McGILL UNIVERSITY

THE EFFECTS OF ADJUNCTIVE METRONIDAZOLE AND ROUTINE PERIODONTAL TREATMENT IN A SAMPLE OF MENTALLY RETARDED ADOLESCENTS WITH DESTRUCTIVE PERIODONTAL DISEASE

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Short Title

EFFECTS OF METRONIDAZOLE THERAPY ON PERIODONTAL DISEASE

ABSTRACT

A double-blind controlled clinical trial was undertaken to determine whether adjunctive metronidazole therapy could significantly increase periodontal improvement achieved by conventional therapy (scaling and polishing) alone. Twenty-three periodontal patients were randomly assigned to conventional therapy plus placebo or conventional therapy plus metronidazole. Disease status and therapeutic effects were assessed by clinical and microbiological measurements at baseline and 1, 3 and 6 months post-baseline).

Spirochete proportions were significantly lower for the metronidazole group at the 1-month and 3-month examinations ($P<0.05$). Significantly higher proportions of cocci were observed for the metronidazole group at 1-month post-baseline ($P<0.05$). Conversely, no differences attributable to treatment group were found for any of the clinical measurements, although there were significant intersubject differences ($P<0.05$). Plausible explanations for the discrepancy between clinical and microbiological findings included inappropriate sample selection, inadequate dosage and the possibility that spirochetes may not be the most crucial etiologic factor in the progression of periodontal disease.

RÉSUMÉ

Une expérience clinique à double insue fut entreprise pour déterminer si l'addition d'un traitement au métronidazole pourrait significativement améliorer les résultats accomplis par le traitement conventionnel (détartrage et polissage). Le traitement conventionnel plus un placébo ou le traitement conventionnel plus le métronidazole furent administrés à 23 patients atteints de maladie péridentaire. L'état de la maladie et les effets du traitement furent évalués au moyen de mesures cliniques et microbiologiques au début du traitement et après 1,3 et 6 mois.

Les proportions de spirochètes furent significativement plus basses à 1 et 3 mois dans le groupe comportant le traitement au métronidazole ($P<0.05$). Une plus grande proportion de cocci fut observée chez le groupe comportant le traitement au métronidazole après un mois ($P<0.05$). Aucune différence attribuable au métronidazole fut observée pour aucune des mesures cliniques; des différences significatives inter-sujets furent cependant observées ($P<0.05$). Un échantillonage inapproprié, un dosage inadéquat, et la possibilité que les spirochètes pourraient ne pas être le facteur étiologique le plus déterminant dans la progression de maladie péridentaire ont pu contribuer au désaccord entre les résultats cliniques et microbiologiques.

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I. INTRODUCTION

Periodontitis is usually a chronic, inflammatory disease which arises and provokes pathological alterations in the periodontium (gingiva, cementum, periodontal ligament, alveolar bone). The periodontal structures: 1) attach and support the teeth; 2) resist forces generated by mastication, speech and deglutition; and 3) adjust for structural changes associated with wear and aging through constant remodeling and regeneration [79]. Figures 1-4 depict the anatomy of the healthy periodontium. Affected persons exhibit gingival inflammation and fibrosis, migration of the junctional epithelium apical to the cementoenamel junction (Figure 5), and destruction to the periodontal ligament and alveolar bone [67,78,92]. These pathological processes eventually lead to tooth mobility and exfoliation. Epidemiological surveys indicate that between 30 and 40 years of age, periodontal disease overtakes caries as a cause of tooth loss [53,73,78,107].

There is general agreement that the inflammatory process begins in childhood as marginal gingivitis, a condition manifested clinically by redness, edema, fibrosis, a tendency to bleed spontaneously or upon probing, and a deepening of the gingival sulcus. This gingival inflammation increases in prevalence and severity with age, invading the subjacent periodontal tissues [67,78,105]. Experimental animal models

have also demonstrated a transition from gingivitis to periodontitis [38,75]. The progression of transient forms of gingivitis is not inevitable, however, and further research may eventually modify the notion of periodontitis as an extension of gingivitis [62,78,86].

The universal prevalence of periodontal disease has been well established by large-scale epidemiologic studies undertaken around the globe [53,66,72,73,107]. While these studies have shown that the situation in underdeveloped or developing countries is far more critical than in the West, periodontal disease nevertheless presents a public health problem of major proportions in North America in terms of treatment needs, resources required to meet those needs, social and economic impact [34,105].

Studies of the prevalence of periodontal disease among the mentally retarded are rather difficult to interpret because of variations in the age and mental level of the subjects, poor patient cooperation, differing methods of assessing the periodontal condition, the uncertainty about whether gingivitis was included in the calculation of statistics on periodontal disease prevalence, and the policy concerning oral hygiene at various institutions. Despite these limitations, one cannot but conclude that destructive periodontal disease is more frequent and has an earlier onset among the mentally retarded, a phenomenon which has been attributed to the lack of oral hygiene practiced by these patients as well as the inadequate dental care they receive

[8,15]. Evaluation of the prevalence and severity of periodontal disease in the mentally retarded may be further complicated by findings that indicate an increased susceptibility in persons with Down's syndrome [15,57,76].

The association between periodontal disease and plaque has been well documented by epidemiologic studies and clinical observations. Plaque is a concentrated and coherent mass of bacteria and intermicrobial substances adherent to soft oral tissues and to tooth surfaces along the gingival margin, apically to the interproximal contact points in fissures and subgingivally [102]. The significance of plaque in the etiology and progression of periodontal disease derives from: 1) experimental gingivitis studies on human subjects [48,103]; 2) animal models of experimental periodontitis [38,75]; and 3) studies indicating that efficacious plaque control achieved through personal hygiene and frequent prophylaxes retards or arrests further periodontal breakdown [3,40,61,71,95]. Since plaque composition is overwhelmingly bacterial in nature [90,100,102], the conclusion drawn from these observations is that suppression of bacterial action is effective in controlling periodontal disease in humans. The recognition that plaque microorganisms constitute the primary etiologic component of periodontal disease does not imply that host resistance to plaque infection and other systemic factors, which may modify the course of the disease, should be overlooked [67,102].

Although clinical observations and evidence from

clinical trials have documented the effectiveness of mechanical plaque control in the prevention and resolution of periodontal disease, such an approach is time-consuming, expensive, labor-intensive and requires a high degree of motivation and dexterity on the part of the patient [3,6,22, 40,49,61,71,95]. Moreover, the beneficial results of such treatment are often short-lived, a finding which attests to the difficulty of completely removing all plaque and calculus at the time of treatment and maintaining plaque-free surfaces subsequent to treatment. Because this mechanical debridement approach was in agreement with the tenets of the Nonspecific Plaque Hypothesis, other therapeutic methods received little attention until quite recently. With increasing acceptance of the Specific Plaque Hypothesis [49,50] together with an appreciation of the drawbacks of the mechanical approach, much interest has been generated for the research of complementary forms of therapy, specifically adjunctive antibiotic therapy. The success of the adjunctive chemotherapeutic approach--and ultimately the rationale for its use in the treatment of periodontal disease--must be demonstrated in terms of its ability to enhance and prolong the effects of traditional periodontal therapy.

A double-blind controlled clinical trial was undertaken to determine the benefits, if any, of adjunctive antibiotic therapy in the treatment of periodontal disease in a sample of mentally handicapped adolescents. Twenty-three patients exhibiting clinically detectable signs of

destructive periodontal disease were randomly assigned to conventional therapy plus placebo (control or placebo group) or to conventional therapy with adjunctive antibiotic therapy (experimental or drug group). Conventional therapy consisted of polishing and scaling; no root planing was required. Therapy was administered immediately after baseline values were recorded, and recall examinations were performed at one month, three months and six months post-baseline. Evaluation of disease status and therapeutic effects included clinical and microbiological measurements.

Metronidazole was chosen over other drugs commonly used in the treatment of periodontal disease (e.g. penicillin, tetracycline) for the following reasons:

1) Its spectrum of activity shows more selectivity for the microbial population implicated in periodontal disease, specifically for gram-negative anaerobic rods [14,60,96] and spirochetes [19,30,32,45,52].
2) Metronidazole appears in the saliva and in the gingival exudate and is also actively secreted by the oral mucosa; it can therefore reinforce its own systemic action [82, 101].
3) Metronidazole is considered to be a safe drug. Infrequent side effects are of a minor nature, are reversible and rarely interfere with the course of therapy [5,9,69].

Several specific objectives were identified at the outset of the study:

1) To determine whether significant differences in clinical

improvement were evident between experimental and control groups.

2) To establish the duration of any significant differences in clinical improvement between experimental and control groups.

3) To compare changes in the subgingival microbiota (as observed by darkfield microscopy) by group and over time.

4) To determine whether changes in the subgingival flora correlated with changes in clinical measurements.

This comprehensive approach to the treatment of periodontal disease could contribute significantly to an area of intense research in dental medicine, i.e., adjunctive antibiotic therapy, and reinforce important research currently in progress. If adjunctive metronidazole therapy were shown to be more effective than conventional therapy alone, the precocious tooth mortality observed in mentally handicapped populations might be delayed and/or reduced. Additionally, this method might permit significant prolongations of recall intervals, theraby reducing treatment time and costs for patients and dental personnel alike. This research might also reveal an innovative treatment regimen that could improve the prognosis in refractory cases. Finally, if changes in the microbiological findings correlated with changes in the clinical measurements, then evidence would be provided for a microbiological definition of periodontal disease, a development which would bear important implications for disease identification, monitoring and evaluation of therapy.

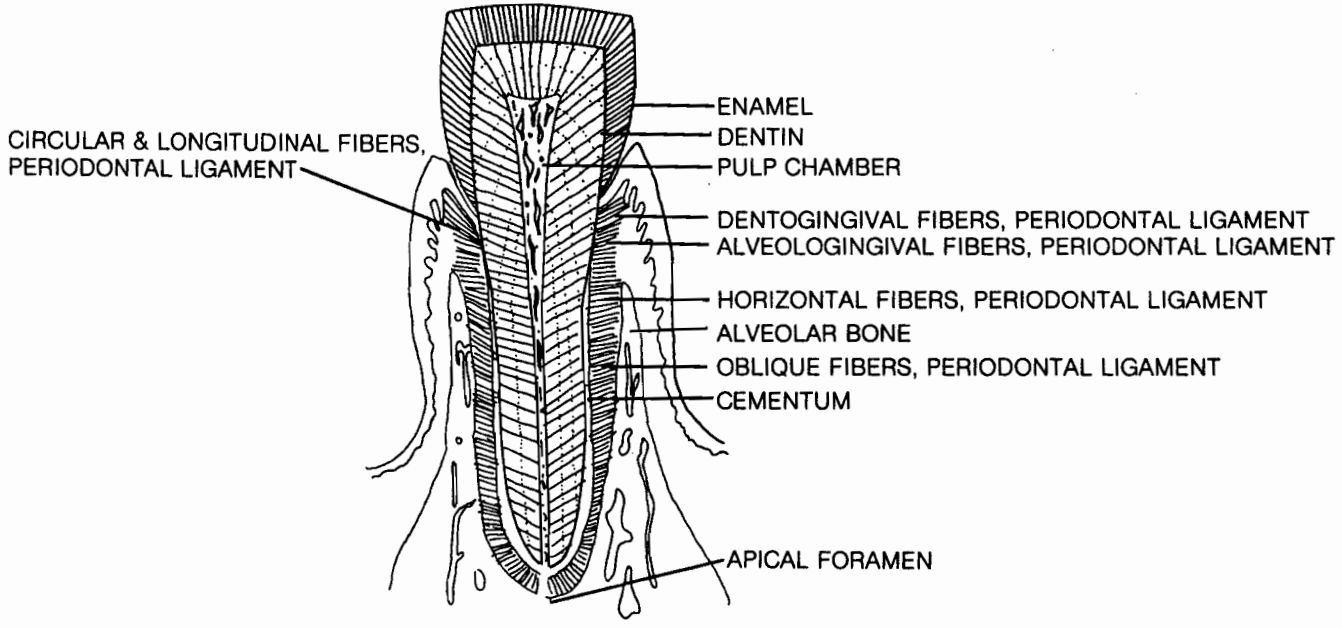

Fig. 1 Buccolingual section of an incisor.

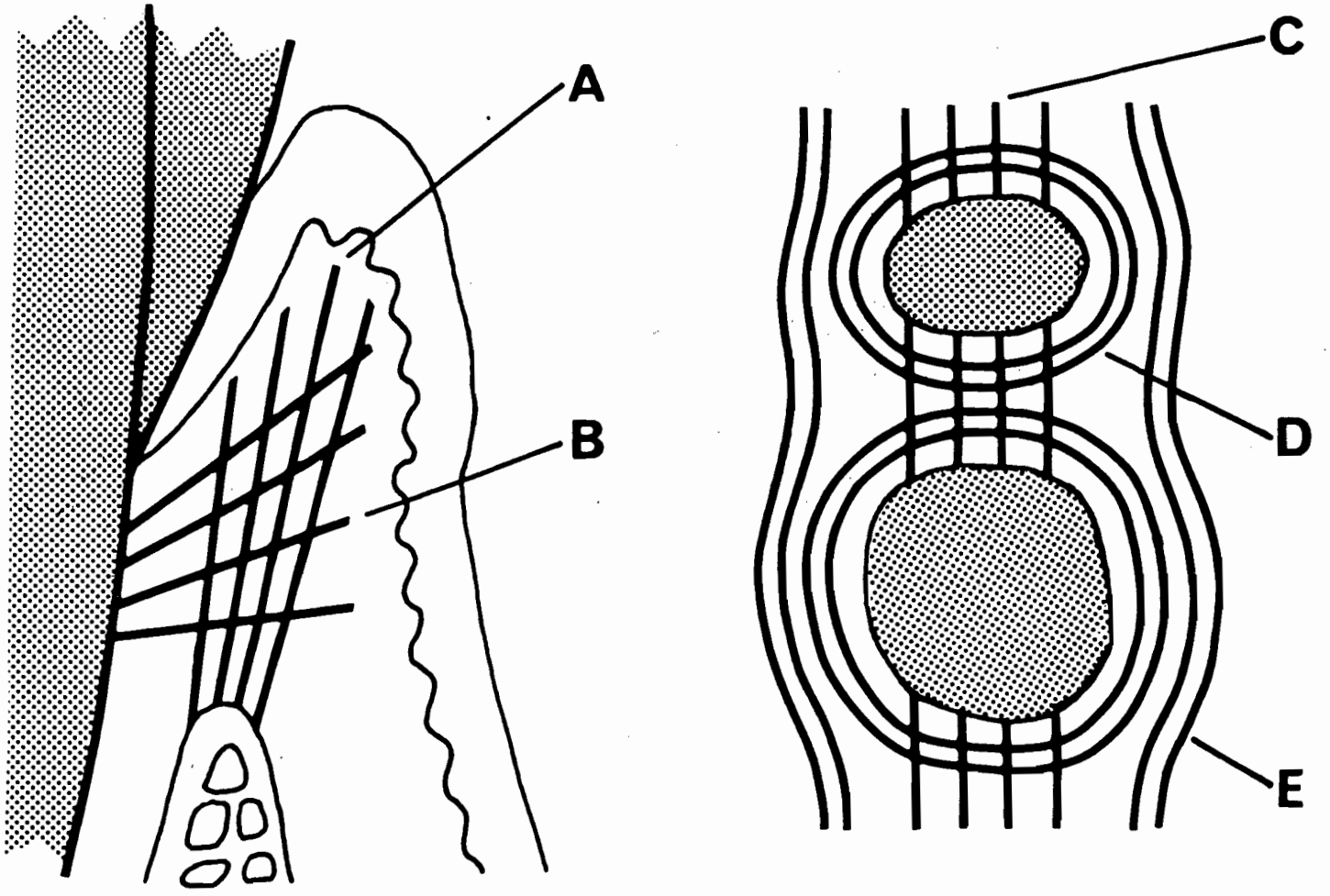

Fig. 2. Gingival Collagen Fibers Shown in Section.*

The collagen fibers of the gingiva contribute to the adaptation of the soft tissue to the tooth. They are classified as:

A) alveologingival C) transeptal E) longitudinal.
B) dento-gingival D) circular

* From Strahan and Waite [92, p. 10].

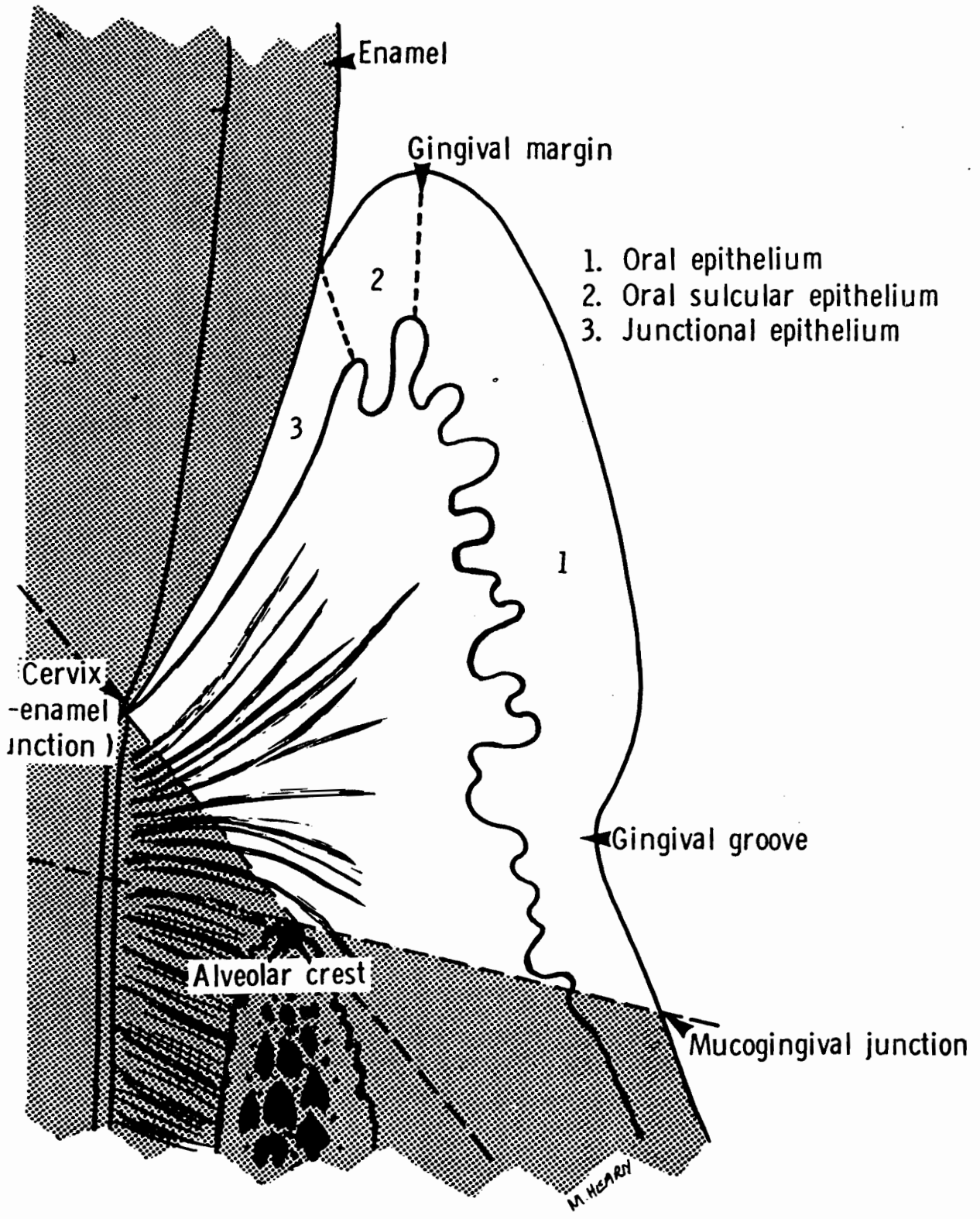

Fig. 3. Labiolingual section showing components of the gingival epithelium.*

*From Listgarten [41, p. 3].

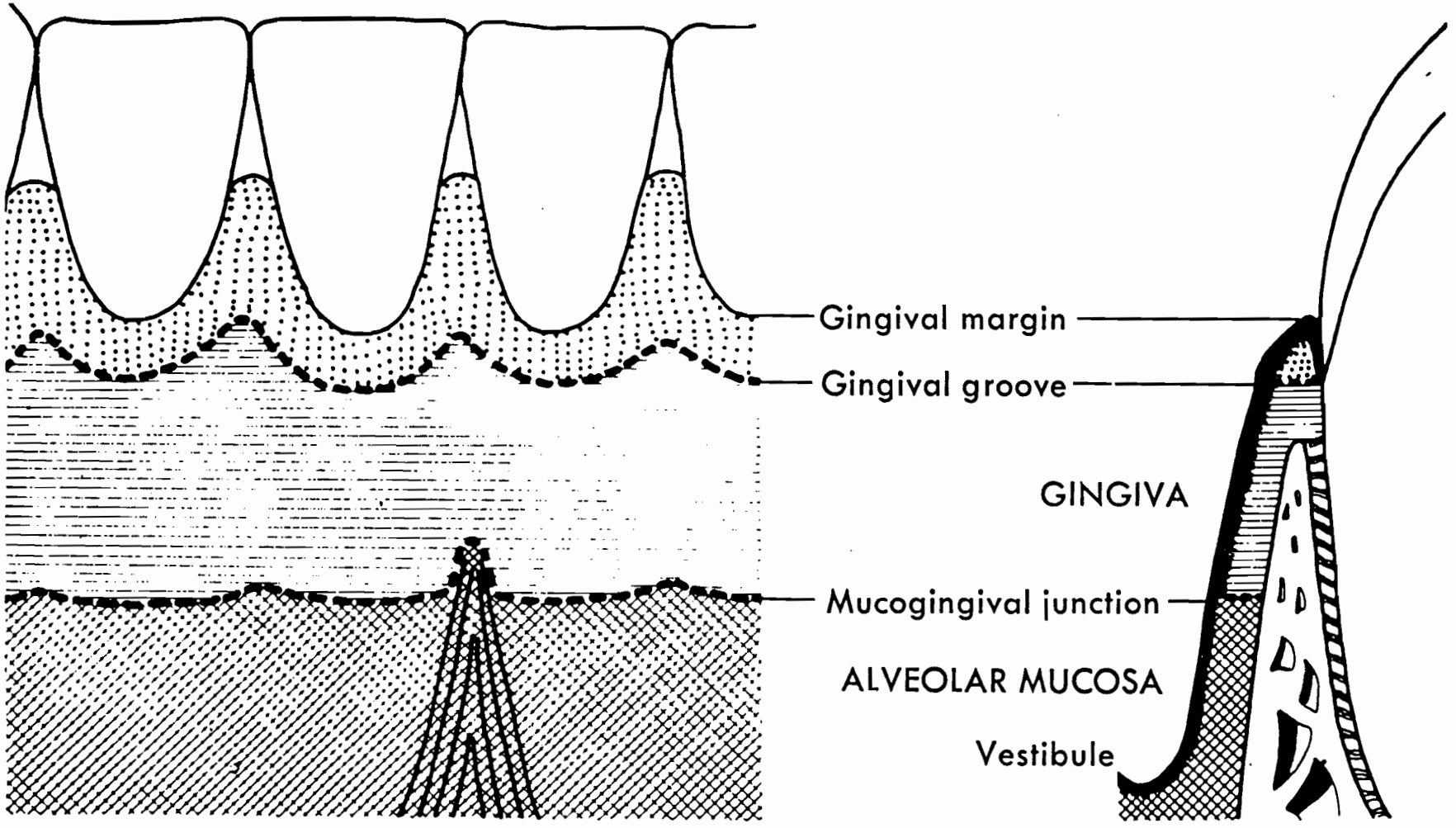

Fig. 4. Anatomic relationships of normal gingiva.* Gingival components include: (A) free gingiva, (B) interdental papilla, (C) marginal gingiva, (D) attached gingiva, (E) alveolar mucosa.

