THE USE OF PHASE CONTRAST MICROSCOPY IN CLINICAL DENTISTRY

by

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SUMMARY PAGE

THE PROBLEM

Phase microscopy has for some time been used as a motivational tool in oral hygiene programs. In such use bacteria plaques are demonstrated to the patient. As a refinement of this use some

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INTRODUCTION

Interest in the bacterial inhabitants of dental plaque dates back to the time of Van Loewenhoek¹. In recent years, the clinical dentist has been stimulated to employ plaque microscopy as a facet of disease control. Arnim^{2, 3, 4} has graphically demonstrated the bacterial nature of the most common dental diseases, caries and periodontal disease. He has also shown how phase microscopy can be used both as a motivational tool and as a means for observing disease control.

The descriptions of plaque inhabitants have been generally qualitative. Dunkin, ⁵ however, devised a microbial index by which he obtained a semiquantitation of selected plaque organisms. His index has been used to follow various hygiene regimens.

The authors had been using phase microscopy as a motivational tool. Patients were allowed to see the nature of the bacterial plaque in their mouths. At the same time they were informed of the relationships of the plaque elements to dental disease and were shown how to keep the plaque off their teeth. The plaque samples were almost always taken from areas of gingival inflammation or necrosis. When explaining the significance of the plaque elements, it soon became apparent that these elements varied considerably. An occasional plaque sample from a clinically normal gingival area was taken and

was, at times, quite similar to those from inflammed sites.

The question naturally arose concerning the possibility of demonstrating to a patient differences in the plaque taken from inflammed and "normal" areas in his mouth.

A rather simple study was designed to answer this question, and thereby, refine our use of phase microscopy in general dentistry.

MATERIALS AND METHODS

The two investigators worked independently of each other. Investigator R.B. drew his subjects from his routine clinical patients while investigator W.R.S. subjects were prospective Submarine School candidates. Approximately 150 of these prospective candidates were examined each week and subjects for the study were selected from the group.

The criteria for subject selection was the presence of non-necrotic gingival inflammation and the presence of apparently healthy gingiva in the same mouth; both areas having grossly discernible plaque on the adjacent tooth surface.

Plaque samples the size of a pinpoint were removed from an area just coronal to the gingival crest and were placed on clean glass slides. A drop of sterile

normal saline was added and a cover slip was immediately placed over the sample and sealed with clear fingernail polish. The two slides were then marked with a coded number by a laboratory technician. A blind evaluation was, therefore, possible. The plaque elements evaluated were: coccal bacteria, short rods, long rods, spirochetes, vibrios, epithelial cells, and white blood cells. A descriptive appraisal of the numbers of each element was made as follows: absent, occasional, few, moderate, many, and predominant. These descriptions were recorded for each slide. These recordings were in numeric scores from 0 to 5, where "0" represented an element's absence and "5" its being the predominant element.

The data were then analyzed both parametrically and non-parametrically for relationships between clinical inflammation and plaque elements.

RESULTS

The overall plaque element assessments are given in Tables 1 and 2. In the case of investigator W.R.S. (Table 1) it is seen that spirochetes and vibrios were associated with clinical inflammation. The distributions for these two elements are not considered likely to be due to chance (P < .01 on basis of chi square analysis). In the case of investigator R.B. (Table 2) no significant relationships were found between inflammation and any plaque element score. The numerical scores (0 - 5) of the plaque elements were treated as actual quantities and from these, means were computed for all elements evaluated and for spirochetes and vibrios combined. In Table 3 it is seen for investigator W.R.S. that the mean numerical scores for all elements and for the spirochetes and vibrios were significantly greater in the inflammed areas than in the normal areas.

These differences are highly significant; P < .001 based on the t test. Closer inspection reveals that the difference in the overall element score is largely due to the larger numbers of spirochetes and vibrios in the inflammed sample. In Table 4 no such differences are indicated in the data from investigator R.B.

Seventeen subjects were seen by investigator W.R.S. a second time three days after the initial samples were taken. Samples from the identical sites were collected and were again blindly evaluated. These data are presented in Table 5. At both examinations the element scores were higher in the inflammed than in the noninflammed samples. Again, closer inspection reveals that most of this difference is due to the spirochete and vibrio scores. Product moment correlation analyses of these data were performed where X = first examination scores and Y = second examination scores. In neither of the four evaluations was a correlation coefficient found to be greater than +.11 thus indicating a great amount of fluctuation in these data even though the means were fairly constant.

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	8	Absent	Occasional or few	Moderate	Many
	Inflammed	0	10	20	9
Cocci	Normal	0	17	20	6
Chart mode	Inflammed	0	19	13	9 🛞
Short rous	Normal	0	22	12	9
T en e vede	Inflammed	5	21	10	. 7
Long rods	Normal	6	16	12	8
Spirochetes	Inflammed	15	9	8	11
	Normal	31	7	2	3
Vibrio	Inflammed	3 👘	19	13	8
	Normal	15	18	7	3
Epithelial cells	Inflammed	4	29	···· 7	3
	Normal	6	23	. 10	3
White blood cells	Inflammed	12	16	8	7
	Normal	17	15	3	7

Table 1. Quantitative Assessment of Organisms - Inflammation Relationship (Investigator W.R.S.) (N=43)

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	- °	Absent	Occasional or few	Moderate	Many
Cooci	Inflammed	0	6	6	8
00001	Normal	0	2	6	12
Short rode	Inflammed	0	11	3	4
Short rous	Normal	0	10	7	3
Long rode	Inflammed	1	12	4	3
Long rods	Normal	0	12	7	1
Spirochetes	Inflammed	5	3	5	7
Spirocheres	Normal	4	7	4	5 .
Vibrio	Inflammed	1	11	5	3
1 10210	Normal	1	9	7	3
Epithelial cell s	Inflammed	0	14	5	1
	Normal	0	17	2	1
White blood	Inflammed	3	10	0	7
cells	Normal	2	12	2	3

Table 2. Quantitative Assessment of Organisms – Inflammation Relationship (Investigator R.B.) (N=20)

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=	N	Inflammed	Normal
All elements	42	12.7* ±.53**	9.52 + .53
Spirochetes and vibrio	42	4.5 ±.44	$2.02 \pm .33$

Table 3. Plaque Element Scores - Inflammation Relationship (Investigator W.R.S.)

