another would lead to the rapid elimination of the affected strain. Because a small degree of inhibition of adherence would be multiplied by the constant need for reattachment, this mechanism of bacterial elimination would be highly efficient, and would require only small amounts of secretory immunoglobulins. Teleologically, this may explain why only low titres of S-IgA are usually detected in secretions.

If these concepts are correct, they imply that secretory antibodies may assist secretions in their cleansing action by specifically inhibiting the adherence of certain bacterial serotypes, thereby facilitating clearance. There are other data which suggest that secretory antibodies can function in this manner. Antibodies in intestinal secretions have been found to block the absorption of macromolecular antigens analogous to the manner proposed for the inhibition of bacterial adsorption[85]. The studies of Freter[28,29] with experimental cholera also substantiate the concept that secretory antibodies may exert a protective action by inhibiting bacterial adherence. He observed that induced coproantibodies, which are primarily S-IgA, were protective against experimental infection by *Vibrio cholerae*. When immune animals were challenged with live vibrios, the organisms tended to remain free in the intestinal lumen, whereas they adsorbed to the mucosal linings of non-immune animals.

There are also data available which suggest that secretory immunoglobulins can affect the colonization of bacteria on mucosal surfaces. Several investigators have reported that there is a continual change-over in the serotypes of *E. coli* colonizing the intestinal canal[16,58], suggesting the existence of an immunologic selection pressure. The conversion and selection of bacterial serotypes has been most extensively studied by Miller and coworkers[55,60]. These investigators observed that when germfree animals were monoinfected with a specific serotype of *Vibrio cholerae*, the inoculated serotype dominated for approximately two weeks, and then was gradually replaced by a different serotype. During colonization, the animals did not develop overt disease, and hence the vibrios acted analogously to indigenous bacteria. The serotypic conversion was found to be accelerated if the animals were previously immunized with the strain inoculated. Conversely, the conversion of serotypes was almost completely eliminated if the animals were treated with cyclophosphamide, an immunosuppresive agent. These studies show clearly that there is an immunological selection pressure imposed upon bacteria colonizing mucosal surfaces [55,60]. Immunoglobulins present in the secretions which bathe these surfaces would seem to be responsible for the effects observed, although the role of cell-mediated immune factors cannot be disregarded.

It is interesting to consider possible effects of immunoglobulins on the colonization of indigenous bacteria on mucosal surfaces. Secretions generally contain low titres of antibodies against indigenous bacteria[10,64], and hence their colonization would also be expected to be influenced. This is also suggested by the changes in serotypes which indigenous bacteria undergo as mentioned above. There are data available which suggest that most indigenous bacteria in the mouth exist in a state of suppressed adherence, likely mediated by secretory immunoglobulins[89]. Epithelial cells scraped directly from human cheeks generally harbor only 10 to 15 bacteria per cell, even though they are continuously exposed to concentrations of *S. salivarius, S. mitis* and other indigenous bacteria approaching 10^8 organisms per mℓ of saliva[35,89]. If buccal epithelial cells are washed and incubated with suspensions of these streptococci grown *in vitro*, hundreds of bacteria attach to each epithelial cell within minutes[35,89]. Thus it appears that the adherence of indigenous streptococci, as they exist *in vivo* in saliva, is suppressed. Brandtzaeg and co-workers have shown that most of the bacteria present in human saliva are coated with S-IgA[10]. Similarly the bacterial deposits present on human teeth also have been found to contain appreciable quantities of S-IgA[74]. The demonstrated ability of S-IgA to inhibit the attachment of bacteria to epithelial cells[89] therefore would appear to be at least partially responsible for the apparent state of suppressed adherence of salivary organisms.

The existence of an immunologic selection pressure, manifested by suppressed adherence of indigenous bacteria, and the phenomenon of serotypic conversion, would seem to be important for understanding the ecology of infectious agents. In most discussions of the acquisition of infectious bacteria, the reader is lead to believe that small numbers of a pathogen are introduced via droplets on a mucous surface. The organisms are then presumed to overgrow most of the indigenous bacteria present so as to attain numerical predominance. However, there are few data concerning most pathogenic bacteria which convincingly suggest that their nutritional requirements, or potential rates of growth, are sufficiently different from those of indigenous bacteria to achieve this effect[56,66]. In fact, in the very few instances where the rate of growth of a pathogen *in vivo* has been estimated, the rate has been slow[53,54], and of the same order as that approximated for indigenous bacteria[31,33]. However, if the colonization of most indigenous bacteria is partially suppressed by secretory antibodies inhibiting their adherence, then exogenously introduced, serotypically distinct patho-

