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THE EFFECT OF ORALLY ADMINISTERED MINOCYCLINE

HYDROCHLORIDE (MINOCIN) ON MODERATE PERIODONTITIS IN HUMANS:

A CLINICAL AND MICROBIOLOGICAL STUDY

By

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A Thesis Submitted to the Faculty of the Graduate School

of Loyola University of Chicago in Partial Fulfillment

of the Requirements for the Degree of

Master of Science

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1982

DEDICATION

To my wife, Gretchen, who helped critique the manuscript
and organize the data. Her love and support made this
all possible and worthwhile. I pledge to give to her
what she has given to me.

ACKNOWLEDGEMENTS

LIFE

The author, Stephen Alan Folson, is the son of Alan and Ruth Folson.
He was born August 8, 1952, in Grand Forks, North Dakota.

His elementary education was obtained in the public schools of Grand
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On August 23, 1980, he married Gretchen Vaaler in the United
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In September, 1980, he entered a two year postgraduate clinical
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Dentistry. In May, 1982, he received a Certificate of Specialty in
Periodontics.

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CHAPTER I

INTRODUCTION

There is abundant evidence implicating microorganisms as the primary cause of the periodontal diseases.[1,2,3,4,5] Loe and coworkers demonstrated that withdrawal of all measures of oral hygiene in healthy subjects resulted in marginal gingivitis. Moreover, upon reinstitution of rigorous oral hygiene the bacterial plaque was reduced to pre-experimental levels and healthy gingival conditions were reestablished. In addition, characteristic changes were observed in the bacterial flora during the development of marginal gingivitis in these subjects.[6,7]

Subsequent studies have shown that specific microorganisms are found in the gingival sulcus in health and in various forms of periodontal disease.[4,5] Accordingly, the proportions of subgingival Gram-negative and anaerobic organisms increase with the severity of periodontal disease.[4,5] As a result of these studies, a rationale has been established for the use of antibiotics in the treatment of periodontal disease. Although many antibiotics have been investigated, tetracycline has received much attention in recent reviews.[8,9,10]

Of special importance is the fact that tetracycline hydrochloride is effective against the majority of the periodontal pathogens.[8] Orally administered tetracycline has also been shown to pass into the gingival crevicular fluid in humans.[11] In addition, minocycline hydrochloride, a

semi-synthetic tetracycline, can be given in lower doses than tetracy-
cline hydrochloride and has been shown by Ciancio and coworkers [12] to be
more concentrated in the gingival fluid than tetracycline and produces a
reduction in subgingival plaque and gingival inflammation. Since the
gingival crevicular fluid is in intimate contact with both the subgingival
and supragingival microflora, this finding suggests that minocycline may
be more effective clinically than tetracycline.[12]

The purpose of this study is to compare a one week regimen of
orally administered minocycline hydrochloride (Minocin) following scaling
and root planing with scaling and root planing alone. The subgingival
microbial population in selected periodontal pockets will be quantitated
and qualitated by darkfield microscopy before and after treatment in five
human subjects with moderate periodontitis.[13] In addition, probing depth
and Gingival Index,[14] as clinical parameters, will be determined from
teeth numbers 3 and 19.[15]

CHAPTER II

LITERATURE REVIEW

A. <u>THE ROLE OF BACTERIA IN THE PERIODONTAL DISEASES</u>

It has been well documented that bacteria are the primary etiologic agents of the various forms of periodontal disease.[1,2,3,4,5] In addition, studies have shown that specific microorganisms are found in the gingival crevice in health, and in the periodontal pockets associated with the different forms of periodontal disease.[4,5,16,17] Review articles by Slots[5] and Socransky[4] summarize the specific groups of organisms that may be etiologically associated with specific forms of periodontal disease.

The predominant cultivable microflora inhabiting the healthy gingival sulcus include Gram-positive facultatively anaerobic cocci and rods such as <u>Streptococcus sanguis</u>, <u>Streptococcus mitis</u>, <u>Actinomyces naeslundii</u> and <u>Actinomyces viscosus</u>. In addition, Slots observed that 15.0% of the isolates from healthy gingival sulci were Gram-negative organisms. The genera identified included <u>Veillonella</u>, <u>Neisseria</u>, <u>Bacteriodes</u>, and <u>Fusobacterium</u>.[5]

In gingivitis, Gram-positive species predominated, but Gram-negative organisms were prevalent.[5] The Gram-positive organisms included <u>Streptococcus mitis</u>, <u>Streptococcus sanguis</u>, <u>Actinomyces israelii</u>, <u>A. Naeslundii</u>, and <u>A. viscosus</u>. Moreover, Gram-negative anaerobic rods averaged 25% of the cultivable organisms and included <u>Fusobacterium nucleatum</u> and

Bacteroides melaninogenicus ss. intermedius.[5] Slots also found that Gram-negative facultative anaerobic rods averaged nearly 15% of the cultivable organisms and were identified as Haemophilus and Veillonella species.[5]

In advanced adult periodontitis, Gram-negative anaerobic rods are the most predominant organisms and include Bacteroides gingivalis, Bacteroides melaninogenicus ss. intermedius, Fusobacterium nucleatum, and strains of the genera Selenomonas, and Campylobacter.[5,8] Moreover, anaerobic vibrios, "corroding" Bacteroides and Eikenalla corrodens are found in the destructive periodontitis pockets.[4] Additionally, Slots found that the Gram-positive anaerobic and facultatively anaerobic rods accounted for about 19% of the isolates. The majority of these organisms were strains of Actinomyces israelii.

As far as juvenile periodontitis (periodontosis) is concerned, the microbial composition of the pocket reveals the presence of a sparse microbiota which is predominated by Gram-negative capnophilic and anaerobic rods.[4] Evidence provided by Newman and Socransky[16] subsequently lead to a significant number of isolates from the juvenile periodontitis pockets to be called Capnocytophaga. In addition, Tanner and coworkers isolated a Gram-negative organism with characteristics consistent with Actinobacillus actinomycetemcomitans. The organisms were isolated from young adult subjects and were physiologically similar to strains isolated from periodontosis lesions.[17]

Listgarten, in a light and electron microscopic study of the structure of the microbial flora on tooth surfaces, also noted that a distinct

microbiota was associated with surfaces grouped according to their perio-
dontal status prior to extraction.[18]   He found that the subgingival bac-
terial flora of the periodontitis samples consisted of relatively fewer
cells adherent to the root surface as opposed to the supragingival bac-
terial deposits. Moreover, there was a concomitant increase in the popu-
lation of Gram-negative, flagellated cells, and spirochetes as compared
to the gingivitis samples.[18]

However, it must be noted that spirochetes, which account for over
one third of the subgingival flora in a severely diseased site, are not
detected by cultural methods in current use for assaying the composition
of the oral flora. However, spirochetes can be morphologically determined
and quantitated by darkfield microscopy.[19]

