INFECTION AND IMMUNITY, Nov. 1970, p. 683-685

Vol. 2, No. 5
Printed in U.S.A.

In Vitro Plaque Formation by Several Oral Diphtheroids Implicated in Periodontal Disease

G. J. HAGEAGE, JR., I. JOHANSSEN,[1] J. M. TANZER[2]

Laboratory of Biological Structure and Laboratory Studies Section, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014

Received for publication 13 July 1970

The in vitro plaque-forming ability of oral diphtheroids is not dependent upon the type of sugar incorporated in the culture medium.

Oral diphtheroid filament-forming bacteria have recently been implicated as infectious agents leading to plaque formation and periodontal disease in conventional hamsters (4, 5, 10, 11, 14) and gnotobiotic rats (2, 9; S. Socransky, *personal communication*). Microorganisms of this type have been shown to form bacterial plaques in vitro. Only limited information concerning the carbohydrate requirements supporting plaque formation is available (12, 13, 16). This paper describes the in vitro formation of bacterial plaques by several strains of oral diphtheroids and its essential lack of dependency upon the nature and concentration of various carbohydrates incorporated into the growth medium.

Ten strains of oral diphtheroids were employed in this study. Among these, two strains of *Actinomyces viscosus* (1), T-6 and HS-69, were isolated from hamsters and induce periodontal disease in hamsters (4, 7, 10). Four strains, 7, 12, and 28 (supplied by S. Socransky, Forsyth Dental Center, Boston, Mass.) and RF-7 (supplied by H. Jordan, Forsyth Dental Center, Boston, Mass.), were isolated from rats and are conducive to periodontal pathosis in rats and hamsters (11; P. Keyes, *personal communication*). Four strains, 5, 6, 8 (15, 16), and N-16 (supplied by H. Jordan), were isolated from institutionalized mentally retarded humans, but their pathogenicity in experimental animals has not been established.

The oral diphtheroids were maintained by biweekly passage in fluid Thioglycollate Medium (BBL) containing 20% horse meat infusion and an excess of $CaCO_3$. For plaque growth study, 0.2 ml of a 24-hr thioglycollate culture was inoculated into test tubes (20 by 150 mm) containing 10 ml of the complex medium of Jordan, Fitzgerald, and Bowler (8), containing 5 mg of Na_2CO_3 per 100 ml and to which various filter-sterilized carbohydrate solutions had been added aseptically to give final concentrations of 0.5 and 5.0% (w/v). Broth containing either dextrose, fructose, sucrose, raffinose, or starch was tested as was basal medium without added carbohydrate.

The method of McCabe et al. (16) was used to grow in vitro plaques on wires. After 5 days of serial wire transfer, the resultant plaque growth was scored (16), serially transferred into tubes of uninoculated broth for an additional 5 days of incubation, and scored again. The purity of cultures was periodically checked by Gram staining and by plating on the medium described by Tanzer and Hageage (18).

Plaque growth ratings after 5 and 10 days are summarized in Table 1. All strains were found to form plaques in the broths tested. Of the strains studied, acknowledged pathogens (strains T-6, HS-69, RF-7, 7, 12, and 28) did not produce more plaques than those strains (5, 6, 8, and N-16) whose pathogenicity had not been established.

There was no clear enhancement of plaque formation by any particular sugar at the concentrations employed, although dextrose-, fructose-, sucrose-, or raffinose-containing broths generally supported higher levels of plaque formation than did basal medium with or without starch. In general, 10-day plaque ratings were one to two grades higher than plaque ratings at 5 days. However, plaque ratings at 10 days sometimes showed a marked increase over those at 5 days, even though inoculation of fresh broth had been discontinued except by means of wire passage. This phenomenon suggests the enrichment by wire passage of the cultures by mutant microorganisms having increased adhesiveness, an enrichment mechanism similar to that suggested for certain streptococci (17). Decreases in the amount of plaque from day 5 to day 10, especially prominent with strain HS-69 when grown

[1] Participant, Program in Dental Research for College Students sponsored by the American Dental Association.

[2] Present address: Veterans Administration Hospital, Newington, Conn. 06111.