gens would be expected to enjoy a temporary immunologic selective advantage whereby they could adhere and colonize unimpeded by secretory antibodies. This selective advantage would be analogous to that imposed on newly converted serotypes of bacteria on mucous surfaces, and it could enable small numbers of a pathogen to attain prominance within a relatively short period of time. If their mass, augmented by any tissue damage produced, evoked a strong secretory antibody response, their adherence would be inhibited to a greater extent than that of the indigenous flora, and the pathogen would be selectively eliminated and an immune state created. However, should their colonization progress slowly and elicit a secretory antibody response comparable to that directed against indigenous bacteria, the pathogen would be affected comparably, and a transient balanced or "carrier state" would result[89]. In this state of partial suppression, they would be expected to colonize for weeks or months, as serotypes of indigenous bacteria do, before being eliminated, or undergoing serotypic conversion.

The concepts discussed can explain how immunoglobulins present in secretions may influence the ecology of indigenous and pathogenic bacteria, without requiring mediation of complement or the phagocytic systems which are fundamental to systemic immunity. Should organisms elude this primary defense, colonize and subsequently invade the host tissues, then systemic defense mechanisms and other recognized immune factors would assume importance.

Natural immunity induced to pathogenic bacteria. There are a variety of data in the literature which suggest that natural immunity to pathogenic bacteria may operate as described above. Several investigations have shown that a state of immunity exists following recovery from an infectious agent during which the host is protected from reinfection. Some of the infectious agents for which this has been observed include beta hemolytic streptococci[49, 52, 87, 88], staphylococci[23], pneumococci[4, 12], *Mycoplasma*[25] species, and *Corynebacterium diphtheriae*[5,41]. The immunity created following these natural infections is usually type specific, and in many cases appears to affect subsequent colonization of the pathogen locally on mucosal surfaces. For example, Watson and co-workers[87] observed that when monkeys were inoculated intranasally with virulent strains of *S. pyogenes*, the organisms colonized the animals for weeks before being eliminated. Such animals became immune. When they were challenged with the same M type of *S. pyogenes*, the inoculated organisms were generally undetectable within 24 hrs. However if the immune animals were challenged with a different M type, the serologically different strain colonized the animals for weeks. These observations suggest that the naturally acquired type specific immunity acted by preventing recolonization of *S. pyogenes* on the mucosal surface. In addition, the basis of this immunity, which was directed against surface proteins involved in the organism's adherence to epithelial cells, appeared to involve facilitated clearance of the affected streptococci.

Respiratory infections induced by *Mycoplasma pneumoniae* are similar[25]. Studies of these infections in humans have shown that previous infection provides immunity to reinfection[15, 70]. Killed vaccines of *M. pneumoniae* administered systemically stimulate complement fixing and growth inhibiting antibodies, yet this vaccination produces only partial resistance to infection[65]. Such vaccinated individuals generally show a rise in serum antibody titre following challenge infection, indicating the infecting organism colonized and provided an antigenic stimulus. However individuals with naturally acquired immunity usually do not show a rise in serum antibody titre following challenge infection, suggesting that their immunity interferred with colonization of the organisms before they could act as an antigenic stimulus.

Studies of *Corynebacterium diphtheriae* provide another example. This organism possesses protein surface components, designated K antigens, which are type specific[5]. However the toxin elaborated by different serotypes of *C. diphtheriae* is serologically homogeneous. The toxoid commonly used for immunization against diphteria is derived from serotype K5. Individuals immunized with this vaccine develop anti-toxin antibodies which are protective against the toxin derived from all serotypes, and they also elicit antibodies against K5 surface antigen. Epidemiological studies have indicated that such vaccinated individuals do not become infected subsequently with type K5 *C. diphtheriae*[5,41]. They may become infected by other serotypes of the organism, but they do not develop toxemia due to the presence of toxin neutralizing antibodies. However, in some cases, the colonization of other serotypes can progress to the point where infants die of the bacterial mat which develops on the mucosal surfaces of the throat.