In a study by Listgarten and Hellden,[19]   they compared the relative
distribution of bacteria from relatively normal and periodontally dis-
eased sites in twelve patients with advanced periodontal disease by dark-
field microscopy. In the "healthy" sites, coccoid cells were the pre-
dominant cell type along with straight rods, but spirochetes were rare.
Conversely, the flora obtained from the "diseased" sites showed marked
differences from that of the relatively healthy sites. That is, coccoid
cells and rods constituted less than one half of the flora. As a result,
spirochetes showed a marked increase both in prevalence and relative pro-
portions compared to the "healthy" sites. They were detected in all sub-
jects with periodontal disease in proportions ranging from 24.5 to 58%
of the subgingival flora. In addition, the ratio of motile to nonmotile

cells was 1:49 in the "healthy" samples, whereas in the diseased sites

this ratio was almost 1:1.[19]

Hence, these findings demonstrated clear-cut differences in the

microbial composition of healthy and periodontally diseased areas in the

same individuals. Moreover, darkfield microscopy has been shown to be an

effective tool in demonstrating distinct differences in the composition

of the microbial flora taken from healthy sulci and periodontally diseased

pockets.[19]

In addition, the potential role of microorganisms as predictors of

disease activity has been studied by many investigators.[20,21,22,23] These

studies suggest that microorganisms associated with certain forms of peri-

odontal disease may be reduced in proportion to other organisms by therapy,

then return to baseline levels prior to detectable deterioration of the

clinical status.[23]

Recently, Listgarten and Levin[23] showed that microbial proportions

determined by darkfield microscopy appeared to provide much better dis-

crimination between disease-resistant subjects and disease-susceptible

individuals as compared to clinical measurements. Their results also

showed an excellent correlation between percentage spirochetes or per-

centage spirochetes and motile rods to human subjects susceptible to

periodontal breakdown.

In another differential darkfield microscopic study, Lindhe and co-

workers[24] reported that the sites of advanced disease were associated

with a flora dominated by motile rods and spirochetes. Their observa-

tions would tend to support the hypothesis that a bacterial change pre-

cedes a change in the periodontal status rather than the other way around.

B. TETRACYCLINE IN THE TREATMENT OF ADULT PERIODONTAL DISEASE

Based upon the concept that the periodontal diseases have a microbial etiology, it is a logical hypothesis that antibiotics could effect a change in the composition of subgingival plaque if adequate levels can be achieved in the gingival fluid and saliva.[8,9] Furthermore, Loesche[25] contends that plaque is not a single nondescript bacteriological entity as is postulated by the nonspecific plaque hypothesis (NSPH), but rather a series of microbial combinations, some of which are associated with clinical disease as is stated by the specific plaque hypothesis (SPH). In other words, the SPH states that only certain plaques cause infection, because of the presence of a pathogen(s) and/or a relative increase in the levels of certain indigenous plaque organisms. Moreover, the SPH requires that a diagnosis of infection be made so that prompt mechanical and/or chemical therapy can be initiated in order to restore the normal plaque flora.[25]

Hence, an ideal antibiotic for use in the prevention and treatment of periodontal disease would be one that would act specifically on periodontal pathogens, would not be allergenic or toxic, would maintain activity in the oral environment or tissue for long periods, would not be in general use for treatment of other disease, and would not be prohibitively expensive.[9] Accordingly, tetracycline hydrochloride, hereafter referred to as tetracycline, a broad spectrum antibiotic used in the treatment of acne vulgaris, has been shown to be inhibitory for bacteria currently implicated in destructive periodontal disease.[8,26] Moreover, tetracycline has a low incidence of hypersensitivity and appears to be one of the

safest antibiotics because severe adverse effects are minimal.[8,9,27]

However, tetracyclines are certainly not without side effects. Their adverse reactions include phototoxicity, oncholysis, allergic reaction, gastrointestinal symptoms including nausea, vomiting, proctitis, and glossitis or stomatitis and staining of the teeth in young children if given during the last half of pregnancy or the first eight years of life. Other side effects include hepatic toxicity, Candida albicans superinfection, resistant staphylococci and Gram-negative bacilli, kidney effects such as azotemia, and the Fanconi syndrome associated with the use of outdated tetracycline. Moreover, tetracyclines may lead to delayed fontanelle closure with increased intracranial pressure in children and their use should be avoided during pregnancy.[8,27,28,29]

In a review by Ciancio,[29] it was found that tetracyclines may alter the production by microorganisms and the absorption of vitamin K in the gastrointestinal tract. Therefore, since vitamin K is important in the formation of prothrombin in the liver, an interaction with oral anticoagulants can be expected.

As far as antibacterial activity is concerned, the tetracyclines are effective against both Gram-positive and Gram-negative organisms both aerobic and anaerobic species.[28,29] These compounds are bacteriostatic in that they are taken up by the cell and interfere with protein synthesis at the 30S ribosomal level. However, if the bacterium is in an area in which there is no further DNA, RNA, or protein synthesis and the bacterium is not phagocytosed, the organism can subsequently start growing again if the tetracycline leaks out of the cell. This is possible

because tetracyclines are not permanently bound to ribosomes.[28]

In terms of efficacy, the tetracyclines have proved useful in a variety of unusual infections. These include the rickettsial and chlamydial infections as well as brucellosis, tularemia and mycoplasma pneumonia. Relapsing fever caused by Borrelia, melioidosis, and cholera are less common, but, again the tetracyclines are the drugs of choice.[28,29]

In recent years, there have been numerous clinical, microbiological and histological studies of the effect of tetracycline on human periodontal disease.[20,21,30,31,32,33,34,56] In a much earlier study, it was shown by Loe and coworkers[35] that a 0.5% mouth rinse of tetracycline used for five days by subjects who ceased all oral hygiene measures, resulted in a pronounced reduction of the gingival bacterial flora.

Most of the recent studies however, have used systemically administered tetracycline in the treatment of adult periodontitis. In a study by Genco[30] et al., it was shown that the use of tetracycline as an adjunct in periodontal therapy significantly enhances healing. Moreover, Reynolds and coworkers[31] reported that changes in the subgingival flora were different and longer lasting in the tetracycline group than those occurring with mechanical therapy alone, and may have been related to the better clinical results observed in the tetracycline group.

Of special importance in considering the pharmocodynamics of antibiotics to be used in periodontal therapy is the concentration of the active drug which reaches the periodontal microflora.[8] This concentration can be estimated by studying the antibiotic levels in gingival fluid.[8,11,36] Since this exudate seems to be a characteristic feature of

gingival inflammation, one could reasonably expect that suitable drugs could be given to a patient and carried from the general circulation to the pathological gingival tissue by the flow of gingival fluid.[36]

Accordingly, tetracycline has been shown to pass into the gingival crevicular fluid in man.[11,37] When Ciancio et al.,[37] quantitated the amount of tetracycline in human gingival fluid they found that tetracycline was measurable in eleven of the fourteen crevices sampled. In addition, the gingival fluid concentration of tetracycline hydrochloride was up to ten times greater than the serum concentration in the twelfth week of drug administration.