*From Goldman [27, p. 2].

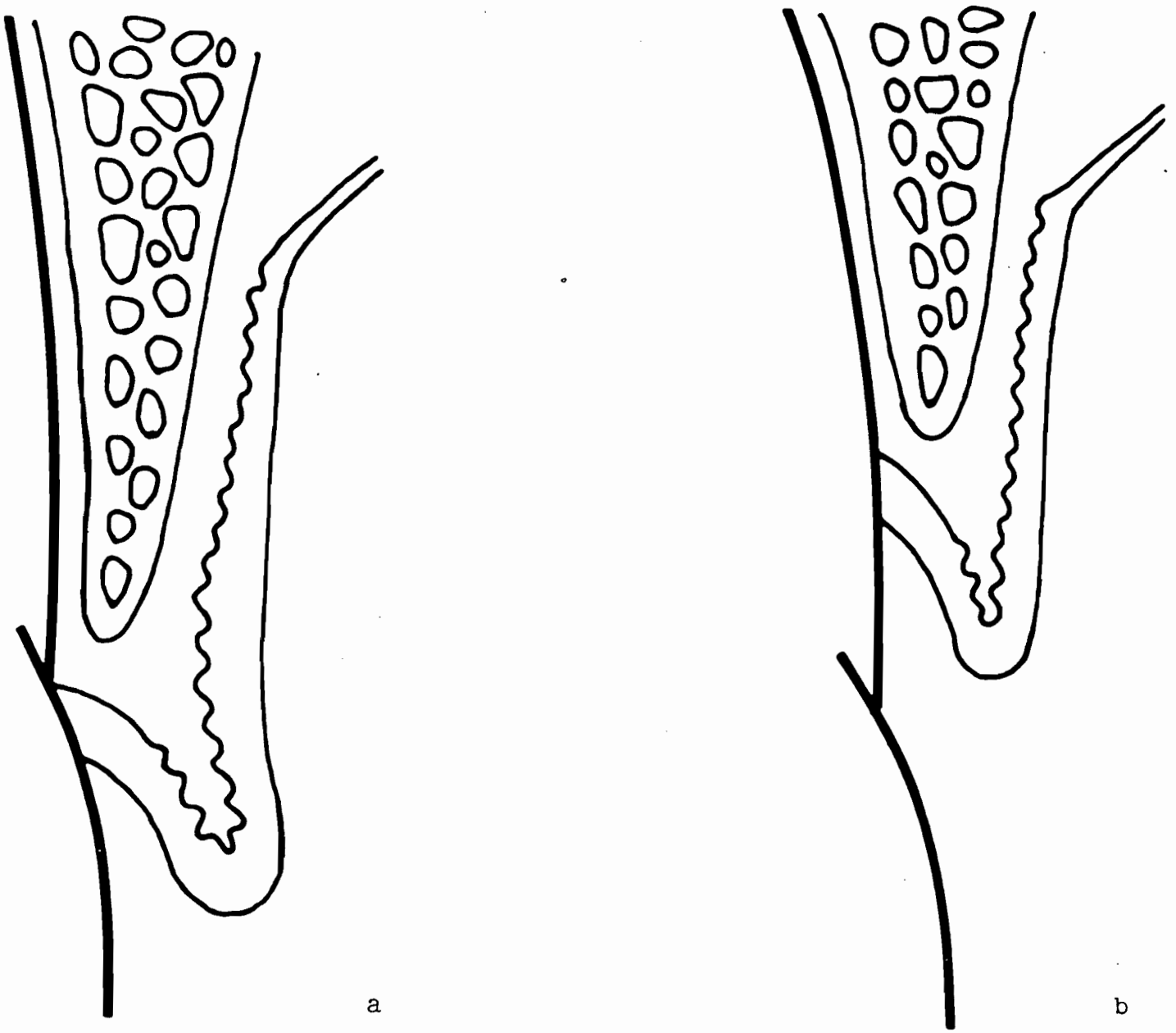

Fig. 5. Tissue destruction in inflammatory periodontal disease.*

[a]In gingivitis there is an apical migration of the junctional epithelium which does not extend beyond the cementoenamel junction.

[b]Periodontitis is characterized by pocket formation which results from a combination of gingival enlargement and loss of attachment. A pocket is a pathological extension of the sulcus apical to the cementoenamel junction.

*From Strahan and Waite [92, p. 19].

II. REVIEW OF THE LITERATURE

A The Association of Plaque and Periodontal Disease: Epidemiologic Surveys

Broadly defined, epidemiology is the science of the occurrence, distribution and determinants of states of health and disease in human populations. As applied to the study of periodontal disease,* the aims of epidemiology include: 1) the description of the prevalence and severity on a world-wide basis; 2) the description of the natural history of the disease; and 3) the search for causal mechanisms, an activity crucial to the development of means for preventing and/or intervening in the disease process.

The development and application of epidemiologic methods in periodontal research has been hampered by a number of factors. The long-term chronic nature of the disease increases the possibility that factors associated with periodontitis are more relevant to the duration rather than to the incidence of the disease. The uncertainty about the homogeneity of periodontal disease as a disease entity, the need to document severity, and the lack of pathognomic features have created further problems.

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*The term periodontal disease as used in this text refers only to gingivitis/periodontitis and not to other inflammatory diseases of the periodontium such as periodontosis (juvenile periodontitis), acute necrotizing ulcerating gingivitis (ANUG), or abscesses.

In response to these difficulties, indices designed to measure the prevalence and severity of periodontal disease in a quantitative fashion were developed. Among these were Shour and Masler's PMA Index for the assessment of gingivitis in 1947 [31], Russell's Periodontal Index in 1956 [74], Ramfjord's Periodontal Disease Index in 1959 [65].

The development of these indices paved the way for the large-scale studies of periodontal disease undertaken during that era [53,66,72,73,107]. In particular, the Periodontal Index and the Oral Hygiene Index have been used extensively. Some basic trends emerge from an inspection of the data generated by these studies. One of the most noteworthy findings concerns the almost universal prevalence of periodontal disease in all population groups examined. Moreover, this prevalence is not confined to adults: gingivitis is common in the primary dentition, and evidence of periodontal destruction (pocket formation, gingival recession, bone loss) may be found from adolescence. Periodontal disease increases in prevalence and severity with age. Rarely, however, is it an important cause of tooth mortality before the age of 30, although it exacts a heavy toll in middle and later life. The association of periodontal disease with age is thought to be due less to the decreased resistance of the host than to the longer exposure of the host to etiologic agents.

Of prime importance in the search for causal mechanisms is the overwhelmingly positive correlation

revealed in these surveys between periodontal disease and poor oral hygiene status. This association is found regardless of the criteria employed to assess periodontal disease: gingivitis scores, Periodontal Index scores, or radiographic estimates of bone loss. In a review of the literature on periodontal disease in children, Stratford concluded that after having examined the possible influences of gender, race, malnutrition, socioeconomic factors, systemic disease and malocclusion, the one positive finding to emerge was the strong correlation between periodontal disease and cleanliness of the dentition [93]. Schei et al grouped 737 male factory workers aged 21-45 by age and efficiency of toothbrushing as determined by oral cleanliness. He concluded that bone loss as measured by radiography increased with age, was greater in every age group for those with poor oral hygiene, and that the discrepancy in bone loss between oral hygiene groups increased with age [77]. Shapiro et al found a high positive correlation, +0.82, between Periodontal Index (PI) scores and Simplified Oral Hygiene Index (OHI-S) scores in a population of incarcerated women [81]. In a review of five surveys sponsored by the World Health Organization and a sixth sponsored by the United States Public Health Service, Ramfjord et al remarked on the strong association between oral cleanliness (as ascertained by calculus, plaque or debris scores) and periodontal disease (as determined by PI or PDI scores). Moreover, he found that differences in scores seemingly attributable to gender, racial or ethnic

group, socioeconomic status, or urban vs. rural living were explainable on the basis of differences in oral hygiene status [66]. Between 1958 and 1961, the International Committee on Nutrition for National Defense sponsored surveys of 21,559 persons aged 5 or older in eight countries. From an analysis of the data collected in South Vietnam and Lebanon, Russell determined that less than 10% of the variance in the PI scores remained to be explained after the influences of oral hygiene (as measured by OHI-S scores) and age had been taken into account [72].

Despite the use of different indices, lack of examiner calibration as well as socioeconomic and ethnic diversity of the populations surveyed, it appears justified to conclude a) that the severity of periodontal disease varies from one population to the next, and b) that it correlates highly with an increasing amount of plaque and calculus on the teeth. North Americans place a high social value on dental status, are more likely to practice good oral hygiene, and enjoy a comparatively high dentist-to-patient ratio; they therefore suffer the end-results of periodontal disease more infrequently and at a later age than do their counterparts in developing nations. Periodontal disease nevertheless poses a serious threat to the dental health of North Americans. The Health and Nutrition Examination Survey (HANES) conducted during 1971-74 on a random sample of 20,000 Americans revealed that pocket formation was present in fully half of the oldest adult group, aged 65-74. Advanced

disease was rare in children and adolescents, although gingivitis was seen in 13.6% of those aged 6-11 and in 32.2% of those aged 12-17. An estimated 14.7% of the adult population aged 18-74 had lost all of their permanent teeth, largely through periodontal disease [105].

B The Prevalence and Severity of Periodontal Disease Among the Mentally Retarded

With respect to poor oral hygiene and inadequate dental care, the dental status of mentally retarded persons may be compared with the dental status of persons in less developed countries. Findings documenting the increased severity and earlier onset of periodontal disease among the mentally retarded are therefore entirely plausible. In a clinical and roentgenographic investigation by Cohen et al of 100 mongoloid patients aged 1-30, 96 manifested signs of periodontal disease. Destructive disease as determined by bone loss and tooth attrition due to periodontal disease was seen as early as 7 to 12 years of age, and was observed in all patients aged 17 to 30 [15]. In an attempt to assess the prevalence of oral diseases in the mentally retarded, Butts examined 1930 children aged 6-18. The subjects were chosen by a combination of random and exhaustive sampling from institutionalized and non-institutionalized populations in Georgia. Extensive baseline data had been collected for dental conditions in normal school-aged children in that state with which Butts was able to compare his data. He concluded that for all ages, PI scores were significantly

higher for mentally retarded children, whether or not they were institutionalized, and that the differences in scores between retarded and normal children became progressively greater with increasing age. OHI-S scores were also significantly higher for the retarded children, whether or not they were institutionalized, a finding in agreement with the previously observed association between lack of oral cleanliness and periodontal disease [8].

C The Association of Plaque and Periodontal Disease: Case Studies and Clinical Trials

To infer a causal relationship between a determinant and a disease, criteria such as biological plausibility, consistency of study results, strength of the relationship, and temporality (i.e., whether the cause preceded the effect) must be demonstrated. As convincing as the association between poor oral hygiene and periodontal disease may appear in light of the epidemiologic studies discussed previously, the cross-sectional designs employed did not establish a cause-and-effect relationship between these two variables. Longitudinal studies of simple conception and design were thus undertaken by investigators such as Löe et al (1965) and Theilade et al (1966) in order to elucidate the temporal connection between oral cleanliness and periodontal disease [48,103]. Following a period of oral hygiene instruction and practice designed to achieve optimal gingival health at baseline, all oral hygiene measures were withdrawn. Within 21 days all subjects developed clinical gingivitis as

determined by whole-mouth Gingival Index scores (GI=1), thus demonstrating unequivocally the etiologic role of plaque formation in the initiation of gingivitis (which was considered to be the initial manifestation of periodontal disease). After oral hygiene measures were reinstituted, clinical signs of gingivitis subsided within one week, thus demonstrating that the removal of plaque produced a resolution of gingival inflammation. Interproximal areas exhibited the greatest plaque accumulation and the most severe gingival inflammation. Also noteworthy among the clinical data was the finding that rapid plaque formers developed gingivitis more quickly than slow plaque formers. The microbiological findings provided additional support for a causal relationship. A marked leukocyte migration was observed over the course of the experimental period, and characteristic changes in the supragingival flora were described by three phases. The establishment of a complex flora (gram-positive and gram-negative cocci and rods, filaments, vibrios and spirochetes) coincided with early clinical signs of gingivitis. Leukocytes and flora quickly returned to baseline levels after oral hygiene measures were resumed.

Experimental periodontitis studies analogous to the aforementioned experimental gingivitis studies cannot be attempted on human subjects for ethical reasons. This restriction has not precluded the undertaking of such studies on animal subjects, however. Owing to the high

prevalence of periodontal disease and to the similarity of clinical and histopathological signs of periodontal disease in Beagles and humans, the Beagle dog is a particularly appropriate animal model [38,67,100]. Clinical trials on Beagles conducted by Saxe et al and Lindhe et al support the notion that gingivitis will progress to periodontitis in the absence of oral hygiene [38,75]. In both studies, oral hygiene procedures were executed in an attempt to ensure optimal gingival conditions at baseline. Oral hygiene procedures were then suspended for designated quadrants, and clinical measurements were taken at specific points in time over an 18-month period. Saxe et al found significant differences in the amount of debris and calculus and in the degree of gingival inflammation between cleaned and uncleaned sites from three months until the end of the trial. Significant differences between groups with respect to loss of attachment was not observed until six months [75]. Lindhe et al reported marked increases in plaque, debris, calculus and gingival inflammation only for the uncleaned sites over the 18-month period. Significant loss of attachment occurred five to seven months after clinical gingivitis had been recognized (four to 12 months after baseline depending on tooth type). No evidence of gingivitis or progression to periodontitis was observed for clean sites [38]. The major discrepancy between these two reports concerns the residual gingival inflammation present at baseline and persisting for the duration of the study by Saxe et al in contrast to the

lack of any evidence of gingival inflammation of the cleaned sites in the dogs studied by Lindhe et al. This may be attributable to the different frequency of toothbrushing followed by the two studies. Saxe et al cleaned the animals' teeth once every other day, while Lindhe et al cleaned the dogs' teeth twice daily.

Further evidence that plaque is the most consequential factor in the etiology of periodontal disease derives from studies which show that mechanical debridement slows or arrests the progress of periodontal disease and retards or prevents recurrence following periodontal therapy. In a three-year clinical trial, Suomi et al offered 11 prophylaxes and repeated instruction in oral hygiene care to 343 experimental subjects, and a baseline prophylaxis to an equal number of control subjects. At follow-up there were substantial differences between the two groups, with the control group exhibiting higher Debris Index, Calculus Index and gingival inflammation scores. In the control group the epithelial attachment (as measured from the cementoenamel junction to the pocket bottom) migrated apically at a rate of 0.30 mm per tooth surface during the study period, in contrast to 0.08 mm per tooth surface in the experimental group. Frequent prophylaxes coupled with oral hygiene instruction therefore resulted in cleaner mouths with less gingival inflammation and a slower rate of attachment loss [95].

In a three-year clinical trial conducted by Axelsson

and Lindhe, age-matched study and control groups were comparable at baseline with respect to oral hygiene status and gingivitis scores. All participants received oral hygiene instruction prior to initial examinations. Thereafter, the study group received 4-6 prophylaxes annually which included scaling and polishing and repeated oral hygiene instructions, while the control group received necessary dental treatment but neither prophylaxes nor oral hygiene instructions. After three years the decrease in plaque scores and the improvement of gingival status were highly significant for the experimental group. No improvement for either of these parameters was noted for the control group. Epithelial attachment levels remained constant for the experimental group, whereas the majority of control patients lost 0.51 to 0.90 mm of attachment per tooth surface during the three-year interval [3].

In two separate studies, Rosling, Nyman and Lindhe followed patients with advanced periodontal disease, most of whom required surgery in all quadrants, for a period of two years. All subjects received oral hygiene instruction before and after surgery, the experimental groups received a professional tooth cleaning every two weeks which did not include scaling, and the control groups received annual or biannual prophylaxes. For the experimental group, Plaque Index and Gingival Index scores remained low for the duration of the trial, and epithelial attachment shifted coronally. These results contrasted sharply with those

obtained for the control group who exhibited increasing PlI and GI scores following surgery as well as a loss of attachment, thus demonstrating recurrent periodontitis [61,71]. As determined from radiographs, considerable bone regeneration was noted for the test patients but not for the controls [71].

In a five-year clinical study of 75 patients selected for treatment compliance, Lindhe and Nyman reported that PlI and GI scores remained low during the post-surgical period and that surgical pocket reductions were maintained. These patients received prophylaxes 2-4 times per year [40].

D The Nonspecific Plaque Hypothesis as a Basis for Treatment Approaches in Periodontal Disease

The studies described above clearly demonstrate: 1) the decisive role played by plaque in the initiation, progression and recurrence of periodontal disease; 2) the success of mechanical plaque control combined with oral hygiene instruction as a preventative measure; and 3) the inadequacy of symptomatic treatment as applied to periodontal disease. These conclusions tend to support the Nonspecific Plaque Hypothesis which has long been a major rationale behind approaches to the treatment of periodontal disease (Table 1). The NSPH states that noxious products elaborated by the entire plaque flora are responsible for manifestations of periodontal disease. Also integral to this hypothesis is the concept of a host threshold; i.e., periodontal destruction results when these noxious products overwhelm host defenses.

Since, in this view, all dental plaques are potentially harmful--and if left undisturbed will precipitate periodontal destruction--the simplest and most effective means of preventing this destruction would be mechanical debridement of cervical tooth surfaces. If periodontal breakdown has already occurred, treatment comprises surgical procedures in addition to mechanical debridement.

This approach to the prevention and treatment of periodontal disease is costly, time-consuming and stressful for the patient. In a British study of patients referred for periodontal treatment to a dental clinic, only 27% of whom required surgery, Ekanayaka and Sheiham found that the average patient required 9.3 ± 3.9 visits over a period of 49.5 ± 16.8 weeks for examination, scaling and polishing, surgery, and patient education; 70% of the time was devoted to prophylaxis and patient education [22]. Although this study cannot be taken at face value, or even be considered representative, due to unresolved issues in estimating treatment times (such as criteria for surgery, proportion of time devoted to oral hygiene instruction, oral conditions of the population under study, availability of equipment and auxiliary personnel, workload, level of operator's skill, etc.), it certainly provides insight into the time-consuming nature of periodontal treatment. An idea of the expense involved may be obtained from the finding that dental disease ranks third in cost behind heart disease and cancer in the U.S. [51]. The short-lived benefits of this treat-

ment approach are evident from studies which show that professional prophylaxes and patient reinstruction in oral hygiene practices must be repeated at least 3-4 times yearly to prevent recurrence and further destruction following surgery [3,40,61,71,95]. Finally, even though estimates of the resources and personnel required to prevent and treat periodontal disease have not been carried out in a systematic fashion, there is little doubt that dental personnel and facilities are insufficient to meet professionally determined treatment needs.

E Support for the Specific Plaque Hypothesis

The SPH (Table 1) states that dental plaques differ in their composition from person to person, from tooth to tooth and from one surface to another on the same tooth, that these plaques have different periodontopathic potentials, and that some plaques even seem to be compatible with healthy periodontal tissues [42,49,50,83]. Evidence in support of the SPH derives from: 1) experiments in which gnotobiotic animals were infected with suspected periodontal pathogens (such as Actinomyces species and gram-negative anaerobic rods) and monitored for manifestations of periodontal disease [67,100,102]; 2) studies of the efficacy of adjunctive chemotherapy in suppressing a select segment of the pocket microbiota [32,33,44,52,85,90]; and 3) microbiological studies of gingival sulcus and periodontal pocket flora in health and various degrees of periodontal disease severity [18,39,42,43,48,83,84,86,90,97,99,103].

TABLE 1

COMPARISON OF THE NONSPECIFIC PLAQUE HYPOTHESIS (NSPH) AND THE SPECIFIC PLAQUE HYPOTHESIS (SPH) AS APPLIED TO PERIODONTAL DISEASE

Criterion	NSPH	SPH
Statement of Hypothesis	All plaques have equal potential for periodontal pathogenesis.	Some plaques are more periodontopathic than others.
Indications for Treatment	Non-discriminatory. Since all persons form plaque, treatment must be universal.	Discriminatory. Precise clinical and microbiological criteria must be met.
Treatment Objective	The prevention or removal of plaque.	The resolution of a specific bacterial infection.
Choice of Chemotherapy	Broad-spectrum chemical antimicrobial agents effective in controlling plaque mass. Antibiotics are not favored because of the development of resistant strains with prolonged use.	Broad-spectrum or more limited spectrum antibiotics effective in the suppression and/or elimination of periodontopathic flora.
Mode of Delivery	Topical or systemic.	Systemic.[a]

Dosage	Lower.	Higher.[a]
Duration	Open-ended or long-term (until) adverse signs prompt the withdrawal of the agent).	Short-term.[a]
Treatment Evaluation	Disease amelioration or resolution as assessed by clinical signs, or the absence of plaque.	Disease resolution as assessed by reduction or disappearance of suspected pathogens coincident with clinical improvement.

[a]Approaches to treatment based on the SPH have as their objective the immediate suppression and/or elimination of periodontal pathogens.

In experiments with gnotobiotic animals, problems of interpretation arise from the recognition that an agent may be pathogenic in the absence of other microorganisms competing for the same ecological niche. The question of comparability of disease etiology and clinical manifestations between different species must also be considered.

Similarly, there are several difficulties inherent in studies of periodontal sulcus/pocket flora and the interpretation of study results including:

1) The complex microbial colonization of the gingival sulcus and periodontal pocket. Individual sites may harbor 20-40 species, and 200-300 species may be recovered from different sites in a single individual [91].
2) Persisting uncertainties in bacterial taxonomy [91,100].
3) Technical problems in the cultivation, dispersion and observation of samples. Many bacteria are nutritionally fastidious or oxygen-sensitive and thus difficult to cultivate. Slots demonstrated that the strictly anaerobic roll tube technique was more efficient than either conventional anaerobic or aerobic methods in maximizing total cultivable isolates from subgingival samples [83,84,86]. Failure to achieve strict anaerobiosis would thus lead to an underestimation of the prevalence of anaerobic microorganisms. Some bacteria cannot be cultivated at all by present methods (e.g., spirochetes). Dispersion enhances homogeneity of the sample which is important for assuming that the sample is representative and hence valid. Approaches to the observation of

samples include direct darkfield microscopy of plaque samples [39,43], light and electron microscopy of in situ plaque [42], and light microscopy of cultivable bacteria using selective and non-selective media [18,83, 84,86,97,99], each of which is associated with certain advantages and disadvantages. This multiplicity of approaches renders comparison of study findings difficult.