Each of the examples selected for discussion indicate that a type of specific immunity exists locally which affects the initial colonization of pathogenic bacteria on the mucosal surfaces of man and animals. The basis of this immunity is likely to involve the secretory immune system, and it would seem to entail the more rapid clearance of infectious bacteria from mucous membranes, thereby preventing their colonization.

The specificity of bacterial adherence to mucosal and other cell surfaces, and the apparent presence of receptor sites for bacterial attachment are analogous to phenomena which are well documented for viral infections. Secretory immunoglobulins are believed to affect viral agents prior to their replication on

mucosal surfaces, whereas systemic antibodies act at the point of viremia and viral dissemination, and prevent their replication in peripheral tissues[78]. Thus the hypotheses proposed concerning the role of the secretory immunoglobulin system in preventing colonization of infectious bacteria has precedent with other infectious agents. It is evident that the role of adherence in bacterial colonization of mucosal surfaces, which was first recognized with indigenous oral streptococci, has broad applicability to a wide range of host-parasite relationships. Knowledge of this ecological parameter may assist in the development of vaccines and modes of immunization which will provide protective immunity against infectious bacteria. For example, in attempts to induce immunity in future studies, it would seem wise to include efforts to stimulate the secretory immune system, and to monitor parameters which relate to the adherence and colonization of bacteria on mucosal surfaces. These concepts are consistant with the observations of several investigators who have reported the induction of an immune state following oral immunization with vaccines of infectious bacteria[24, 27, 45, 57, 84].

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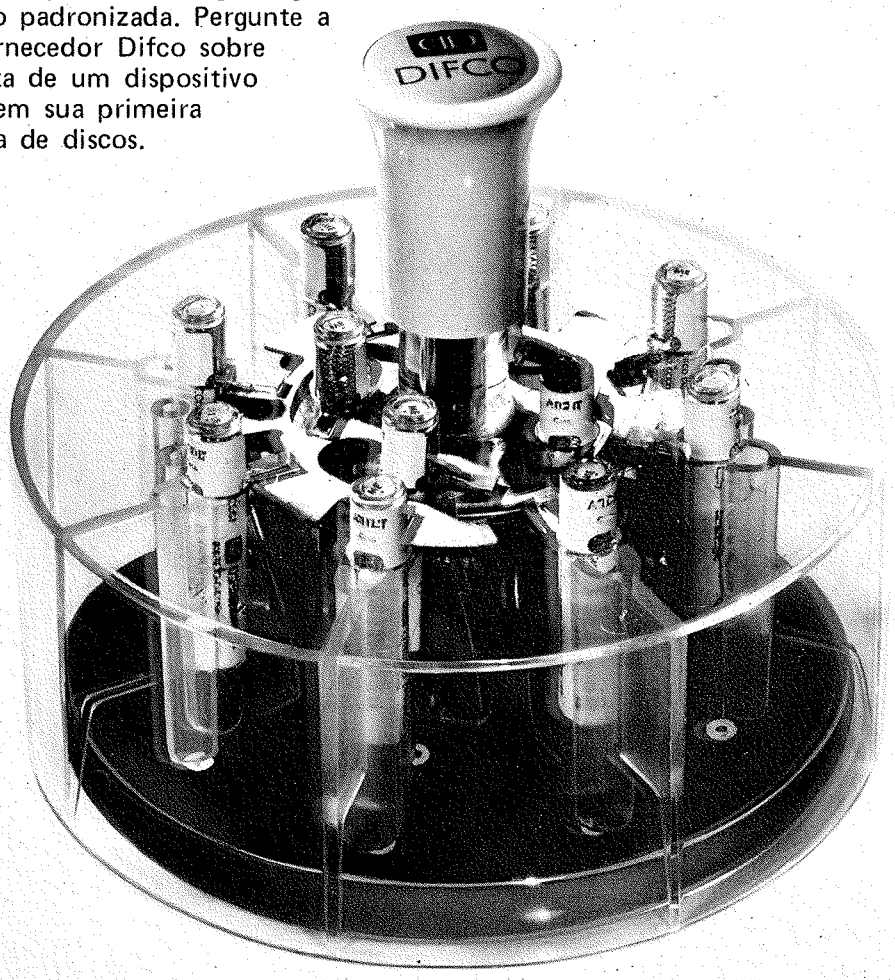

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