Furthermore, studies by Gordon and coworkers[38] reported that the levels of tetracycline in gingival fluid were two to ten times blood levels after a single oral dose of either 250 mg or 500 mg. Using the same sensitive bioassay after oral administration of repeated 250 mg doses of tetracycline, the same investigators[39] demonstrated that the crevicular fluid levels of tetracycline were typically two to four times the blood levels.[39] In the repeated dosage study,[39] volunteers given 250 mg of tetracycline hydrochloride every six hours had average crevicular fluid concentrations between four to eight micrograms per milliliter and blood concentrations between two to two and one half micrograms per milliliter. Consequently, significantly higher concentrations of tetracycline in the gingival fluid as compared to blood may alter dosage and administration schedules of the antibiotic as well as affect the clinical efficacy of the antibiotic in periodontal therapy.[39]

As a result of these studies, the antimicrobial activity of

tetracycline on subgingival organisms was investigated by Walker and co-
workers.[26] In an _in vitro_ study, they compared the susceptibility of
the bacteria associated with periodontal disease to tetracycline levels
achieved at the infection site. Minimum inhibitory concentrations (MICs)
were determined using tetracycline by an agar dilution technique. The
MIC was defined as the lowest concentration of the antibiotic that gave
no growth.[26] With the exception of the streptococci and a few strains of
lactobacilli, most isolates examined were inhibited by four to eight mi-
crograms per milliliter of tetracycline.[26]

As a result of the study by Gordon et al.,[39] four to eight micro-
grams per milliliter is the concentration of the drug that can be ex-
pected in crevicular fluid following oral administration of one gram per
day. However, there is a limitation to these _in vitro_ studies. Namely,
spirochetes are presently not cultivable and hence, cannot be tested _in_
vitro.[8]

Although recent interest has focused on the local delivery of tetra-
cycline with the use of hollow fiber devices,[40,41,42,57] the subsequent re-
view will concern itself with systemically administered tetracycline.
Correspondingly, Listgarten and coworkers[20] studied the effect of tetra-
cycline and/or scaling on 12 adult periodontitis patients for 25 weeks.
Six subjects received one gram per day of tetracycline during weeks one
and two, and again during weeks seven and eight, while the others did
not. Moreover, all subjects were scaled and root planed on only one side
of their dentition, and all were instructed in oral hygiene.

The results of this study showed that in the tetracycline-treated patients, sites which were scaled showed changes in the clinical, microbiological and histological parameters that were similar to changes seen in the scaled sites in the nontetracycline-treated patients at all intervals. However, in the presence of the antibiotic the eighth week proportions of coccoid cells were higher and those for motile rods and spirochetes lower. Furthermore, the sites that were not scaled in the tetracycline-treated patients showed improvement in the clinical and microbiological parameters at eight weeks, but at 25 weeks the microbial composition showed a significant rebound toward the values observed at baseline.[20]

In a subsequent report on the same patient groups, Hellden, Listgarten and Lindhe[32] showed that the only beneficial effect of tetracycline was significant gains in the attachment level in the tetracycline-treated patients between the zero-week and 25-week examinations. However, the clinical significance of the results obtained is unclear since the magnitude of the gain of attachment was very small. However, their results did agree with Genco et al.,[30] in that tetracycline as an adjunct in periodontal therapy significantly enhances healing.

In a recent case report, Osterberg and others[43] studied the long-term effects of tetracycline on the subgingival microflora in a 33 year old female with advanced periodontitis. In this study, 1000 mg per day of tetracycline was administered orally for 14 days followed by 500 mg per day for 14 days. Within the scope of this investigation, it was noted

that small spirochetes had begun to recolonize 16 weeks after therapy but Bacteroides asaccharolyticus and the "corroding" anaerobic rods remained inconspicuous. In addition, many of the Gram-positive cocci isolated during treatment had high levels of resistance to tetracycline.

In a subsequent study by the same investigators,[34] samples of subgingival plaque were collected from periodontal patients receiving two different tetracycline regimens following conventional periodontal therapy. In this study, four patients received 1000 mg/day for 14 days and nine patients received 1000 mg/day for one week followed by 250 mg/day for extended time periods (21-114 days). For both treatment groups, Streptococci and the branching, filamentous Gram-positive rods, Actinomyces and Rothia were the predominant organisms cultured. However, the microflora of the group receiving 250 mg a day had a greater complexity and included many fastidious Gram-negative organisms presently implicated in the etiology of periodontal disease. Therefore, it was concluded that the prolonged lower dose treatment was no more effective than the two week, 1000 mg/day treatment and that it resulted in the generation of greater numbers of tetracycline resistant organisms.[34]

Accordingly, bacterial resistance demonstrates the need for critical evaluation of the long term, low dose use of tetracycline.[34,43,44] Transmission of antibiotic resistance among bacterial strains, mediated by extrachromosomal DNA molecules called plasmids, is most frequently encountered in association with the continual presence of tetracycline.[34] These plasmids, or R-factors, may carry resistance genes for nearly all

the known antibiotics.[45,46]

Antibiotic resistance then, is transferred from one bacterium to another by the process of conjugation which requires close contact between bacteria.[46] R plasmid-tetracycline resistance is often the result of a new active transport system that "pumps" the drug out of the cell so as to prevent it from accumulating intracellularly and inhibiting growth.[8] Hence, antimicrobial therapeutic approaches, such as systemically administered tetracycline for the treatment of periodontal disease, must be tempered with knowledge of the risks associated with the spread of bacterial drug resistance.[8]

In another microbiological and clinical study of six periodontitis patients, Slots and coworkers[21] investigated the kinetics of repopulation of the periodontal microflora after a single course of scaling and root planing and by the adjunctive systemic use of tetracycline. A main finding of this six month study was the demonstration of major differences in the composition of the pre- and post-treatment subgingival flora. Furthermore, the most pronounced and long lasting differences were found with the total number of organisms and the proportion of spirochetes, both of which were markedly reduced in most pockets after therapy as monitored by phase contrast microscopy.

In their study, major adjunctive effects of tetracycline on the subgingival flora were not seen. However, there was a more consistent and earlier reduction in the Gram-negative anaerobic organisms with the use of tetracycline as compared to conventional therapy alone. Of possible

significance however, were the effects of tetracycline observed in the two "refractory" patients in whom reduction of the flora was not achieved by repeated conventional therapy. A distinct reduction in the gingival microflora and a clinical improvement in soft tissue inflammation were found in both patients after systemic administration of tetracycline.[21]

From these results, Slots and coworkers[21] proposed a model for the treatment of periodontal disease consisting of the following three steps: (1) conventional therapy including thorough periodontal scaling and root planing; (2) monitoring the subgingival flora and the clinical course; and (3) use of antimicrobial therapy in refractory cases when an adequate microbiological subgingival and/or clinical response is not taking place.

In a more recent double-blind crossover study, Scopp et al.,[33] compared a group of patients treated with conventional therapy and adjunctive tetracycline to a group treated with conventional therapy alone. At the end of three months, the groups were switched and the study was repeated for an additional three months. Although microbiological parameters were not studied, their data suggested that adjunctive tetracycline therapy does not appreciably alter either the Gingival Index, Debris Index, or the Papillary Bleeding Index as compared to that achieved by oral hygiene instruction and root planing alone.

