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The influence of denture hygiene and the bacterial flora on the condition of the oral mucosa in full denture cases.

By

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Introduction.

There have been many investigators whose clinical observations have led them to the opinion that inadequate denture hygiene may result in the appearance of inflammatory changes of the soft tissues beneath removable dentures. Inefficient cleansing of the plate permits the accumulation of food particles and detritus between it and the mucosa, providing conditions favourable to the formation of bacterial media. The protection against temperature changes renders the environment still more suitable. The products of decomposition from food remains and detritus may set up irritation of the mucosa.

In this connection there has been much discussion of the porosity of the denture base and the consequent retention of bacteria, food particles, desquamated epithelial cells and other detritus.

The mechanical effect of the denture on the mucosa is generally considered conducive to bacterial infection.

Survey of the literature.

The literature provides little in the way of experimental material in support of the theories advanced.

Hygienic care of dentures.

W. H. WRIGHT (1933) states that for the 100 patients cited the average extent to which cleansing was performed was $2^2/_3$ times a day. There is no discussion of any relation between the frequency of cleansing and the condition of the mucosa.

LEIMEISTER (1942) and VON WEISSENFLUH (1945) accept the general aspects of the importance of denture hygiene dealt with summarily in the introduction, and proceed to a treatment of the bactericidal and mechanical cleansing efficiency of some commercial preparations.

The author has been unable to find mention in the literature of any worker who has presented experimental case series or has been concerned with a possible relation between inadequate denture hygiene and the frequency of the inflammation of the soft tissues commonly termed denture sore mouth.[1]

Bacteriological studies.

SCHMENGLER (1922) mentions an attempt to examine the antihygienic properties associated with the rubber denture. This he effected by calculating the number of micro-organisms per field of vision on a number of stains, observing a case series and using an experimental denture of rubber which he himself wore. His conclusions were that the number of bacteria is greater the longer a denture is worn, and that the mucosa beneath a rubber plate invariable exhibits pronounced inflammatory changes.

PRYOR published in 1924 the results of an investigation on bacterial growths on dentures. In the first series of tests he attached various sorts of metal foil to halves of plate surfaces; no details are given of the types of foil used. After some time samples were taken with a sterile glass rod of 4 mm diameter, from both the foil-covered area and the rubber base itself. He stated that after 48 hours cultures from all the metal surfaces yielded a lower number of colonies than those from the rubber surfaces.

In a second series he laid different metal foils in an unspecified number of cultures of staphylococcus pyogenes aureus. Judging from his photographs he obtained a bacteriostasis. The bac-

[1] Denture sore mouth will in the following pages be abbreviated to DSM.

teriostatic effect was in his opinion best for 20 carat gold and worst for tin. He concluded that the growth of bacteria has probably some significance in the occurrence of hyperaemia sometimes found under dentures: it might be a direct effect or due to irritation.

Yeast-like organisms.

At the end of the 1920s attention was directed on the different kinds of yeast-like organisms and their importance as sources of disease. It was perhaps CASTELLANI and OKABE who were principally responsible for this through their comprehensive work of systematization and diagnostics.

CAHN states, in a study "The Denture Sore Mouth" (1936), that in cultivation of samples from dentures by a technique he does not mention he invariably found on, for example, Loeffler plates a yeast fungus of the type Monilia (Candida) albicans. No mention is made of the manner of differentiating between the types. As far as the material is concerned, it is certain only that it consisted of four patients in an extremely low state of health (two anaemic, one nephritic and one diabetic.) CAHN himself is very cautious when drawing conclusions from his work: "I have no decisive proof that the yeast is the responsible factor." It appears from the text that he was working with BARTELS, who issued an original paper in 1937 where the method is mentioned in greater detail. Sabouraud medium was used for the cultivation. Identification and differentiation of the fungus types were effected from the macroscopic and microscopic appearance of the colonies. Of the case series he says: "During the past year a few patients have come under my observation." Only two are mentioned in detail, both of whom recovered after treatment with a 5% aqueous solution of gentian violet; furthermore, they kept their dentures in hypochlorite during the night, as did CAHN's subjects. BARTELS is also cautious in his conclusions: "It is important to emphasize ... that not all cases of denture sore mouth are due to infection by yeast-like organisms."

KNIGHTON (1939) reckons to have shown that in his material consisting of 146 patients there was no correlation between the occurrence of yeast-like organisms and the health of the gingiva. The author performed several cultivations from patients (both edentulous and with teeth) wearing plates. Twelve of these

suffered from subjective irritation of the mucosa while 11 presented no such symptoms. According to the author the series is too small to justify the drawing of inferences. Comparing cases with and without dentures, he found that Monilia (Candida) albicans occurs with equal frequency in both groups. The difference in incidence of growths other than Monilia albicans was, however, significantly higher for those who wore dentures.

It would appear, then, that the question of the significance of yeast-like organisms, and perhaps of Monilia (Candida) albicans in particular, in the occurrence of DSM is by no means resolved. The hitherto published investigations say only that while these organisms should not be neglected entirely, too great importance should not be attributed to them.

Spirochaetes and fusiforms.

Spirochaetes and fusiforms play some rôle in a number of pathological conditions of the gingiva. Their occurrence in the edentulous mouth has been studied by ROSENTHAL & GOOTZEIT (1942), among others. They investigated 212 edentulous cases, apparently without dentures (92 males, 120 females, of ages 21—87 years). The mucosa is stated to have been intact and without clinically observable inflammation. Stains from these cases are characterized by certain difficulties in colouring and a low number of micro-organisms. Staining and ultramicroscopic study of direct preparates revealed the presence of spirochaetes and fusiforms in 2 cases (0.9 %) and spirochaetes alone in 7 cases (3·3%). Unaccompanied fusiforms appeared in 23 cases (10·8%).

The same authors studied 46 edentulous patients with complete dentures of rubber, acrylic, metal, or combinations of these materials. The denture hygiene is reported to have varied from "very clean to extremely dirty". Neither fusiforms nor spirochaetes could be detected in these cases.

The authors studied 21 surfaces exhibiting traumatic injuries of the oral mucosa beneath full dentures. The character of the lesions of the soft parts varied from mild hyperaemia to ulceration. In this series, too, spirochaetes and fusiforms were absent. Both of these could, however, be identified in cases where some teeth remained if the preparates were taken in the neighbourhood of those teeth.

Studies of the bacterial flora of edentulous adults.

The bacterial flora has been studied experimentally with material which, as far as can be judged, comprised only edentulous adults with and without dentures. G. M. PEJRONE (1934, 1935) and A. VIVANCO, A. VILA & SR. J. ASENJO (1946) seem, however, to be the only workers to have dealt with this aspect. PEJRONE required his subjects to swill their mouth with 75 cc sterile water in three portions, each for 15 seconds. The liquid was then diluted to 1 : 100,000 and 1 cc poured on to blood agar plates, evidently for aerobic cultivation only. Two cultivations were performed for each subject. The average of the calculated numbers of colonies after 48 hours growth was termed the bacterial index for the individual concerned.

Thirty women and twenty men of 65—85 years, all edentulous subjects without plates, comprised the material for the study. The figures for the mean values of the index from PEJRONE's study point to a probable difference in bacteria index between cases with and without residual teeth. The cases with teeth would thus be associated with a greater number of bacteria.

The South American research group (VIVANCO, VILA and ASENJO) used a similar method in which the media were broth alone, broth with acetic acid, a tomato broth, an acid, a neutral and a glucose broth; and in addition, media of ordinary agar, ascitic agar, blood agar, tomato agar, an acid, a neutral and an acetic agar.

The case series consisted of 50 edentulous subjects with and 60 without dentures.

Their studies led them to the conclusions that edentulous persons fitted with dentures have a higher index (mean 3,364 · 6) than those without such appliances (mean 600 · 6), and that wearers of plates who neglect denture hygiene have higher indexes than more heedful persons.

The types of bacteria have been divided into permanent and occasional. The authors observed a constant recurrence, among those not wearing dentures, of certain types of bacteria, all of them aerobic: they were staphylococcus pyogenes, streptococcus pyogenes and lactobacillus acidophilus. Occasional types included B. subtilis, Sarcinae, Pneumococci and Diplococci, these also being aerobic. Practically the same flora were found where dentures were worn. Plates in poor hygienic condition were accom-

panied in particular by Leptothrix buccalis and B. fusiformis, their presence being confirmed by staining.