TABLE 1. *In vitro plaque rating*

Microorganisms	Dextrose				Fructose				Sucrose				Raffinose				Starch				No sugar added	
	0.5%		5%		0.5%		5%		0.5%		5%		0.5%		5%		0.5%		5%			
	5 days[a]	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
Associated with periodontal pathosis in hamsters																						
Isolated from hamsters																						
T-6	3	3[b]	3	3[b]	2	5[b]	1	5[b]	3	5[b]	4	5[b]	2	3[e]	2	3[e]	<1	0[e]	2	4[d]	1	<1[e]
HS-69	3	4[b]	3	4[b]	5	1[e]	4	1[e]	5	4[c]	4	3[c]	3	5[d]	2	3[e]	<1	<1[e]	<1	3[b]	<1	<1[e]
Isolated from rats																						
7	3	5[c]	2	3[c]	2	4[c]	3	5[c]	2	3[c]	3	3[c]	1	3[b]	2	3[c]	2	2[b]	<1	4[a]	1	2[d]
12	3	5[c]	3	5[c]	3	4[c]	3	4[d]	3	3[c]	2	3[c]	3	3[c]	3	3[d]	1	2[d]	1	4[c]	1	2[b]
28	1	4[c]	2	4[c]	2	4[c]	1	4[e]	2	3[c]	2	3[c]	1	3[d]	1	4[d]	<1	3[e]	<1	3[c]	1	2[e]
RF-7	2	5[c]	2	4[c]	3	3[c]	2	3[e]	3	4[c]	2	4[c]	2	3[e]	1	4[c]	<1	2[e]	<1	2[d]	<1	1[d]
Pathogenicity not established in test animals																						
Isolated from mentally retarded humans																						
5	4	5[b]	4	5[b]	3	5[b]	<1	<1[b]	5	5[b]	4	5[b]	4	5[b]	4	5[b]	<1	2[c]	<1	3[b]	<1	2[e]
6	3	4[b]	4	4[c]	2	3[c]	4	3[c]	4	5[c]	3	5[c]	3	4[b]	4	5[b]	3	3[b]	<1	4[e]	<1	1[b]
8	3	5[b]	4	4[c]	2	3[c]	<1	<1[b]	4	5[b]	<1	3[b]	3	4[b]	4	5[b]	<1	<1[b]	2	2[d]	<1	<1[b]
N-16	4	5[c]	3	4[c]	<1	3[b]	3	2[b]	4	4[b]	4	4[c]	3	4[c]	3	3[c]	<1	2[b]	<1	2[b]	<1	2[b]

[a] Days of incubation at 37 C.
[b] Hard, rough-appearing plaque which is not lost at transfer (*see* Fig. 1a).
[c] Soft, rough-appearing plaque some of which may be lost at wire transfer (*see* Fig. 1b).
[d] Firm, smooth-appearing plaque which is not lost at wire transfer (*see* Fig. 1c).
[e] Loose, smooth-appearing plaque which may be lost at wire transfer.

in fructose-containing medium, were due to the loss of plaques from the wires during transfer and reflected their poor adhesiveness.

The in vitro plaques varied in consistency and morphology (Table 1). Four general types of plaque were observed (Fig. 1): a hard, rough-appearing plaque which was not lost in transfer (a); a soft rough-appearing plaque some of which was lost in transfer (b); a firm, smooth-appearing plaque which was not lost in transfer (c); and a smooth-appearing plaque of such consistency that it tended to completely slide off the wire during transfer. It was impossible to satisfactorily photograph the latter. Preliminary electron microscopic observations of these plaques failed to reveal any ultrastructural correlation between extracellular bacterial products and the morphological types observed.

By contrast with the dependency of *Streptococcus mutans* plaque formation upon the presence of sucrose in the culture medium (3), no common correlation existed between the carbohydrate incorporated into the culture medium and either the quantitative rating of plaque formed by the oral diphtheroids or its qualitative consistency. *A. viscosus* strains T-6 and HS-69 have been described as forming levans but not dextrans when grown in the presence of sucrose or raffinose (6). However, neither levan nor dextran formation was observed in the presence of fructose or dextrose, or both (6). Thus, the present observations indicate that the plaque-forming ability of the diphtheroids tested cannot be simply ascribed to the synthesis of extracellular dextran or levan by these cells. The formation of plaques in the presence of various carbohyrates and the variable consistency of these plaques suggest that the composition of extracellular bacterial products germane to the process of adhesion is likely to be complex and perhaps variable.