C. <u>MINOCYCLINE IN THE TREATMENT OF ADULT PERIODONTAL DISEASE</u>

Minocycline hydrochloride, hereafter referred to as minocycline, is a semi-synthetic derivative of tetracycline. Introduced in 1967, it is chemically known as 7-dimethylamino-6-deoxy-6-demethyltetracycline

hydrochloride. [8,12,27,28]

Like its parent compound, minocycline is primarily bacteriostatic and exerts its antimicrobial effect by the inhibition of protein synthesis. Accordingly, minocycline has antibacterial activity against a wide range of Gram-negative and Gram-positive organisms. In addition, it is effective against staphylococci, particularly those resistant to other tetracyclines such as S. aureus. [12,28]

Pharmacologically, minocycline has a longer serum half life (17-20 hours) and a lower urinary excretion rate than most other tetracyclines. Those characteristics permit the use of smaller and less frequent doses. Minocycline reaches a greater concentration in tears and saliva, due to its increased solubility in lipid, than other tetracyclines. [12,27,47]

Additionally, minocycline is well absorbed from the stomach and upper small intestine. More specifically, food and dairy products that contain calcium do not interfere with its absorption, as in the case with tetracycline. However, the absorption of all oral tetracyclines is impaired by the concomitant administration of antacids containing divalent cations (aluminum, calcium, magnesium) and iron preparations. [27,28] The mechanisms responsible for the decreased absorption appear to be chelation and an increase in gastric pH. [27]

Regarding excretion, minocycline is recoverable both from urine and feces in significantly lower amounts than are the other tetracyclines. It appears to be metabolized to a considerable extent and hence, renal clearance is low. Moreover, minocycline persists in the body after its

administration is stopped, most likely due to retention in fatty tis-
sues.[27]

As reported earlier, side effects associated with tetracycline ther-
apy are varied.[28,29] However, minocycline shows less phototoxicity and
less renal toxicity as compared to tetracycline.[8] Conversely, patients
receiving minocycline may experience vestibular toxicity, characterized
by dizziness, ataxia, nausea, and vomiting.[8,27,28] Those symptoms occur
soon after the initial dose and generally disappear within 24-48 hours
after drug administration is stopped. The frequency of this side effect
is directly related to the dose and has been noted more often in women
than in men.[12,27]

Recently, there have been numerous studies[12,48,49] evaluating min-
ocycline in the treatment of human periodontal disease. Ciancio and
others[12,50] have shown that minocycline is concentrated in gingival crev-
icular fluid at much higher levels than in serum and saliva. The mech-
anism responsible for the elevated levels of oral tetracyclines, including
minocycline, in gingival fluid is presently not known, but may be related
to the elevated calcium content of gingival fluid.[36] Accordingly, the
elevated concentration of minocycline in gingival crevicular fluid must
be considered when determining the antibiotic susceptibility of the micro-
biota inhabiting the periodontal pocket.

In one study, Ciancio et al.,[12] determined the passage and con-
centration of minocycline in gingival crevicular fluid (GCF), and the re-
lationship between its concentration in saliva, GCF, serum and changes in

periodontal health. Over an eight day period, a group of patients with gingivitis and/or periodontitis was given either 200 mg (Group 1) or 150 mg (Group 2) per day of minocycline (Minocin). The results of the study showed that Minocin administration resulted in no significant changes in blood chemistry, blood counts and prothrombin time, and was effective against oral microorganisms as shown by decreased Gingival and Plaque Index scores.

In addition, it was determined that the concentration of minocycline in saliva is far below that in serum, and the concentration in GCF is at levels five times as high as serum. Over the eight days, the GCF concentrations ranged from 3.98 ± 0.62 to 15.89 ± 3.12 µg/ml, while the blood concentrations ranged from 1.02 ± 0.10 to 3.26 ± 0.30 µg/ml. Since the concentration of minocycline in serum remained in the bacteriostatic range (above 1 µg/ml) in both dosage groups, a dose of 150 mg per day should be adequate for use in dental patients.[12] Moreover, vertigo was reported in four of nine patients in Group 1 but not in Group 2. No other adverse side effects were reported or observed in this study.[12]

In a subsequent clinical and microbiological study, Ciancio and co-workers[48] compared minocycline to a placebo concomitant with half mouth scaling and root planing in 26 periodontitis patients. By phase contrast microscope monitoring of the subgingival flora, all the minocycline patients had marked reductions in total bacterial counts and complete elimination of spirochetes for periods up to two months. Moreover, the clinical parameters were reduced after antibiotic therapy, especially on the scaled side.

Reynolds et al.,[49] in an _in vitro_ study, compared minimal inhibitory concentrations (MICs) of minocycline with 25 other antimicrobial agents. In all test strains of Bacteroides gingivalis, Bacteroides melaninogenicus subspecies, Fusobacterium nucleatum, Capnocytophaga and Actinomyces, the MIC values for minocycline were maximally 1 µg/ml. Moreover, several strains exhibited much lower MIC values for minocycline than for other tetracyclines. Hence, minocycline is highly active against most periodontopathic organisms.[49]

Finally, in a case report by Bartolucci and Parkes,[51] minocycline was used adjunctively in the hygienic phase of periodontal treatment in an uncontrolled diabetic. Minocin in a dose of 150 mg/day was prescribed for two cycles of eight days each, separated by an interval of three weeks. The authors felt that Minocin, when used as an adjunct to scaling and root planing, contributed to the success of the initial periodontal therapy in this systemically compromised patient.

CHAPTER III

MATERIALS AND METHODS

A. <u>SELECTION OF PATIENTS</u>

Six adult human subjects, two females and four males, ranging in age from 36 to 48 years were selected for this study. Those patients will hereafter be referred to as JK, BM, BP, BF, RS or TS. They were diagnosed as having Type III moderate periodontitis according to the American Academy of Periodontology's definition of case types.[52] Five of the subjects had no antibiotic therapy or scaling within the previous four months. One of the patients, BP, had a prophylaxis four months previous to her participation in the study. In addition, she completed a seven day regime of erythromycin (1 gm/day) for the treatment of pneumonia two months prior to her involvement in this study. She was included in the study because she had an abundance of subgingival plaque and met the other diagnostic criteria.

To participate in the study, the patients were required to have teeth numbers 3 (maxillary right first molar) and 19 (mandibular left first molar). They were selected because they were among the representative teeth determined by Ramfjord[15,53] to provide an accurate assessment of the periodontal status of an individual. In addition, these teeth must have been in at least mesial contact with an adjacent tooth in order that an interproximal periodontal pocket could be studied.

Each subject received an oral written explanation of the study and was asked to sign a consent form (Fig. 1). This study was reviewed by the Loyola University Medical Center Institutional Review Board (IRB) for the Protection of Human Subjects and was approved prior to the initiation of the study.

The control group A consisted of two subjects, BF (Table 1) and TS, who did not take the drug. The experimental group B consisted of three subjects, JK, BM and BP (Table 1), each of whom requested to take the antibiotic, minocycline. The sixth subject, RS, started in the control group but finished as experimental group C. The experimental group C became necessary when that patient underwent penicillin therapy for an acute periodontal abscess during the experimental period. Before commencing the study, the three subjects in the experimental groups received a sequential multiple analysis (SMA-24) and a complete blood count with differential. In all cases the laboratory results were unremarkable and within normal limits. Moreover, none of the patients had a history of hepatitis or liver disease, rheumatic fever, renal impairment, or hypersensitivity to any of the tetracyclines. The two females in the study were neither pregnant nor lactating.