Neither PEJRONE nor VIVANCO and collaborators have described the condition of the mucosa, nor did they ascertain the significance of the bacterial flora concerned.

Surface porosity.

In the introduction it was stated that some importance was attributed to surface porosity. This will be dealt with more fully in a paper under preparation.

Aim of the present investigation.

The principle aim of the present investigation was thus to supply an answer to the question: *Is there any evidence that inadequate denture hygiene is a factor influencing the occurrence of inflammatory changes of the soft tissues under complete maxillary dentures?*

Within the framework of the main problem there have arisen a number of secondary questions. These will serve as a basis for the study of *denture hygiene practice* and its influence on the condition of the mucosa.

1. Has the frequency of cleansing any influence on the frequency of DSM?
2. Does the method of cleansing effect the frequency of DSM?

As a consequence of the discussions in the literature, subsidiary problems have arisen in connection with the *bacteriological studies*. The problems are stated in the propriate part of the text.

A study of denture cleansing practice.

Details of the frequency and method of cleansing the denture were registered on the punch card as a part of the case history record.[1] Two groups of patients were recognized: those who 'seldom' cleaned their dentures — that is, not more than twice a week —, and the others who gave frequencies of from once to more than three times a day.

[1] NYQUIST, G.: A study of denture sore mouth. Acta Odont. Scand., Supplementum 9; folded leaf between pp. 38 and 39.

Replies to such a question must of course be accepted with reserve, and there is some risk of a *classification error*. To eliminate this as far as possible the patients were questioned on two different occasions, the female patients often by the dental nurse. If contradictory information was obtained the patients were requested to attend a third time, when those points of the denture history of special interest were dealt with again. The magnitude of the classification error cannot be estimated.

The method of statistical analysis was the original of which an account is to be found elsewhere.[1]

Case series.

The material comprised the same full denture cases as constituted the case series of the author's investigation on denture sore mouth[2] (pages 33—38; 50—60; 127—140).

Of the original material of 1,301 cases 609 remained after sifting demanded by the statistical analysis. The frequency of DSM for this number was 27·1%. In addition to the tabulation described on p. 127—140 of the thesis a grouping was effected with respect to denture cleansing practice.

Results.

The significance of the frequency of cleansing.

One would expect to find a lower frequency of DSM among patients who often practise denture hygiene. The statistical analysis revealed a λ-value of 1·81 for the 'seldom' group against the others, and an estimated difference of 14%, with more DSM in the group with poorer cleansing habits — thus in the direction expected. The results of the analysis show, however, no statistically significant difference in the material.

The 'seldom' group is very small, consisting of only 12 cases. Any existing tendency would therefore have been difficult to detect. Since the λ-value was relatively high (the lower limit for the almost significant difference is 1·96, as the test can be considered two-tailed) it appeared desirable to try to collect a larger case series in the group with poorer oral hygiene. Those patients who cleaned their dentures but once a day were there-

[1] Ibid. pp. 143—154.
[2] See footnote to p. 25.

fore combined with those of the 'seldom' group. This is justified by the fact that any classification error must be constituted by some cases properly belonging to the 'seldom' group who are registered, on account of false statements, in the group who cleaned their dentures once a day. The combination gave two groups of roughly equal size. The statistical analysis gave a λ-value of $\cdot 50$ and an estimated difference of 2%. In this case the higher DSM frequency was found to be in the group who cleansed their dentures more diligently.

No significant difference in the frequency of DSM was found between the two groups in this classification either. The tendency was, moreover contrary, to that of the previous analysis.

Conclusions.

The material provides no support for the supposition that the number of times the denture is cleaned has any real effect on the occurrence of DSM under full upper dentures.

The significance of the various modes of cleansing.

The manner in which the denture is cleaned varies a good deal from one patient to another. Any one patient even does not always use the same cleansing agent and methods. On examination note was taken only of the mode of cleansing usually practised by the patient.

A closer study of these habits showed that a large number of patients use only *mechanical* means of cleaning in their daily denture hygiene: namely running water and some sort of brush — frequently a tooth or nail brush. Another large group of patients found it necessary to employ some form of *chemical cleanser in addition to the mechanical method.* Tooth paste, soap, scouring powder and disinfectant fluids were among the substances used.

It would be supposed that the most thorough cleansing would be obtained where both chemical and mechanical methods were used. If deficient denture hygiene played any decisive part in the occurrence of inflammations of the mucosa beneath dentures these cases should probably present a lower frequency of denture sore mouth than those that used only mechanical means.

A statistical analysis following the original method mentioned

above gave a λ-value of 0·98 and an estimated difference of 4% with the higher frequency of DSM for mechanical cleansing only.

There was thus no statistically significant difference in the frequency of DSM between the two groups.

Conclusions.

The material provided no support for the assumption that the mode of cleansing of dentures is of any essential importance in the occurrence of DSM under full upper dentures.

Discussion.

It has been suggested that it is the cleansing of the mucosa rather than of the denture that is the vital point. An experimental series of 50 cases of DSM who during 3 months cleansed both denture and mucosa twice a day have convinced the author that this view is false. For cleaning the mucosa a tooth brush with soft nylon fibres was used. No definite improvement in DSM could be observed. In this connection it might be of value for the avoidance of misunderstanding to repeat that the case series of the investigation consisted only of cases of full dentures and that the conclusions are true only for those cases. As soon as remaining natural teeth with periodontias and interproximal spaces come into the picture it must be realized that quite new factors have to be considered.

Bacteriological study.

Method.

All tests were made in the morning before the patients had taken food or drink.

After the denture had been rinsed under running water small pieces of sterile unsized paper were laid between the plate and the mucosa. The pieces were all punched in the same apparatus. Two pieces were laid initially in the glandular zones, two in the fatty zones and two in the regions of the alveolar process and torus.

Experience showed that results in satisfactory agreement could

be obtained with one piece of paper in the ventral area, generally in the region of the alveolar process, and one in the dorsal area in either the fatty or the glandular zone. This technique was therefore adopted throughout.

The paper was left between the plate and the mucosa for 10 minutes, after which it was removed with sterile tweezers and placed in 1 ml of ordinary broth of pH 7 · 2. After shaking, the liquid was diluted to 1 : 100, 1 : 1,000, and 1 : 10,000. The dilution 1 : 1,000 proved in preliminary tests to give the most consistent results. Further dilution sometimes resulted in no growth at all, while a lower degree of dilution rendered the calculation of colonies difficult on account of too dense a growth. The standard dilution of 1 : 1,000 was thus used in all the tests described here, with the exception of one or two cases of a control cultivation, when the greatest dilution of 1 : 10,000 was taken.

The cultivation was performed by spreading 1/10 cc of the broth on blood agar in Petri dishes. From each broth tube two applications were made for aerobic and two for anaerobic cultivation. The plates remained in the thermostat at 37°C for 48 hours.

The *anaerobic conditions* of growth were created by adding sodium carbonate to pyrogallol in Petri dishes rendered airtight with plasticine.

The *number of colonies* was calculated and multiplied by the dilution factor. If the number of colonies did not exceed 500, the whole plate was counted. As may be seen from Tables 7 a—7 d, this applied to the majority of the determinations. In the case of the very highest values, at least 5 sectors were counted, each comprising one eighth of the circumference. The *practice of counting* in sectors was often followed for the sake of clearness even when considering the whole plate. The average of the two cultivations from each broth tube was taken as the actual number of bacteria yielded by the test. By performing the tests two at a time, two figures were obtained, both of them average values.

Identification of the types of bacteria was effected by examining the macroscopic appearance of the colony on the plate, and by studying the morphology under the microscope after Gram staining. In this way it was easy to identify the various types of Staphylococci as well as Sarcina, Gaffkya tetragena, Bacillus subtilis, Proteus vulgaris and yeast fungi. No differentiation was made between the various strains of pseudodiphterial bacteria.

Tables 1.

Distribution of patients for bacteriological tests.

