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POSSIBLE ROLE OF MYCOPLASMAS IN PERIODONTAL DISEASE

BY

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ABSTRACT

Seventy-five subjects were examined for the incidence of isolation of mycoplasmas from their oral cavities, identification of mycoplasma strains isolated, the number of viable mycoplasma organisms in oral cavities, and antibody response to M. salivarium and M. orale 1. The subjects were divided into 21 edentulous and 54 dentulous ones. Dentulous subjects were subdivided tentatively into normal, gingivitis, and periodontitis groups on the basis of their periodontal score. Mycoplasmas were isolated from 7 of 21 edentulous subjects, all of whom did not use complete dentures, 18 of 22 subjects of normal group, and all of the subjects in gingivitis and periodontitis groups.

The number of viable mycoplasma organisms in oral cavities was significantly greater in gingivitis and periodontitis groups than edentulous and normal groups. There was a tendency for subjects in the periodontitis group to have a greater number of viable mycoplasma organisms than those in the gingivitis group, although there was no significant difference between these two groups.

The number of M. salivarium and M. orale 1 strains isolated from edentulous, normal, gingivitis and periodontitis groups was in the ratio 1:1, 1:1, 2:1, and 4:1, respectively.

On the other hand, as a result of titration of IHA antibodies to M. salivarium and M. orale 1 antigens in dentulous subjects, the incidence of detecting antibodies to M. salivarium antigen was lower in patients than in healthy persons, while to M. orale 1 antigen increased in the order of normal, gingivitis, and periodontitis groups.

INTRODUCTION

On the basis of a number of results accumulated so far, it is recognized in general that mycoplasmas are common inhabitants in oral cavities.

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Therefore, a variety of data concerning oral mycoplasmas are available such as the incidence of isolation, distribution, biological properties, and so on (Inoue[1], Morton[2,3], Burnett[4], Takeuchi[5], Shklair[6], Razin[7], Taylor-Robinson[8,9], Watanabe[10], Fox[11], Kumagai[12], Ohashi[13]).

The incidence of isolation of mycoplasma from oral cavities is very high and most of mycoplasma strains isolated are identified with M. salivarium and M. orale 1. It may not be unreasonable that oral mycoplasmas are regarded as M. salivarium and M. orale 1 in most cases. At the moment, the role of oral mycoplasmas in oral microbial flora or oral diseases has not been clarified. However, it may suggest a certain relation between oral mycoplasmas, especially, M. salivarium and periodontal disease that preferential sites of oral mycoplasmas are dental plaque, dental calculus, and gingival pocket, and that all mycoplasma strains isolated from dental calculus and gingival pocket are identified with M. salivarium. In addition, this speculation may be supported by a finding that the incidence of detecting neutralyzing antibodies to M. salivarium is overwhelmingly high in patients suffering from periodontal disease in comparison with persons of a control group (Kumagai[12]).

On the other hand, there are several indirect evidences for the conjecture that oral mycoplasmas may play a pathological role in oral cavities. First of all, it is demonstrated that M. salivarium, M. orale 1, M. orale 2, and M. hominis degrade arginine actively by the arginine dehydrolase pathway, which is not found in mammalian cells, involving arginine deiminase, ornithine transcarbamylase, and carbamyl phosphokinase (Schimcke[14], Barile[15]). Most of contaminants in tissue cultures are shown to be arginine-splitting mycoplasmas, which sometimes inhibit the growth of monolayer cells (Hayflick[16], Rouse[17]). Besides, some investigators noted that these mycoplasmas inhibited DNA and RNA synthesis in lymphocytes (Barile[18], Spitler[19]), and changed human cell chromosomes (Paton[20]). In addition, ammonia, an end product in the process of degradation of arginine, is toxic to animal cells, and possibly will raise the pH value of an affected region to produce an optimal atomosphere for formation of dental calculus which is closely associated with periodontal disease.

Furthermore, some M. salivarium strains are found to have lipase activities and some of them decompose egg yolk (Cole[21,22]). It is not confirmed yet which of lipase, lethicinase, and a proteolytic enzyme is causative in the egg yolk reaction, but the lipolytic activities will surely have a harmful effect on a cell membrane of animal cells.