Although six subjects agreed to participate in the experiment, five completed it. One of the male patients in the control group, TS, withdrew from the study two months after baseline measurements were obtained due to lack of interest.

B. EXPERIMENTAL DESIGN

The subjects in both groups (control and experimental) had initial clinical and microbiological measurements taken from teeth numbers 3 and 19 to establish a baseline (Table 1). Subsequently, the five patients were each given oral hygiene instructions and full mouth scaling and root planing during several appointments. Two to three weeks after the hygiene phase (Day 0), both groups again had clinical and microbiological measurements taken. On Day 1, the three subjects in the experimental group B began taking a seven day regimen of minocycline (Minocin, Lederle Laboratories Div., American Cyanamid Co., Pearl River, N.Y.). This seven day Minocin regime[54] consisted of 50 mg in the morning and 100 mg in the evening. During this time, the control group A practiced oral hygiene only. Selected sites from teeth numbers 3 and 19 were monitored clinically and microbiologically for nine weeks from Day 0 in Groups A and B.

The clinical parameters studied were the Gingival Index (GI), of Loe and Silness,[14,55] to assess the qualitative changes in the gingival soft tissue and the probing depth[13] measured from the gingival margin of selected periodontal pockets. In the Gingival Index, each of the four gingival areas of the tooth is given a score from zero to three. The scores from the four areas of the tooth are added and divided by four to give the GI for the tooth. Table 2 gives the criteria for this index system.[14]

Table 2. Criteria for the Gingival Index System.[14]

0 = Normal gingiva

1 = Mild inflammation - slight change in color, slight edema, no bleeding on probing

2 = Moderate inflammation - redness, edema, glazing, bleeding on probing

3 = Severe inflammation - marked redness, edema, ulceration, tendency to spontaneous bleeding

The probing depth of the periodontal pockets was measured with a calibrated University of Michigan #0 probe (Hu-Friedy Manufacturing Corp., Chicago, IL.). The probe had graduated markings at each millimeter except the fourth and sixth millimeter position. The probing depth was measured from the gingival margin to the bottom of the periodontal pocket and always at the same site at each tooth. Measuring the probing depth rather than the sulcus or pocket depth is consistent with the studies of Listgarten. Moreover, investigations on the histopathology of the periodontal lesion and the histological features of the healing lesion, together with histological studies on the relationship of the probe to periodontal tissues, have shed new light on periodontal probing. In accordance with studies by Listgarten, probing depth measured from the gingival margin seldom corresponds to sulcus or pocket depth. The discrepancy decreases in the absence of inflammatory changes and increases with increasing degrees of inflammation.[13]

The microbiological parameters studied were the qualitative and quantitative measurements of microbial forms obtained by darkfield

microscopic examination of plaque samples from selected periodontal pockets associated with teeth numbers 3 and 19. Figure 2 gives a schematic summary of the experimental design.

C. MICROBIOLOGICAL PROCEDURES

The subgingival microflora was monitored by darkfield microscopy subsequent to obtaining a two microliter plaque sample from each periodontal pocket. The two periodontal pockets selected per patient included one from tooth number 3 and one from tooth number 19.

Before obtaining a plaque sample however, a sampling device had to be prepared. It consisted of a five microliter Hamilton syringe (Hamilton Co., Reno, Nev.) with a blunted tip to which a two microliter capillary tube (Micro Pipets, Curtin Matheson Scientific, Inc. Houston, Tx.) was affixed with heat shrinkable tubing. Next, two microliters of sterile heparinized water (5 USP units heparin sodium per 1 ml water) were drawn into the syringe. A stream of air into the attached capillary tube kept it empty and dry.

The tip end of the sampling device was then inserted into the periodontal pocket of the tooth under study. The two microliters of sterile heparinized water were slowly injected from the syringe into the pocket while the tip was moved carefully in all directions. During this time the capillary tube should have filled completely due to capillary attraction. The subgingival fluid sample contained microorganisms that inhabited the periodontal pocket.

Subsequently, the two microliter sample was dispersed by a modified

eye dropper (Curtin Matheson Scientific, Inc. Houston, Tx.) on to a plain microscopic slide (Scientific Products, McGaw Park, IL.) and mixed with an additional three microliters of heparinized water. A cover glass (Scientific Products, McGaw Park, IL.) was then placed over the five microliter mixture and its edges were sealed with paraffin.

Next, the slide was focused under a darkfield microscope (Balplan Microscope, Bausch & Lomb Inc., Rochester, N.Y.) utilizing a 100X objective lens and a 10X wide field eyepiece on oil immersion. As each slide was examined by darkfield microscopy at a magnification of 1000X, the organisms were enumerated from randomly selected microscopic fields. In each field, the organisms were counted according to the following morphologic groups: spirochetes (small, medium, large), motile rods (small, large, curved), and non-motile organisms (short rods, cocci, fusiforms and filaments) (Figure 3).[19] The counting continued until either 12 fields were examined or at least 100 organisms were counted, whichever came first. Any observed neutrophils were counted but did not enter into the total microbial count.

Upon completion of the data collection, the raw microbial counts per number of fields were converted into the actual microbial counts per microliter by calculations made on a 3033-S IBM computer. The counts per microliter were plotted against the time period of the study by an S-100 microcomputer with a Hi-Plot DPM-3 plotter (Houston Instruments, Houston, Tx.) according to the following microbial groups: spirochetes, motile rods, and the combined non-motile organisms. Figure 4 lists and

interprets the darkfield microscopic calculations made to determine the
microbial counts per microliter of those groups.

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CHAPTER IV

RESULTS

A. <u>CLINICAL PARAMETERS</u>

The effect of scaling and root planing on the Gingival Index (GI)
and probing depth (PD) is shown for the single completed patient in the
control group A, B.F., in Table 3 and Figures 5 and 6. Although both clin-
ical parameters decreased after scaling and root planing, the PD reduc-
tion was more pronounced at the completion of the study. The GI tended
to return to pre-scaling levels by week nine for both teeth numbers 3
and 19.

The effect of scaling and root planing and a one week regimen of
minocycline (Minocin) on the GI and PD is presented in Tables 4-6 and
Figures 7-12 for the patients in the experimental group B, J.K., B.M. and
B.P. The GI dropped noticeably subsequent to the minocycline regime in
all patients. The GI for J.K. returned to pre-treatment levels by week
eight. However, the post-Minocin GI scores for B.M. and B.P. remained
lower than the pre-scaling scores throughout the experimental period.

The PD, as measured in the same experimental group, was reduced in
four out of six periodontal pockets after the seven day Minocin regimes.
In addition, three of these four PD measurements remained below the post-
scaling levels throughout the experimental period. One of the post-
scaling pocket depths of B.M. remained at a constant level throughout

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the term of the study. In only one instance, a PD of subject J.K., was the final PD level greater than the day zero PD level.

The fifth subject, R.S., started in the control group, but finished as experimental group C. At the completion of the scaling and root planing phase, R.S. took 500 mg of penicillin (V-Cillin K, Eli Lilly and Co., Indianapolis, In.) orally every six hours for five days for treatment of a lateral periodontal abscess associated with tooth number 14. Two weeks later, he commenced a second oral regime of penicillin for treatment of a palatal space abscess adjacent to the extraction site of tooth number 15.