Rubber dentures

♂

Age of denture (in years)	Age of patient (in years)								
	0—49			50—59			60—		
	N	DSM	Σ	N	DSM	Σ	N	DSM	Σ
½—3	—	—	—	1	—	1	—	1	1
4—7	—	1	1	—	—	—	1	2	3
8—12	—	—	—	1	—	1	1	—	1

N	DSM	Σ
4	4	8

♀

Age of denture (in years)	Age of patient (in years)								
	0—49			50—59			60—		
	N	DSM	Σ	N	DSM	Σ	N	DSM	Σ
½—3	1	—	1	2	1	3	3	3	6
4—7	2	3	5	2	1	3	3	3	6
8—12	1	1	2	1	4	5	3	3	6

N	DSM	Σ
18	19	37

This is true also of Neisseria strains, which were probably for the most part catarrhalis.

Differentiation of streptococci was performed according to Scherman's scheme with some small modifications.

Each patient was tested for *oral spirochaetes*. Samples were taken directly from the mucosa with flame-sterilized platinum loops. These samples were stained by KLEIN's (1943) method and examined through an ultramicroscope.

Case series.

The total material consisted of 58 cases with clinically normal mucosa and 56 cases with denture sore mouth. The distributions with respect to sex, patient and dentures ages, and type of base material are clear from Table 1. The selection is probably fairly representative of the complete re-examined material.

Tables 1 (cont.).

Acrylic dentures

♂

Age of denture (in years)	Age of patient (in years)								
	0—49			50—59			60—		
	N	DSM	Σ	N	DSM	Σ	N	DSM	Σ
½—3	1	1	2	—	—	—	3	1	4
4—7	—	—	—	1	1	2	1	—	1
8—12	—	—	—	—	—	—	—	—	—

N	DSM	Σ
6	3	9

♀

Age of denture (in years)	Age of patient (in years)								
	0—49			50—59			60—		
	N	DSM	Σ	N	DSM	Σ	N	DSM	Σ
½—3	4	6	10	2	7	9	3	3	6
4—7	1	—	1	9	8	17	10	4	14
8—12	—	1	1	—	1	1	1	—	1

N	DSM	Σ
30	30	60

For aerobic bacteria there are values from all these cases.

In the case of the anaerobic cultivations reliable values were obtained in 53 normal cases and 55 of DSM.

Tables 2 a—2 d show the frequencies of the two samples taken on the same occasion (r_1 and r_2) for both aerobic and anaerobic bacterial strains.

All cases were taken in serial order with the exception of No. 64 of the aerobic normal cases. This was taken from the fixation dentures of an edentulous patient operated on for mandibular prognathism and should not therefore be considered as a full denture case.

Of the anaerobic cases with clinically normal mucosas, tests Nos. 40, 43, 44, 45 and 52 have been excluded, since only one value was obtained from the cultivations.

Of those cases which had DSM, aerobic test No. 7 has been excluded on the grounds of contamination.

Table 2 a.

Number of aerobic bacteria in cases with clinically normal mucosa.

Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$	Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$
33	S. L.	36	32	34	61	E. L.	5	8	6.5
34	G. N.	32	48	40	62	A. C.	22	30	26
35	A. L.	11	10	10.5	63	A. G.	600	800	700
36	A. E.	100	80	90	65	H. J.	120	80	100
37	T. E.	100	120	110	66	H. W.	200	150	175
38	M. B.	2	4	3	67	A. R.	700	650	675
39	E. A.	20	24	22	68	A. T.	8	8	8
40	A. P.	240	208	224	69	P. M.	50	60	55
41	V. L.	160	128	144	70	B. J.	28	25	26.5
42	M. K.	8	6	7	71	S. D.	50	46	48
43	O. P.	80	120	100	72	F. Ö.	133	120	126.5
44	M. G.	40	44	42	73	E. D.	600	550	575
45	M. E.	8	8.8	8.4	74	I. B.	10	11	10.5
46	S. L.	8	12	10	75	S. K.	10	15	12.5
47	M. L.	120	96	108	76	S. G.	75	81	78
48	E. S.	96	32	64	77	E. K.	22	15	18.5
49	E. R.	8	8.8	8.4	78	H. A.	46	60	53
50	A. R.	4	24	14	79	A. S.	30	26	28
51	H. R.	48	80	64	80	H. J.	7	10	8.5
52	H. S.	6.4	16	11.2	81	A. B.	5	8	6.5
53	A. S.	40	20	30	82	L.	50	70	60
54	H. S.	64	24	44	83	H. J.	95	86	90.5
55	N. S.	30	24	27	84	S. P.	75	80	77.5
26: 2	E. K.	30	36	33	85	K. G.	130	150	140
56	E.	10	9	9.5	86	P. N.	50	40	45
57	H. K.	24	24	24	87	A. A.	80	90	85
58	H. B.	10	15	12.5	88	A. N.	120	88	104
59	O. E.	200	200	200	89	M. S.	40	40	40
60	H. K.	1000	1500	1250	90	T. J.	150	200	175

n = 58

Number of bacteria given in 1000s.

Results.

1. Comparison between the numbers of bacteria in cases of DSM and clinically normal mucosas.

An examination was made to determine whether the number of bacteria was greater for DSM than normal cases. For this purpose the case series was divided into groups with geometric intervals. Using the mean value of the two samples taken on the same occasion the following table is obtained.

See table 3.

It seems that there is no trend towards a higher bacterial

Table 2 b.

Number of anaerobic bacteria in cases with clinically normal mucosa.

Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$	Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$
34	G. N.	20	20	20	65	H. J.	110	60	85
35	A. L.	10	10	10	66	H. W.	30	40	35
36	A. E.	80	90	85	67	A. R.	400	400	400
37	T. E.	120	90	105	68	A. T.	7	8	7.5
38	M. B.	2	6	4	69	P. M.	83	60	71.5
39	E. A.	36	30	33	70	R. I.	20	20	20
33: 2	A. L.	40	52.8	46.4	71	S. D.	40	42	41
41	V. L.	80	160	120	72	F. Ö.	110	135	122.5
42	M. K.	1.6	3.2	2.4	73	E. D.	500	500	500
46	S. L.	8.8	8	8.4	74	I. B.	10	10	10
47	M. L.	120	108	114	75	S. K.	5	6	5.5
48	E. S.	48	24	36	76	S. G.	200	130	165
49	E. R.	4	12	8	77	E. K.	24	16	20
50	A. R.	8	8	8	78	H. A.	80	85	82.5
51	H. R.	12	80	46	79	A. S.	20	18	19
53	A. S.	40	40	40	80	H. J.	15	10	12.5
54	H. S.	80	72	76	81	A. B.	4	3	3.5
55: 2	N. S.	20	22	21	82	L.	200	120	160
26	E. K.	36	20	28	83	H. J.	80	70	75
56	E.	7	3	5	84	S. P.	50	56	53
57	H. K.	23	21	22	85	G.	160	150	155
58	H. B.	16	11	13.5	86	P. N.	30	28	29
59	O. E.	86	120	103	87	A. A.	60	75	67.5
60	H. K.	50	90	70	88	A. N.	100	100	100
61	E. L.	30	10	20	89	M. S.	22	26	24
62	A. C.	20	15	17.5	90	T. I.	150	140	145
63	A. D.	500	400	450					

n = 53

Number of bacteria given in 1000s.

population — either aerobic or anaerobic — for DSM than for normal mucosa cases.

This may be explained at least in part, however, by assuming that in bacteriological tests of this kind it is impossible to exclude quite appreciable *experimental errors*. To examine these variations more closely the results were treated statistically.

To obtain an approximate normal distribution of the observed values in the statistical analysis a logarithmic transformation was made. Analyses of significance of the mean values were performed by the t-test (HOEL 1948). For each subject double determination values, r_1 and r_2 were found. The influence of the method error was diminished by using $\frac{1}{2}$ (log r_1 + log r_2).

Table 2 c.

Number of aerobic bacteria in cases of DSM.

Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$	Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$
1	G. Y.	28	32	30	30	S. K.	68	50	52
2	A. W.	11	6	8.5	31	E. W.	14	13	13.5
3	E. A.	82	50	66	32	A.	20	24	22
4	L. C.	45	20	32.5	33	H. G.	90	85	87.5
5	A. S.	7	9	8	34	H. S.	90	95	92.5
6	F. N.	40	50	45	35	E. E.	200	200	100
8	A. G.	25	26	25.5	36	V.	75	75	75
9	E. L.	400	400	400	37	M. S.	53	65	59
10	E. I.	70	65	67.5	38	L.	6	7	6.5
11	E. G.	20	22	21	39	E. A.	80	60	70
12	E. Y.	10	20	15	40	W.	850	1000	925
13	A.	400	380	390	41	H. S.	400	460	430
14	E. G.	8	8	8	42	E. I.	225	230	227.5
15	E. L.	10	8	9	43	A. S.	40	42	41
16	T. K.	150	170	160	44	L. A.	30	25	27.5
17	E. M.	6	10	8	45	G. N.	16	20	18
18	I. S.	58	50	54	46	S. I.	11	16	13.5
19	S. P.	12	15	23.5	47	G. G.	200	160	180
20	Y. K.	24	22	23	48	H. A.	26	30	28
21	M. L.	110	100	105	49	A. K.	3	5	4
22	A. I.	1000	800	900	50	A. E.	60	63	61.5
23	A. E.	40	49	44.5	51	K. A.	500	400	450
24	E. N.	65	40	52.5	52	K. Z.	27	20	23.5
25	A. E.	12	10	11	53	A. A.	600	700	650
26	E. L.	15	17	16	54	B. K.	200	250	225
27	S. H.	50	50	50	55	H. A.	31	36	33.5
28	K.	60	50	55	56	A. P.	65	85	75
29	M.	3	5	4	57	A. D.	5	5	5

$n = 56$

Number of bacteria given in 1000s.

This gives, for *aerobic bacteria*

$$\bar{x} = 1.623 \qquad s_x = 0.576; \qquad n_x = 58$$
$$\bar{y} = 1.649 \qquad s_y = 0.605; \qquad n_y = 56$$

where $x = \frac{1}{2}$ (log r_1 + log r_2) for normal cases and $y = \frac{1}{2}$ (log r_1 + log r_2) for DSM cases.

It was found that the differences between the standard variations were negligible. The difference between the mean values is then $t = \cdot 235$ and $P = \cdot 81$. The difference is not significant.

Before any attempt is made to draw conclusions from this result the magnitude of the experimental error should be examined. The error for a single observation of log r_1 or log r_2 is constituted by:

Table 2 d.

Number of anaerobic bacteria in cases of DSM.

Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$	Pat. no.	Pat. in.	r_1	r_2	$\frac{r_1 + r_2}{2}$
3	E. A.	70	55	62.5	31	E. W.	10	8	9
2	A. W.	10	16	13	32	A.	20	20	20
4	A. C.	41	26	33.5	33	H. G.	50	55	52.5
5	A. G.	3	8	8.5	34	H. S.	100	80	90
1	G. I.	18	22	20	35	E. E.	200	150	175
6	F. N.	700	750	725	36	W.	80	90	85
8	A. G.	7	13	10	37	M. S.	70	58	64
9	E. L.	300	200	250	38	L.	4	6	5
10	E. I.	72	70	71	39	E. A.	15	20	17.5
11	E. G.	15	20	17.5	40	W.	400	500	450
12	E. I.	7	10	8.5	41	H. S.	300	380	340
13	A.	350	360	355	42	E. I.	150	150	150
14	E. G.	7	6	6.5	43	A. G.	60	55.5	57.75
15	E. L.	4	3	3.5	44	L. A.	10	8	9
16	T. K.	120	130	125	45	G. N.	20	22	21
17	E. M.	10	8	9	46	G. Y.	20	15	17.5
18	A. S.	40	40	40	47	G. S.	150	150	150
19	N. P.	8	6	7	48	H. A.	40	50	45
20	Y. K.	15	18	16.5	49	A. K.	3	4	3.5
21	M. L.	65	80	72.5	50	A. E.	60	60	60
23	A. E.	30	36	33	51	K. A.	400	400	400
24	E. N.	40	50	45	52	K. Z.	29	25	27
25	A. E.	10	8	9	53	A. A.	800	600	700
26	E. L.	12	10	11	54	B. A.	180	160	170
27	S. H.	60	50	55	55	H. A.	25	30	27.5
28	K.	50	40	45	56	A. P.	55	60	57.5
29	M.	4	3	3.5	57	A. D.	4	3	3.5
30	S. K.	30	28	29					

$n = 55$

Number of bacteria given in 1000s.

statistical fluctuations in the calculated bacteria population, the experimental error in the selected volumes, and fluctuations in the proportion of the number of bacteria adhering to the pieces of paper.

It can be seen from Table 4 that only an insignificant part of the spread in the material is subject to experimental error. It is thus possible to conclude that the difference between the populations in clinically normal and DSM cases is negligible.

In the same way as for the aerobic we have for *anaerobic bacteria*

$$\bar{x} = 1.543 \qquad s_x = 0.554 \qquad n_x = 53$$
$$\bar{y} = 1.551 \qquad s_y = 0.605 \qquad n_y = 55$$

Table 3.

Distribution of the numbers of bacteria in cases of clinically normal mucosa (N) and denture sore mouth (DSM).

1000s per ml of broth	Bacterial growth			
	Aerobic		Anaerobic	
	N	DSM	N	DSM
—3.5	1	—	2	4
4—7.5	3	4	4	3
8—15.5	12	10	7	9
16—31.5	8	10	11	10
32—63.5	11	13	8	12
64—127.5	13	7	14	6
128—255.5	6	5	4	5
256—511.5	—	4	3	4
512—1023.5	3	3	—	2
1024—2047.5	1	—	—	—
2048—	—	—	—	—
	58	56	53	55

Table 4.

Experimental error incurred in the aerobic bacteria tests.

	Tot. var.	Experimental error	Tot. var. if exp. error is removed
	S_x	$\frac{S_d}{2}$	$\sqrt{S_x^2-\left(\frac{S_d}{2}\right)^2}$
Clinically normal mucosa	·576	·093	·569
Denture sore mouth	·605	·060	·597

Table 5.

Experimental error incurred in anaerobic bacteria tests.

	Tot. var.	Experimental error	Tot. var. if exp. error is removed
	S_x	$\frac{S_d}{2}$	$\sqrt{S_x^2-\left(\frac{S_d}{2}\right)^2}$
Clinically normal mucosa	·554	·106	·542
Denture sore mouth	·620	·053	·615

The table indicates that again only an insignificant part of the spread of the material is subject to experimental error. It may be deduced that the difference between the anaerobic bacteria populations in clinically normal mucosa and DSM is negligible.

Conclusions.

The material provides no statistically significant difference between the numbers of bacteria counted in cases of DSM and clinically normal mucosas. The material is, from the statistical standpoint, so devoid of trend that the probability that a larger case series would yield a different result is small.

2. Comparison between the numbers of bacteria for any one subject on different days.

Case series and results.

To examine the individual fluctuations in the number of bacteria, 10 cases were tested 10 days in succession. The results are set out in Table 6 which gives the highest and lowest mean values of the double determinations, expressed in 1000s of bacteria per cc broth.

Table 6.

Subjects	Aerobic bacteria		Anaerobic bacteria	
	Max.	Min.	Max.	Min.
K. F. A.	500	135	410	140
G. S.	230	49	200	50
R. L.	225	93	175	55
L. A.	105	20	55	20
I. S.	70	11	47.5	7
I. W.	74	8.5	77.5	17
E. J.	73.5	11	21	5.5
E. L.	400	5.5	250	7
R. N.	71	13.5	67.5	9
E. A.	24	10	20	6

Conclusions.

Taking into account the previously calculated experimental error, the tables would seem to indicate that the average numbers

of bacteria are different from one person to another. Furthermore, the fluctuations for any one person would seem to apply within only a part of the region over which the values for the normal and DSM material are spread. The number of bacteria appears thus, in spite of very large variations, to be in some degree characteristic of the individual.

3. Comparison between the aerobic and anaerobic populations for an individual.

Case series.

For this comparison the values r_1 and r_2 for 50 normal and DSM cases were used.

No selection of the material was made. All cases with reliable values for both aerobic and anaerobic cultures were included.

Statistical analysis.

In spite of the skew distribution it was still possible to use the t-test because of the large number of observations ($n = 100$).

It is found that

$$t = 2.99 \qquad P \sim 0.005$$

There thus exists in the material a significant tendency towards a higher aerobic than anaerobic bacterial population for any one subject. Bearing in mind the comparatively unreliable method of anaerobic cultivation, however, this result should be accepted with reservation.