We examined subjects with and without periodontal disease for the incidence of isolation of mycoplasmas from their oral cavities, the number

of viable mycoplasma organisms in them, and antibody response to M. salivarium and M. orale 1. As a result, it was proved that patients suffering from periodontal disease harbored a greater number of viable mycoplasma organisms in the oral cavities than healthy persons and that M. salivarium is predominant in ones with periodontal disease although M. salivarium and M. orale 1 are isolated almost at an equal frequency from normal oral cavities. However, any significant relationship between antibody response to oral mycoplasmas and periodontal disease could not be established (Watanabe[23], Horikawa[24], Ohashi[13]).

In the present study, edentulous subjects, and dentulous subjects who were divided into a normal group, a gingivitis group, and a periodontitis group on the basis of Russell's Periodontal Index, were examined for the above-mentioned items.

Materials and Methods

Subjects tested: Subjects were patients who had visited the clinics of the Department of Periodontology and the 3rd Department of Prosthetic Dentistry, Tokyo Medical and Dental University, and students of dental hygienist school attached to the same University. The oral cavities of 75 subjects, both male and female, were examined. The subjects, ranging in age from 18 to 70 years, were composed of 21 edentulous ones, 3 of whom used complete dentures, and 54 dentulous ones. Dentulous subjects were divided tentatively into normal group, gingivitis group, and periodontitis group on the basis of the periodontal score determined for each of dentulous subjects in accordance with the criteria of Russell's Periodontal Index (Russell[25], Table 1). The score was calculated by the following formula on the authority of the observation of gingiva around six teeth $\frac{6|14}{41|6}$ and

Table 1. Classification of dentulous subjects

Classification	Periodontal score	Number of subjects	
Normal	0 —1.0	6	
	1.1—2.0	16	22
Gingivitis	2.1—3.0	3	
	3.1—4.0	3	
	4.1—5.0	4	
	5.1—6.0	8	18
Periodontitis	6.1—7.0	6	
	7.1—8.0	8	14
Total		54	54

observation on the full mouth X-ray films. The general health of all subjects was satisfactory.

$$\text{Periodontal score} = \frac{\text{Sum of individual score}}{\text{Number of teeth observed}}$$

In this paper, patients and healthy persons correspond to patients suffering from gingivitis or periodontal disease and subjects who have clinically healthy gingiva, respectively.

Media: The fluid medium was composed of Difco 'PPLO' broth (70%), horse serum (20%), and 25% yeast extract (10%); this was supplemented with arginine-HCl (1%), phenol red (0.002%), penicillin (1000 u/ml), and thallium acetate (1:2000). The medium was adjusted to pH 7.0. The solid medium was composed of the same components without arginine-HCl and phenol red, and with agar (1%) added. The medium was adjusted to pH 7.8.

Collection of samples: Samples were collected by making subjects gargle vigorously with 10 ml of trypticase soy broth (BBL) containing 0.5% bovine albumin, limitedly in the oral cavity for 1 min. At the same time, 10 ml of peripheral blood was drawn from only the dentulous subjects. Serum was separated and preserved at $-20°C$.

Determination of the number of viable mycoplasma organisms in oral cavities and isolation of mycoplasmas: The samples were diluted in serial 10-fold steps with the fresh medium. An amount of 0.1 ml of undiluted sample and each dilution were inoculated on agar plates, which were incubated anaerobically at 37°C for 5 days. After incubation, the number of colonies produced on agar plates were counted and the number of viable mycoplasma organisms in oral cavities was recorded as the number (CFU, colony-forming unit) in 1 ml of gargle. Then the isolation of mycoplasmas was attempted. A pure culture of each isolate was obtained after 5 times of cloning.

Identification of mycoplasma strains isolated: The isolates were identified by growth inhibition test (Clyde[26]). The antisera to M. salivarium (a standard strain obtained from Dr. R. M. Chanock, NIH, U.S.A.), M. orale 1 (strain Ch19299 obtained from Dr. R. M. Chanock), and M. hominis (strain PG-21 obtained from Dr. D. Taylor-Robinson, Clinical Research Centre, Middlesex, England) were prepared according to Taylor-Robinson's method (Taylor-Robinson[27]). Reciprocal antibody titres of prepared antisera to homogeneous mycoplasma antigens were 10240 by IHA (Taylor-Robinson[8]) and 2024 by metabolic inhibition test (Purcell[28]). The antisera produced 7-mm growth inhibition zone on the agar medium on which 10^6 CFU of mycoplasma organisms were inoculated. Additionally, the anti-

sera were highly specific.