4) Sample selection. Samples collected from one site per individual may not be representative of the flora associated with the periodontal disease state of the individual. Samples pooled from several sites in the same individual may mask possible between-site differences in flora composition [84]. Since it is biologically plausible that microorganisms at the advancing front of the lesion are the most likely to be involved in the etiology and progression of periodontal disease, care should be taken to obtain the sample from the most apical portion of the sulcus/pocket and to reduce contamination by debridement of supragingival plaque adjacent to the site prior to sample collection [84,91]. Finally, individuals to be studied should exhibit similar clinical signs in order to avoid distortion of results due to misclassification error [91].

5) Determination of disease activity. Whether progression of periodontitis proceeds at a constant rate, or is marked by exacerbations followed by periods of remission, has yet to be determined. If this latter course is more

characteristic of the progression of the disease, then one might expect the microbiota to undergo secular change, a situation which could compromise the representativeness of plaque samples [90,91].

6) The likelihood that several microbial species or groups of species, either singly or together, may initiate and figure in the progression of a single disease entity, e.g., periodontitis [90].

Despite these barriers to interpretation, certain patterns have emerged from recent studies on the sulcus/pocket flora. Studies of healthy gingiva reveal the existence of a scanty (1 to 20 cell layers) supragingival plaque predominated by gram-positive cocci and rods, representing roughly 90% of the total flora [18,48,83,90,103]. Species typically found in this flora include Streptococcus mitis, Streptococcus sanguis, Staphylococcus epidermides, Micrococcus and Peptococcus species, Actinomyces viscosus, Actinomyces naeslundii, Rothia dentocariosa, Arachnia propionica.

In the experimental gingivitis studies, there was an increase in total cell mass (100 to 300 cells in thickness), in the proportion of filamentous Actinomyces species, and in gingival inflammation following cessation of oral hygiene procedures. Löe et al and Theilade et al observed a sharp increase in the proportion of fusiform and filamentous microorganisms at 2-4 days post-baseline and the appearance of motile cells and spirochetes at 6-10 days post-baseline

[48,103]. Syed et al noted that Streptococcus species predominated in 0- to 1-week-old plaques, while Actinomyces species predominated in 2- to 3-week-old plaques [97].

Slots conducted a series of studies designed to characterize and enumerate the cultivable subgingival flora in the absence of periodontal disease, in chronic gingivitis and in advanced periodontitis [83,84,86]. He found that proportions of both gram-negative microorganisms and obligate anaerobes increased with severity of disease (Table 2). Gram-positive cocci and rods dominated the flora of all 7 subjects in the healthy sulci group, while gram-negative rods (e.g., Bacteroides melaninogenicus, Fusobacterium nucleatum, other Bacteroides and Fusobacterium species, and a minor group of motile cells including Campylobacter sputorum, and Selenomonas sputigenum) dominated the flora in 6 of the 8 subjects with advanced periodontitis. Crawford et al reported that asaccharolytic gram-negative anaerobic rods such as Bacteroides and Fusobacterium species made up an average of 70% of the predominant cultivable flora in sites exhibiting signs advanced disease, a finding which substantiates Slot's figure of 74.3% [18].

The primary limitations to light microscopy of cultivated bacterial samples to which the gram stain has been applied include: distortion of proportions due to the inability to cultivate some microorganisms--notably spirochetes--and the impossibility of assessing motility. These limitations may be avoided by direct darkfield

TABLE 2

RESULTS OF BRIGHTFIELD MICROSCOPY STUDIES OF CULTIVABLE ISOLATES OBTAINED FROM SUBGINGIVAL PLAQUE SAMPLES [a]

	Slots 1977 [83] Healthy Sites (n=7)[b]	Slots et al 1978 [86] Gingivitis (n=9)[b]	Slots 1977 [84] Advanced Periodontitis (n=8)[b]
Criteria used to determine health/disease status	GI=0, pocket depth ≤ 3 mm	pocket depth ≤ 4 mm, no alveolar bone loss, GI=1,2, or 3	pocket depth ≥ 6 mm, radiographic bone loss in 5 of the 8 Ss
gram-negative obligately anaerobic rods	12.7%	25.0%	74.3%
gram-negative facultatively anaerobic rods	----	14.8%	----
gram-negative obligately anaerobic cocci	2.0%	4.3%	0.6%
gram-negative facultatively anaerobic cocci	0.3%	----	----

gram-positive obligately anaerobic rods	9.5%		9.2%		15.1%	
gram-positive facultatively anaerobic rods	35.1%	44.6%	16.9%	26.1%	3.9%	19.0%
gram-positive obligately anaerobic cocci	0.8%		3.0%		----	
gram-positive facultatively anaerobic cocci	39.6%		26.8%		6.2%	
total gram-negative cocci and rods	15.0%		44.1%		74.9%	
total obligately anaerobic cocci and rods	25.0%		41.5%		90.0%	

[a]Results are expressed as a percentage of total cultivable isolates.

[b]Multiple sites were samples per subject.

microscopy, although this method cannot differentiate between gram-positive and gram-negative cells. Electron microscopy permits the viewer to distinguish cell wall structures characteristic of gram-positive or gram-negative organisms, motility by the presence of flagella, and spirochetes. Thus, one might expect studies using either darkfield or electron microscopy to generate results different from those obtained through light microscopy of cultivated samples.

In a light and electron microscope study of in situ plaque, Listgarten found the supragingival plaque from healthy specimens to be dominated by gram-positive cocci. Some filamentous and gram-negative organisms were also observed, but spirochetes and motile rods were not seen. Supragingival plaque from gingivitis and periodontitis samples (as determined by probing depth, alveolar bone loss and GI scores) was remarkably similar to that of healthy specimens, although it was denser and exhibited a higher proportion of filamentous forms and gram-negative organisms. Motile rods and spirochetes were observed in the subgingival plaque from gingivitis and periodontitis specimens, with the presence of intermediate-sized spirochetes being very marked in advanced disease [42].

The findings from two darkfield mircoscopy studies of subgingival plaque (Table 3) clearly show a decrease in the proportion of cocci and straight rods as well as an increase in the proportions of both motile rods and spirochetes

TABLE 3

RESULTS OF DARKFIELD MICROSCOPY STUDIES OF SUBGINGIVAL PLAQUE SAMPLES [a]

	Listgarten and Helldén 1978 [43] (n=12)[b]		Lindhe, Liljenberg, Listgarten 1980 [39] (n=22)[b]		
	Healthy* sites	Diseased** sites	Healthy sites	Established gingivitis	Advanced disease
Cocci, Straight Rods	90.0%	40.0%	76.0%	40.1%	16.4%
Filaments, Fusiforms	7.2%	7.5%	19.2%	42.9%	10.8%

Spirochetes	1.8%	37.7%	2.1%	8.1%	57.2%
Motile Rods	0.3%	12.7%	2.9%	7.9%	15.3%

[a]Results are expressed as a percentage of total enumerated cells.

[b]Multiple sites were sampled per subject.

Criteria for site selection:

*GI=0 or 1; mean pocket depth = 1.9 mm.

**Pocket depths of at least 5 mm; alveolar bone loss of at least 25%.

†No criteria given.

††Pocket depths of at least 8 mm; alveolar bone loss of at least 50%.

with increasing severity of disease. Reasons for the large discrepancy between study results with respect to percentages of filamentous and fusiform cells remain obscure, although criteria for site selection and small sample size may provide partial explanations. Listgarten and Helldén found statistically significant differences in the proportions of cocci, motile rods, and spirochetes between healthy and diseased sites. Also noteworthy was their finding that the ratio of motile to nonmotile cells was 1:49 for the healthy sites compared to 1:1 for the diseased sites [43]. Lindhe, Liljenberg and Listgarten found the proportion of cocci and straight rods to be significantly greater than the proportion of other morphological types in healthy sites, while the proportion of spirochetes was significantly greater than the proportion of other morphological types in sites affected by advanced disease [39]. Listgarten and Levin studied the subgingival flora in 19 patients with chronic periodontal disease who received no further professional treatment for one year following a baseline polishing and scaling. For ethical reasons, teeth which showed signs of disease progression were removed from the study and given appropriate treatment. Significantly higher proportions of both spirochetes and motile cells were found at all recall examinations for those patients with 2 or more 'exited' teeth, while significantly higher proportions of coccoid cells were demonstrated for those patients with no 'exited' teeth. Based on these findings, the authors proposed the use of

microbiological profiles as a predictor of periodontal breakdown [44].

The research efforts described here strongly suggest that different microbiotas seem to be associated with healthy, chronic gingivitis and advanced disease sites in humans. Moreover, in view of the difficulties involved in the interpretation of such studies, the results appear to be remarkably consistent, although further studies will be required to substantiate these results. Healthy sites are characterized by a preponderance of gram-positive cocci, straight rods and filaments. Spirochetes, motile and nonmotile gram-negative rods are absent or detected only rarely. In established gingivitis there is an increase in filamentous forms, spirochetes, and motile and nonmotile gram-negative rods with a concomitant decrease in gram-positive cocci and straight rods. Gingivitis is considered to be an antecedent to further periodontal breakdown. However, transitory episodes may resolve without sequelae, and in some patients chronic gingivitis may persist for long periods of time without progressing to periodontitis. Whether or not progression to periodontitis occurs may be explained by differences in host response to the causative bacteria and/or an alteration in the bacterial component either by the overgrowth of one or more existing species or by the appearance of new pathogenic species in the established gingival lesion. Existing data tend to support the second explanation. Advanced periodontitis is associated with:

1) an obligately anaerobic gram-negative population of asaccharolytic rods; 2) a smaller proportion of saccharolytic rods such as Eikenella corrodens and corroding Bacteroides; 3) a group of gram-negative anaerobic motile rods; and 4) spirochetes. Unfortunately, due to the use of different technical methods and sampling criteria, the relative proportions of these groups cannot be established at the present time.

The similarity of the subgingival flora in humans and Beagles with respect to the high proportions of Bacteroides asaccharolyticus, Fusobacterium nucleatum and spirochetes found in diseased sites [45,89,98] provides evidence for the possible pathogenicity of these microorganisms and further substantiates the suitability of the Beagle model for the study of human periodontal disease.

Although the data indicate a pronounced shift towards an obligately anaerobic gram-negative population of non-motile rods, motile rods and spirochetes in association with periodontitis, caution must be exercised when attributing an etiologic role to these bacteria. Instead of playing a major role in the pathogenesis of the disease, it may be that this distinctive flora appears secondarily in response to nutritional and anaerobic conditions. This possibility underlines the necessity of establishing criteria for determining the periodontal pathogenicity of a given microorganism.

Since the late 19th century, Koch's postulates have been employed to assess the etiological role of infectious

disease agents. Briefly, these are:

1) The organism is regularly found in lesions of the disease;
2) The organism can be isolated and cultured;
3) When injected into experimental animals, pure cultures of this organism will produce the same or similar signs of disease; and
4) The organism can be recovered from the lesions in these animals.

Because of the complex array of bacteria inhabiting the sulcus/pocket and the questionable comparability of culturing and implanting certain microorganisms, Koch's postulates may be inadequate as criteria for the etiologic role of a given organism in periodontal disease. Socransky has suggested that the following criteria may be more relevant to dental researchers [91]:

1) The suspected organism is frequently found at sites of pathology and may account for a large proportion of the total flora. At healthy sites or sites with different forms of disease, the implicated organism is found in smaller proportions or may be entirely absent.
2) Elimination or suppression of the organism by mechanical debridement or chemotherapy should coincide with a termination of progression in the active lesion.
3) An increased or decreased cellular or humoral immune response to an organism is suggestive of an etiologic role.
4) Animal pathogenicity testing as embodied by Koch's

postulates should continue to serve as a basis for the determination of a possible etiologic role.

5) The demonstration of the biochemical or immunological mechanisms whereby a suspected organism could contribute to the pathogenesis of the disease represents important confirmatory evidence.

Socransky also devised a weighting scale for evaluating these criteria, placing the greatest emphasis on the first two. The present study will likewise focus on a) establishing the association of certain microorganisms with periodontitis, and b) determining the effect of suppressing or eliminating these organisms in terms of clinical improvement in periodontal status.

In summary, recent microbiological findings provide much support for the Specific Plaque Hypothesis as it applies to destructive periodontal disease.

F The Pathogenesis of Periodontal Disease

On the basis of histopathologic findings from animal and human studies, the sequence of changes which occur during the development of periodontal disease has been delineated in four stages. Initially, there is an increase of polymorphonuclear leukocytes (PMN'S) in the junctional epithelium, a decrease in collagen density in the most coronal portion of the underlying connective tissue, an apical shift of the sulcus without an associated loss of connective attachment, and an increasing capillary dilation and permeability. These changes are macroscopically

invisible: gingiva exhibiting these histopathological alterations would be considered clinically healthy. The early lesion is marked by an inflammatory infiltrate in the subjacent connective tissue with lymphocytes being the most numerous cell type, an increasing gingival exudate and continuing collagen destruction. The preponderance of plasma cells in the inflamed connective tissue, the proliferation of epithelium into the connective tissue (=rete peg formation), continued collagen destruction, and morphological vascular changes are characteristic of the established lesion. Progression through these first three histopathological stages--which correspond to the descriptive terms 'subclinical' and 'clinical' gingivitis--may occur in 2-3 weeks following cessation of oral hygiene procedures. The established lesion may remain indefinitely confined to the free gingiva or may extend laterally and apically after a period of weeks or years. The advanced lesion is synonymous with periodontitis: clinical manifestations include the migration of the junctional epithelium apical to the CEJ; acute vasculitis; chronic, fibrotic inflammation; destruction to the periodontal ligament and alveolar bone; tooth mobility, migration and attrition [62].

Mechanisms of disease production have been postulated on the basis of the morphological alterations described above and the results from immunological studies. The pathogenic mechanisms of periodontal disease represent a series of complex interactions between bacterial effects and

host responses to these bacteria (Table 4). Bacterial effects include:

1) Induction of neutrophil chemotaxis by low molecular weight peptides and endotoxins elaborated by the plaque bacteria.
2) Tissue destruction due to bacterial enzymes such as collagenase, hyaluronidase, chondroitin sulfatase, or to the action of endotoxins or toxic metabolic products such as hydrogen sulfide, ammonia, amines.
3) Antigenic stimulation of lymphocytes.
4) Release of endotoxins which stimulate phagocytosis by PMN'S, collagenase production by macrophages, bone resorption, activation of complement.

The lymphocytic infiltrate of the early lesion suggests that cellular immunity plays a role. In response to antigenic challenge, T cells proliferate and generate lymphokines, pharmacologically active substances which marshal and amplify cellular immune responses. The presence of plasma cells as well as intra- and extracellular antibodies suggests that humoral immune responses also play a role. Antibody-mediated immunity mechanisms may include anaphylactic reactions, Arthus reactions and complement-fixing cytotoxic reactions. The release of lysosomal enzymes from PMN's and macrophages contribute to tissue damage. What substances, if any, initially penetrate the healthy gingiva have not yet been determined, but it is likely that bacterial antigens become localized in the gingiva at some point

TABLE 4

ETIOLOGY OF PERIODONTAL DISEASE

I BACTERIAL EFFECTS

- Endotoxins
- Enzymes
- Antigens
- Other cytotoxic products

II HOST RESPONSE

- Inflammation
- Vascular response
- Cellular response

A Humoral Immune Response

- Production of antibodies with specificity for bacterial toxins, enzymes, antigens
- Formation of antigen-antibody complexes which may be phagocytosed by neutrophils or macrophages or which may activate complement
- B cell production of lymphokines physiochemically similar to those manufactured by T cells

1) Activation of Complement by Ag-Ab Complexes or Endotoxins

- PMN chemotaxis
- Enhancement of phagocytosis
- Cell lysis
- Increased vascular permeability

TABLE 4 — Continued

B Cellular Immune Response

- Chemotaxis of macrophages
- Inhibition of monocyte and macrophage migration from the inflamed area
- Stimulation of collagenase production by macrophages
- Nonspecific cell destruction by lymphotoxins
- Activation of osteoclasts
- Mediation of humoral immune response
- Cell lysis either directly by activated T cells or by macrophages which have acquired antigen specificity

during the progression of the disease [28,67,78,92].

In conclusion, it appears that both inflammatory and immunological reactions contribute to the pathogenesis of periodontal disease with more emphasis being placed on the latter as a cause of tissue damage. The inflammatory responses present early in the development of gingivitis as well as part of the inflammation seen in more advanced disease result from non-immunologic, inflammatory effects of endotoxins, enzymes and other bacterial products. Immunosensitization probably develops shortly after plaque accumulation, a phenomenon which amplifies the inflammatory response. Specific mechanisms essential to the initiation and/or progression of chronic inflammatory periodontal disease have not yet been determined.

G Adjunctive Antibiotic Therapy in the Treatment of Periodontal Disease

The NSPH is prejudiced to the use of antibiotics because their universal, open-ended use increases the likelihood of patient sensitization, antibiotic toxicity and the selection of resistant bacterial strains. Recent research findings documenting discrete microbial profiles associated with different periodontal disease entities have lent support to the SPH. Treatment approaches based on the premises of the SPH are aimed at the immediate suppression and/or elimination of these suspected periodontal pathogens. With increasing acceptance of the SPH, there has been renewed interest in the use of antibiotics in periodontal therapy.

Although the definitive treatment remains to be established, current thinking suggests that this might include intensive mechanical plaque control measures and adjunctive antibiotic therapy. Since treatment would be initiated in the presence of specific clinical bacteriological signs and terminated upon their disappearance, the drawbacks of promiscuous use of antibiotics would be largely avoided.

An antibiotic considered for use in the treatment of periodontal disease according to the SPH should possess certain properties [49]:

1) Specificity. The selected agent should demonstrate efficacious action against the suspected microbial pathogens.
2) Substantivity. The agent must remain in contact with the substrate sufficiently long to exert its bactericidal action.
3) Safety. Side effects should be minor, infrequent and reversible. Neither should the drug be implicated in human carcinogenesis or mutagenesis, nor should it encourage the development of resistant strains.
4) Stability. The drug should have a reasonably long shelf life.

The suitability of metronidazole with respect to the above criteria as well as clinical studies of its efficacy in the treatment of periodontal disease will be discussed in the following sections.

H Metronidazole: Development, Mode of Action, Metabolism, Toxicity, Tumorigenicity, Teratogenicity

Research for an effective antitrichomonal agent was undertaken by the Rhone-Poulenc Laboratories (France) in 1954. The identification of the weak trichomonicidal activity of azomycin (2-nitroimidazole) in 1956 stimulated the synthesis of almost 150 analogs.

Metronidazole (1-hydroxymethyl-2-methyl-5-nitroimidazole) emerged as one of the most effective and least toxic compounds studied (Fig. 6). Clinical testing was begun in 1958, and the drug was first marketed in 1960 primarily for the treatment of genitourinary tract infections caused by Trichomonas vaginalis [36]. Subsequent testing revealed its effectiveness against Entameba histolyica (amebiasis) and Giardia lamblia (giardiasis) [36,69]. In 1962 Shinn observed that a patient suffering simultaneously from trichomoniasis and Vincent's gingivitis (ANUG), and receiving metronidazole for the first condition, was cured of both [82]. Because Vincent's gingivitis was considered to be caused be anaerobic bacteria, research efforts to determine the effectiveness of metronidazole against anaerobic bacteria were initiated.

Chow et al examined the bactericidal activity of metronidazole against 1054 strains of anaerobic bacteria. Sensitivity was determined by the minimal inhibitory concentration (MIC), the lowest concentration that yielded no growth on culture plates. The results confirmed metronidazole's broad-spectrum activity against anaerobes. At a

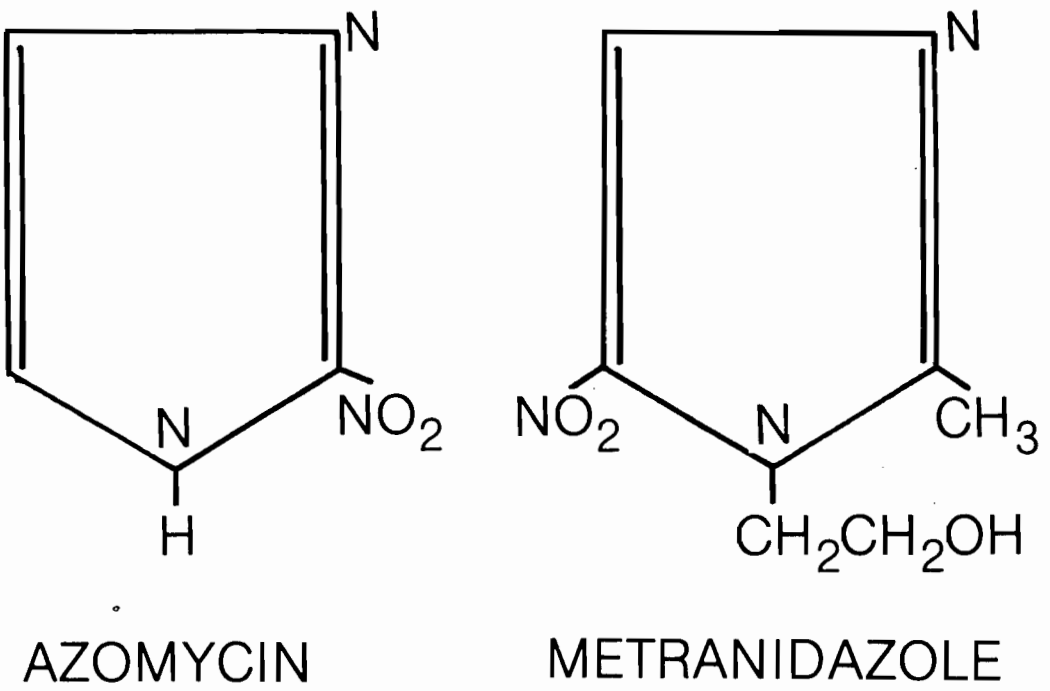


Fig. 6. Chemical Formulas for Azomycin and Metronidazole.

concentration of 6.25 mcg/ml, Fusobacterium was the most sensitive group (95% inhibition), followed by Glostridium (87%), Bacteroides (85%), Veillonella (77%) and Peptostreptococcus (65%). Among the Bacteroides species, Bacteroides melaninogenicus was the most sensitive (95%). Thus, organisms which have been implicated in the etiology of periodontitis were found to be highly susceptible to the bactericidal action of metronidazole. In contrast, gram-positive rods and cocci such as Propionibacterium, Eubacterium, Lactobacillus, Actinomyces and Streptococcus species, all of which are found in high proportions in the healthy gingival sulcus, were moderately resistant to the antibiotic. In a separate analysis of the resistant anaerobic strains, it was discovered that these were the very ones most likely to develop

aerotolerance upon subculture; e.g., Eikenella corrodens and Bacteroides corrodens [14]. In vitro studies of 730 anaerobic strains by Sutter and Finegold substantiated the findings of Chow et al. At 4 mcg/ml 100% inhibition was noted for Bacteroides melaninogenicus, Bacteroides asaccharolyticus, Fusobacterium nucleatum, Veillonella parvula, Veillonella alcalescens [96]. Metronidazole's ability to rapidly eliminate spirochetes has also been documented [19, 30,32,45,52].