Conclusions.

The material exhibits a significant tendency towards larger aerobic than anaerobic populations for any one individual. The actual importance of this trend is however probably small, since the technique of anaerobic cultivation, although the best available, cannot be considered really satisfactory.

4. The dependence of the numbers of bacteria on the length of the testing period.

To study the importance of the time factor to the number of bacteria, tests were performed with 20 patients, using 10

Table 7.

Dependence of number of bacteria on the testing period.

Aerobic bacteria.

r_{10m}	r_{2-4h}
36	800
32	960
32	320
48	200
100	200
80	240
20	40
24	50
240	960
208	1280
56	160
40	200
160	960
128	640
8	90
9	80
48	640
80	480
6	64
16	40

minute and 2—4 hour periods in each case. Throughout the test the subjects fasted and refrained from drinking. The numbers of bacteria, expressed in 1000s are given in Table 12. Only the aerobic populations were used in this analysis, since earlier calculations showed that the actual differences between aerobic and anaerobic bacteria with the described method of cultivation were small.

Statistical analysis.

In all 20 cases the numbers of bacteria were considerably higher in the 2—4 hour than in the 10 minute tests.

The probability that the experimental error will result in a second value greater than the first is $\frac{1}{2}$, and the probability of obtaining the same result 20 times in succession is $\frac{1}{2^{20}}$. The probability of this event is thus approximately $\sim 0 \cdot 000{,}001$.

Discussion.

The high significance revealed may be due in part to the fact that during the testing period the unsized paper was able to absorb greater quantities of saliva and, with it, bacteria. It is

also conceivable that the bacteria associated with the 2—4 hour tests had an opportunity to multiply. As a check, tests were performed with the usual time (10 minutes) using fixation dentures after operations for mandibular protrusion. The surface of the plate facing the mucosa was not cleansed for 8 weeks nor immediately before the test. These 5 cases all presented considerably higher numbers of bacteria than were obtained from the other material. There is thus a slight possibility that the bacteria increase in number when the plate is worn for a period without cleansing. Whether this might influence the occurrence of inflammation of the mucosa is difficult to judge.

Conclusions.

Using the described testing procedure it was found that the bacteria increased in number when the length of the testing period was increased. There is, however, no justification for drawing any definite conclusion from this fact.

5. Comparison of the number of bacteria cultured from cases with rubber and acrylic dentures.

A number of authors have voiced the opinion that DSM is more frequent under rubber than under acrylic plates. It might be due to the greater porosity of the rubber. This would render it more difficult to maintain the denture in a hygienic condition, so that the number of bacteria would be greater in cases of patients wearing such dentures. It would account for the higher incidence of inflammation.

None of these authors offer descriptions of material in support of their statements, so that they can be of little real scientific value.

An analysis was performed to throw light on the problem of whether there is any difference in the bacteria populations beneath rubber and acrylic plates. The material was well suited to such a treatment.

Case series.

As has already been shown, there was no difference between bacteria populations in cases of normal mucosa and DSM. It

Table 8 a.

Comparison between bacteria frequency for rubber and acrylic dentures.

Aerobic bacteria. Acrylic dentures.

Pat. no.	r_1	x_1	r_2	x_2	$\frac{x_1+x_2}{2} = y$
35 N	11	1.041	10	1.000	1.020
36 N	100	2.000	80	1.903	1.952
38 N	2	0.301	4	0.602	0.452
39 N	20	1.301	24	1.380	1.340
41 N	160	2.204	128	2.107	2.156
43 N	80	1.903	120	2.079	1.991
44 N	40	1.602	44	1.644	1.623
45 N	8	0.903	9	0.954	0.929
46 N	8	0.903	12	1.079	0.991
47 N	120	2.079	96	1.982	2.031
48 N	96	1.982	32	1.505	1.744
49 N	8	0.903	9	0.954	0.929
50 N	4	0.602	24	1.380	0.991
51 N	48	1.681	80	1.903	1.792
52 N	6	0.778	16	1.204	0.991
54 N	64	1.806	24	1.380	1.593
55 N	30	1.477	24	1.380	1.429
26 N	30	1.477	36	1.556	1.517
56 N	10	1.000	9	0.954	0.977
58 N	10	1.000	15	1.176	1.088
62 N	22	1.342	30	1.477	1.409
63 N	600	2.778	800	2.903	2.840
68 N	8	0.903	8	0.903	0.903
71 N	50	1.699	46	1.663	1.681
74 N	10	1.000	11	1.041	1.020
76 N	75	1.875	81	1.909	1.892
77 N	22	1.342	15	1.176	1.259
84 N	75	1.875	80	1.903	1.889
85 N	130	2.114	150	2.176	2.145
86 N	50	1.699	40	1.602	1.650
87 N	80	1.903	90	1.954	1.929
90 N	150	2.176	200	2.301	2.239
4 I	45	1.653	20	1.301	1.477
5 I	7	0.845	9	0.954	0.899
6 I	40	1.602	50	1.699	1.650
11 I	20	1.301	22	1.342	1.321
12 I	10	1.000	20	1.301	1.150
14 I	8	0.903	8	0.903	0.903
15 I	10	1.000	8	0.903	0.952
16 I	150	2.176	170	2.230	2.203
17 I	6	0.778	10	1.000	0.889
18 I	58	1.763	50	1.699	1.731
19 I	12	1.079	15	1.176	1.128
20 I	24	1.380	22	1.342	1.361
22 I	1000	3.000	800	2.903	2.952
23 I	40	1.602	49	1.690	1.645
24 I	65	1.813	40	1.602	1.708
26 I	15	1.176	17	1.230	1.203
29 I	3	0.477	5	0.699	0.588
31 I	14	1.146	13	1.114	1.130
32 I	20	1.301	24	1.380	1.340

Table 8 a.

(cont.)

Pat. no.	r_1	x_1	r_2	x_2	$\frac{x_1+x_2}{2} = y$
33 I	90	1.954	85	1.929	1.942
34 I	90	1.954	95	1.978	1.966
35 I	200	2.301	200	2.301	2.301
36 I	75	1.875	75	1.875	1.875
37 I	53	1.724	65	1.778	1.751
38 I	6	0.778	7	0.845	0.812
39 I	80	1.903	60	1.778	1.840
40 I	850	2.929	1000	3.000	2.964
41 I	400	2.602	460	2.663	2.633
42 I	225	2.352	230	2.362	2.357
45 I	16	1.204	20	1.301	1.253
46 I	11	1.041	16	1.204	1.123
47 I	200	2.301	160	2.204	2.253
49 I	3	0.477	5	0.699	0.588

was therefore possible, for the purpose of this analysis, to combine these two classes of material. Values were obtained for 65 cases of aerobic and 62 of anaerobic cultivations using acrylic plates, and 44 aerobic and 40 anaerobic using rubber. Tables 8 a—8 d give the frequencies and averages.

So as to approach as near as possible to the real population both r_1 and r_2 were considered in the analysis.

Statistical analysis.

A student's test was performed.

Thus, for the aerobic bacteria $t = -1.751$, $P \sim 0.08$
and, for the anaerobic bacteria $t = -1.460$, $P \sim 0.15$.

Conclusions.

For the aerobic bacteria an almost significant trend is obtained towards a larger population for rubber than for acrylic dentures. The actual magnitude of this difference is small, the mean of the aerobic bacteria for rubber being about 1.5 times greater than for acrylic. Taking into account the wide spread, one should not attribute much importance to this difference. For the anaerobic bacteria no significant value was obtained.

Table 8 b.

Comparison between bacteria frequency for rubber and acrylic dentures.

Aerobic bacteria. Rubber dentures.