Titration of antibodies to M. salivarium and M. orale 1 in subjects' sera: Subjects' sera were tested for IHA antibodies to M. salivarium and M. orale 1 antigens using microtitration systems described by Taylor-Robinson[8].

Results

Incidence of isolation of mycoplasmas: Mycoplasmas were isolated from 7, who did not use dentures, of 21 (33%) edentulous subjects, 18 of 22 (82%) subjects belonging to normal group and all (100%) the subjects belonging to both gingivitis and periodontitis groups (Tables 2–5).

Number of viable mycoplasma organisms in oral cavities: The number of viable mycoplasma organisms in oral cavities was recorded as CFU (colony-forming unit) in 1 ml of gargle (Tables 2–5). From Table 6 and Fig. 1, it is obvious that a greater number of mycoplasmas inhabit in dentulous oral cavities than in edentulous ones. Furthermore, the results

Table 2. Number of viable mycoplasma organisms in oral cavities (Edentulous group)

Subject No.	CFU	Log_{10} CFU
57	1.5×10^4	4.1761
58	0 (1)*	0
59	5.0×10	1.6990
60	4.0×10	1.6021
61	0 (1)*	0
62	0 (1)*	0
63	0 (1)*	0
64	0 (1)*	0
65	0 (1)*	0
66	0 (1)*	0
67	0 (1)*	0
68	0 (1)*	0
69	0 (1)*	0
70	3.7×10^4	4.5682
71	0 (1)*	0
72	9.0×10^2	2.9542
73	0 (1)*	0
74	3.8×10^3	3.5798
75	0 (1)*	0
76	1.0×10^2	2.0000
77	0 (1)*	0

* Instead of 0, 1 was given for the convenience of the logarithmic manifestation of CFU.

Table 3. Number of viable mycoplasma organisms in oral cavities (Normal group)

Subject No.	CFU	Log_{10} CFU
2	7.6×10^3	3.8808
3	0 (1)*	0
4	0 (1)*	0
5	7.8×10^2	2.8921
6	1.3×10^2	2.1139
7	0 (1)*	0
8	9.0×10	1.9542
9	1.0×10^3	3.0000
10	1.1×10^3	3.0414
11	0 (1)*	0
27	3.7×10^3	3.5682
28	1.8×10^3	3.2553
29	1.5×10^3	3.1761
30	5.0×10^2	2.6990
31	1.8×10^4	4.2553
32	1.2×10^3	3.0792
36	2.7×10^4	4.4314
37	2.0×10^4	4.3010
38	5.0×10^2	2.6990
47	8.0×10^4	4.9031
50	4.0×10^4	4.6021
56	1.4×10^3	3.1461

* Instead of 0, 1 was given for the convenience of the logarithmic manifestation of CFU.

indicate that mycoplasmas grow better in patients' oral cavities than in healthy persons' (Table 6, Fig. 1). When the mean of CFU of each group was compared, there was a statistically significant difference between edentulous group and normal group, and between normal group and each of gingivitis and periodontitis groups, but there was no significant difference between gingivitis group and periodontitis group (Table 7).

Isolation of mycoplasmas: Five hundred and three mycoplasma strains (169 from normal group, 172 from gingivitis group, 130 from periodontitis group, and 32 from edentulous group) were obtained (Tables 8–11).

Identification of mycoplasma strains isolated: By growth inhibition test, 318 strains, 182 strains, and 3 strains were identified with M. salivarium, M. orale 1, and M. hominis, respectively (Tables 8–11). In consequence, it was reconfirmed that oral mycoplasmas were mostly M. salivarium and M. orale 1, and that M. hominis, M. orale 2, and M. orale 3 were extremely rare to be detected from oral cavities. Additionally, M. salivarium and M. orale 1 were isolated at an almost equal frequency from

Table 4. Number of viable mycoplasma organisms in oral cavities (Gingivitis group)