In spite of the extensive testing and use of this drug, its precise mode of action is not completely understood. It has been hypothesized that sensitive organisms possess ferrodoxin- or flavodoxin-type electron transport proteins capable of reducing the nitro group of metronidazole, thereby establishing a concentration gradient which increases cellular uptake. The reduction process produces derivatives which are apparently responsible for killing the microorganisms, probably by disturbing mechanisms of protein synthesis. The diminished sensitivity of aerobic bacteria may be attributed to the competition for electrons between molecular oxygen and metronidazole: if the electron transport protein is oxidized by molecular oxygen (or any other oxidizing agent), fewer electrons will be available for the reduction of the nitro group [60].

Metronidazole is usually well absorbed (80% within one hour) after oral administration [101]. Although a few patients may fail to respond to treatment due to poor

gastrointestinal absorption of the drug, effective serum levels can usually be attained in these patients by increasing the dosage [9]. Equilibration between the blood and regions of therapeutic importance is rapid: metronidazole has been detected in vaginal secretions, seminal fluid, bile, cerebrospinal fluid, brain and hepatic abscesses. Judicious use is recommended in pregnant and nursing mothers since metronidazole crosses the placental barrier and is excreted in milk. The renal pathway is the major route of elimination.

For the purposes of the present study, it should be emphasized that metronidazole is actively secreted by the salivary and oral mucous glands; it is detectable in the saliva and gingival exudate [101]. One investigation showed a mean concentration of 15.32 mcg/ml of saliva by the fourth day of a standard therapeutic regimen consisting of 250 mg metronidazole t.i.d. [82]. And for a course of 200-250 mg given t.i.d. for seven days, a plateau of about 6 mcg/ml of blood is reached by the third day of dosing [101]. Based on the assumption that many tissues and body fluids will have a concentration equal to or exceeding the measurable blood concentration in the first hours following administration, and considering the low minimal inhibitory concentrations characteristic of most anaerobic bacteria, 250 mg t.i.d. over a 7-day period was selected as the appropriate therapeutic dose for this study.

Metronidazole has been tested for toxicity in a

number of different species and at a multitude of dose levels. In a review of the topic, Bost summarized the results of several studies which have been performed on mice, rats, dogs and monkeys. In mice and rats, both acute dose and long-term administration of metronidazole suggested a sizable margin of safety. Toxic changes included reduced spermatogenesis and decreased weight gain. These signs of toxicity were present only occasionally and were confined to animals receiving high multiples of the therapeutic dose recommended for humans. A considerable degree of safety was also demonstrated in monkeys: histological liver changes without accompanying changes in liver function were seen at very high doses. In comparison with other species, dogs were sensitive to metronidazole. At levels of 150 and 225 mg/kg/day, ataxia, muscular rigidity and tremors were observed; however, all signs disappeared within one week following termination of treatment [7]. It is worth remarking that 150 mg/kg/day represents the equivalent of 7500 mg/day in a 50-kilogram (small adult) human, or ten times the recommended therapeutic dose for humans.

Perhaps the most convincing evidence for the safety of metronidazole is provided by the twenty years of extensive use during which it has been shown to be non-toxic at recommended dosages. Occasional side effects include nausea, an unpleasant taste in the mouth, furring of the tongue, and gastrointestinal upsets. Headache, dizziness, sleepiness, depression, ataxia, and skin eruptions have been only

infrequently reported, and a disulfiram-like reaction may occur if alcohol is taken during therapy [9,69].

Roe reviewed the tumorigenicity studies in mice, rats and hamsters and found that positive results had been reported for a single study of Swiss mice which were fed a daily diet containing at least 420 times the standard dose for humans. In this study, an increased incidence of lung tumors was demonstrated for both sexes, and female mice showed a significant increase in malignant lymphomas. Several points are salient with respect to the interpretation of these findings. First, the incidence of lung tumors found in untreated control animals for both sexes was approximately 20%. It seems plausible that other factors, either genetic or environmental, could have significantly influenced the occurrence of these lesions. Secondly, there is a lack of any clear dose-related trend for lung tumor occurrence. Thirdly, no explanation has been given for the finding that the incidence of malignant lymphomas was elevated only for female mice [70].

In response to the study just described, to studies which have demonstrated the mutagenic activity of metronidazole in bacteria, and to the lack of case reports and epidemiologic data to complement animal studies, Beard et al undertook a retrospective cohort study to assess the risk of cancer after the use of metronidazole for the treatment of trichomoniasis. The medical reports of all women with a first diagnosis of vaginal trichomoniasis from January, 1960,

through December, 1969, were extracted and analysed. After excluding women with doubtful diagnoses and those who had already developed cancer, there were 771 women with a documented exposure to metronidazole and 237 with no exposure. The 24 observed cancers in the exposed group were compared with the expected numbers of 21.7 (Connecticut Tumor Registry) and 18 (Third National Cancer Survey). Calculations of risk ratios (observed/expected) and their 95% confidence intervals showed that tumor incidence in the exposed group was not significantly higher than that cited in the general population surveys. In a separate analysis of 10 types of cancer, only the risk ratio for lung cancer was found to be statistically significant ($P<0.05$). However, all four women in the exposed group who developed lung cancer were smokers, and three were over 60 years old. In yet another analysis, the incidence of in situ carcinoma of the uterine cervix was examined for exposed and non-exposed women. Findings revealed that non-exposed women actually had a greater incidence of cancer than exposed women. This was interpreted as indicating that trichomoniasis may be associated with carcinoma of the cervix, a situation which could be potentially confounding and lead to spuriously positive results in other studies of this sort [5].

Bost reviewed the embryotoxic and teratogenic potential of metronidazole in two mouse studies, six rat studies, four rabbit studies and one guinea pig study. Positive results were reported in one mouse, one rat and one

guinea pig study, all of which were performed by a single laboratory [7]. It is important to note that Beard et al failed to demonstrate a significant increase in fetal death in pregnant women in the exposed group when compared to pregnant women in the non-exposed group [5].

In summary, the effectiveness of metronidazole in treating protozoal and anaerobic infections, together with the lack of evidence that it exerts toxic, carcinogenic or teratogenic effects in humans at recommended doses, justifies its use as an antibiotic and antiprotozoal agent.

I Metronidazole: Applications in Dentistry

Shinn's fortuitous observation that a woman receiving treatment for trichomonal vaginitis underwent a spontaneous cure of her ulcerating gingivitis led to clinical studies of the efficacy of metronidazole in treating ANUG, (a periodontal disease characterized by ulceration of the interdental papillae, and sometimes accompanied by halitosis, lymphadenopathy, pyrexia and malaise). Metronidazole was subsequently shown to resolve hemorrhage and ulceration within two days on a regimen of 200 mg t.i.d. [82]. This discovery marked the beginnings of the drug's applications in dentistry.

In response to the positive results obtained from uncontrolled clinical trials of metronidazole reported by Shinn and Davies [19,82], Duckworth undertook a double-blind randomized controlled clinical trial. Two groups of patients were ascertained to be comparable at baseline with respect to age, sex and severity of disease. The 32 subjects in

the control group received 250 mg phenoxymethylpenicillin, an antibiotic commonly used in the treatment of ANUG, q.i.d. for three days; the 33 experimental subjects received 200 mg metronidazole t.i.d. for two days. Subjective assessment of pain and bleeding, bacteriologic smears and percentage of affected interdental papillae were recorded at baseline and 48 hours later. In terms of all three parameters, metronidazole was shown to be as effective as penicillin. Interestingly, of the 41 persons who responded to a questionnaire sent to patients one year after treatment, all 8 who reported recurrences had been treated with penicillin [21].

In further clinical trials the beneficial results of metronidazole therapy in ANUG have been demonstrated when tested against placebo [30] and spiramycin [56]. Metronidazole has also proven as effective as penicillin in the management of pericoronitis and periodontal abscesses [35, 55].

Although the evidence presented in this review suggests the potential usefulness of metronidazole in the treatment of other inflammatory periodontal diseases such as gingivitis and periodontitis, preliminary reports have been published only recently. Heijl and Lindhe studied the effects of metronidazole on the development of plaque and gingivitis in Beagles. Five Beagles were brought to optimal oral health by scaling and twice daily brushing. After baseline GI and PlI scores were recorded and subgingival

plaque samples were procured, brushing was discontinued for the right maxillary quadrant. Repeat examinations for the control period were conducted on days 7, 14 and 28 for this quadrant. This was followed by a 28-day test period during which brushing was discontinued for the left maxillary quadrant and 20mg/kg/day (about 200 mg/day) metronidazole was administered. By day 14, 100% of the control sites harbored gross plaque accumulations compared with only 12% of the test sites. By day 28, 100% of the control sites demonstrated moderate to severe inflammation compared with only 30% of the test sites. These results indicate that metronidazole alone was effective in reducing plaque formation and gingivitis in Beagles. Additionally, differential flora counts taken at baseline for both control and test groups showed a predominance of cocci+straight rods (90%), a finding which conforms to bacterial proportions indicative of relative gingival health in humans [39,43]. During the control period a pronounced shift towards a flora typically found in diseased sites was manifested: proportions of cocci and straight rods were markedly reduced, while those of spirochetes and motile rods were elevated. In contrast, bacterial proportions remained stable throughout the test period: the flora was dominated by cocci+rods, while spirochetes and motile cells were virtually absent [32].

Listgarten, Lindhe and Parodi demonstrated the effects of different systemic antibiotics on plaque formation and gingivitis in dogs. One pair of dogs was given 250 mg

tetracycline b.i.d., one pair received 200 mg metronidazole b.i.d., and one dog served as a control over a 4-week test period. These drugs were selected on the basis of their successful application in treating periodontitis and ANUG in humans [19,21,82,85]. PlI scores, GI scores and differential counts of the sulcular flora were taken at baseline, 2 weeks, and 4 weeks. When baseline GI scores were grouped and their respective differential bacterial counts were examined, the percentage of coccoid cells tended to decrease while that of spirochetes and motile cells increased with increasing GI scores. These observations are in agreement with those of other darkfield microscopy studies of sulcular flora [39,43]. Both drugs exerted a similar effect of the sulcular flora: the proportion of coccoid cells increased in relation to baseline values, while spirochetes and motile bacteria were drastically reduced. However, metronidazole appeared to be slightly more effective than tetracycline in eliminating small spirochetes and in lowering GI scores [45].

Some caution should be exercised in interpreting the data from the studies by Listgarten et al and Heijl et al [32,45]. Since the microbiotic changes described were accompanied by decreased GI and PlI scores, it was not possible to determine whether improved GI scores during and following treatment were attributable to a specific antimicrobial effect or to a generalized decrease in the microbial mass, although circumstantial evidence points to the former explanation. Furthermore, it is uncertain whether

drugs which reduce gingival inflammation in dogs would be equally effective in treating periodontitis in humans.

Loesche et al monitored various clinical and microbiological parameters for six months or longer in five selected periodontal patients [52]. All received 250 mg metronidazole t.i.d. for seven days at the beginning of the study; scaling and root planing were carried out for three. Pretreatment bacteriological samples indicated that Bacteroides asaccharolyticus comprised 40% of the total cultivable bacteria, while spirochetes accounted for 25% of the direct microscopic counts. The high proportions of these suspected periodontal pathogens found by Loesche et al are consistent with findings of other investigators [39,43, 84,100]. Proportions of both Bacteroides asaccharolyticus and spirochetes were significantly reduced for at least 6 months after treatment. A 50-75% reduction in Papillary Bleeding scores, a reduction in mean pocket depth of 2.5 mm and a mean gain in epithelial attachment in excess of 1.4 mm were also evident in test sites 6 months post-treatment. Given that the spectrum of metronidazole is limited to obligate anaerobes, the reduction in proportions of Bacteroides asaccharolyticus and spirochetes coincident with improved clinical status following antibiotic therapy implied an etiologic role played by these microorganisms. The dramatic and long-lasting changes in clinical and microbiological parameters following a single course of therapy suggested the potential value of this drug as adjunctive therapy in the

treatment of periodontitis. However, the small sample size limited the generalizability of the findings, and observer bias may have distorted the results. A randomized controlled double-blind study such as the present one was clearly indicated to evaluate the efficacy of adjunctive metronidazole therapy in the treatment of periodontitis.

J Clinical Measures Used in the Determination of Disease Status and the Evaluation of Therapy

(i) Determination of Disease Status: Patient Selection

Periodontal indices have been utilized in large population surveys to ascertain the prevalence and severity of periodontal disease, but these have not generally been considered sensitive enough for clinical use [65,74]. The most commonly employed methods for determining the presence and severity of periodontal disease in individuals have been roentgenography and clinical probing of pocket depths. Less commonly used methods have included measurements of tooth mobility, gingival crevicular fluid, gingival biopsy, etc. [12]. A true morbidity index for periodontal disease has yet to be devised: these traditional clinical diagnostic methods neither determine the rate of disease progression nor distinguish between active and inactive disease at the time of examination.

The difficulty of projecting a 3-D image on a 2-D screen without distortion, problems with standardizing angulations, distances and film exposure, factors such as inflammation which affect the radiodensity of

the bone, underdetection of certain osseous lesions and general underestimation of bone loss, and ethical concerns regarding unnecessary x-ray exposure were factors in the decision not to employ radiography as a method of patient selection or evaluation in this study [12,64,68]. Perhaps most importantly, the slow rate of bone loss and the supposedly irreversible nature of this loss precludes the use of radiography as a sensitive measure of response to therapy. Clinical probing was therefore chosen as a convenient, rapid and accurate means of patient selection.

(ii) Goals of Periodontal Therapy

The primary objective of periodontal therapy is to arrest or reverse the disease process. Many dependent variables may be used to evaluate the success of therapeutic endeavors. Perhaps the most salient outcome measure is the preservation or gain in support for affected teeth. Other indicators of therapeutic efficacy include pocket elimination, reduction of soft tissue inflammation, physiologic gingival contour and firm gingival consistency [64]. Because no single dependent variable or parameter provides a total appraisal of therapeutic success or failure, most studies of therapeutic agents/procedures have utilized several parameters for the evaluation of disease status and treatment. Typically these studies employ:

1) a measure of periodontal support such as probing

epithelial attachment levels [33,40,44,61,71,85];

2) a measure of gingival inflammation [32,33,40,44,45, 61,71,85]; and

3) a measure of oral hygiene status [32,33,40,44,45, 61,71,85].

This last measure is usually included because the intimate association of oral cleanliness with periodontal disease necessitates some system for evaluating the influence of oral cleanliness on therapeutic outcome.

(iii) Use of Indices for Clinical Measurements in Periodontology

An index is a composite measure--a variable of variables requiring theoretical assumptions and occasionally complicated calculations--which attempts to provide a relative numerical estimate for the purposes of comparison. Index construction is indicated when there are multiple dependent variables to be measured simultaneously and which are most appropriately or practically recorded on either an ordinal or a nominal scale. Many parameters used in the evaluation of periodontal disease therapy fit this description and may be more precisely measured by specifically conceived indices than by subjective evaluation.

In selecting an index, the following characteristics should be considered [13,31,68]:

1) Validity. The criteria should be biologically valid indicators of the parameter to be measured.

2) Reproducibility. Clearly defined objective criteria, avoidance of items which are too divergent in a single index, reduction of investigator decision-making to a minimum and examiner calibration all foster reproducibility.
3) Amenability to statistical analysis.
4) Speed, simplicity (in terms of equipment and methods), minimal cost.
5) Sensitivity. As the number of scoring categories increases, sensitivity may also be increased, but reproducibility may be consequently diminished.
6) General applicability and comprehensiveness. These may be achieved at the cost of sensitivity.
7) Popularity. Widely used indices furnish a basis for interstudy comparisons.

Depending on the type of study and its objectives, some characteristics may be more desirable than others. In epidemiologic surveys, the purpose is to identify associations between two or more factors, the sample size is large, lesions have usually developed over a period of time so detection is easier, and examination time is often at a premium. Under such circumstances speed, simplicity, minimal cost and general applicability assume greater importance, while less emphasis is placed on sensitivity. An example is provided by Russell's Periodontal Index [74]. Clinical trials, on the other hand, are oriented toward

establishing causal relationships, the sample size is usually smaller, lesions develop over a short period of time so detection is more difficult. For clinical trials, index sensitivity and completeness of data are essential; thus, costlier, more complex and time-consuming procedures may be required to achieve these ends.

Drawbacks to most of the clinical periodontal indices currently in use include:

1) the fact that an index score is an abstraction and has meaning only when scores are being compared;
2) the subjective criteria employed by many indices increase both random and systematic observer error; and
3) the questionable validity between the criteria and the disease process. Newer methods such as crevicular gingival fluid measurements, determination of collagenase activity, and microbiological counts may eventually provide more objective criteria or a true morbidity index.

However, the indices currently in use represent large improvements in terms of validity, reproducibility and suitability for statistical analysis over the descriptive (non-numerical) ordinal indices which prevailed until the late 1950's.

(iv) Measurement of Periodontal Support

Several outcome measures have been employed in

various clinical studies to determine the status of periodontal support [12,25,64,65,68,78]:

1) Epithelial attachment levels, i.e., the distance from a fixed landmark on the tooth to the bottom of the sulcus/pocket;
2) Measurements of bone loss made from the cementoenamel junction to the alveolar crest using a periodontal probe;
3) Radiographic evaluation of bone loss;
4) Tooth mobility;
5) Gingival recession, i.e., the change in the distance from the CEJ to the free gingival margin;
6) Pocket depths (probing depths), i.e., the distance from the free gingival margin to the bottom of the sulcus/pocket.

The investigators selected epithelial attachment levels as the most meaningful and practical measure of periodontal support after considering the objectives of the study and the advantages/disadvantages of the various measures. Measurements from the CEJ to the alveolar crest entail the use of anesthetics. Radiographic evaluation is more suited to trials involving loss or gain of alveolar bone support. When measuring tooth mobility concerns about practicality, accuracy, and the relationship of mobility to support must be addressed. Gingival recession and probing depths are both indirect measures of attachment levels. For example, if a probing depth is recorded at 5 mm, this

does not imply that a 5 mm loss of attachment has taken place: gingival hyperplasia may be partially responsible. Conversely, a pocket depth of 2 mm may actually represent a loss of attachment exceeding 2 mm if gingival recession has occurred concomitantly with loss of attachment.

The rationale for using attachment levels as an outcome measure is as follows: the accumulation of subgingival plaque precipitates an inflammatory response which engenders a disruption of epithelial and connective tissue attachment manifested clinically by the formation of a periodontal pocket. Mechanical debridement aims to reverse this process by removing subgingival plaque (which provokes the inflammatory response), calculus (which is a retention factor for plaque), and toxic cementum (which may inhibit reattachment). Adjunctive antibiotic therapy may supplement this effect through its action on specific plaque bacteria. Although a gain in attachment may be observed following therapy [61,71], the predictability of this reversal has not been determined. Moreover, whether this reattachment is epithelial or both epithelial and connective in nature is not known.

Measurements of epithelial attachment levels record the distance from a fixed landmark to the most apical portion of the sulcus/pocket. Listgarten et al have shown that the clinical bottom of the sulcus/pocket

is deeper than the histological one (Fig. 7): the tip of the probe tends to be located near the demarcation line between the junctional epithelium and the connective tissue attachment to the root rather than at the coronal extension of the junctional epithelium [46].

Various reference points have been employed to measure attachment levels. In canine studies, notches or amalgam fillings have been placed for this express purpose [38,75]. In most human studies, the measurement has been obtained indirectly by subtracting the measurement of the free gingival margin to the CEJ from the probing depth [3,52,61,71,95]. Intraexaminer agreement for this method has been estimated at 95% [87]. However, measurements cannot be made for some teeth since the CEJ may be obliterated by carious lesions, restorations or scaling.

The use of acrylic occlusal templates as landmarks for attachment level measurements was first reported by Helldén et al [33]. This method should enhance reproducibility because:

1) it requires one, rather than two measurements;
2) it avoids the use of the CEJ as a reference point; and
3) template markings allow repeated measurements to be taken at the same sites every time.

Other sources of error inherent in the probing technique include [12]:

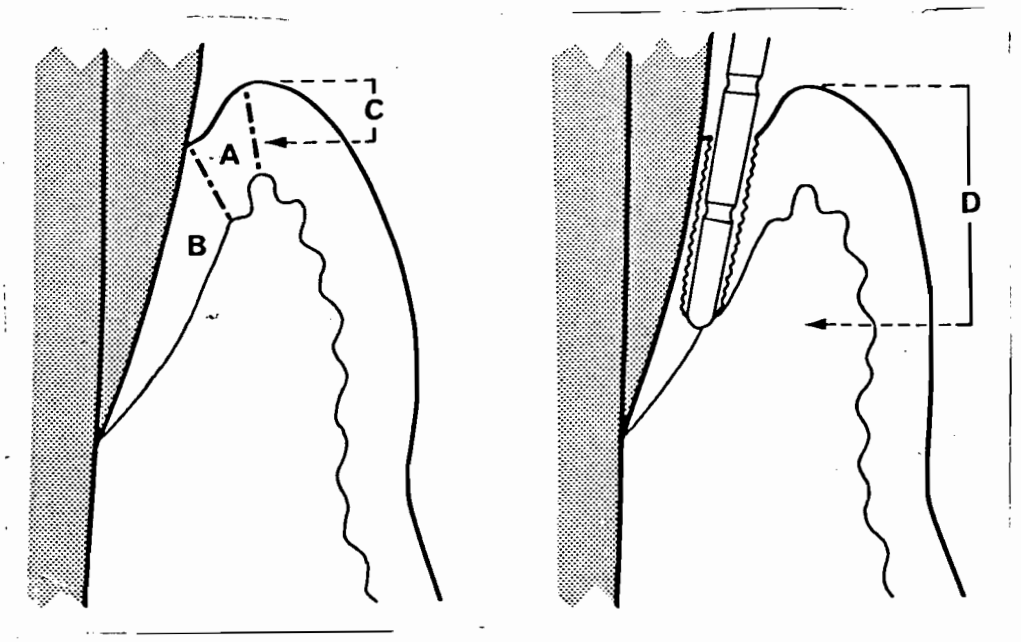


Fig. 7. Measurement of Attachment Levels: Location of the Probe Tip.* A=sulcular epithelium; B=junctional epithelium; C=histological sulcus; D=clinical sulcus.

1) The angle of probe insertion. The greater the angulation away from the long axis of the tooth, the higher the reading.

2) Variations in probing force. The greater the force, the deeper the penetration.

3) Variations in tissue consistency. Periodontally diseased tissues exhibit a less firm consistency than healthy tissues, which permits deeper penetration of the probe.

4) The size of the probe. The thinner the probe, the

* From Strahan and Waite [92, p. 10].

deeper the penetration.

5) Inaccurate probe markings.