Pat. no.	r_1	x_1	r_2	x_2	$\frac{x_1+x_2}{2} = y$
37 N	100	2.000	120	2.079	2.040
40 N	240	2.380	208	2.318	2.349
42 N	8	0.903	6	0.778	0.840
53 N	40	1.602	20	1.301	1.452
57 N	24	1.380	24	1.380	1.380
59 N	200	2.301	200	2.301	2.301
60 N	1000	3.000	1500	3.176	3.088
61 N	5	0.699	8	0.903	0.801
65 N	120	2.079	80	1.903	1.991
67 N	700	2.845	650	2.813	2.829
69 N	50	1.699	60	1.778	1.738
70 N	28	1.447	25	1.398	1.423
72 N	133	2.124	120	2.079	2.102
73 N	600	2.778	550	2.740	2.759
75 N	10	1.000	15	1.176	1.088
79 N	30	1.477	26	1.415	1.446
80 N	7	0.845	10	1.000	0.923
81 N	5	0.699	8	0.903	0.801
83 N	95	1.978	86	1.935	1.957
88 N	120	2.079	88	1.945	2.012
89 N	40	1.602	40	1.602	1.602
1 I	28	1.447	32	1.505	1.476
2 I	11	1.041	6	0.778	0.909
3 I	82	1.914	50	1.699	1.807
8 I	25	1.398	26	1.415	1.407
9 I	400	2.602	400	2.602	2.602
10 I	70	1.845	65	1.813	1.829
13 I	400	2.602	380	2.580	2.591
25 I	12	1.079	10	1.000	1.040
27 I	50	1.699	50	1.699	1.699
28 I	60	1.778	50	1.699	1.738
30 I	68	1.833	50	1.699	1.766
43 I	40	1.602	42	1.623	1.612
44 I	30	1.477	25	1.398	1.438
48 I	26	1.415	30	1.477	1.446
50 I	60	1.778	63	1.799	1.789
51 I	500	2.699	400	2.602	2.650
52 I	27	1.431	20	1.301	1.366
53 I	600	2.778	700	2.845	2.812
54 I	200	2.301	250	2.398	2.349
55 I	31	1.491	36	1.556	1.524
56 I	65	1.813	85	1.929	1.871
37 BI	25	1.398	22	1.342	1.370
57 I	5	0.699	5	0.699	0.699

6. Comparison between types of bacteria cultured in the DSM and normal cases.

Case series.

Differentiation of the bacterial types was effected on the 112 cultivations described above.

Results.

By far *the most common type* of bacteria was *Neisseria catarrhalis*. It appeared in 54 of 58 normal and in 54 of 56 DSM cases. Staphylococcus albus came next in order of frequency, with 37 of the 58 normal and 39 of the 54 DSM.

The rareness of streptococci is remarkable. The Streptococcus salivarius that so often appears in mouths with natural teeth was found in only 16 normal and 17 DSM cases.

All other types of bacteria were sparsely represented. Since several authors, *e. g.* CAHN (1936), attribute some importance to the yeast-like organisms of the type Candida (Monilia) albicans in respect of the occurrence of DSM beneath plates in cases of persons in poor general condition, it might be of value to note that these appear in 11 normal and 16 cases with DSM. None of the colonies, however, exhibited such morphology that would warrant consideration of Candida. The frequency is in close agreement with the figure of 33.8 % given by VIRTANEN (1952).

The problem of the frequency of occurrence of Monilia albicans and its possible importance to the condition of the mucosa beneath full dentures is one which the author intends to take up later.

Conclusions.

There does not appear to be any difference in the types of bacteria occurring in the clinically normal and DSM cases of the material.

7. Comparison between the types of bacteria occurring beneath rubber and acrylic dentures.

Case series.

As in the previous comparison the material comprised 112 cases. 67 of the tests were concerned with acrylic plates and 45 with rubber.

Table 8 c.

Comparison between bacteria frequency for rubber and acrylic dentures.

Anaerobic bacteria. Rubber dentures.

Pat. no.	r_1	x_1	r_2	x_2	$\frac{x_1+x_2}{2} = y$
35 N	10	1.000	10	1.000	1.000
36 N	80	1.903	90	1.954	1.928
38 N	2	0.301	6	0.778	0.540
39 N	36	1.556	30	1.477	1.517
41 N	80	1.903	160	2.204	2.054
44 N	16	1.204	16	1.204	1.204
46 N	9	0.954	8	0.903	0.928
47 N	120	2.079	108	2.033	2.056
48 N	48	1.681	24	1.380	1.530
49 N	4	0.602	12	1.079	0.840
50 N	8	0.903	8	0.903	0.903
51 N	12	1.079	80	1.903	1.491
54 N	80	1.903	72	1.857	1.880
55 N	28	1.447	24	1.380	1.413
26 N	36	1.556	20	1.301	1.428
56 N	7	0.845	3	0.477	0.661
58 N	16	1.204	11	1.041	1.123
62 N	20	1.301	15	1.176	1.238
63 N	500	2.699	400	2.602	2.650
68 N	7	0.845	8	0.903	0.874
71 N	40	1.602	42	1.623	1.612
74 N	10	1.000	10	1.000	1.000
76 N	200	2.301	130	2.114	2.208
77 N	24	1.532	16	1.204	1.368
78 N	80	1.903	85	1.929	1.916
82 N	200	2.301	120	2.079	2.190
84 N	50	1.699	56	1.748	1.723
86 N	30	1.477	28	1.447	1.462
87 N	60	1.778	75	1.875	1.826
90 N	150	2.176	140	2.146	2.161
4 I	41	1.613	26	1.415	1.514
5 I	9	0.954	8	0.903	0.928
6 I	700	2.845	750	2.875	2.860
11 I	15	1.176	20	1.301	1.238
12 I	7	0.845	10	1.000	0.923
14 I	7	0.845	6	0.778	0.812
15 I	4	0.602	3	0.477	0.540
16 I	120	2.079	130	2.114	2.097
17 I	10	1.000	8	0.903	0.951
18 I	40	1.602	40	1.602	1.602
19 I	8	0.903	6	0.778	0.840
20 I	15	1.176	18	1.255	1.215
23 I	30	1.477	36	1.556	1.517
24 I	40	1.602	50	1.699	1.650
26 I	12	1.079	10	1.000	1.040
29 I	4	0.602	3	0.477	0.540
31 I	10	1.000	8	0.903	0.951
32 I	20	1.301	20	1.301	1.301
33 I	50	1.699	55	1.740	1.719
34 I	100	2.000	80	1.903	1.951
35 I	200	2.301	150	2.176	2.238

Table 8 c.

(cont.).

Pat. no.	r_1	x_1	r_2	x_2	$\frac{x_1+x_2}{2} = y$
36 I	80	1.903	90	1.954	1.928
37 I	70	1.845	58	1.763	1.804
38 I	4	0.602	6	0.778	0.690
39 I	15	1.176	20	1.301	1.238
40 I	400	2.602	500	2.699	2.650
41 I	300	2.477	380	2.580	2.528
42 I	150	2.176	150	2.176	2.176
45 I	20	1.301	22	1.342	1.322
46 I	20	1.301	15	1.176	1.238
47 I	150	2.176	150	2.176	2.176
49 I	3	0.477	4	0.602	0.540

Results and conclusions.

No difference could be established between the types of the bacterial flora occurring in the cases of acrylic and rubber dentures.

8. Comparison between the types of bacteria associated with any one patient on different days.

Case series.

The case series comprised 10 persons tested on 10 days in succession, and is thus the same as that described under heading no. 2.

Result.

As with previous comparisons between the bacterial flora, Neisseria catarrhalis and Staphylococcus albus were found to occur with great regularity. It was, in addition, of interest to note how the other types changed from day to day. There were two exceptions, where Streptococcus salivarius was present in every examination. This difference from one occasion to another should be noted, in particular for the yeast-like organisms.

9. Oral spirochaetes.

Case series.

In all 112 cases where bacterial cultivation was performed tests were made, as detailed under "Method", to determine the

Table 8 d.

Comparison between bacteria frequency for rubber and acrylic dentures.

Anaerobic bacteria. Rubber dentures.