Subject No.	CFU	Log_{10} CFU
12	6.0×10^4	4.7782
13	8.0×10^3	3.9031
15	1.8×10^3	3.2553
16	5.1×10^3	3.7076
17	2.6×10^3	3.4150
18	1.0×10^4	4.0000
19	1.8×10^4	4.2553
21	2.2×10^4	4.3424
23	2.0×10^3	3.3010
35	3.0×10^3	3.4771
41	1.2×10^6	6.0792
43	1.0×10^4	4.0000
44	1.8×10^4	4.2553
45	4.0×10^5	5.6021
46	4.0×10^3	3.6021
48	1.5×10^3	3.1761
49	6.0×10^4	4.7782
51	1.6×10^3	3.2041

Table 5. Number of viable mycoplasma organisms in oral cavities (Periodontitis group)

Subject No.	CFU	Log_{10} CFU
14	2.8×10^3	3.4472
20	2.5×10^4	4.3979
22	1.8×10^5	5.2553
24	1.0×10^4	4.0000
25	7.5×10^3	3.8751
26	3.6×10^4	4.5563
33	2.7×10^6	6.4314
39	1.1×10^3	3.0414
40	8.0×10^6	6.9031
42	3.0×10^3	3.4771
52	9.0×10^3	3.9542
53	5.0×10^4	4.6990
54	1.0×10^6	6.0000
55	6.0×10^4	4.7782

the edentulous group (Table 8) and normal group (Table 9), while the number of M. salivarium and M. orale 1 strains isolated from gingivitis group (Table 10) and periodontitis group (Table 11) were in the ratio of 2:1 and 4:1, respectively. Another thing is that 16 of 18 (89%) subjects in the gingivitis group and 13 of 14 (93%) subjects in the periodontitis

Table 6. Distribution of subjects against the number of viable mycoplasma organisms in oral cavities

Log_{10} CFU	Edentulous	Normal	Gingivitis	Periodontitis
0.0000—1.0000	14	4	0	0
1.0001—2.0000	3	1	0	0
2.0001—3.0000	1	5	0	0
3.0001—4.0000	1	7	11	6
4.0001—5.0000	2	5	5	4
5.0001—6.0000	0	0	1	2
6.0001—7.0000	0	0	1	2
Total	21	22	18	14

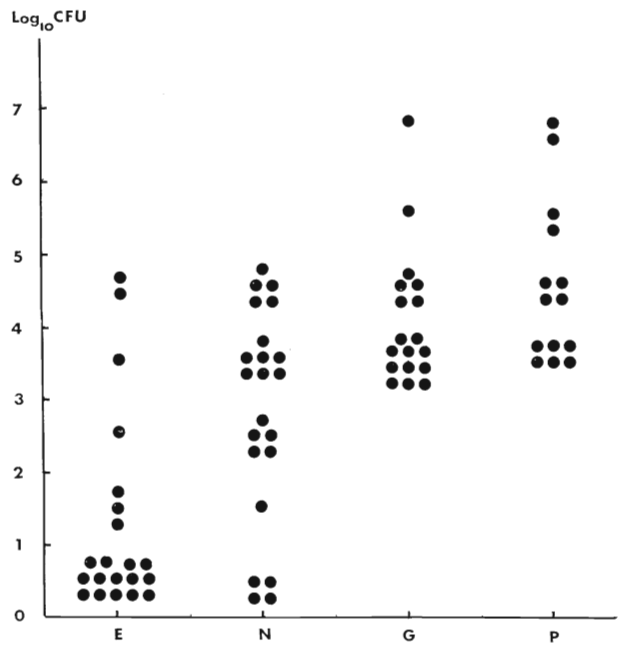

Fig. 1. Distribution of subjects against the number of viable mycoplasma organisms in oral cavities.

group harbored M. salivarium while 11 of 22 (50%) subjects in the normal group and 3 of 21 (14%) subjects in the edentulous group did (Table 12, Fig. 2). The number of subjects harboring both M. salivarium and M. orale 1 increased in the order of edentulous, normal, gingivitis, and periodontitis groups (Table 13); that is, no (0%) subjects of edentulous group, 3 of 22

Table 7. Mean of $\log_{10}$ CFU in each group

Groups	Mean
Edentulous	1.3777
Normal	2.7726
Gingivitis	4.0629
Periodontitis	4.6233

Table 8. Identification of mycoplasma strains isolated (Edentulous group)