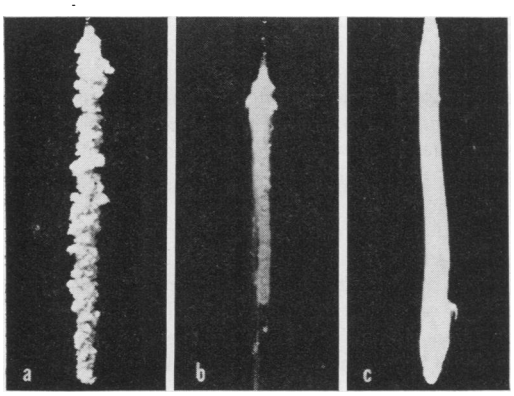

FIG. 1. *Photograph showing morphology of plaque formed on wires by oral diphtheroids. (a) Hard, rough-appearing plaque which is not lost at transfer of wires. (b) Soft, rough-appearing plaque some of which is lost in transfer of wires. (c) Firm, smooth-appearing plaque which is not lost in transfer of wires.*

LITERATURE CITED

1. Georg, L. K., L. Pine, and M.A. Gerencser. 1969. *Actinomyces viscosus*, comb. nov., a catalase positive, facultative member of the genus Actinomyces. Int. J. Syst. Bacteriol. 19:291–293.
2. Gibbons, R. J. 1969. Bacteriology of peridontal disease, p. 49–56. *In* Proc. 26th Annu. Meeting Amer. Inst. Oral Biol., Palm Springs, Calif.
3. Gibbons, R. J., and M. Nygaard. 1968. Synthesis of insoluble dextran and its significance in the formation of gelatinous deposits by plaque-forming streptococci. Arch. Oral Biol. 13:1249–1262.
4. Howell, A. 1963. A filamentous microorganism isolated from periodontal plaque in hamsters. I. Isolation, morphology, and general culture characteristics. Sabouraudia 3:81–92.
5. Howell, A., and H. V. Jordan. 1963. A filamentous microorganism isolated from periodontal plaque in hamsters. II. Physiological and biochemical characteristics. Sabouraudia 3:93–105.
6. Howell, A., and H. V. Jordan. 1967. Production of an extracellular levan by *Odontomyces viscosus*. Arch. Oral Biol. 12:571–573.
7. Howell, A., H. V. Jordan, L. K. Georg, and L. Pine. 1965. *Odontomyces viscosus*, gen. nov., spec. nov., a filamentous microorganism isolated from periodontal plaque in hamsters. Sabouraudia 4:65–68.
8. Jordan, H. V., R. J. Fitzgerald, and A. E. Bowler. 1960. Inhibition of experimental caries by sodium metabisulfite and its effect upon the growth and metabolism of selected bacteria. J. Dent. Res. 39:116–123.
9. Jordan, H. V., R. J. Fitzgerald, and H. R. Stanley. 1965. Plaque formation and periodontal pathology in gnotobiotic rats infected with an oral actinomycete. Amer. J. Pathol. 47:1156–67.
10. Jordan, H. V., and P. H. Keyes. 1964. Aerobic, gram-positive filamentous bacteria as etiological agents of experimental periodontal disease in hamsters. Arch. Oral Biol. 9:401–414.
11. Jordan, H. V., and P. H. Keyes. 1965. Studies in the bacteriology of hamster periodontal disease. Amer. J. Pathol. 46:343–357.
12. Jordan, H. V., and P. H. Keyes. 1966. *In vitro* methods for the study of plaque formation and carious lesions. Arch. Oral Biol. 11:793–801.
13. Jordan, H. V., P. H. Keyes, and S. Lim. 1969. Plaque formation and implantation of *Odontomyces viscosus* in hamsters fed different carbohydrates. J. Dent. Res. 48:824–831.
14. Keyes, P. H., and H. V. Jordan. 1964. Periodontal lesions in the Syrian hamster. III. Findings related to an infectious and transmissible component. Arch. Oral Biol. 9:377–400.
15. Littleton, N. W., R. M. McCabe, and C. H. Carter. 1967. Studies on oral health in persons nourished by stomach tube. II. Acidogenic properties and selected components of plaque material. Arch. Oral Biol. 12:601–609.
16. McCabe, R. M., P. H. Keyes, and A. Howell. 1967. *In vitro* method for assessing the plaque forming ability of oral bacteria. Arch. Oral Biol. 12:1653–1656.
17. Tanzer, J. M., and R. M. McCabe. 1968. Selection of plaque-forming streptococci by the serial passage of wires through sucrose-containing broth. Arch. Oral Biol. 13:139–143.
18. Tanzer, J. M., and G. J. Hageage, Jr. 1970. Polyphosphate inhibition of growth of plaques formed by streptococci and diphtheroids implicated in oral disease. Infec. Immun. 1:604–606.