6) Difficulty in taking certain measurements especially from the lingual face.

7) The angle at which the observer reads the probing measurement.

It is evident from the above discussion that examiner calibration and standardization of equipment are important factors in reducing observer error.

Attachment level values have sometimes been converted to index scores [65]. While this is satisfactory for field surveys, the loss of precision entailed by such a transformation makes the use of numerical values preferable in clinical trials.

(v) Measurement of Gingival Inflammation

In clinical studies of periodontal disease, the following types of indices have been used to evaluate gingival inflammation:

1) Descriptive indices with an underlying ordinal scale (e.g., a scoring system for which a greater number of plusses '+' indicates more severe disease). These indices are less sensitive, less amenable to statistical analysis and more subject to inter- and intra-examiner error than other currently available ones.

2) 'Present or absent' indices, e.g., Ainamo and Bay's Gingival Bleeding Index [1]. The GBI score represents the percentage of locations with gingival bleeding, or

site prevalence. Such an index reduces examiner decision-making to a minimum, thereby improving the comprehension and reproducibility of the results. Another advantage concerns the suitability of index scores for parametric statistical analysis. A serious drawback is presented by the inability of the index to determine the severity of gingival inflammation. The sensitivity of gingival bleeding as a sole criterion of gingival inflammation has also been questioned.

3) Numerical severity indices. Among the first numerical indices to be developed were Russell's Periodontal Index [74], Ramfjord's Periodontal Disease Index [65], and Masler and Schour's PMA Index [31]. The PI and the PDI are composite indices which record both gingival inflammation and deeper periodontal pathology. The use of either of these indices to evaluate gingival inflammation is contraindicated in clinical trials because a) geographical extent of the inflammation is used as a measure of severity, b) incomparable parameters with different underlying scales of measurement are combined to produce a single score, and c) more sensitive indices are available.

More recently developed indices have distinguished between severity of the gingival lesion (quality) and its geographical extent of localization

(quantity). The Löe and Silness Gingival Index was the first to make this distinction which was achieved by changing the unit of evaluation from the gingival tissue surrounding an individual tooth to a gingival surface--buccal, lingual, mesial, distal. The degree of color change, edema and bleeding on probing are assessed and a score of 0 to 3 is assigned for each unit; color change is considered to be the initial sign. This index has been used extensively in field surveys, clinical trials and individual patient management. Sufficiently high inter- and intraexaminer reliability may be attained with calibration [47].

The Sulcular Bleeding Index also assesses the severity of inflammation for each gingival unit [59]. This index differs from the Gingival Index in that sulcus bleeding, rather than color changes, is considered to be the initial sign of gingivitis, and inflammation is ranked on a scale of 0 to 5 rather than 0 to 3. The validity of the premise that sulcus bleeding, hyperemia, and edematous swelling appear in that order with increasing severity of gingivitis has not been established. The issue of which of these signs, if any, appears initially and the relative weights each should be accorded in a severity index remains a critical one for the validity of all gingival indices. Even if bleeding is not the initial sign, if it occurs soon after another sign such as hyperemia and is easier to detect, its use

as a criterion may give more accurate results. Investigators should also consider that subgingival bleeding may more accurately reflect subgingival disease activity than does marginal inflammation [25].

Suomi and Barbano's index examines only the facial and lingual gingival surfaces [94]. This index possesses only three scoring categories based primarily on the degree of color change. While it is considered to be less sensitive than either the GI or the SBI, it is quick and simple to use.

The monitoring of gingival crevicular fluid may prove to be a more sensitive and objective measure of early gingival disease than any of the indices available. Unfortunately for this and other newer methods of detecting gingival inflammation, no standards have been devised for the interpretation of results. Other methods having potential diagnostic value include measurement of collagenase activity, oral leukocytes and the presence and proportions of specific plaque bacteria [12].

(vi) Measurement of Oral Hygiene Status

Assessment of soft deposits--plaque and debris--is important in a) epidemiologic studies examining the association between oral cleanliness and periodontal disease, b) clinical trials of the efficacy of various anti-plaque agents and procedures, and c) individual patient management [54]. As was the case for other

types of indices, index selection should be based on the objectives of the study, sample size, the duration of the study, and the type and extent of changes anticipated [24]. The present study does not fall into any of the above named categories. However, an assessment of oral hygiene status was deemed advisable in order to evaluate soft accumulations as a possible confounding influence as well as any anti-plaque effect which might appear.

Most indices of soft deposits have been classified as plaque or debris indices. Although plaque may someday be characterized more objectively--for example, in terms of its periodontopathic potential--it is presently defined in descriptive terms such as 'bacterial masses and intermicrobial substances adherent to soft tissues and tooth surfaces.' Debris is defined as 'loosely adherent, structurally unorganized matter composed of food particles, mucin, bacteria, and desquamated epithelial cells.' While plaque is considered to have a far greater periodontopathic potential, debris may also be capable of initiating an inflammatory response in the gingiva.

With the exception of Silness and Löe's Plaque Index [47], the most commonly encountered oral debris indices (such as the debris component of Greene and Vermillion's OHI-S, Podshadley and Haley's modification of this index, and Glass' method of scoring debris) and

plaque indices (such as the Schick and Ash index and the Turesky modification of the Quigly-Hein index) suffer from two serious flaws [54]. First, the basis for scoring is the extent of a tooth surface occupied by plaque or debris. The rationale for such a scoring criterion is that the more extensive the area covered by soft accumulations, the poorer the oral hygiene. Even if the geographical extension of debris is indicative of debris thickness (and therefore is sensitive to the efficacy of oral hygiene), one must question the relevance of assessing plaque of debris anywhere except at the gingival margin. The second defect concerns the failure of these indices to consider subgingival plaque which presumably plays a more significant role in deeper periodontal destruction than does supragingival plaque.

The Plaque Index, like the Gingival Index, distinguishes between severity and location [47]. No attention is paid to the coronal extension of the plaque, thus imparting a greater biological validity to this index. Moreover, consideration is given to subgingival plaque, although not exclusively. Plaque thickness at the gingival margin is scored from 0 to 3, average scores may be calculated for individual teeth, groups of teeth, or patients. The Plaque Index is probably the index of choice in small clinical trials, especially when used to complement the Gingival Index.

Although more sensitive indices (in terms of having more scoring categories with which to assess debris at the gingival margin) are available, the debris component of the OHI-S was used in this study because of its simplicity, rapidity, high reproducibility, extensive use, and the examiner's familiarity with this method [29,87]. Additionally, the OHI-S is appropriate for the assessment of calculus [106]. Scores of 0 to 3 are assigned to selected surfaces on the basis of geographical extension; an individual's score is the mean surface score. Because calculus and debris scores may be summed to arrive at an overall assessment of oral hygiene, the OHI-S is called a 'composite' index.

III. METHODS

A Sample Selection

Patients recruited for participation in the study comprised mentally retarded adolescents screened at the Dental Hygiene Unit of the Montreal Children's Hospital and at outside institutions (Appendix A). Patient selection aims to include only genuine cases of the study disease (in order to avoid a misclassification bias and consequent dilution of effect) unassociated with any possible confounding factors. Thus, to ensure accuracy of results, the following criteria for patient selection were established:

1) Clinical evidence of destructive periodontal disease (i.e., pocket depths of 5 mm or greater) in at least two sites.
2) No indication of history of any conditions listed as precautions or contraindications to metronidazole, e.g., active neurological disorders, blood dyscrasias, hypothyroidism, hypoadrenalism, anticonvulsant therapy with diphenylhydantoin [17].
3) No clinical evidence indicating a diagnosis of ANUG or periodontosis.
4) At least two quadrants of natural dentition.
5) No surgical periodontal treatment, scaling or root planing during the six months prior to initiation of the study.
6) No antibiotic therapy within the past 6 months.

7) No routine use of antiseptic or fluoridated mouthrinses.

8) Parental consent. (Appendix B).

B Study Procedures

(i) A design with prolonged entry and random allocation on subjects to one of two treatment groups was used. As each subject became eligible for inclusion, his/her name was placed on a master list. Odd-numbered patients received one regimen; even-numbered patients received the other. Both assignment to treatment group and assessment of results were conducted double-blind: i.e., neither patients nor investigators were aware of patients' treatment status. The similarity of the study and control procedures permitted double-blind assessment to be maintained throughout the study.

(ii) All patients received a scaling and polishing with prophy paste. For the study group this routine periodontal treatment was supplemented by 250 mg metronidazole taken orally t.i.d. for one week. The control group received 250 mg placebo t.i.d. for one week. Identical capsules containing either metronidazole or placebo were prepared by the pharmacy of the Montreal Children's Hospital. Packets of 21 capsules were assembled, coded and distributed to patients' guardians accompanied by written and oral instructions. Both routine periodontal treatment and adjunctive antibiotic therapy (or placebo) were delivered only once--immediately after baseline examinations.

(iii) Baseline clinical examinations and microbiological sampling were performed prior to the administration of treatment procedures and were repeated at 1,3 and 6 months following baseline measurements (Appendix C).

(iv) Two investigators performed the perioprobing for attachment levels (SRS,CC). These investigators were also responsible for recording oral hygiene status (SRS) and gingival inflammation (CC). A third investigator was charged with microbiological sampling and evaluation (PS). A fourth investigator devised methods of statistical analysis and data presentation and recorded clinical measurements during data collection sessions (SCS). A part-time research assistant managed patient scheduling and assisted with the recording of the data. The sequence of procedures was as follows: microbiological sampling, assessment of gingival inflammation, perioprobing (CC), assessment of oral hygiene, perioprobing (SRS).

(v) Efforts aimed at the reduction of biases inherent in clinical research included:

1) random allocation;
2) double-blind assessment;
3) standardization of methods, procedures and equipment; and
4) recording of different measurements by different investigators.

C Clinical Measurements

(i) Gingival inflammation was evaluated using the Löe and Silness Gingival Index [47]. The methodology of Löe and Silness was adhered to closely with the exception of tooth selection. Modification of the GI involved substituting the teeth immediately adjacent to the two most severely affected periodontal sites for the more commonly used teeth: 12,16,24,32,36,44. Thus, four teeth per patient were selected for assessment of soft tissue inflammation.

A site was defined as any interproximal area that fulfilled the following criteria:

1) Demonstrates periodontal probing depths of 5 mm or greater.
2) Is in functional occlusion.
3) Is vital.
4) Does not possess large interproximal caries or restorations that adversely affect the contour of the tooth.
5) Does not demonstrate extensive furcation involvement.
6) Does not demonstrate any other pathological condition.

After gently air drying the gingival areas to be examined (any 'spontaneous' bleeding at this point warranted a score of '3'), the four gingival units of each tooth--facial, lingual, mesial, distal--were scored with the aid of a mirror and a periodontal probe according to the criteria developed by Löe and Silness

(Table 5). Surface scores for all teeth were summed, divided by the total number of surfaces, and rounded off to the nearest hundredth. The resulting GI score was an average value for the two most severely affected periodontal sites.

TABLE 5

SCORING CRITERIA FOR THE GINGIVAL INDEX SYSTEM

0 = Normal gingiva

1 = Mild inflammation —— slight change in color, slight oedema. No bleeding on probing

2 = Moderate inflammation —— redness, oedema and glazing. Bleeding on probing

3 = Severe inflammation —— marked redness and oedema. Ulceration. Tendency to spontaneous bleeding.

(ii) Oral hygiene status was evaluated by the Simplified Oral Hygiene Index (OHI-S) developed by Greene and Vermillion [29]. The only modification to their methodology concerned the use of a disclosing solution. After painting an erythrosine solution on the surfaces to be assessed--11 facial, 16 facial, 26 facial, 31 facial, 36 lingual, 46 lingual--debris scores (DI) and calculus scores (CI) were determined separately for each surface. Scoring was carried out according to the criteria established by Greene and Vermillion

(Tables 6,7) with the aid of a mirror and an explorer [29]. In the absence of an anterior tooth, the central incisor on the opposite side of the midline was scored, and if a first molar was absent, a second molar was scored instead. The DI and CI for each patient was calculated by summing the surface scores and dividing by the number of surfaces. Individual scores were calculated to one decimal place, and group scores were calculated to two decimal places.

TABLE 6

SCORING CRITERIA FOR THE DEBRIS COMPONENT OF THE OHI-S

0 — No debris or stain present

1 — Soft debris covering not more than one-third of the tooth surface being examined or the presence of extrinsic stains without debris regardless of surface area covered

2 — Soft debris covering more than one-third but not more than two-thirds of the exposed tooth surface

3 — Soft debris covering more than two-thirds of the exposed tooth surface

TABLE 7

SCORING CRITERIA FOR THE CALCULUS COMPONENT OF THE OHI-S

0 — No calculus present

1 — Supragingival calculus covering not more than one-third of the exposed tooth surface being examined

2 — Supragingival calculus covering more than one-third but not more than two-thirds of the exposed tooth surface, or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth

3 — Supragingival calculus covering more than two-thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth

(iii) Attachment levels were measured according to a technique described by Helldén et al [33]. Acrylic occlusal templates (splints) spanning several teeth were fabricated in a dental laboratory for the two selected sites per patient. To improve measurement reproducibility, notches were cut into the template to indicate interproximal sites, and indelible pen markings identified the positions of facial and lingual measurements. The margin of the template was located somewhat coronal to the cervix of the tooth (Fig. 9).

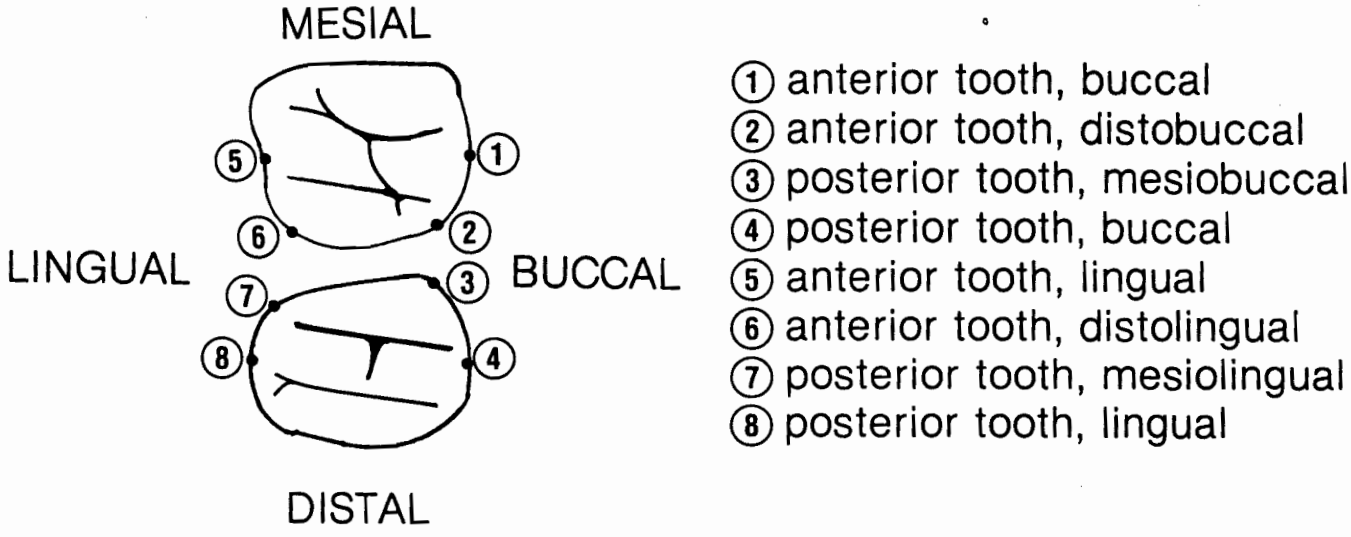
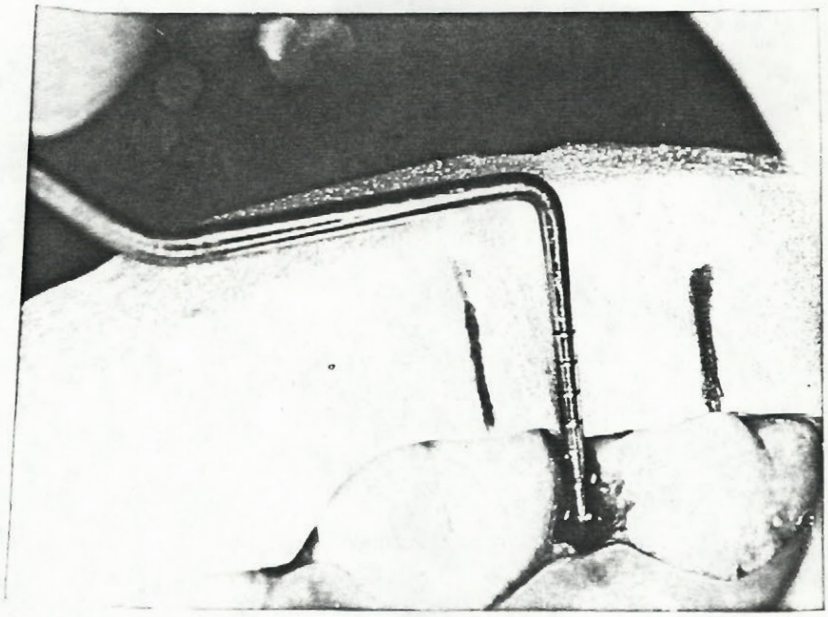
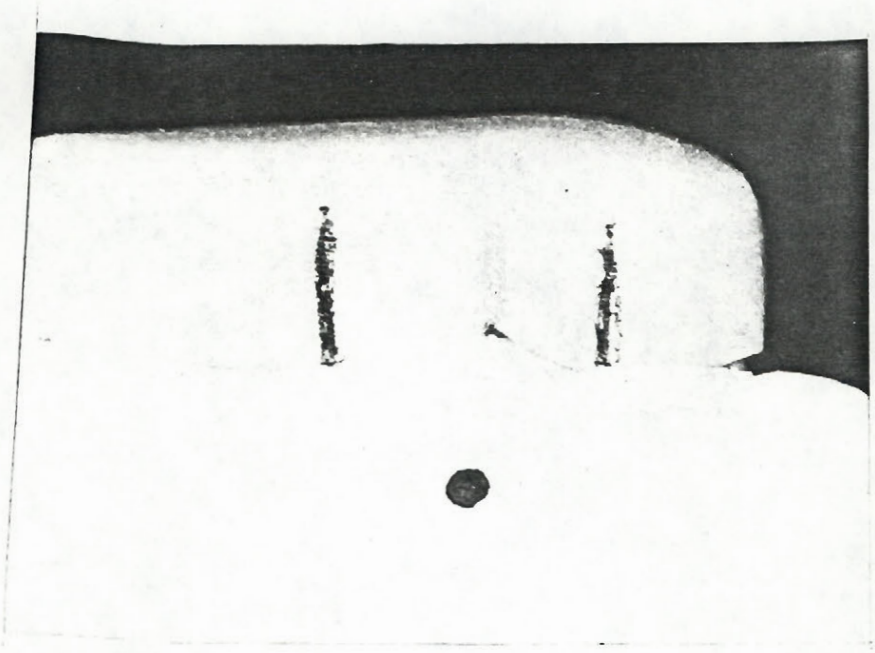


Fig. 8. Occlusal View of an Interproximal Site: Locations of Attachment Level Chartings.



A



B

Fig. [illegible] Use of Templates for Measurement of Epithelial Attachment.

A) Probing at an interproximal site.

B) Template on plaster model.

Attachment levels were recorded at 8 probing points for each site. One of the interproximal points was designated as the deepest initial probing point (MAX).

For buccal and lingual measurements, a Michigan periodontal probe was inserted into the pocket parallel to the long axis of the tooth until resistance was met. The attachment level was indicated by the intersection of the probe and the apical border of the template. Measurements were recorded to the nearest millimeter. For interproximal chartings, the probe was angled slightly in order to locate the tip of the probe under the contact area--a technique which represents a deviation from the principle of probing parallel to the long axis of the tooth. The rationale for this method concerns the observation that the vertical osseous defect is usually deepest midway between the facial and lingual cortical plates interproximally; thus, failure to penetrate the probe deeply enough into the interproximal site would result in an underestimation of tissue destruction. Although the bulk of the template precluded the full interproximal penetration of the probe, the investigators felt that this method was more valid (i.e., less likely to underestimate attachment levels) than the more frequently used vertical probing. According to Schluger, the actual linear discrepancy between vertical probing and the angulated probing just described is actually less than 0.25 mm--an acceptably small error considering the return in measure-

ment validity [78, p.292].

Interexaminer variability, in contrast to intraexaminer variability, tends to be of a systematic nature and thus constitutes a more serious bias. In an attempt to reduce measurement error from this source, two examiners (SRS,CC) were calibrated prior to any actual data collection. Informal calibration occurred throughout the remainder of the study: after the attachment levels for each patient were recorded by both examiners, a discrepancy of 2 mm or more (which seldom occurred) required rereadings from both examiners.

The selection of periodontal probes to be utilized during the trial represented a further effort to decrease measurement error. With a Boley gauge, five probes which had the most comparable millimeter markings were chosen from a batch of probes.