It was during that second regimen of 250 mg of penicillin every six hours for seven days that coincided with week one of the experimental period. For that reason, R.S. continued participation in the study so that the adjunctive effect of penicillin could be monitored clinically and microbiologically. Table 7 and Figures 13-14 show the effect of scaling and root planing and two oral regimes of penicillin on the GI and PD for R.S.

The results of the experimental group C show a noticeable drop in both the GI and PD after the second regime of penicillin. The GI scores for both teeth stayed below the pre-treatment levels through week nine. Conversely, the PD levels returned to the pre-scaling and root planing measurements for both periodontal pockets by week eight.

B. MICROBIOLOGICAL PARAMETERS

The effect of scaling and root planing on the counts/μl of

spirochetes, motile rods and non-motile forms of the two selected perio-
dontal pockets in the single control group A patient, B.F., is shown in
Tables 8-9 and Figures 15-16. The spirochetes in both pockets were re-
duced to zero after scaling, root planing and oral hygiene. However,
an increase in the spirochete levels of both pockets was evident by
week four. In the pocket associated with tooth number 19, the spiro-
chete count returned to zero at week eight before exceeding the pre-
scaling count at week nine. In addition, the motile rods decreased notice-
ably after treatment in both pockets but exceeded the day zero counts by
week four. Conversely, the non-motile forms tended to increase in both
periodontal pockets between weeks one and eight.

   In the experimental group B, the microbial counts/μl for the pa-
tients J.K., B.M. and B.P are shown in Tables 10-15 and Figures 17-22.
In each pocket the spirochetes were dramatically reduced after the
Minocin regime. Moreover, in three of the six pockets they were totally
eliminated by week one. The spirochetes/μl in four of the six pockets
remained below the pre-treatment counts at the completion of the study.
The spirochetes in the two remaining pockets in patients J.K. and B.P.
eventually exceeded the pre-scaling spirochete counts by weeks eight and
nine respectively.

   In the same experimental group, the motile rods also decreased in
number in all six pockets following the seven day minocycline regimen.
In J.K. and B.M. the post-antibiotic motile rods/μl remained below the
post-scaling counts at weeks four and nine respectively. In contrast,
the post-Minocin motile rod counts in B.P. exceeded the post-scaling

counts at week four. The non-motile forms also decreased after the anti-biotic administration in five of those six periodontal pockets, but to a much lesser degree than that of the spirochetes. In addition, the non-motile form counts/μl were noticeably lower than either the spirochetes or the motile rods when the study was completed.

The microorganism counts/μl in the experimental group C, for the single patient R.S., are shown in Tables 16-17 and Figures 23-24. All organisms were shown to decrease at week one but the effect of penicillin, scaling and root planing was less pronounced on the spirochetes. The spirochetes/μl in both pockets exceeded their post-scaling counts at week four, whereas the total non-motile forms/μl did not exceed their post-scaling counts until week eight. The motile rod counts returned to their post-scaling levels in the pockets associated with teeth numbers 3 and 19 by weeks eight and four respectively.

Neutrophils were also counted from the plaque samples but their raw counts were not converted into actual counts/μl. Table 18 gives the neutrophil count of each plaque sample obtained from the five subjects in groups A, B and C during the time of the study. No noticeable trends could be observed from the sparse and intermittent neutrophil count.

CHAPTER V

DISCUSSION

The present investigation demonstrated that in five patients with

moderate periodontitis, treatment that consisted of either oral hygiene

instruction and scaling and root planing alone or in combination with

orally administered minocycline or penicillin, resulted in a decrease in

the nine week post-treatment GI and PD of selected periodontal pockets

associated with teeth numbers 3 and 19. It was also demonstrated that

these treatment modalities had quantitative and qualitative effects on

the subgingival microbial flora associated with the selected periodontal

pockets.

Because of the small sample size, statistics were not utilized in

this investigation. Furthermore, statistics should be applied with great

caution to clinical material where the basic data are dependent upon the

ability of the examiner to observe, assess, and record rather subtle

clinical variation.[58]   Such subtleties appear to apply to the results of

the clinical and microbiological parameters utilized in this study.

The observed post-treatment Gingival Index scores were higher in

this investigation than those found in other comparable studies. For

example, in the study by Listgarten and coworkers[20]   the observed median

GI scores at 8 and 25 weeks were 0.5 and 0 respectively for both the

scaling and the scaling and tetracycline groups. In another study,

Ciancio et al.[12] reported reduced mean GI scores during an eight day Minocin regime that did not include scaling and root planing. The mean GI score at day 8 was 1.16.

The sampling technique utilized in this study has confirmed the subgingival microbial repopulation of selected periodontal pockets. In their research, Hoerman and Hryhorczuk found consistent repopulation patterns by the subgingival microbial flora nine weeks after scaling and root planing selected periodontal pockets. Utilizing this sampling technique whereby a standard volume is secured in a micropipet and diluted to a standard volume, they collected over 250 samples in 30 patients.[81]

The decrease in gingival inflammation and probing depths in the control group A patient, B.F., after oral hygiene instruction, scaling and root planing is in agreement with several studies. More specifically, Lovdal and coworkers[59] found that the combined effect of subgingival scaling and controlled oral hygiene reduced the incidence of gingivitis. In 1975, Tagge et al.[60] conducted an eight week study evaluating the soft tissue response of suprabony periodontal pockets treated by root planing and oral hygiene or by oral hygiene measures alone. They found that root planing accompanied by oral hygiene reduced the mean pocket depth and the incidence and severity of gingivitis more than oral hygiene measures alone. More recent studies by Morrison et al.,[61] Hill et al.,[62] and Lindhe and coworkers[63] have shown that scaling, root planing and oral hygiene instruction resulted in reduced probing depth and gingival inflammation. Morrison et al., found that for pockets 7 mm or

greater, the mean reduction was 2.22 ± 1.35 mm (P < .0001) one month following the hygienic phase of periodontal therapy.

The decrease in the GI scores and probing depths after the seven day Minocin regime in the experimental group B confirm data presented by Ciancio and coworkers[12] in 1980. In a subsequent investigation, Ciancio et al.[48] confirmed their earlier study and found that minocycline signi-ficantly reduced the GI scores in 13 periodontitis patients with or with-out scaling and root planing. The findings in the present study are also in accordance with the 1978 investigation by Listgarten et al.[20] Their results from the scaling and tetracycline sites showed decreased GI scores and reduced probing depths after 8 and 25 weeks. It should be noted that none of the experimental group B patients experienced any adverse reactions to Minocin.

In experimental group C, R.S. had reduced clinical parameters after penicillin administration. But, the PD reduction was not as long-lasting as that experienced by experimental group B after minocycline administration. Since the penicillin was taken systemically, some en-tered the oral cavity via saliva and/or gingival crevice.[25] The reduced post-penicillin GI scores observed in patient R.F. are in contrast to the observations of Pendrill and Reddy.[64] They studied the efficacy of prophylactic penicillin taken for five days by patients who had under-gone periodontal surgery. Throughout the four week study there was no statistical difference between the GI scores for the penicillin and placebo groups.