Subject number	Number of isolates	Mycoplasma		
		salivarium	orale 1	hominis
57	6	0	6	0
59	4	4	0	0
60	4	0	4	0
70	4	4	0	0
72	4	0	4	0
74	4	0	4	0
76	6	6	0	0
Total	32	14	18	0
Ratio		1 :	1.3 :	0

Table 9. Identification of mycoplasma strains isolated (Normal group)

Subject number	Number of isolates	Mycoplasma		
		salivarium	orale 1	hominis
2	9	0	9	0
5	10	10	0	0
6	10	1	9	0
8	10	0	8	2
9	9	0	9	0
10	10	10	0	0
27	10	0	10	0
28	9	9	0	0
29	9	0	9	0
30	7	7	0	0
31	10	4	6	0
32	10	10	0	0
36	9	0	9	0
37	9	9	0	0
38	8	2	6	0
47	10	10	0	0
50	10	0	10	0
56	10	10	0	0
Total	169	82	85	2
Ratio		1 :	1 :	0

Table 10. Identification of mycoplasma strains isolated (Gingivitis group)

Subject number	Number of isolates	Mycoplasma		
		salivarium	orale 1	hominis
12	10	10	0	0
13	9	9	0	0
15	10	10	0	0
16	9	9	0	0
17	10	6	4	0
18	9	9	0	0
19	10	2	8	0
21	8	3	5	0
23	9	3	6	0
35	10	7	3	0
41	10	10	0	0
43	10	0	10	0
44	10	10	0	0
45	10	10	0	0
46	10	2	8	0
48	8	8	0	0
49	10	10	0	0
51	10	0	10	0
Total	172	118	54	0
Ratio		2.2 :	1 :	0

Table 11. Identification of mycoplasma strains isolated (Periodontitis group)

Subject number	Number of isolates	Mycoplasma		
		salivarium	orale 1	hominis
14	10	10	0	0
20	7	4	2	1
22	6	5	1	0
24	10	9	1	0
25	9	8	1	0
26	10	8	2	0
33	10	10	0	0
39	8	7	1	0
40	10	0	10	0
42	10	10	0	0
52	10	10	0	0
53	10	10	0	0
54	10	10	0	0
55	10	3	7	0
Total	130	104	25	1
Ratio		4.2 :	1	0

Table 12. Ratio of the number of M. salivarium-positive subjects to the total of the subjects in each group

Group	Ratio
Edentulous	3/21 (14%)
Normal	11/22 (50%)
Gingivitis	16/18 (89%)
Periodontitis	13/14 (93%)

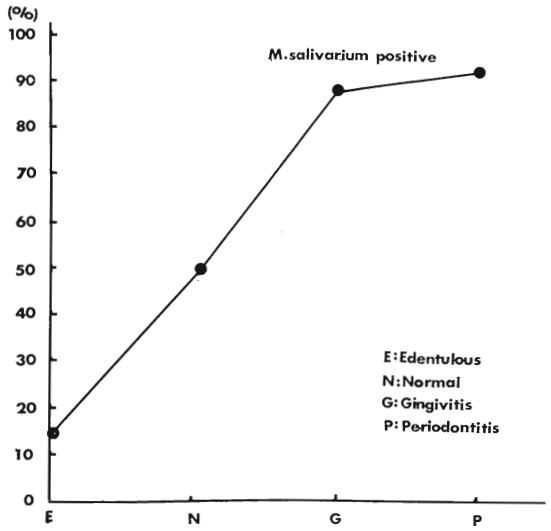

Fig. 2. Ratio of the number of M. salivarium-positive subjects to the total of the subjects in each group.

Table 13. Ratio of the number of M. salivarium and M. orale 1-positive subjects to the total of the subjects in each group

Group	Ratio
Edentulous	0/21 (0%)
Normal	3/22 (14%)
Gingivitis	6/18 (33%)
Periodontitis	7/14 (50%)

(14%) subjects of normal group, 6 of 18 (33%) subjects of gingivitis group, and 7 of 14 (50%) subjects of periodontitis group were demonstrated to harbor both M. salivarium and M. orale 1. However, it is not clear whether or not this phenomenon has any meaning.