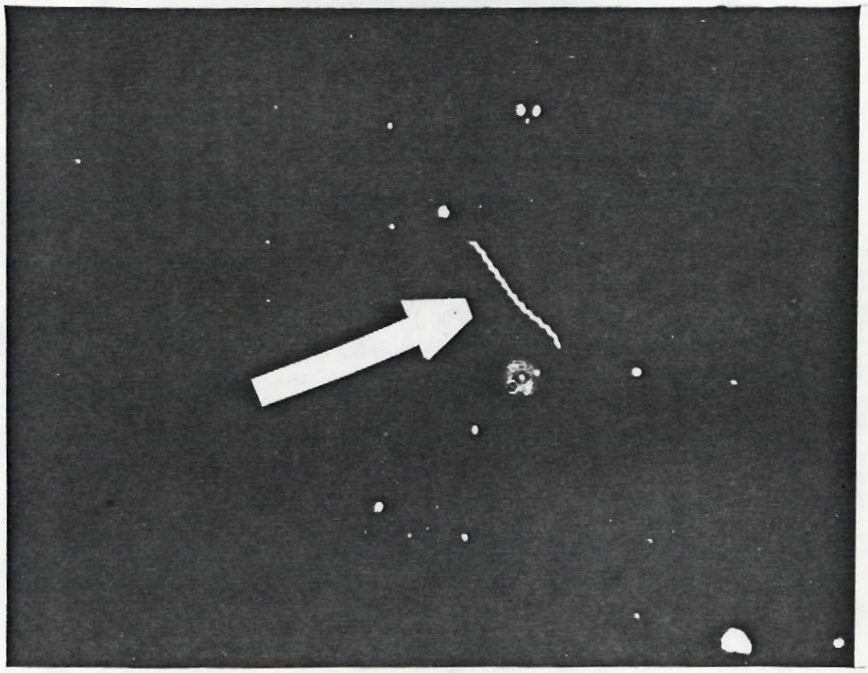
D Microbiological Techniques

Subgingival flora sampling was carried out before any clinical measurements in order to avoid disturbing the flora and reducing the validity of the bacterial counts. For each patient, samples were taken from the sites identified as exhibiting the most extensive disease involvement at the time of the initial screening session. Sampling and microscopic evaluation of samples adhered closely to techniques described by Listgarten and Helldén [43]. Since the objective of the sampling procedure was the procurement of a sample representative of the most apical portion of the

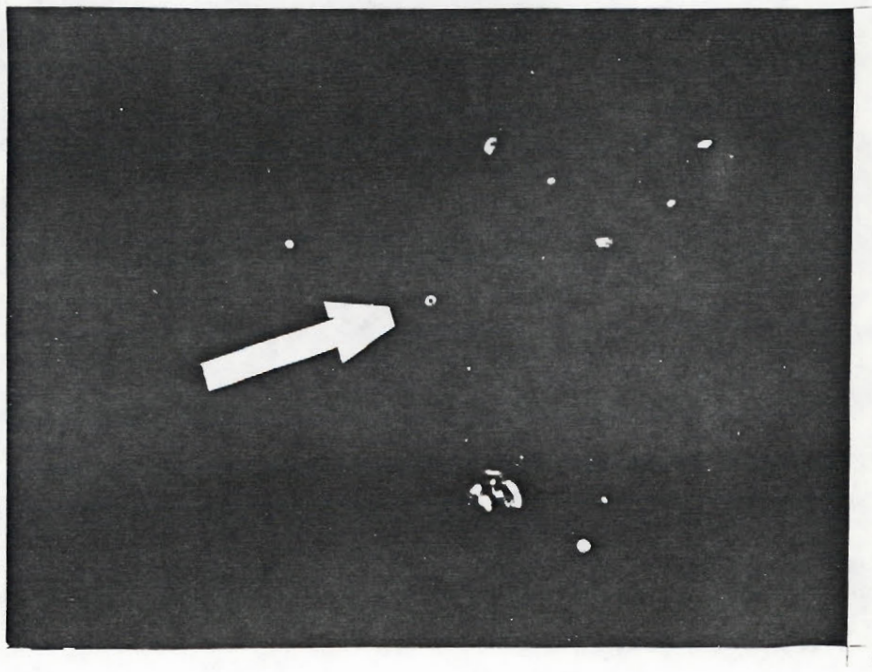
pocket, supragingival plaque was first removed by curette and one absorbent paper point was inserted into the pocket and kept in place for 10 seconds in an effort to reduce sample contamination. Three paper points were subsequently inserted into the pocket until resistance was met, and each was left in place approximately 10 seconds [85]. The points were then clipped at 3-4 mm from the tip and deposited in a small screwcap vial containing 0.4 ml normal saline solution. Several investigators have obtained samples by introducing a curette as far as possible into the pocket and removing the bacterial contents [32,39,43]. The sampling procedure employed in this study was preferred because of the possibility of gingival laceration when obtaining samples by curette from non-compliant patients.

Samples were examined within one hour of their collection so as to maintain cell viability, a crucial factor in obtaining valid counts of motile cells. Vials were vortexed for 1 minute to facilitate dispersion of the bacteria in the solution, i.e., to foster solution homogeneity. A drop of the solution was then placed on a slide, cover slipped and observed by darkfield microscopy (x1560).

In general, 150-200 bacteria were examined from fields chosen at random and were recorded with the aid of a manual cell counter. Clumps of cells in which all cells were not clearly distinguishable were disregarded. Bacteria were classified according to the nine morphological types described by Listgarten and Helldén [43]:



A



B

Fig. 10. Morphologic Bacterial Types.

A) Spirochete

B) Coccoid cell

1) Cocci--comparatively small circular forms showing a bright outline and a dark interior.
2) Straight rods--non-flagellated cells with a bright outline, a dark center, rounded ends, and at least twice as long as they are wide.
3) Filaments--cells with a bright outline and a dark center, at least six times as long as they are wide, and occasionally appearing as branched forms.
4) Fusiforms--long rods with tapered or pointed ends which have a more solid appearance than the aforementioned types.
5) Curved rods--cells similar to straight rods but with a crescent shape.
6) 7) 8) Small, intermediate and large spirochetes--helically coiled, motile cells with no visible dark interior. Determination of size is based on both thickness and length.
9) Motile cells--all cells other than spirochetes, generally straight or curved rods, which exhibit motility as distinct from Brownian motion or motion attributable to streaming of the solution between the slide and cover slip.

E Statistical Analysis

Descriptive and inferential statistical analyses were undertaken for both clinical (epithelial attachment levels, gingival inflammation and oral debris) and microbiological variables using SAS package programs [4]. Means

and standard deviations (raw scores) were calculated by treatment group (drug vs. placebo) and examination (baseline, 1 month, 3 months, 6 months) for all variables. Additionally, mean differences from baseline values (raw scores) were examined for clinical measurements by group and over time. (Mean differences from baseline cannot be generated directly from the raw data because sample sizes changed over time due to missing values).

Prior to any parametric inferential analyses, microbial measurements (proportions) were subjected to angular transformation such that

$$P^1 = \text{arc sin } \sqrt{p}$$

in order to stabilize the variances [33,44,63,85]. Since whole-mouth index scores (GI, DI) exhibited a relatively normal distribution, these values were not tranformed. Previous investigators have demonstrated the equivalence of results obtained using either raw or transformed scores [43].

All analyses of variance (ANOVA) and multivariate analyses of variance (MANOVA) were performed using differences from baseline (e.g., 6-month value minus baseline value) as the data for either raw or transformed scores. Multivariate analyses of variance using quadratic and linear models were undertaken for all probing points together (i.e., posterior mesiobuccal, anterior distobuccal, posterior mesiolingual, anterior distolingual, maximum initial probing).

A quadratic model was used to assess the presence of a non-linear trend attributable to time (i.e., time^2) as well as a time-group interaction (i.e., time^2-group). After ruling out quadratic relationships, a linear model was employed to assess the significance of treatment and time effects, and time-group interaction. In an analogous fashion, quadratic and linear ANOVA's were carried out for each clinical and microbiological variable.

Owing to the striking pattern observed for most variables in the first month following treatment, the paired t-test was used to assess the magnitude of pretreatment-first examination changes, by treatment group, for all variables. Since this was, in fact, a post hoc analysis, one would be less likely to conclude significance at the conventional probability level of 0.05. The student's t-test was employed to assess between-group differences at 1 and 3 months post-baseline for coccoid cells and spirochetes.

Finally, rank correlations (Kendall's tau) were computed to measure the association between various clinical and microbiological variables. Since the untransformed microbial proportions were used in the correlation analyses, this non-parametric method was preferred to the more commonly utilized Pearson's correlation coefficient.

IV RESULTS

A Epithelial Attachment Levels

Due to the lack of pocket formation at facial and lingual probing points in our sample, data analysis was restricted to the following interproximal probing points: posterior mesiobuccal (PMB), anterior distobuccal (ADB), posterior mesiolingual (PML) and anterior distolingual (ADL). Because it was highly probable that measurements for different probing points were correlated, an a priori decision was made to consider the deepest initial probing point per site (MAX) as the focus of this analysis. An average of all measurements at a particular site or any single interproximal point could have been arbitrarily selected instead. The maximum initial probing point was chosen in view of the proposed correlation analysis between attachment levels and microbial flora: since plaque samples were retrieved from the most apical portion of the interproximal site, microbial data would be most representative of the deepest initial point. It was also hypothesized that the most striking attachment gains due to treatment would most likely be observed at sites of more advanced disease.

An examination of maximum initial probing depths over time (Table 8, Figure 11) and the mean differences from baseline (Figure 12) showed a substantial gain in attachment at

1 month for both treatment groups, followed by a gradual attachment loss. This initial gain was statistically significant for both groups ($P < 0.05$, placebo group; $P < 0.01$, drug group). After excluding the possibility of quadratic relationships, the ANOVA of maximum probing depth at each site (MAX) demonstrated: 1) the similarity of the pattern of attachment level change for treatment regimens; 2) the failure of the adjunctive antibiotic to achieve any statistically significant reattachment gains beyond those achieved by traditional therapy (i.e., prophylaxis and scaling); and 3) a significant recidivism over the period between the first and final examinations ($P < 0.05$).

As a matter of theoretical interest, analyses for the four original probing points were also conducted. A quadratic MANOVA for all probing points (PMB, ADB, PML, ADL, MAX) revealed no significant time^2 trend or time^2-group interaction. A linear MANOVA revealed a significant time trend ($P < 0.01$), but neither a group effect nor a time-group interaction. From Table 9 and Figures 11-12 one readily sees that the pattern of change was remarkably similar for all points: a respectable gain in attachment recorded at 1 month was followed by a gradual loss, no matter which treatment regimen was received. Analyses of variance on these data produced results comparable to those for the deepest initial probing point: neither time-group interactions nor group differences, but significant annulment of gains occurred from 1 to 6 months (Table 10). One may further

observe that despite the similarity in the pattern of attachment level changes, initial gains were most remarkable for the deepest initial point. Because the pattern of attachment loss was comparable for all points, considerable attachment gains for MAX were maintained throughout the study period. In contrast, a net loss of attachment was actually witnessed for some points. Thus, the hypothesis that the most affected site would demonstrate the most benefit from treatment--if indeed such benefits occurred--would appear to be borne out by these results.

Finally, it is noteworthy that the randomization procedure was somewhat less than optimal in that the placebo group (P) exhibited more advanced disease than the drug group (D) at baseline (Table 9). This phenomenon was remedied by analysing mean differences from baseline rather than raw data. The differences method of adjustment implicitly assumes that improvement of less diseased sites will be of the same order of magnitude as for more affected sites. If one acknowledges that sites of more advanced disease might be expected to show differential improvement, then a spurious failure to have demonstrated between-group differences--a Type II error--might have occurred in this particular situation. The observation that a greater (though not significantly greater) attachment gain was observed for all probing points for the drug group (which exhibited less disease at baseline) tends to dispel reservations concerning the use of the differences method of adjustment in this study.

B Gingival Inflammation

Raw mean Gingival Index (GI) scores and mean differences from baseline, plotted by treatment group and over time (Table 11, Figures 13-14), demonstrated a clinical improvement in gingival status which was maintained for the duration of the study. An ANOVA based on a quadratic model ruled out the possibility of a significant time^2-group interaction or a time^2 effect. A linear ANOVA based on differences data failed to show either a significant time-group interaction or main effects attributable to treatment group or time. There were, however, significant differences between subjects ($P < 0.05$): some subjects had consistently higher or lower scores than others.

The above results concur with those obtained for epithelial attachment in that there were no between-group differences between the first and final examinations. A significant decrease in gingival inflammation was observed at the first examination for the metronidazole group only ($0.05 < P < 0.10$, placebo group; $P < 0.05$, drug group). In contrast to the gradual recidivism observed from 1 to 6 months for attachment levels, improved gingival status persisted throughout the study period.

C Oral Hygiene

The Debris Index (DI) component of the OHI-S Index was chosen as the focus of this analysis because: 1) debris scores have been repeatedly shown to be highly correlated with periodontal health; and 2) considerable debris was present at all examinations, while comparative-

ly little calculus was recorded subsequent to post-baseline treatment.

For the placebo group, an initial drop in debris scores (not statistically significant) was followed by a steep increase between 3 and 6 months, such that 6-month scores surpassed baseline levels (Table 11, Figures 15-16). For the drug group, a net increase in debris scores was also observed, but unaccompanied by an initial decrease.

Analyses of variance using both quadratic and linear models uncovered neither time^2-group or time-group interactions, nor main effects due to time, time^2 or treatment group. As for previously discussed variables, there was significant intersubject variability ($P < 0.01$). However, in consideration of the bizarre plot of DI scores obtained for the drug group (Figures 15-16), these results must be viewed with skepticism.

D Microbiological Measurements

Two multivariate analyses of variance performed for all bacterial categories together ruled out quadratic relationships, a time-group interaction and group differences. An overall time trend was significant ($P < 0.01$).

Previous research has demonstrated that spirochetes and motile rods are particularly implicated in periodontal disease; proportions of coccoid cells, on the other hand, are conspicuously reduced [39,43,44,52,58]. Further analyses were therefore confined to these morphological types. Analyses of variance failed to show quadratic trends for any

of these bacterial categories.

For both treatment groups, the proportion of spirochetes plummeted during the first post-treatment month and rose gradually thereafter, but never regained baseline levels (Table 13, Figure 17). This initial drop was statistically significant for both treatment groups ($P < 0.01$). A linear ANOVA indicated: 1) the similarity of secular change in proportions of spirochetes for both groups; 2) the significant increase in spirochetes between the first and last examinations; and 3) the nearly significant between-group differences for all examinations combined (Table 12). The proportion of spirochetes for the metronidazole group was, in fact, significantly lower at 1 month ($P < 0.05$) and 3 months ($P < 0.05$), but not at 6 months.

The proportion of motile rods decreased significantly from baseline regardless of treatment group ($P < 0.01$). While motile rods continued to decrease over the remaining study period ($P < 0.01$), no additional decrease was [illegible] experienced by the metronidazole group (Tables 12-13, Figure 17).

Proportions of coccoid cells increased dramatically during the first month for both treatment groups ($0.05 < P < 0.10$, placebo group; $P < 0.1$, drug group). The subsequent decrease from 1 to 6 months was not significant for either group ($P > 0.10$), indicating a long-lasting treatment effect. The pattern of secular change in the proportions of cocci was comparable for both groups (Tables 12-13, Figure 17). Although there

rods; these were significant for the metronidazole group only (Table 16).

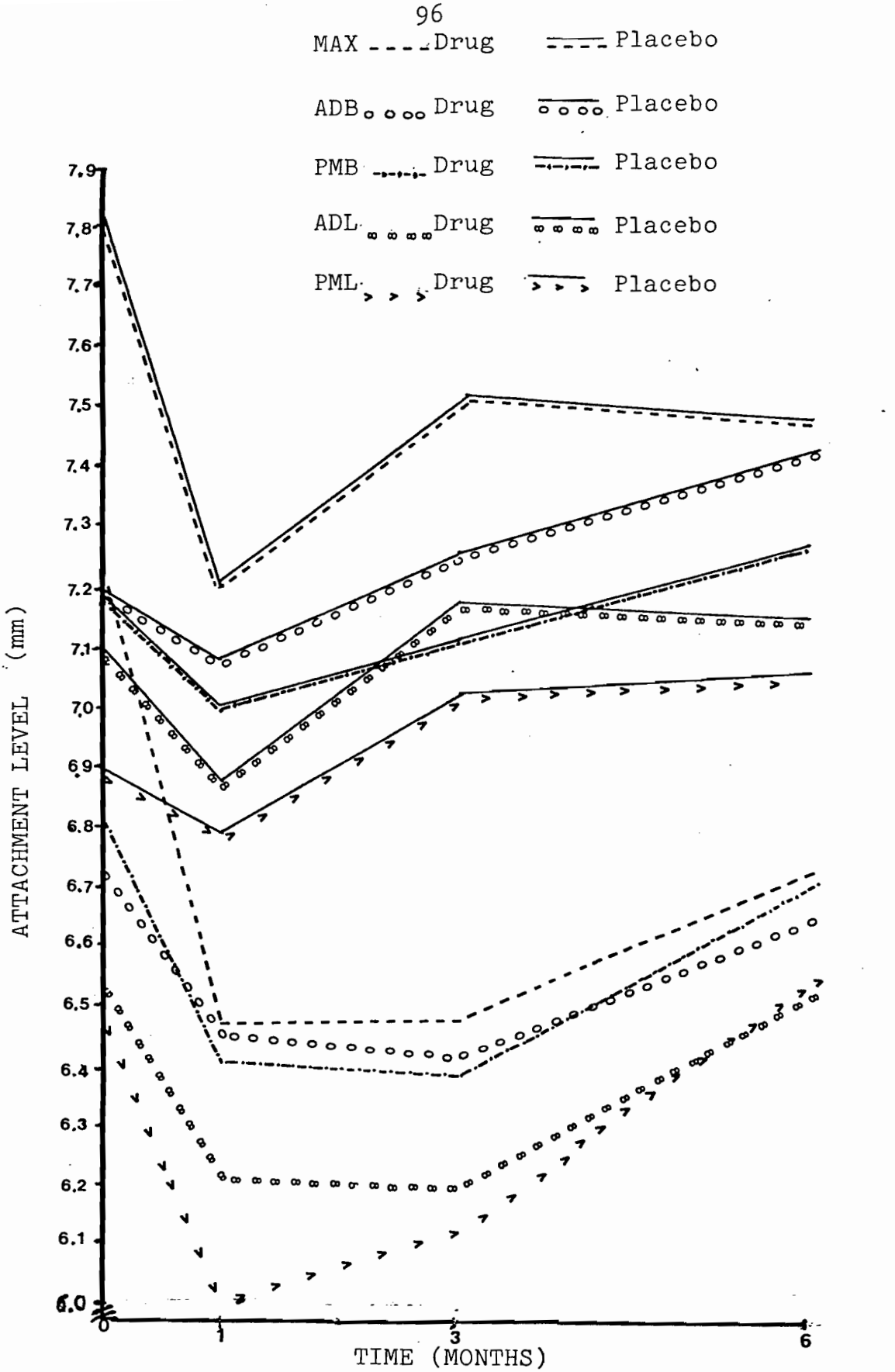


Fig. 11. Raw epithelial attachment levels (means) at baseline and at follow-up examinations for all interproximal probing points and the deepest initial probing point, by treatment group. Values were averaged for the two sites.

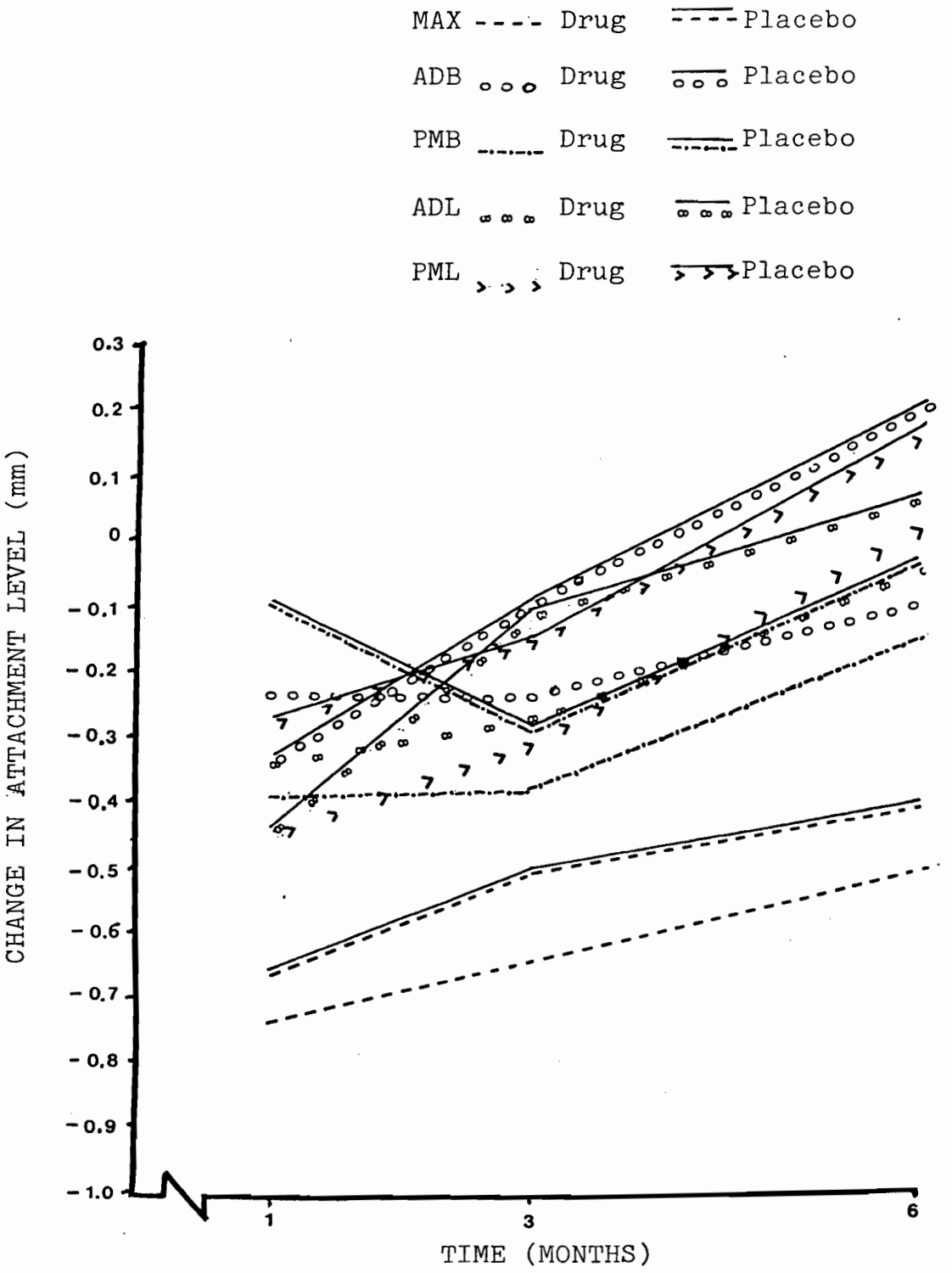


Fig. 12. Change in epithelial attachment levels from baseline (means) by treatment group. Values were averaged for the two sites.

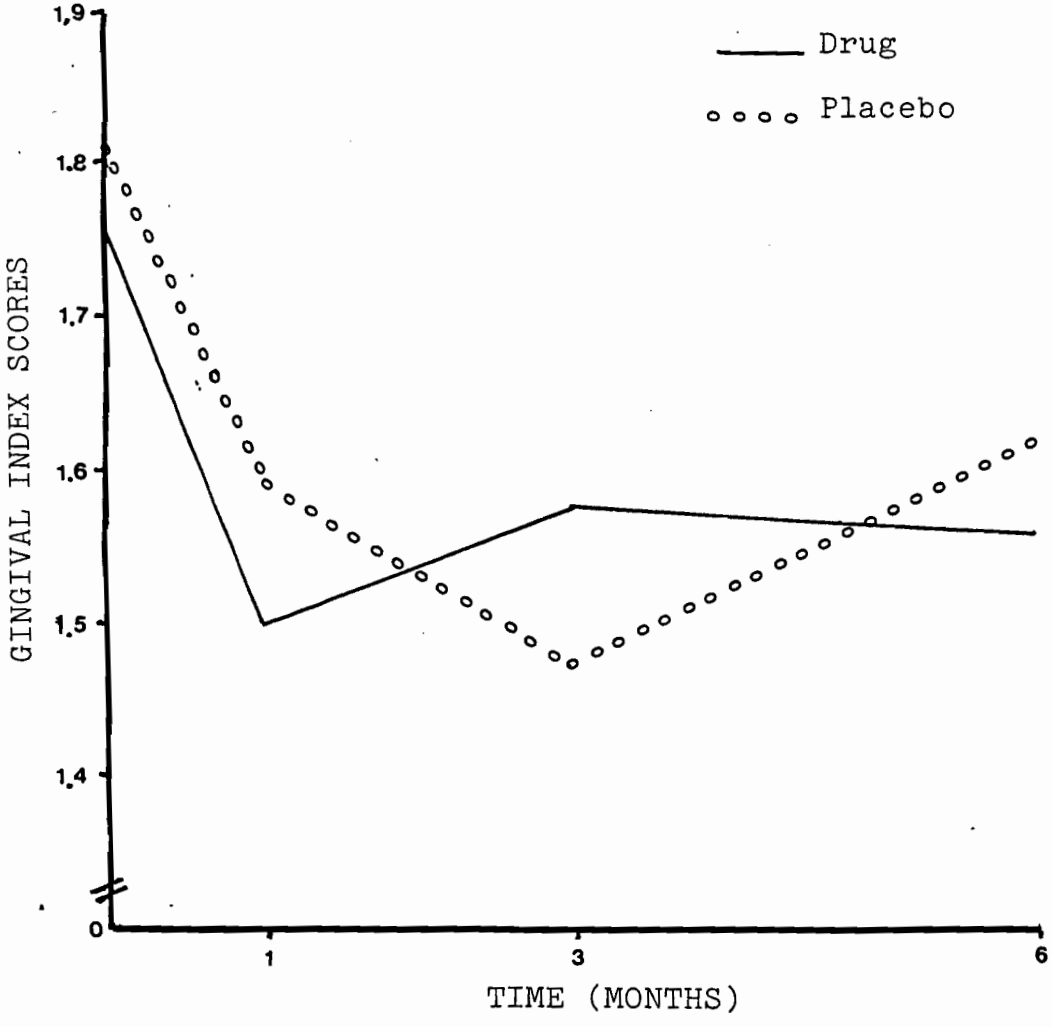


Fig. 13. Gingival Index scores at baseline and at follow-up examinations (means), by treatment group.