As antimicrobial agents, the penicillins are one of the most important of the antibiotics. Their basic structure consists of a thiazolidine ring connected to a beta-lactam ring, to which is attached a side chain R. The beta-lactam antibiotics can kill susceptible bacteria by inhibition of peptidoglycan synthesis. Peptidoglycan is a heteropolymeric component of the cell wall that provides rigid mechanical stability by virtue of its highly cross-linked latticework structure. Moreover, the cell walls of bacteria are essential for their normal growth and development.[65]

In the present investigation, R.S. took penicillin V, the acid-stable congener of penicillin G. Its antimicrobial spectrum includes Streptococcus species, Neisseria species, many anaerobes and spirochetes. However, penicillin G and its phenoxymethyl derivative, penicillin V, are hydrolyzed by staphylococcal penicillinase. After oral ingestion, penicillin V escapes destruction in gastric juice, since it is both insoluble and stable at a low pH. It goes into solution in the more alkaline medium of the duodenum and is well but incompletely absorbed from the upper portion of the small intestine. The peak concentration in the blood of an adult after an oral dose of 500 mg is nearly 3 µg/ml. Once absorbed, penicillin V is distributed in the body and excreted by the kidney. Because penicillin V is rapidly eliminated, its half-life in the body is typically between 30 to 60 minutes and its concentration in urine is high.[65]

Hypersensitivity reactions are by far the most common adverse

effects noted with the penicillins. Although they are relatively non-toxic, a large percentage of the population is allergic to them.[8,65]
Unquestionably, penicillin is the drug of choice when a potent bacteri-cidal agent is required. However, the therapist must always remember that penicillin is the most allergenic drug in current use and should never be used arbitrarily. Its use should be selected only on the basis of an established need for penicillin.[66] In the present investigation R.S. did not experience any hypersensitivity or toxicity to penicillin V.

The effectiveness of scaling alone or in combination with minocy-cline on the microbiological parameters was assessed by monitoring the microbial repopulation kinetics of the subgingival area by darkfield microscopy. In the control group A patient, B.F., the spirochete and motile rod counts were noticeably reduced after scaling and root planing. These results are in agreement with the findings of Listgarten et al.[20] Slots et al.[21] and Mousques et al.[22] Those studies showed that mechanical debridement can produce a relatively long-lasting alteration in certain microbial proportions, and that the microbial proportions are shifted from those generally associated with untreated periodontal defects to-ward those seen at periodontally healthy sites.[19,24] In patient B.F., the spirochetes/μl did not return to the pre-treatment level by week nine in the periodontal pocket associated with tooth number 3. However, the spirochete counts/μl exceeded the pre-treatment level by the completion of the study in the periodontal pocket associated with tooth number 19. These spirochete shifts follow similar patterns in other studies. Slots

et al.[21] found that the proportions of spirochetes did not reach their pre-treatment levels even after six months, whereas Mousques et al.[22] reported a return to baseline levels by day 42.

Moreover, there was a tendency for the GI scores and PD measurements in B.F. to vary directly with the counts/μl of spirochetes or motile rods. These findings confirm data reported by Rosenberg and co-workers[67] in 1981. In general, they found that the GI and PlI (Plaque Index) scores and PD measurements varied directly with the proportions of spirochetes or motile rods after scaling and root planing. In patient B.F., the dramatic post-scaling reduction in spirochetes/μl in the periodontal pockets associated with teeth numbers 3 and 19 is also in accordance with the findings of a recent study by Syed et al.[68] They found that the effects of initial, nonsurgical periodontal therapy resulted in a significant reduction of spirochetes.

An aberration occurred in the result of the spirochete counts/μl in the periodontal pocket associated with tooth number 19 of patient B.F. during week eight. Due to an error in the sampling technique the spirochete count was zero. The effect of sampling on the composition of the human subgingival microbial flora was studied by Mousques, Listgarten and Stoller.[69] They observed clear-cut changes from baseline to day 3 in the proportions of spirochetes and motile cells after sampling of periodontal pockets in 18 adults with chronic periodontitis. The subsequent lack of change from the 3 day through the 42 day intervals suggested that sampling per se may have contributed only to the changes

from baseline noted in the first few days. Thus, the aberration that occurred in the current study during week eight was not a result of the previous sampling, but rather a result of human error in the sampling technique during week eight.

In the experimental group B, the spirochetes/μl were totally eliminated in three of the six periodontal pockets after scaling, root planing, and minocycline therapy. These findings are enhanced by data presented by Ciancio et al.[48] who enumerated the subgingival microbial flora in 26 periodontitis patients by phase contrast microscopy. In the 13 patients treated by half mouth scaling, root planing and orally administered Minocin, they observed complete elimination of spirochetes for periods up to two months. In the current investigation, the spirochete counts in four of the six pockets from experimental group B remained below the pre-treatment counts at the completion of the study. However, in no instance were the spirochetes eliminated completely for as long as two months.

When comparing control group A to experimental group B there was a tendency for the experimental group to further prolong the subgingival repopulation of motile rods. Because of the small sample size such a trend could not be extrapolated from the subgingival repopulation of the spirochetes in groups A and B. This trend is strengthened by the 1978 study of Listgarten, Lindhe and Hellden.[20] Their tetracycline and scaling sites showed essentially similar microbiological changes as the scaling only sites at the 0-, 8- and 25-week intervals. However, in the

presence of the antibiotic the 8-week proportions of motile rods and spirochetes were lower than in the scaling only sites.[20] Experimental group B, like control group A, showed a tendency for the GI scores and PD measurements to correspond directly with the counts/μl of spirochetes or motile rods.

In the experimental group C patient, R.S., the motile rods were more effectively inhibited by the penicillin than were the spirochetes. Moreover, the spirochetes were never totally eliminated by scaling, root planing and penicillin in group C as they were by scaling, root planing and minocycline in three pocket sites in group B. There was also a tendency for the subgingival repopulation of the motile rods to be more prolonged in the minocycline experimental group than in the penicillin experimental group. However, in vitro studies by Mashimo et al.[70,71] have shown that penicillin is inhibitory to most periodontopathic bacteria. In their 1981 study, Mashimo et al.[71] found that penicillin was the most effective agent to control subgingival plaque flora at concentrations of .1, 1 and 5 μg/ml. In another in vitro study, Walker and coworkers[72] determined that most potential periodontopathic bacteria were sensitive to penicillin. They also encountered several strains of Selenomonas sputigena and Bacteroides melaninogenicus subspecies intermedius that produced beta-lactamases which could destroy penicillin in the periodontal pocket.