Detection of IHA antibodies to M. salivarium and M. orale 1 antigens

Table 14. Titration of IHA antibodies to M. salivarium and M. orale 1 (Normal group)

Subject number	IHA antibody titre to	
	M. salivarium	M. orale 1
2	∠8	∠8
3	16	∠8
4	∠8	∠8
5	≧512	≧512
6	∠8	∠8
7	≧512	∠8
8	∠8	∠8
9	∠8	∠8
10	∠8	∠8
11	∠8	∠8
27	32	∠8
28	8	∠8
29	32	∠8
30	128	8
31	∠8	∠8
32	256	∠8
37	8	∠8
38	128	∠8
47	∠8	∠8
50	32	∠8
56	∠8	∠8

Table 15. Titration of IHA antibodies to M. salivarium and M. orale 1 (Gingivitis group)

Subject number	IHA antibody titre to	
	M. salivarium	M. orale 1
12	∠8	∠8
13	∠8	64
15	∠8	∠8
16	∠8	∠8
17	∠8	∠8
18	∠8	∠8
19	∠8	∠8
21	≧512	32
23	∠8	∠8
35	16	∠8
41	∠8	8
43	∠8	16
44	16	∠8
46	∠8	∠8
48	∠8	∠8
49	∠8	∠8
51	∠8	∠8

Table 16. Titration of IHA antibodies to M. salivarium and M. orale 1 (Periodontitis group)

Subject number	IHA antibody titre to	
	M. salivarium	M. orale 1
14	<8	<8
20	128	<8
22	≧512	≧512
24	<8	<8
25	<8	<8
26	≧512	≧512
33	≧512	64
39	<8	<8
40	<8	<8
42	<8	<8
52	16	64
53	<8	<8
54	<8	<8
55	<8	8

Table 17. Incidence of detecting IHA antibodies to M. salivarium and M. orale 1 in each group

Subjects	Incidence of detecting IHA antibodies to	
	M. salivarium	M. orale 1
Normal	52.38% (11/21)	14.29% (3/21)
Gingivitis	16.67% (3/17)	22.22% (4/17)
Periodontitis	35.71% (5/14)	35.71% (5/14)

in dentulous subjects (Tables 14–16): IHA antibody to M. salivarium antigen was detected from 11 of 21 (52%) subjects in the normal group, 3 of 18 (17%) subjects in the gingivitis group, and 5 of 14 (36%) subjects in the periodontitis group. On the other hand, IHA antibody to M. orale 1 antigen was detected from 3 of 21 (14%) subjects in the normal group, 4 of 18 (22%) subjects in the gingivitis group, and 5 of 14 (36%) subjects in the periodontitis group (Table 17).

Discussion

Razin[7] demonstrated that natural dentitions were essential for mycoplasmas to inhabit in oral cavities. In the present study, however, mycoplasmas were isolated from edentulous subjects without dentures, although it is apparent, judging from a very much lower incidence of isolation of mycoplasmas and smaller number of viable mycoplasma organisms in eden-

tulous oral cavities than in dentulous ones, that natural dentitions will provide better conditions for mycoplasmas to inhabit in oral cavities.

The number of viable mycoplasma organisms was significantly greater in the oral cavities of gingivitis and periodontitis groups than normal and edentulous groups. At the same time, there was a tendency for subjects in the periodontitis group to harbor a little larger number of mycoplasma organisms than those in the gingivitis group, although there was not any significant difference between them. Besides, the incidence of isolation of mycoplasmas from dentulous subjects was extremely high and 500 of 503 (99.4%) isolates were M. salivarium and M. orale 1. They were in the ratio of 1:1 in the normal group, which is in agreement with the data we published before (Watanabe[23]), and edentulous group, 2:1 in the gingivitis group, and 4:1 in the periodontitis group. In addition, it was revealed that most of the patients harbored M. salivarium in their oral cavities.

These results seem to imply that mycoplasma organisms in oral cavities continue to propagate in parallel with the advancement of gingivitis and that the speed of the propagation becomes slow at the stage of periodontitis permitting that mycoplasma flora of oral cavities shifts from the equilibrium of M. salivarium and M. orale 1 to M. salivarium predominancy.

Moreover, the afore-mentioned findings, in combination with the preferential sites for mycoplasmas to inhabit, predominancy of M. salivarium in the areas and biological activities of arginine-splitting mycoplasmas described in INTRODUCTION will support the speculation that mycoplasmas, especially M. salivarium, may play a certain role in periodontal disease.