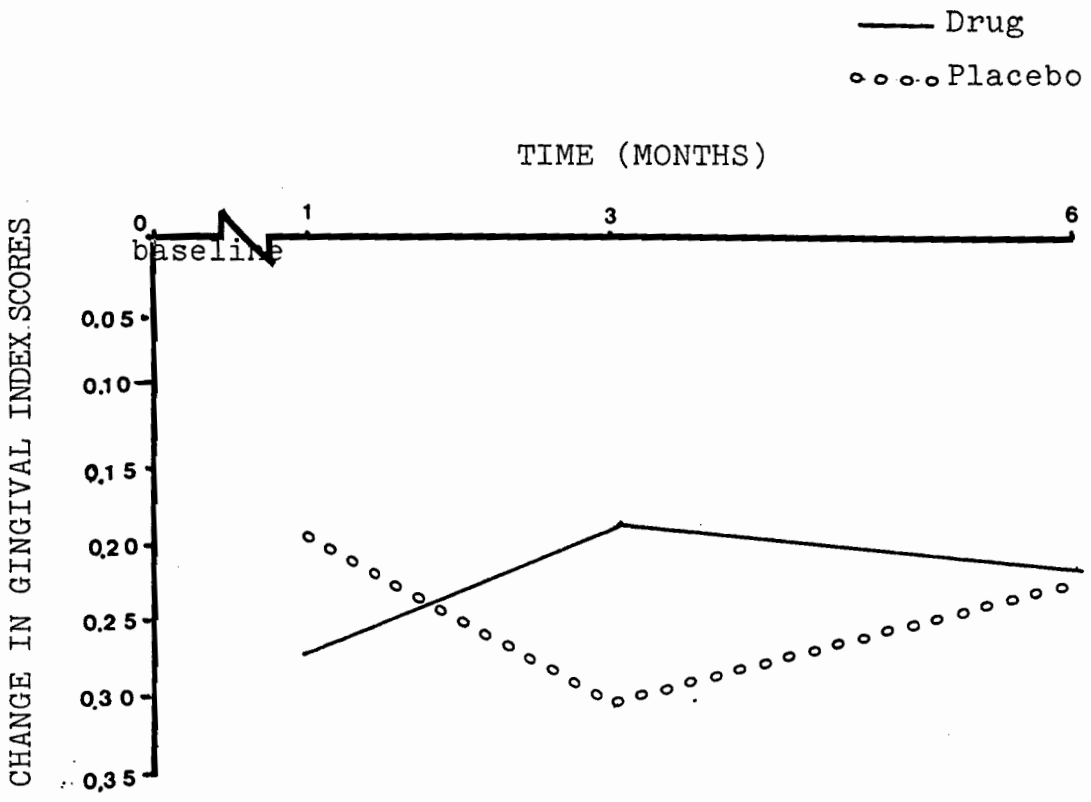


Fig. 14. Change in Gingival Index scores from baseline (means), by treatment group, averaged for the two sites.

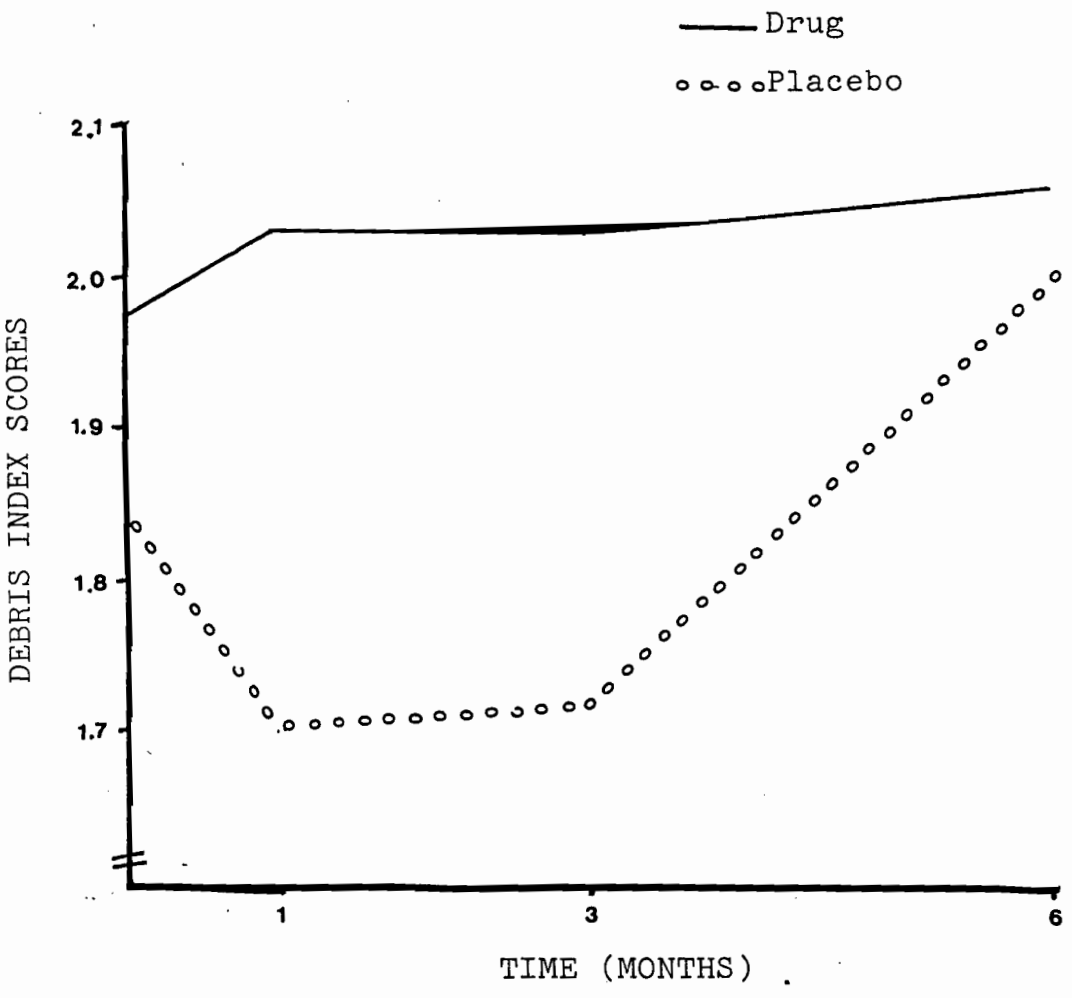


Fig. 15. Debris Index scores at baseline and at follow-up examinations (means), by treatment group, averaged for the two sites.

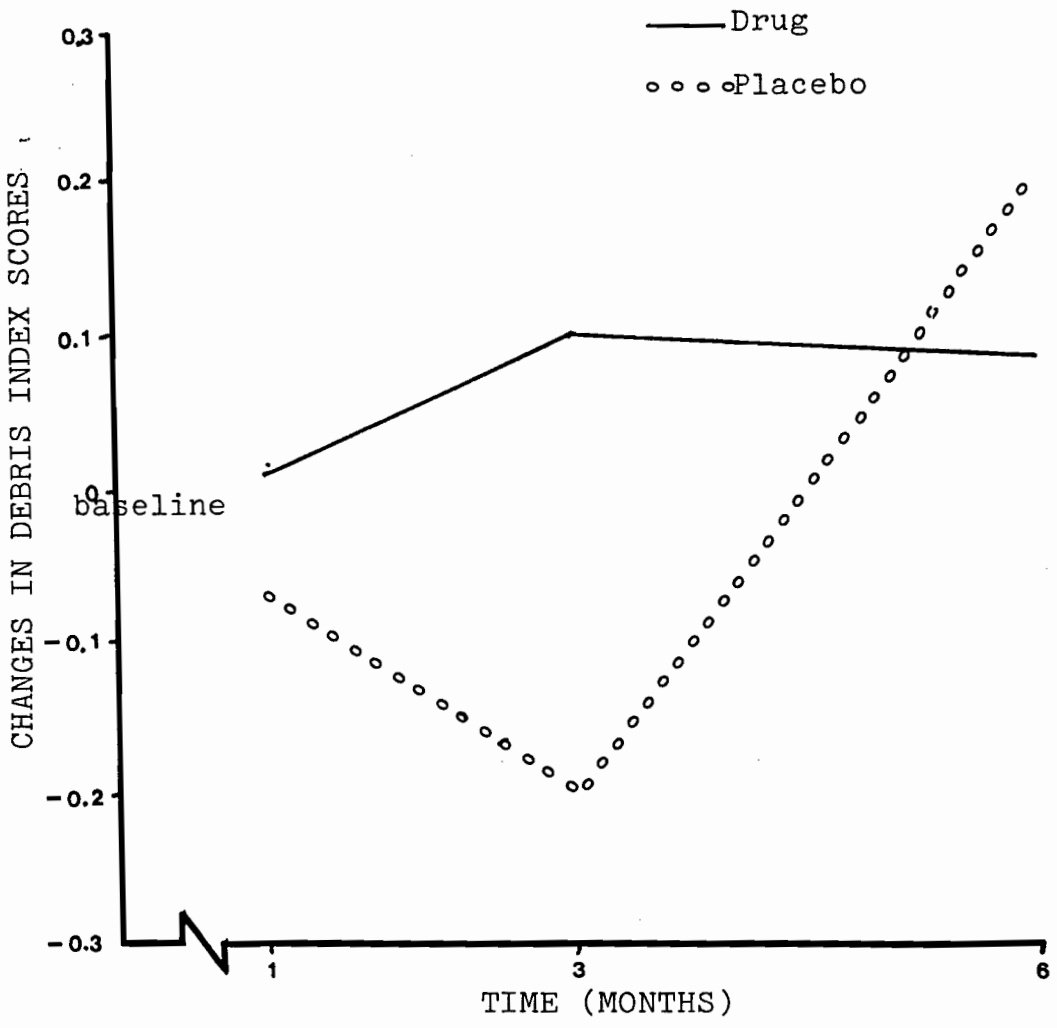


Fig. 16. Change in Debris Index scores from baseline (means), by treatment group, averaged for the two sites.

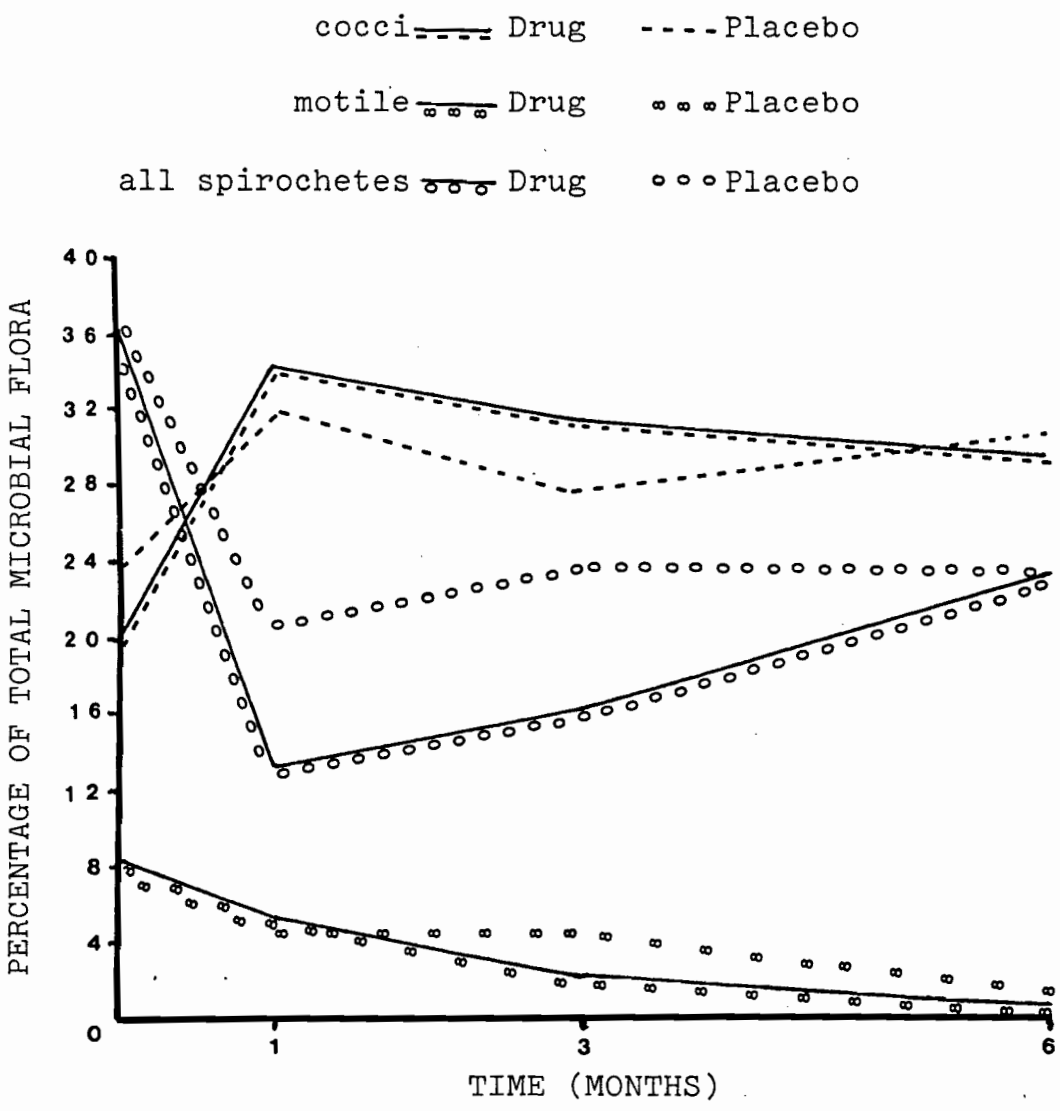


Fig. 17. Mean percentages of morphologic bacterial types at baseline and at follow-up examinations, by treatment group, averaged for the two sites.

TABLE 8

ANALYSIS OF VARIANCE TABLE FOR THE DEEPEST INITIAL PROBING POINT (DIFFERENCES FROM BASELINE)

Source	Degrees of Freedom	Sums of Squares	F-Value	Probability
Group	1	0.08417629	0.21	0.6507
ID (Group)	21	31.34924008	3.66	0.0001
Site	1	0.00153893	0.00	0.9512
Time	1	1.86359856	4.57	0.0352
Time-Group	1	0.33047525	0.81	0.3704

TABLE 10

ANALYSIS OF VARIANCE RESULTS FOR ALL PROBING POINTS: PROBABILITY VALUES (DIFFERENCES FROM BASELINE)

Dependent Variable	Group Main Effect	Time Main Effect	Group-Time Interaction
PMB	0.7235	.0260	.7999
ADB	.5495	.0399	.2494
PML	.2904	.0008	.7818
ADL	.9505	.0298	.7031
MAX	.6507	.0352	.3704

TABLE 9

EPITHELIAL ATTACHMENT LEVELS (mm) AT BASELINE AND AT FOLLOW-UP EXAMINATIONS FOR ALL PROBING POINTS

Probing Point	Group	Baseline	1 Month	3 Months	6 Months
PMB	Placebo				
	Mean (±S.D.)	7.19(1.15)	7.06(1.25)	7.11(1.31)	7.26(1.32)
	n	21	17	18	19
	Drug				
	Mean (±S.D.)	6.83(0.70)	6.41(0.73)	6.38(0.76)	6.69(0.88)
	n	24	22	21	24
ADB	Placebo				
	Mean (±S.D.)	7.19(1.42)	7.09(1.18)	7.25(1.47)	7.42(1.76)
	n	21	17	18	19
	Drug				
	Mean (±S.D.)	6.73(0.77)	6.45(0.83)	6.40(0.83)	6.63(0.82)
	n	24	22	21	24

TABLE 9 — Continued

PML	Placebo				
	Mean (±S.D.)	6.90(1.17)	6.79(1.25)	7.03(1.02)	7.05(1.63)
	n	21	17	18	19
	Drug				
	Mean (±S.D.)	6.50(0.99)	6.05(0.94)	6.12(0.79)	6.52(0.87)
	n	24	22	21	24
ADL	Placebo				
	Mean (±S.D.)	7.10(1.41)	6.88(1.34)	7.17(1.21)	7.16(1.57)
	n	21	17	18	19
	Drug				
	Mean (±S.D.)	6.54(1.06)	6.20(1.01)	6.19(0.83)	6.50(0.85)
	n	24	22	21	24
MAX	Placebo				
	Mean (±S.D.)	7.81(1.29)	7.21(1.17)	7.53(1.37)	7.47(1.63)
	n	21	17	18	19
	Drug				
	Mean (±S.D.)	7.21(0.85)	6.48(0.82)	6.48(0.70)	6.71(0.98)
	n	24	22	21	24

TABLE 11

GINGIVAL INDEX AND DEBRIS INDEX SCORES AT BASELINE AND AT FOLLOW-UP EXAMINATIONS

Index	Group	Baseline	1 Month	3 Months	6 Months
GI	Placebo				
	Mean (±S.D.)	1.81(0.16)	1.60(0.30)	1.47(0.14)	1.62(0.19)
	n	11	9	9	10
	Drug				
	Mean (±S.D.)	1.77(0.22)	1.50(0.27)	1.58(0.17)	1.57(0.21)
	n	11	11	11	12
DI	Placebo				
	Mean (±S.D.)	1.85(0.54)	1.70(0.42)	1.72(0.65)	2.00(0.55)
	n	11	9	9	9
	Drug				
	Mean (±S.D.)	1.97(0.54)	2.03(0.43)	2.03(0.77)	2.06(0.61)
	n	12	11	11	12

TABLE 12

ANALYSIS OF VARIANCE RESULTS FOR MORPHOLOGIC BACTERIAL TYPES: PROBABILITY VALUES

Dependent Variable	Group Main Effect	Time Main Effect	Group-Time Interaction
Coccoid Cells	.1597	.1757	.5296
Spirochetes	.0577	.0066	.1458
Motile Rods	.9268	.0001	.3009

TABLE 14

CORRELATIONS BETWEEN EPITHELIAL ATTACHMENT LEVELS AND MICROORGANISMS [a,b]

Morphological Type	Deepest Initial Probing Depth	
	Placebo Group (n=74)	Drug Group (n=91)
Coccoid Cells	-0.20464*	-0.23075*
Spirochetes	0.18551*	0.16542*
Motile Rods	0.07513	0.17312*

*Significant at $\alpha = 0.05$

[a]Kendall's tau correlation coefficients.

[b]All times combined.

TABLE 13

MICROBIAL PROPORTIONS AT BASELINE AND AT FOLLOW-UP EXAMINATIONS

Microbial Type	Group	Baseline	1 Month	3 Months	6 Months
Coccoid Cells	Placebo				
	Mean (±S.D.)	23.30(14.70)	31.41(10.60)	27.05(11.14)	30.14(12.38)
	n	21	17	18	18
	Drug				
	Mean (±S.D.)	19.70(7.73)	33.92(10.60)	31.92(10.60)	29.12(11.24)
	n	24	22	22	24
Spirochetes	Placebo				
	Mean (±S.D.)	36.71(16.50)	20.11(13.30)	23.38(20.72)	22.89(14.94)
	n	21	17	18	18
	Drug				
	Mean (±S.D.)	36.18(.4.00)	12.96(12.36)	16.13(11.33)	22.80(15.89)
	n	24	22	22	24

TABLE 13 — Continued

Motile Rods	Placebo				
	Mean (±S.D.)	7.33(3.77)	4.19(2.20)	3.98(3.40)	2.08(2.60)
	n	21	17	18	18
	Drug				
	Mean (±S.D.)	8.00(4.65)	4.65(2.63)	1.84(1.44)	1.26(1.10)
	n	24	22	22	24

TABLE 15

CORRELATIONS BETWEEN CLINICAL INDEX SCORES AND MICROORGANISMS [a,b]

Comparison	Placebo Group Site 1 (n)	Placebo Group Site 2 (n)	Drug Group Site 1 (n)	Drug Group Site 2 (n)
GI vs. DI	0.00465 (38)	0.00465 (38)	0.11676 (45)	0.11676 (45)
GI vs. Coccoid Cells	-0.11668 (38)	-0.16333 (36)	-0.28530* (45)	-0.18556 (45)
GI vs. Spirochetes	0.25404* (38)	0.15319 (36)	0.14055 (45)	0.29879* (45)
GI vs. Motile Rods	0.18149 (38)	0.10353 (36)	0.27403* (45)	0.27990 (45)
DI vs. Coccoid Cells	0.14748 (38)	0.04603 (35)	-0.08986 (46)	-0.02095 (46)
DI vs. Spirochetes	-0.03647 (38)	-0.16968 (35)	0.00200 (46)	-0.01902 (46)
DI vs. Motile Rods	-0.25246* (38)	-0.17738 (35)	0.00704 (46)	0.05750 (46)

*Significant at $\alpha=0.05$

[a]Kendall's tau correlation coefficients.

[b]All times combined.

TABLE 16

CORRELATIONS BETWEEN MICROBIAL TYPES [a,b]

Comparison	Placebo Group Site 1 (n)	Placebo Group Site 2 (n)	Drug Group Site 1 (n)	Drug Group Site 2 (n)
Spirochetes vs. Coccoid Cells	-0.59117** (38)	-0.56916** (36)	-0.59080** (46)	-0.54343** (46)
Spirochetes vs. Motile Rods	0.06456 (38)	0.20436 (36)	0.23622* (46)	0.18548 (46)
Coccoid Cells vs. Motile Rods	-0.13917 (38)	-0.10799 (36)	-0.25489* (46)	-0.27972* (46)

*Significant at $\alpha = 0.05$.