Pat. nr.	r_1	x_1	r_2	x_2	$\frac{x_1+x_2}{2} = y$
37 N	120	2.079	90	1.954	2.017
42 N	2	0.301	3	0.477	0.389
53 N	40	1.602	40	1.602	1.602
57 N	23	1.362	21	1.322	1.342
59 N	86	1.935	120	2.079	2.007
60 N	50	1.699	30	1.954	1.827
61 N	30	1.477	10	1.000	1.239
65 N	110	2.041	60	1.778	1.910
66 N	30	1.477	40	1.602	1.539
67 N	400	2.602	400	2.602	2.602
69 N	83	1.919	60	1.778	1.849
70 N	20	1.301	20	1.301	1.301
72 N	110	2.041	135	2.130	2.086
73 N	500	2.699	500	2.699	2.699
75 N	5	0.699	6	0.778	0.739
79 N	20	1.301	18	1.255	1.278
80 N	15	1.176	10	1.000	1.088
81 N	4	0.477	3	0.602	0.540
83 N	80	1.903	70	1.845	1.874
88 N	100	2.000	100	2.000	2.000
89 N	22	1.342	26	1.415	1.379
3 I	70	1.845	55	1.740	1.793
8 I	7	0.845	13	1.114	0.979
9 I	300	2.477	200	2.301	2.389
10 I	72	1.857	70	1.845	1.851
13 I	350	2.544	360	2.556	2.550
25 I	10	1.000	8	0.903	0.952
27 I	60	1.778	50	1.699	1.738
30 I	30	1.477	28	1.447	1.462
43 I	60	1.778	56	1.748	1.763
44 I	10	1.000	8	0.903	0.952
48 I	40	1.602	50	1.699	1.650
50 I	60	1.778	60	1.778	1.778
51 I	400	2.602	400	2.602	2.602
52 I	29	1.462	25	1.398	1.430
53 I	800	2.903	600	2.778	2.840
54 I	180	2.255	160	2.204	2.229
55 I	25	1.398	30	1.477	1.438
56 I	55	1.740	60	1.778	1.759
57 I	4	0.602	3	0.477	0.539

occurrence of oral spirochaetes, since there are many authors who attribute great importance to them in the setting up of an inflammatory condition of the oral mucosa.

Results and conclusions.

In none of the 112 cases it was possible to find oral spirochaetes.

As this result might lead one to question the reliability of the methods, control tests were made with 10 cases of complete dentures with cleft palates. In all these cases spirochaetes were proved to be present in the region of the cleft but nowhere beneath the maxillary denture. It would seem justifiable, then, to conclude that the methods were satisfactory.

That oral spirochaetes could not be found in the other material was due to the fact that they do not occur in edentulous mouths of persons fitted with plates.

BRAILOVSKY-LOUNKEVITCH (1915), SCHENK (1929) and others have shown previously that oral spirochaetes are not to be found in the mouths of infants before the deciduous teeth have erupted. SCHENK points out that this must generally be the case, implying that these bacteria will not be found before the interdental spaces — delimited by the contact points — have formed.

The results of the oral spirochaetes tests with persons wearing complete dentures thus lend support to the view that the presence of spirochaetes is due to gingival pockets and interdental spaces which provide conditions favourable for anaerobic growth. The author's findings confirm the results of GOOTZEIT & ROSENTHAL.

General discussion and summary.

In the study of the importance of denture cleansing practice to the state of the mucosa, the conclusions are based on so large a case series — analysed also from other aspects — that they may be considered significant.

It might be said of the material for the bacteriological study that it is, in parts, too small to warrant the drawing of a definite negative inference. It is, however, probably large and representative enough to establish a reliable absence of trend which might be of value to further studies. It might be profitable to examine a more detailed quantitative analysis. The case series is probably large enough to serve as a basis for answers to the questions dealt with in this paper. In summary form:

1. The material provided no support for the assumption that the frequency with which dentures are cleaned plays any considerable rôle in the occurrence of DSM.

2. The case series provided no proof for the belief that the mode of cleansing has any real influence on the occurrence of DSM.

3. With the method used there was no statistically confirmed difference between the bacteria populations occurring in connection with clinically normal and inflamed states of the mucosa.

4. The average population varied from one person to another. With respect to any one subject the variations in the number of bacteria applied only to a part of that region over which the values for the whole material were spread. The population seemed thus, in spite of very wide variations, to be generally characteristic of the individual.

5. There was a significantly larger aerobic than anaerobic population where the described method of cultivation is used. The real importance of this significance must, however, be considered small, since 65% of the cases had a higher number of aerobic bacteria while 35% had a higher number of anaerobic.

6. It seems in some degree probable that the bacterial populations between the denture and the mucous membrane increased with the time the denture was worn without cleansing — a relationship which in itself is perhaps of little importance.

7. There existed a larger aerobic population in cases of rubber dentures than of acrylic (statistically "almost significant"). The mean was about 1.5 times greater. Taking into account the wide spread, one should not attribute much importance to this difference. The method of cultivation used cannot be considered really satisfactory, but was the best available. The anaerobic bacterial flora yielded no statistically demonstrable difference between the numbers for the two base materials.

8. With the method used no difference existed between the types of bacterial flora from the point of view of clinically normal mucosa and DSM.

9. For rubber and acrylic dentures the same types of bacterial flora were identified. This fact still further emphasizes the relatively small degree of importance attaching to the significance referred to under 7.

10. For any one person the types of bacteria found were very constant.

11. Oral spirochaetes were not found in the case of adult edentulous persons fitted with dentures.

In addition to those types of bacteria recognized in the oral flora, there were certainly a number present for which the medium employed — blood agar — did not possess the requisite

conditions of growth. Moreover, no tests for pathogenesis were carried out on the bacteria types that did grow on the plates. For these reasons the bacteriologic section of the investigation cannot be considered as complete. Those definite negative findings concerning the correlation of the denture cleansing practice with denture sore mouth, together with the essential result of the author's previous investigation — namely that trauma (instability, traumatizing occlusion and traumatizing articulation) is the dominant factor in the occurrence of DSM, render it probable that, even if in certain cases one or several strains of bacteria might be proved to be the direct cause of these inflammations of the mucous membrane, there is generally no causal connection between denture sore mouth and the bacterial flora under full upper dentures.[1]

Zusammenfassung.

Über die Bedeutung der Prothesenhygiene und der Bakterienflora für den Zustand der Mundschleimhaut bei Patienten, die eine totale Prothese tragen.

Das Material ist bei dem Studium der Prothesenhygiene dasselbe, wofür ich in einer früheren Arbeit (NYQUIST 1952) im Detail Bericht erstattet habe, d. h. 609 Fälle von totalen Prothesen mit einer Frequenz von Stomatitis prothetica von 27.1 %. Bei den bakteriologischen Studien wurde ein Teil des oben erwähnten Materials verwendet, der 58 Fälle mit klinisch normaler Mukosa und 56 Fälle mit Stomatitis umfasst. Die Auswahl wurde gutdünklich vorgenommen.

Methoden.

Anamnestische Angaben liegen den statistischen Berechnungen der Bedeutung der Prothesenhygiene für den Zustand der Mundschleimhaut zu Grunde. Die gleiche statistische Originalmethode wurde angewendet, für die in oben erwähnter Arbeit im Detail Bericht erstattet worden ist.

Bei den bakteriologischen Studien wurden Kulturen auf Blutagar gezüchtet, nachdem mit sterilen ungeleimten Papierschnitzeln, die für gewöhnlich zehn Minuten zwischen Prothese und Schleimhaut gelegen hatten, eine Probe genommen worden war.

[1] The statistical treatment has been performed by M. SANDELIUS, Fil. lic. and Mr. A. RAUD. For their kind assistance I tender my sincere thanks.

Im Artikel wird Einzelbericht über das Verfahren bei dem Züchten, Rechnen und Differenzieren der Bakterien, die auf dem erwähnten Medium gewachsen sind, erstattet.

Spirochaeten wurden teils in Dunkelfeldbeleuchtung teils nach dem Züchten nach der Methode KLEIN (1943) studiert.

Ergebnis.

1. In dem vorliegenden Material spricht nichts für die Annahme, dass die Anzahl der Reinigungen der Prothese pro Tag eine wesentliche Rolle bei dem Entstehen von inflammatorischen Veränderungen in der unter ihr liegenden Mukosa spielen würde.

2. In dem Material deutet nichts darauf hin, dass die Art auf welche die Prothese gereinigt wird, eine wesentliche Rolle bei dem Entstehen von inflammatorischen Veränderungen in der Mukosa spielen würde.

3. Ein statistisch festgelegter Unterschied in der Bakterienfrequenz beim Vergleichen von Fällen mit klinisch normaler und inflammatorisch veränderter Schleimhaut liegt nicht vor.