On the other hand, limited information is available concerning antibody response to M. salivarium and M. orale 1 in patients. Above all, Kumagai[12] detected neutralyzing antibodies to M. salivarium from 62% of the patients while from 8% of the subjects in the control group. Such an exceedingly high incidence of detection of neutralyzing antibodies to M. salivarium from patients in comparison with a control group will give ground in part for the argument that M. salivarium may participate in periodontal diseases.

In the past, we attempted to determine IHA and CFT antibody titres to M. salivarium and M. orale 1 in healthy persons' sera. This attempt resulted in the detection of IHA antibodies to M. salivarium from 48% of healthy persons, although IHA antibodies to M. orale 1 and CFT antibodies to M. salivarium and M. orale 1 could not be detected (Watanabe[23]). Ohashi[13] in our laboratory detected IHA antibodies to M. salivarium from 58% of patients, but could not detect IHA antibodies to M. orale 1 and CFT antibodies to M. salivarium and M. orale 1. Consequently, no signifi-

cant relationship could be established between antibody response to M. salivarium and periodontal disease.

In the present study, the incidence of detecting IHA antibodies to M. salivarium was lower in patients than in healthy persons. We do not know its reason, but there are some reports which will support our data. For instance, Kennedy[29] titrated antibodies to cariogenic streptococci in caries-free and caries-rampant subjects' sera. It was demonstrated that caries-free subjects have antibodies at a higher incidence and greater titre than caries-rampant subjects. This is supposed to suggest that it is because they have antibodies of greater titre more frequently that caries-free subjects have no caries.

If this is also the case with mycoplasmas, it may be supposed that hosts can produce antibodies to mycoplasmas, a member of common inhabitants in oral cavities, depending on their congenital ability or constitution. As a result, hosts who produced antibodies to mycoplasmas may be protected from their infection in most cases, and this may be the reason why the incidence of detecting IHA antibodies was lower in patients than in healthy persons.

Finally, in the present study, it was shown that the incidence of detecting IHA antibodies to M. orale 1 increased in the order of normal, gingivitis, and periodontitis groups. This remains to be explained.

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References

1) Inoue, T.: Über die Peripneumonia-Virus ähnlichen, filtrierbaren Mikroorganismen, isoliert von der Mundhöhle des Menschen. (in Japanese). Osaka Igakukai Zasshi (Mitt. Med. Ges. Osaka), 39: 1795–1814, 1940.

2) Morton, H. E., Smith, P. F., Williams, N. B., and Eickenberg, C. F.: Isolation of PPLO from human saliva: A newly detected member of the oral flora. J. Dent. Res., 30: 415–422, 1951.

3) Morton, H. E., Smith, P. F., and Keller, R.: Prevalence of PPLO and the evaluation of media and methods for their isolation from clinical material. Amer. J. Pub. Health, 42: 913–924, 1952.