**Significant at $\alpha = 0.001$.

[a]Kendall's tau correlation coefficients.

[b]All times combined.

TABLE 17

MEAN MICROBIAL PROPORTIONS, BY GROUP AND EXAMINATION, EXPRESSED AS A PERCENTAGE OF TOTAL ENUMERATED CELLS

		Baseline	1 month	3 months	6 months
Coccoid Cells and Straight Rods	P	42.11	64.05	65.86	72.06
	D	45.18	71.83	75.67	72.07
Filaments and Fusiforms	P	10.35	9.42	6.56	3.07
	D	8.38	9.26	6.65	2.73
Spirochetes	P	36.18	20.11	23.38	22.89
	D	36.18	12.96	16.13	22.88
Motile Rods	P	7.33	4.19	3.98	2.08
	D	8.00	4.65	1.84	1.26

V. DISCUSSION

Previous studies have shown that professional prophylaxes and patient reinstruction in oral hygiene practices must be repeated at least 3-4 times yearly to maintain attachment levels [3,40,61,71,95]. The persistence of attachment gains for the deepest initial probing point--given the poor oral hygiene practiced by most study participants and the lack of further treatment following the first prophylaxis and scaling--was therefore somewhat surprising. To what extent this improvement reflects an actual gain in attachment--as opposed to merely an increase in tissue consistency--is uncertain. Although Helldén et al documented significant gains in attachment levels at 6 months compared to pre-treatment levels, both for patients who received scaling plus tetracycline and for those who received either treatment alone, interpretation of this study is complicated by the small sample size and the fact that additional treatment was administered to some patients prior to study's end [33]. In a study of five periodontal patients who received an initial scaling, root planing and course of metronidazole, (two of whom received extra treatment during the study), diseased sites achieved attachment gains of 1.5-2.0 mm over a 9-month period [52]. On the other hand, Listgarten and Levin were unable to observe any changes in

attachment levels at bimonthly examinations carried out for one year on 19 periodontal patients after an initial cleaning and scaling [44].

The precision of the data collected for attachment levels is supported by the small standard deviations in the measurements (Table 9) as well as the similarity in attachment level changes seen for all probing points. The use of an acrylic template as a standard reference for measurement no doubt contributed greatly to this measurement accuracy.

Before undertaking further studies similar to the present one, the following points should be considered:

1) The failure to find significant differences between treatment groups for any probing point.
2) The lack of clinically significant disease at facial and lingual sites.
3) The fact that the probing point should correspond to the microbiological sampling point (i.e., the deepest point interproximally).
4) The possibility that the most diseased (deepest) point may benefit to a greater degree than less diseased points and thus show more treatment-related improvement.

In view of these considerations, it would seem redundant to monitor attachment level changes for any but the deepest initial point per site.

From a review of the literature, an initial decrease for both debris and gingival inflammation was expected

[32,33,45,58]. However, studies of secular trends have produced conflicting results. Listgarten and Levin reported no change in Plaque Index (PlI) scores and an increase in GI scores during the 12 months following initial mechanical debridement [44]. Helldén et al observed a decrease in PlI and GI scores for all patients except those who had received no treatment at all [33]. In a descriptive study of 14 patients following an initial scaling and planing, Mousquès et al showed that PlI and GI scores had reverted to baseline levels at 1 month post-treatment [58].

The hypothesized initial decrease in gingival inflammation was realized for both treatment groups. This decrease reflects a generalized improvement in periodontal status, whereas gains in epithelial attachment reflect more localized changes--specifically those occurring at the apical boundry of the pocket. In the sense that 1) both variables indicated initial periodontal improvement, and 2) no additional benefits attributable to metronidazole administration were apparent for either variable, the results for attachment levels and GI scores were congruent.

Why no parallel recidivism between the first and final examinations was seen for GI scores is a matter of conjecture. The removal of large amounts of calculus from the majority of subjects which did not recur to any appreciable extent during the study period provides a plausible hypothesis. Another possible explanation is that the examiner's criteria for assessing gingival inflammation

changed over time. A third, but less probable, explanation derives from the observation that spirochete levels (which correlated positively with gingival inflammation) never reverted to baseline levels: the gradual increase in the proportion of spirochetes from 1 to 6 months may have been sufficient to reverse attachment gains but not to exacerbate gingival inflammation. Finally, it should be emphasized that while GI scores were not calculated in the usual manner, they nevertheless reflect a combination of phenomena at relatively healthy (facial and lingual surfaces of teeth adjacent to sites) and diseased sites (interproximal surfaces). Thus, recidivism at diseased sites might have been considerably diluted by the inclusion of relatively healthy sites (for which no or little change occurred) in the calculation of GI scores.

The Debris Index is rarely employed in small clinical trials; the Plaque Index is usually prefered because of its greater sensitivity and biological validity [47]. The DI results reported in this study (e.g., the disparity of DI score changes over time by treatment group, the lack of significant correlations between DI and GI scores, the confusing correlations between microbial types and DI scores, the large standard deviations about the mean values) should be viewed with suspicion. Indeed, the only expected finding was the initial improvement in oral cleanliness seen for the placebo group during the first month. Because the DI, which was calculated in the prescribed manner, 1) is a whole mouth

measurement which does not necessarily include any diseased sites, 2) does not evaluate subgingival plaque, and 3) evaluates plaque coronal to the cervical margin, it probably bears few implications for subgingival phenomena at diseased sites while it may be of academic interest to establish that metronidazole does or does not differentially decrease oral debris in comparison with mechanical debridement alone, such a finding is probably irrelevant to the central thesis of this study--namely, whether adjunctive metronidazole can significantly increase the periodontal amelioration achieved by mechanical therapy.

A final caveat to the interpretation of clinical indices concerns their relative lack of measurement accuracy. Certainly their ordinal nature and limited scoring categories precludes sensitivity, thereby reducing precision. Accuracy is also hampered by examiner subjectivity and dubious biological validity (especially true for the Debris Index).

According to theory, mechanical debridement--alone or in combination with antibiotic therapy--reduces or eliminates harmful periodontal flora (e.g., spirochetes and motile rods). Treatment thus disrupts the disease process, which consequently permits a gain in epithelial attachment (whether connective or epithelial) and a repopulation of the pocket with flora indicative of periodontal health (e.g., gram-positive cocci and rods). The secular changes in bacterial flora following initial treatment observed in this study agree with general theoretical expectations. Less well

documented, however, are the duration and magnitude of these changes. Mousquès et al reported only transitory microbial changes following once-only mechanical debridement: cocci reverted to baseline levels at 1 month, spirochetes at 6 weeks, and motile rods within one week [58]. Slots et al documented three types of post-treatment shifts. Spirochetes underwent a rapid reduction followed by a slow rise without regaining baseline levels at 6 months. Cocci increased rapidly and slowly reverted to baseline levels by 6 months. Motile rods decreased sharply, then underwent a rebound increase surpassing baseline levels before returning to baseline levels [85]. Loesche et al showed that spirochete proportions remained significantly lower than baseline levels after 6 months [52]. Conversely, Listgarten and Levin were unable to demonstrate a significant time trend for any bacterial groups [44].

Given the paucity of studies and their limitations (such as no control groups, small sample sizes, administration of additional treatment within the study period, technical difficulties inherent to darkfield microscopy), the findings of the present study with respect to cocci and spirochetes are entirely credible. This credibility is enhanced by significant correlations between microbial types and attachment levels. Findings for motile rods are less comprehensible, however. Although a post-treatment decrease was expected, no other study has documented a continuing reduction of motile rods over time.

Correlations between microbiological and clinical variables and between microbial types concurred with expectations except for PI vs. GI and DI vs. microbes comparisons. Discrepancies in DI results have already been discussed: it appears that the measurement of oral debris in this study was less than optimal and perhaps irrelevant as well.

VI. CONCLUSIONS

The primary objectives of this research were:

1) To determine whether significant differences in clinical improvement and microbial proportions could be shown between experimental and control groups during the 6-month period following a single course of therapy; and
2) To compare clinical and microbiological results.

Administration of metronidazole significantly reduced the spirochete population over and above the reduction achieved through mechanical means alone--at least for 3 months following treatment--thus demonstrating the specificity of the drug for these bacteria. This microbial reduction was, however, insufficient to produce clinical differences between groups as measured by epithelial attachment, gingival inflammation or oral debris. How may we best reconcile these results?

One possible explanation is that spirochetes may not be the most crucial determinant of periodontal disease. It has been suggested that they appear secondarily in response to favorable anaerobic and nutritional conditions characteristic of diseased sites. Strong positive correlations between spirochetes and attachment levels cannot discount this possibility. Even positive correlations between periodontal improvement and spirochete reduction could be explained by

this view of spirochetes as opportunistic pathogens: improved periodontal status would reduce those favorable conditions which encourage the secondary population of site by spirochetes.

A second explanation concerns the possibility that although spirochetes were reduced, this statistically significant reduction was not of sufficient magnitude to engender observable clinical differences. If one compares the data from this study with bacterial profiles reported for healthy and diseased periodontia (Tables 3,17), the striking similarity between the bacterial profile for our study subjects at baseline and that of Listgarten and Helldén's diseased sites is evident. Despite having received one of two supposedly effective treatment regimens, the bacterial profile of our patients--for either group at any examination--never resembled a profile characteristic of healthy sulci [39,43]. Had spirochetes been eliminated rather than only partially reduced by the administration of metronidazole, clinical differences between groups might have been observed.

Yet another explanation concerns the choice of study subjects. Our sample was, for the most part, incapable of maintaining a desired level of oral hygiene. It is entirely plausible that in conjunction with a high level of oral hygiene, metronidazole therapy might have been more efficacious. Moreover, this group exhibited mild to moderate disease. More advanced cases might have shown differential improvement with metronidazole therapy.

In view of the latter two explanations, the potential usefulness of metronidazole as adjunctive therapy in the treatment of periodontal disease cannot be ruled out at this time. Further clinical trials should be undertaken after investigators have carefully considered: 1) which populations might be most likely to benefit from metronidazole therapy; 2) dosage adequacy; and 3) procedures to reduce measurement error for clinical indices and microbial counts.

APPENDIX A

SOURCES OF PARTICIPANTS

Peter Hall Lasalle
7676 Central Street
Ville Lasalle, P.Q.

Peter Hall East
127 St. Cyr
Montreal, P.Q.

Peter Hall St. Laurent
11880 Michel Sarrazin
Ville St. Laurent, P.Q.

Miriam School
1750 Deguire
Ville St. Laurent, P.Q.

APPENDIX B

March 10, 1981

Dear Parents and Guardians:

Your child or the child you sponsor has been selected to participate in a study aimed at controlling periodontitis or "gum disease" (because he or she is affected with this disease). Attached to this letter is a patient consent form which must be signed and returned to your school nurse before starting the study.

The treatment in this study includes periodic teeth cleanings, oral hygiene instructions and a single course of antibiotic treatment with metronidazole (or flagyl). This antibiotic has been used for other types of infections for years, and is considered safe and effective by the medical profession. This study is expected to establish the beneficial effects of metronidazole in treating periodontal disease. Although your child may originally be assigned to the group which doesn't receive the antibiotic, he or she will receive the benefits of this antibiotic treatment after a period of observation.

The study will require periodic dental examinations to observe changes in the severity of the disease. Your help and cooperation in the scheduling of these appointments will be extremely helpful. If you have any questions concerning this study or the antibiotic metronidazole, please feel free to contact Dr. Schwartz, Dr. Stulginski or your school nurse for further information.

Thank you for your help and concern.

Sincerely,

Dr. Chris Clark | Dr. Philip Stulginski | Dr. Stephane Schwartz

INFORMED CONSENT

I give my consent to the participation of my child or the child I sponsor in a study aimed at investigating the effect of an antibiotic called Metronidazole (or Flagyl) used in conjunction with routine periodontal treatment (cleaning, scaling and root planing of teeth).

I understand that my child or the child I sponsor may receive a placebo drug instead of Flagyl, depending on which group she/he is assigned. I also understand that she/he will receive periodic dental examinations, teeth cleanings and oral hygiene instructions, regardless of group assignment.

I understand that my child or the child I sponsor will not encounter any significant risk to her/his health as a result of this periodontal therapy (cleaning and scaling of teeth). If she/he takes the antibiotic, she/he might experience minor and reversible side effects that she/he would experience in taking any drug. These side effects include: metallic taste, furry tongue and dry mouth; gastrointestinal disturbances such as diarrhea, anorexia, nausea, vomiting, epigastric distress, constipation; occasional flushing and headaches, especially with concomitant ingestion of alcohol.

I understand that my child or the child I sponsor will have her/his teeth cleaned every 6 months and that a clinical and microbiological examination (collection of plaque and observation under a microscope) will be carried out at periodic intervals throughout the study.

I understand that I am free to have my child or the child I sponsor stop her/his participation in this study at any time.

I understand all the explanations given to me and I know that I can reach Dr. S. Schwartz or Dr. P. Stulginski at any time, should the child develop any reaction to the antibiotic or should I have any other questions. (937-8511, extension 686).

DATE: _____

SIGNATURE: _____

Montréal , Mars 1981.

FORMULE DE CONSENTEMENT

Je consens à ce que mon enfant ou l'enfant dont j'ai la garde participe à une étude destinée à l'observation de l'effet thérapeutique d'un antibiotique appelé Métronidazole (ou Flagyl) lorsqu'employé en conjonction avec les traitement périodontiques de routine (Nettoyage, détartrage et aplanissement radiculaire des dents).

Je comprends que mon enfant ou l'enfant dont j'ai la garde peut reçevoir un placebo au lieu du Flagyl,selon le groupe où elle/il est assigné. Je comprends également qu'elle/il recevra des examens dentaires périodiques, des nettoyages de dents et des instructions d'hygiène buccale, quelque soit le groupe où elle/il sera assigné.

Je comprends que mon enfant ou l'enfant dont j'ai la garde ne courra pas de risque particulier pour sa santé en reçevant des traitements de périodontie (nettoyage et détartrage des dents). Si elle/il reçoit l'antibiotique, elle/il peut ressentir des effets secondaires mineurs et réversibles qu'elle/il pourrait ressentir à la suite de n'importe quel médicament. Ces effets secondaires comprennent : goût metallique, langue pâteuse et bouche sèche; dérangement gastro-intestinaux comme diarrhée, anorexie, nausée, vomissements, douleurs stomaccales, constipation; rougeurs occasionnelles et maux de tête, surtout lorsqu'il y a ingestion simultanée d'alcool.

Je comprends que mon enfant ou l'enfant dont j'ai la garde aura ses dents nettoyées à chaque 6 mois et qu'un examen clinique et microbiologique (collection et examen de la plaque dentaire au microscope) sera accompli à intervalles réguliers pendant toute la durée de l'étude.

Je comprends que je suis libre d'annuler la participation de mon enfant ou de l'enfant dont j'ai la garde à n'importe quel moment de cette étude.

Je comprends toutes les explications qui m'ont été données et je sais que je peux rejoindre Dr Philip Stulginski ou Dr Stephane Schwartz n'importe quand si l'enfant présentait des effets secondaires ou pour n'importe quelle autre raison.

_____

Montreal, march 1981

Chers Parents ou Gardiens,

Votre enfant, ou l'enfant dont vous avez la garde a été sélectionné pour participer à une étude sur la périodontite ou "maladie des gencives" (Parce que elle/il présente cette maladie).

Attachée à cette lettre, vous trouverez une formule de consentement qui doit être signée et retournée à l'infirmière de l'école avant de commencer l'étude.

Les traitements inclus dans cette étude comprennent le nettoyage périodique des dents, des instructions d'hygiène buccale et une seule prescription d'antibiotique appelé Métronidazole (ou Flagyl). Cet antibiotique est déjà employé depuis des années dans le traitement d'infections diverses et le corps médical le considère comme efficace et dépourvu de risques. Nous attendons de cette étude qu'elle démontre l'amélioration additionnelle apportée au traitement de la périodontite par l'antibiotique Métronidazole. Bien que votre enfant soit peut-être assigné à un groupe qui ne prenne pas l'antibiotique, elle/il pourra quand même le recevoir plus tard après la période d'observation nécessaire.

Cette étude comprend des examens dentaires périodiques qui nous permettront d'observer l'évolution de la maladie. Votre assistance et votre coopération quant à l'exactitude des rendez-vous seront essentielles.

Si vous avez des questions à poser à propos de cette étude ou de l'antibiotique utilisé, n'hésitez pas à rejoindre Dr Stephane Schwartz, Dr Philip Stulginski ou encore l'infirmière de l'école !

Merci de votre aide et de votre intérêt,

Dr Philip Stulginski

Dr Stephane Schwartz

Hôpital de Montréal pour les Enfants
937-8511, local 685

APPENDIX C

PATIENT EXAMINATION RECORD

METRONIDAZOLE STUDY

Name _____

Address _____

_____

_____

Telephone Number _____

Name of Guardian _____

Medical Diagnosis _____

Regular Medications _____

Anticholinergic Drugs _____

Date _____

CARD NUMBER — 1 ☐

A. PERSONAL AND DEMOGRAPHIC DATA

I.D. Number — 2-4 ☐☐☐

Group I.D. Number — 5 ☐

Medicare Number — 6-17 ☐☐☐☐☐☐☐☐☐☐☐☐

Sex — 0. Male, 1. Female — 18 ☐

Birthdate _____/_____/_____ (Day / Month / Year) — 19-24 ☐☐☐☐☐☐ (DA. MO. YR.)

Age _____ — 25-26 ☐☐

Examiner — 1. Dr. Clark, 2. Dr. Schwartz, 3. Dr. Stulginski — 27 ☐

Duplicate Examination — 0. No, 1. Yes — 28 ☐

Ethnic Group — 0. Caucasian, 1. Negro, 2. Oriental, 3. Other — 29 ☐

Examination Number

0. Baseline
1. 1st examination
2. 2nd examination
3. 3rd examination
4. 4th examination

30 ☐

CARD NUMBER — 1 ☐

B. TEETH PRESENT

Number of permanent teeth — 2-3 ☐☐

Name _____

Date _____

H. MICROBIOLOGICAL FINDINGS

Bacterial Counts (site number one)

Fusiform		22-24 ☐☐☐
Coccoid		25-27 ☐☐☐
Spirochete	- small	28-30 ☐☐☐
	- medium	31-33 ☐☐☐
	- large	34-36 ☐☐☐
Filament		37-39 ☐☐☐
Rod	- motile	40-42 ☐☐☐
	- curved	43-45 ☐☐☐
	- straight	46-48 ☐☐☐

Bacterial Counts (site number two)

Fusiform		49-51 ☐☐☐
Coccoid		52-54 ☐☐☐
Spirochete	- small	55-57 ☐☐☐
	- medium	58-60 ☐☐☐
	- large	61-63 ☐☐☐
Filament		64-66 ☐☐☐
Rod	- motile	67-69 ☐☐☐
	- curved	70-72 ☐☐☐
	- straight	73-75 ☐☐☐

Name _____

Date _____

C. CARIES EXPERIENCE

Total DMFT (baseline and final examination only)	4-5	☐☐

D. GINGIVAL ASSESSMENT

G.I. index modified — site evaluation only.

0. Normal gingiva.
1. Mild inflammation - slight change in colour, slight edema, no bleeding on probing.
2. Moderate inflammation - redness, edema and glazing, bleeding on probing.
3. Severe inflammation - marked redness and edema, ulceration, tendency to spontaneous bleeding.

Site number 1			
Tooth number		6-7	☐☐
Surfaces	- distal	8	☐
	- buccal	9	☐
	- mesial	10	☐
	- lingual	11	☐
Tooth number		12-13	☐☐
Surfaces	- distal	14	☐
	- buccal	15	☐
	- mesial	16	☐
	- lingual	17	☐
Site number 2			
Tooth number		18-19	☐☐
Surfaces	- distal	20	☐
	- buccal	21	☐
	- mesial	22	☐
	- lingual	23	☐
Tooth number		24-25	☐☐
Surfaces	- distal	26	☐
	- buccal	27	☐
	- mesial	28	☐
	- lingual	29	☐
G.I. score: Total divided by number of surfaces scored. (Round off to nearest hundredth)		30-33	☐☐☐☐

Name _____

Date _____

E. TEST SITE LOCATIONS

Interproximal probing locations between following two teeth:

Site 1	Tooth #	34-35	☐☐
	Tooth #	36-37	☐☐
Site 2	Tooth #	38-39	☐☐
	Tooth #	40-41	☐☐

F. PERIODONTAL PROBING SCORES

Number in millimeters of perio probing depths in 2 designated sites:

Site 1 probings

- posterior tooth, buccal probe location	42-43	☐☐
- posterior tooth, buccal proximal probe location	44-45	☐☐
- anterior tooth, buccal probe location	46-47	☐☐
- anterior tooth, buccal proximal probe location	48-49	☐☐
- posterior tooth, lingual proximal probe location	50-51	☐☐
- posterior tooth, lingual probe location	52-53	☐☐
- anterior tooth, lingual proximal probe location	54-55	☐☐
- anterior tooth, lingual probe location	56-57	☐☐

Site 2 probings

- posterior tooth, buccal probe location	58-59	☐☐
- posterior tooth, buccal proximal probe location	60-61	☐☐
- anterior tooth, buccal probe location	62-63	☐☐
- anterior tooth, buccal proximal probe location	64-65	☐☐
- posterior tooth, lingual proximal probe location	66-67	☐☐
- posterior tooth, lingual probe location	68-69	☐☐
- anterior tooth, lingual proximal probe location	70-71	☐☐
- anterior tooth, lingual probe location	72-73	☐☐

Name _____

Date _____

CARD NUMBER 1 ☐

G. ORAL HYGIENE ASSESSMENT (One examiner only. Patient's teeth are disclosed.)

OHI-S Index

DI-S

0. No debris present.
1. Soft debris covering not more than one third of the tooth surface being examined or the presence of extrinsic stains without debris regardless of surface area covered.
2. Soft debris covering more than one third but not more than two thirds of the exposed tooth surface.
3. Soft debris covering more than two thirds of the exposed tooth surface.

CI-S

0. No calculus present.
1. Supragingival calculus covering not more than one third of the exposed tooth surface being examined.
2. Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface, or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth.
3. Supragingival calculus covering more than two thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth.

Tooth Selection

- Upper and lower molars - 1st permanent molar distal to second permanent bicuspid or 2nd primary molar.

- Upper and lower centrals - contralateral central incisor is used if specified tooth is missing.

			DI	CI
Maxillary right first molar	- buccal	2-3	☐	☐
" " central incisor	- labial	4-5	☐	☐
" left first molar	- buccal	6-7	☐	☐
Mandibular left first molar	- lingual	8-9	☐	☐
" " central incisor	- labial	10-11	☐	☐
" right first molar	- lingual	12-13	☐	☐

OHI-S score: Total of tooth surface scores divided by 6, round off to nearest hundredth. 14-17 ☐☐☐

Total debris index scores divided by 6, round off to nearest hundredth. 18-21 ☐☐☐

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