4. Verschiedene Individuen haben verschieden grosse Bakterienfrequenz. Die Variationen in der Anzahl der Bakterien bei dem gleichen Individuum, halten sich nur innerhalb einen Teil des Gebiets, über welches sich die Werte des ganzen Materials verteilen. Die Anzahl der Bakterien scheint daher, trotz sehr grosser Variationen, in gewissem Masse für das Individuum charakteristisch zu sein.

5. Bei der angeführten Züchtungstechnik liegt eine signifikante höhere Frequenz der aeroben als der anaeroben Bakterien vor. Die reelle Bedeutung dieser Signifikanz kann jedoch als klein bezeichnet werden, indem 65 % der Fälle höhere Frequenz der aeroben und 35 % höhere Frequenz der anaeroben Bakterien haben.

6. Eine gewisse Wahrscheinlichkeit scheint vorzuliegen, dass die Bakterienfrequenz zwischen Prothese und Schleimhaut zunimmt in Relation zu der Zeit, in der die Prothese getragen wird, ohne gereinigt zu werden. Ein Verhältnis, das jedoch an und für sich ohne Bedeutung sein dürfte.

7. Bei Kautschukprothesen liegt eine fast signifikante höhere aerobe Bakterienfrequenz im Vergleich mit Prothesen aus Akryl-Kunststoff vor. Der Mittelwert ist etwa 1.5 Mal grösser. Auf Grund der grossen Variation darf man diesem Unterschied keine

grössere Bedeutung zumessen. — In der anaeroben Bakterienflora liegt kein statistisch festlegbarer Frequenzunterschied bei den beiden Prothesenwerkstoffen vor.

8. Ein Unterschied in dem Typus der Bakterienflora liegt bei dem Material nicht vor, wenn man Fälle mit klinisch normaler und mit inflammatorisch veränderter Schleimhaut vergleicht.

9. Träger einer totalen Prothese aus Kautschuk und Träger einer totalen Prothese aus Akryl-Kunststoff haben den gleichen Typus der Bakterienflora. Dieser Befund unterstreicht nochmals die geringe Bedeutung der unter Punkt 7 gefundenen Signifikanz.

10. Der Typus der Bakterienflora is bei dem gleichen Individuum sehr konstant.

11. Mundspirochaeten kommen bei zahnlosen, adulten Individuen, die eine Prothese tragen, nicht vor.

Ganz gewiss umfasste die Mundflora der untersuchten Fälle auch Bakterientypen, die auf dem Substrat, Blutagar, das zur Anwendung kam, nicht ausreichende Lebensbedingungen fanden. Auch wurden keine Pathogenitätsbestimmungen der auf den Platten wachsenden Bakterientypen ausgeführt. Aus diesem Grunde kann der bakteriologische Teil der Untersuchung als unvollständig betrachtet werden. Die in dieser Untersuchung sicher festgelegten negativen Befunde betreffend der Korrelation zwischen dem Reinigen der Prothese und Stomatitis prothetica und das wesentliche Ergebnis der früheren Untersuchungen des Verfassers, nämlich, dass ein Trauma (Instabilität, traumatisierende Okklusion, und traumatisierende Artikulation) der ganz vorherrschende Faktor bei dem Entstehen von derselben ist, machen es wahrscheinlich, dass, selbst wenn bewiesen werden könnte, dass in gewissen Fällen der eine oder der andere Bakterienstamm die direkte Ursache dieser Inflammation in der Mundschleimhaut gewesen ist, als Regel kein kausaler Zusammenhang zwischen Stomatitis prothetica und der Bakterienflora unter totalen Prothesen im Oberkiefer besteht.

Résumé.

L'importance de l'hygiène des prothèses et de la flore de microbes pour l'état de la muqueuse buccale des sujets avec des prothèses dentaires complètes.

Les sujets des études sur l'importance de l'hygiène des prothèses sont les mêmes que ceux dont l'auteur a rendu compte

détaillé dans un travail antérieur (NYQUIST 1952), c'est à dire 609 cas de prothèses complètes avec une fréquence de stomatite prothétique de 27.1 %. Aux études bactériologiques, 58 des cas mentionnés ci-dessus avec muqueuse clinicalement normale et 56 cas de stomatite ont été considérés. La sélection a été faite au hasard.

Méthodes.

Des renseignements anamnestiques servent de base pour les calculs statistiques de l'importance de l'hygiène des prothèses pour l'état de la muqueuse buccale. La même méthode originale statistique a été employé dont l'auteur a rendu compte en tout détail dans le travail mentionné ci-dessus.

Aux études bactériologiques des cultures ont été faites sur des agars de sang après avoir fait des prises avec des bouts de papier non collé et stérile mis entre la prothèse et la muqueuse, généralement pendant 10 minutes. Dans l'article un compte détaillé a été rendu de la manière de cultiver, compter et différencier les microbes ayant crû au milieu mentionné.

Des spirochètes ont été étudiés, et dans une illumination de champ sombre et après culture selon KLEIN (1943).

Résultats.

1. Les sujets traités ne prêtent pas d'appui à l'hypothèse que *le nombre* de nettoyages par jour de la prothèse joue de rôle essentiel pour la formation de changements inflammatoires de la muqueuse au-dessous de la prothèse.

2. Les sujets traités ne prêtent pas d'appui à l'hypothèse que *la manière* de nettoyer la prothèse joue de rôle essentiel pour la formation de changements inflammatoires de la muqueuse.

3. Il n'existe pas de différence de fréquence de microbes statistiquement garantie entre les cas avec muqueuse clinicalement normale et ceux avec muqueuse changée par inflammation.

4. La fréquence moyenne de microbes varie chez les sujets divers. Les variations du nombre de microbes chez le même sujet ne tournent qu'en une partie du rayon sur lequel les valeurs de tous les sujets sont répandues. Le nombre de microbes semble donc, à un certain degré, être caractéristique au sujet.

5. Avec la technique de culture décrite par l'auteur, l'on trouve une fréquence significativement plus grande de microbes

aérobies que de microbes anaérobies. Cependant, on pourrait dire que l'importance réelle de cette signification compte peu, parce que 65 % des cas ont une fréquence plus grande de microbes aérobies tandis que 35 % ont une fréquence plus grande de microbes anaérobies.

6. Une certaine vraisemblance semble exister pour que la fréquence de microbes entre la prothèse et la muqueuse augmente en rapport avec le temps que la prothèse est portée sans être nettoyée, une condition qui importe probablement peu en soi.

7. Il existe une fréquence de microbes presque significativement plus grande chez les prothèses de caoutchouc en relation aux prothèses de résine acrylique. La moyenne est environ 1.5 fois plus grande. A cause de la grande variation on ne doit pas attacher trop d'importance à cette différence. — Dans la flore de microbes anaérobies il n'existe aucune différence de fréquence statistiquement démontrable entre les deux matières de base.

8. Il n'existe aucune différence de type en la flore de microbes chez les sujets si comparaison est faite entre les cas avec la muqueuse clinicalement normale et celle changée par inflammation.

9. Les porteurs de prothèses dentaires complètes de caoutchouc et de résine acrylique, ont le même type de flore de microbes. Cette découverte accentue encore le peu d'importance de la signification trouvée selon paragraphe 7.

10. Le type de flore de microbes montre chez le même sujet une grande constance.

11. Il n'y a pas de spirochètes buccaux chez les sujets adultes édentés portant des prothèses dentaires.

La flore de microbes buccales en les cas examinés a certainement aussi compris les types de microbes dont les conditions de culture n'ont pas été satisfaisantes sur la base employée c'est à dire agar de sang. L'on n'a non plus fait de déterminations de pathogénie chez les types de microbes qui ont crû sur les plaques. Pour cette raison l'on pourrait dire que la partie bactériologique de l'investigation est incomplète. Les résultats décidément négatifs de cette investigation concernant la corrélation des habitudes de nettoyage avec la stomatite prothétique et le résultat essentiel des investigations antérieures de l'auteur — à savoir que le trauma (instabilité, occlusion traumatisante et articulation traumatisante) est le facteur tout dominant pour l'origine de stoma-

tite prothétique — portent à croire que même si, dans certains cas, on pourrait prouver qu'un ou deux troncs de microbes soient la cause immédiate de ces inflammations de la muqueuse, il n'existe guère, ordinairement, de relation causale entre la stomatite prothétique et la flore de microbes au-dessous des prothèses dentaires complètes.

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