*Mean

**Standard error of the mean.

Table 4.	Plaque Element Scores – Inflammation Relationship
	(Investigator R.B.)

	N	Inflammed	Normal
All elements	20	12.8* ±.78**	12.9 ± .68
Spirochetes and vibrio	20	5.0 ± .53	4.7±.50

*Mean

**Standard error of the mean.

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8 8 8	# 75	N	First Examination	Second Examination
All elements	Inflammed	17	11.7* ±.65**	$12.4 \pm .74$
	Normal	17	8.9 ±.82	8.2 ± .91
Spirochetes	Inflammed	17	$3.9 \pm .52$	4.1 ± .71
and vibrio	Normal	17	1.9 ± .60	2.0 ± .54

Table 5. Plaque Element Scores - InflammationRelationship at Two Examination Periods

*Mean

**Standard error of the mean.

DISCUSSION

The plaque evaluation by investigator W.R.S. showed close relationship between spirochete/vibrio occurrence and inflammation. This was not really surprising. It was somewhat surprising that no other significant relationships were found; such as in the case of white blood cells and in the case of all elements evaluated by investigator R.B. The answer can probably be found by considering the design of this study.

First and foremost, the clinical criteria of disease were macroscopic. Arnim⁶, for one, has amply demonstrated that a rather extensive severe gingival disease state can be present microscopically before the clinician and the patient would be aware of it. Since the determination pairs in this study were from the same mouth, it would certainly seem likely that a subclinical disease state would be present in some of the gingival areas judged "normal." The fact that investigator W.R.S. found significant differences in the occurrence of spirochetes and vibrios between clinically normal and inflammed areas probably point up the fact that these organisms are present in great numbers in rather well advanced stages of gingivitis. The fact that investigator R.B. did not find this relationship can again be explained on the basis of experimental design. His cases were drawn from a rather small number of routine operative dentistry patients and consequently the areas of inflammation were probably not as severe as were those of investigator W.R.S. The subjects in the latter instance were drawn from a population of almost a thousand young

incoming Submarine School students not under dental treatment at the time of examination. As a result the inflammed areas evaluated by investigator W.R.S. were quite severe.

Perhaps the chief value of this study is to point out limitations in the use of phase microscopy in clinical dentistry. It must be remembered that one is looking at a very small part of the system under study; the qualitative nature of a pinpoint amount of plaque. This necessarily means that projections of disease course on the basis of these observations must be extremely imprecise.

The fact that a relationship was seen between spirochetes and vibrios and gingivitis might lead one to the use of these elements as indicators for the demonstration to the patient the presence of disease causing elements and the microscopic proof of improvement with treatment. This use is possible with some limitations. The authors have found several instances in which the gross clinical picture did not correspond to the microscopic picture of the plaque. Like most areas of dentistry, proper interpretation of health and disease both by the patient and the dentist depends upon complete understanding of the pathological processes involved. The phase microscope gives an excellent view of one of the pathological components and it certainly remains one of the great motivational tools available to the dentist.

SUMMARY

1. The phase microscope is a useful tool in enabling the dentist and the

patient to observe the bacterial plaque elements.

- 2. In areas of overt gingival inflammation, spirochete and vibrio microorganisms are observed in greater numbers in the plaque than found in plaque associated with clinically normal tissue.
- 3. An understanding of the complete pathological processes is necessary in order to make intelligent use of the phase microscope in clinical dentistry.
- 4. It must be emphasized that this study in no way casts doubt on the idea that gingivitis is caused by the bacterial plaque. It was not designed to even consider this question.

REFERENCES

- 1. Dobell, Clifford. Antony Van Loewenhoek and his "Little Animals," Diver, New York, 1960.
- Arnim, S. S. Microcosms of the Mouth - Role in Periodontal Disease. <u>Texas Dent. J.</u> 82:4-10, 1964.
- Arnim, S. S. An Effective Program of Oral Hygiene for the Arrestment of Dental Caries and the Control of Periodontal Disease.
 J. So. Cal. Dent. A. 35:264-280, 1967.
- 4. Arnim, S. S. The Effect of Thorough Mouth Cleansing on Oral

Health - Case Report. <u>Periodon-</u> tics 6:41-52, 1968.

- 5. Dunkin, R. T. A New Approach to Oral Physiotherapy With a New Index to Evaluation. J. Periodont. 36:55-61, 1965.
- Arnim, S. S. and Holt, R. T. The Defense Mechanism of the Gingiva. J. Periodont. 26:79-90, 1955.

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As a refinement of this use some infor	mation on rel	ationships	between disease		
status and plaque elements were needed	. A blind ev	aluation w	as performed of the		
plague elements taken from inflammed a	nd from clini	cally norm	al gingival areas		

of the same mouth. The only elements related significantly to the clinical

picture were spirochetes and vibrios and these only in the more severe gingivitis cases. It is concluded that while the phase microscope remains a highly useful motivational tool, its use in following gingival disease course is possible only if one recognizes that microscopic plaque evaluation will not necessarily mirror

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macroscopic tissue evaluation.

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