4) Burnett, G. W., and Gilmore, E.: Incidence and distribution of oral PPLO and

L-organisms. J. Dent. Res., 38: 632, 1959.
5) Takeuchi, T.: Isolation of PPLO from the human oral cavity. (in Japanese, with English summary). Shika Gakuho (J. Tokyo Dent. Coll. Soc.), 62: 305–315, 1962.
6) Shklair, I. L., Mazzarella, M. A., Gutekunst, R. R., and Kiggins, E. M.: Isolation and incidence of PPLO from the human oral cavity. J. Bacteriol., 83: 785–788, 1962.
7) Razin, S., Michmann, J., and Shimshoni, Z.: The occurrence of Mycoplasma (pleuropneumonia-like organisms, PPLO) in the oral cavity of dentulous and edentulous subjects. J. Dent. Res., 43: 402–405, 1964.
8) Taylor-Robinson, D., Canchola, J., Fox, H., and Chanock, R. M.: A newly identified oral Mycoplasma (M. orale) and its relationship to other human mycoplasmas. Amer. J. Hyg., 80: 135–148, 1964.
9) Taylor-Robinson, D., Fox, H., and Chanock, R. M.: Characterization of a newly identified Mycoplasma from the oropharynx. Amer. J. Epidemiol., 81: 180–191, 1965.
10) Watanabe, T.: Biological and serological studies on oral Mycoplasma. Bull. Tokyo Med. Dent. Univ., 14: 447–460, 1967.
11) Fox, H., Purcell, R. H., and Chanock, R. M.: Characterization of a newly identified Mycoplasma (Mycoplasma orale Type 3) from the human oropharynx. J. Bacteriol., 98: 36–43, 1969.
12) Kumagai, K., and Iwabuchi, T.: Kōkū biseibutsu to shite no Mycoplama—tokuni shisōnōro tono kanren ni oite (Mycoplasma as a member of oral microorganisms—especially, relation between Mycoplasma and periodontal disease). (in Japanese). Shikai Tembo (Dent. Outlook), 33: 785–794, 1969.
13) Ōhashi, Y.: Studies on oral Mycoplasma in relation to periodontal disease. (in Japanese, with English summary). J. Japan. Stomatol. Soc., 38: 363–374, 1971.
14) Schmicke, R. T., and Barile, M. F.: Arginine metabolism in pleuropneumonia-like organisms isolated from mammalian cell culture. J. Bacteriol., 86: 195–206, 1963.
15) Barile, M. F., Schimcke, R. T., and Riggs, D. B.: Presence of the arginine dehydrolase pathway in Mycoplasma. J. Bacteriol., 91: 189–192, 1966.
16) Hayflick, L., and Stanbridge, E.: Isolation and identification of Mycoplasma from human clinical materials. Ann. N. Y. Acad. Sci., 143: 608–621, 1967.
17) Rouse, H. C., Bonifas, V. H., and Schlesinger, R. W.: Dependence of Adenovirus replication of arginine and inhibition of plaque formation by pleuropneumonia-like organisms. Virology, 20: 357–365, 1963.
18) Barile, M. F., and Leventhal, B. G.: Possible mechanism for Mycoplasma inhibition of lymphocyte transformation induced by phytohaemagglutinin. Nature, 219: 751–753, 1968.
19) Spitler, L., Cochrum, K., and Fudenberg, H. H.: Mycoplasma inhibition of phytohemagglutinin stimulation of lymphocytes. Science, 161: 1148–1149, 1968.
20) Paton, G. A., Jacobs, J. P., and Perkins, F. T.: Chromosome changes in human diploid-cell cultures infected with Mycoplasma. Nature, 207: 43–45, 1965.
21) Cole, B. C., and Pease, P.: Lipolytic activity by oral pleuropneumonia-like (Mycoplasma) organisms. J. Gen. Microbiol., 47: 171–174, 1967.
22) Cole, B. C., Ward, J. R., Golightly-Rowland, L., and Graham, C. E.: Characterization of mycoplasmas isolated from the great apes. Can. J. Microbiol., 16: 1331–1339, 1970.
23) Watanabe, T., Ohashi, Y., Takamori, K., and Horikawa, T.: Kōkū kara bunri shita Mycoplasma ni tsuite (Studies on mycoplasmas isolated from oral cavities). (in Japanese). Japan. J. Bacteriol., 24: 578–579, 1969. (Proceeding).
24) Horikawa, T., Takamori, K., Watanabe, T., and Ōhashi, Y.: Shisōnōrosho to Mycoplasma ni tsuite (Relationship between periodontal disease and Mycoplasma). (in Japanese). Japan. J. Oral Biol., 12: 268, 1970. (Proceeding).

25) Russell, A. L.: A system of classification and scoring for prevalence surveys of periodontal disease. J. Dent. Res., 35: 350–359, 1956.
26) Clyde, W. A.: Mycoplasma species identification based upon growth inhibition by specific antisera. J. Immunol., 92: 958–965, 1964.
27) Taylor-Robinson, D., Somerson, N. L., Turner, H. C., and Chanock, R. M.: Serological relationships among mycoplasmas as shown by complement-fixation and gel diffusion. J. Bacteriol., 85: 1261–1273, 1963.
28) Purcell, R. H., Taylor-Robinson, D., Wong, D., and Chanock, R. M.: Color test for the measurement of antibody to T-strain mycoplasmas. J. Bacteriol., 92: 6–12, 1966.
29) Kennedy, A. E., Shklair, I. L., Hayashi, J. A., and Bahn, A. N.: Antibodies to cariogenic streptococci in humans. Arch. Oral Biol., 1: 1275–1278, 1968.