In this investigation no trends were observed from the sporadic neutrophil count. These findings confirm data presented by Claffey et

al.[73] in 1982. They found no significant difference between deterior-
ating periodontal pocket sites and stable or improving sites in the leu-
kocyte counts taken from subgingival washings. Of all the parameters
evaluated at 12 months, only probing pocket depth showed significant
differences between those sites. Conversely, in a recent study by Keyes
and coworkers,[74] they concluded that the assessed change in pattern and
amount of plaque microbiota and white blood cells is clinically useful
in determining efficacy of therapy in destructive periodontitis. Their
subgingival root surface plaque samples were removed with a curette and
quickly transferred to either a drop of tap water or physiologic saline
on clean microscopic slides. Attempts to disperse, dilute or stain the
samples were not made. Every effort was made to examine the bacterial
complexes "intact" after removal from the circumradicular spaces by phase-
contrast microscopy. In another study, Keyes et al.[75] examined the high-
ly organized aggregations of aquatic microlife found in samples removed
from root surfaces of pockets in patients with chronic destructive perio-
dontitis. Besides the highly organized motile rods and spirochetes, the
vast accumulation of leukocytes in the too-numerous-to-count range was
another predominant feature found in destructive periodontitis. The dis-
crepancy in the leukocyte counts between the current investigation and
the studies by Keyes et al. appears to be due to the different sampling
techniques performed.

In this study it was shown that the seven day Minocin regime no-
ticeably reduced the spirochetes and motile rods. By means of darkfield

microscopic counts of subgingival microbial samples, distinct patterns

in the counts/µl of motile rods and spirochetes were demonstrated before

and after scaling, root planing and minocycline therapy. Moreover, it

has been shown by Listgarten and Hellden[19] that the proportion of motile

rods and spirochetes was significantly higher at diseased sites as com-

pared to healthy sites. In a subsequent study, Listgarten and Levin[23]

found that a subgingival microbiota rich in spirochetes, with or without

motile rods, tended to precede a clinically detectable deterioration of

the periodontium as determined by subsequent increases in probing depth

measurements. Thus, spirochetes and motile rods may not be primary path-

ogens, but may act simply as indicator organisms of a pathogenic micro-

biota and to some extent as predictors of future clinical deteriora-

tion.[76] Listgarten and Schifter[76,77] feel that these organisms can be

monitored by darkfield microscopy so as to facilitate the optimal sched-

uling of recall visits for the maintenance of periodontal health.

The series of darkfield and electron microscopic studies by List-

garten and coworkers[18,19,23] and the phase-contrast microscopic observa-

tions by Keyes et al.[74,75] have shown that spirochetes are abundant in

the periodontal pockets characteristic of periodontitis. This finding

is confirmed by the present study in which spirochetes were the pre-

dominant microorganism found in the majority of the pre-treatment perio-

dontal pockets (Tables 8-17). Accordingly, Loesch and Laughon feel

that such bacterial specificity is evidence in support of the role of

spirochetes in periodontal disease. In an excellent 1982 review

article,[78] they cite several association and response-to-treatment studies

which make a strong argument for the involvement of spirochetes in all

forms of periodontal disease except possibly localized juvenile periodon-

titis. Because spirochetes account for 35 to 55% of the flora before

treatment, and then drop significantly to below 10% as a result of treat-

ment which restores periodontal health, they must be considered periodon-

topathic organisms.[78]

This study showed that the seven day Minocin regime which commenced

2 to 3 weeks after full mouth scaling and root planing was effective in

reducing clinical and microbiological parameters in three patients with

moderate periodontitis. However, an antibiotic should not be chosen

arbitrarily but should be selected to meet the needs of the case. This

selection must be based upon a knowledge of (1) the state of the pa-

tient's general health, (2) the nature of the specific infection in-

volved, (3) the pharmacology of the drugs available, and (4) the host-

bacterium response to treatment.[66,79]

Chemotherapeutic treatment, without due regard for these basic

parameters, usually turns into a superficial preoccupation with the iso-

lation of supposed pathogens and the dispensing of antibacterial agents.

This "magic bullet" approach ignores the fact that these agents do not

completely eliminate bacteria but merely give the host an opportunity to

remove the microorganisms, or to reestablish a balance which is compat-

ible with health.[79] Consequently, minocycline treatment for moderate

periodontitis is most effective when used as an adjunct to conventional

periodontal therapy. The optimal dose regimen and the optimal route of administration of this antibiotic remain to be established in subsequent studies.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present study demonstrated that in five patients with moderate periodontitis, treatment that consisted of either scaling and root alone or in conjunction with orally administered minocycline or penicillin, resulted in a decrease in the nine week post-treatment Gingival Index scores and decreased probing depths. In addition, the kinetics of the subgingival bacterial repopulation were effected by these three treatment modalities quantitatively and qualitatively.

Minocycline hydrochloride is a semi-synthetic tetracycline that concentrates in the gingival crevicular fluid and can be given in lower and less frequent doses than tetracycline hydrochloride. Minocin treatment for moderate periodontitis is most effective when used as an adjunct to scaling and root planing.

Darkfield microscopy is an excellent technique to monitor the recolonization patterns of the subgingival microbial flora after periodontal therapy. In the present investigation, darkfield microscopic examination showed that the counts/μl of motile rods and spirochetes before and after treatment corresponded directly with changes in the clinical parameters. In the Minocin group there was a tendency to further prolong the subgingival repopulation of motile rods as opposed to the control group. Each of these treatment modalities was shown to be more

effective against spirochetes than the adjunctive penicillin group. Statistics were not utilized in this study because of the small sample size.

Any antibiotic should not be chosen arbitrarily but should be selected to meet the needs of the case. This selection must be based upon a knowledge of (1) the state of the patient's general health, (2) the nature of the specific infection involved, (3) the pharmacology of the drugs available, and (4) the host-bacterium response to treatment.[66,79]

Table 1. Profile and Baseline Measurements of Study Participants.

Patient	Age	Race	Sex	Periodontal Pocket and Plaque Sample Site	Probing Depth	Gingival Index (GI)	Treatment Proposed
JK	36	Indian	M	3 MB*	5	2	OHI, Sc & RP, Mc**
				19 DB	4	2	
(Experimental Group B)							
BM	48	Caucasian	M	3 MB	4	2	OHI, Sc & RP, Mc
				19 DB	6	2	
(Experimental Group B)							
BP	40	Caucasian	F	3 DB	7	2	OHI, Sc & RP, Mc
				19 MB	4	2	
(Experimental Group B)							
BF	42	Indian	F	3 DP	7	1.75	OHI, Sc & RP
				19 ML	4	1.75	
(Control Group A)							
RS	38	Caucasian	M	3 MB	7	2	OHI, Sc & RP
				19 ML	7	2	
(Experimental Group C)							

* M refers to mesial, B to buccal, D to distal, L to lingual, P to palatal
** OHI refers to oral hygiene instruction, Sc & RP to scaling and root planing, Mc to minocycline

Table 3. Gingival Index (GI) and Probing Depth (PD) of Selected
Periodontal Pockets Associated with Teeth Numbers 3 and
19 of Control Group A, Patient B.F.

TIME	GINGIVAL INDEX (GI)		PROBING DEPTH (PD)	
	3	19	3DP*	19ML*
Pre-scaling and root planing	1.75	1.75	7	4
2-3 weeks post-scaling and root planing = Day 0	1.25	1.25	5	3
Week 1	1.25	1.25	5	2
Week 4	1.5	1.5	4	2
Week 8	1.5	1.25	4	2
Week 9	1.5	1.75	4	3

* D refers to distal, P to palatal, M to mesial, L to lingual, B to buccal

Table 4. GI and PD of Selected Periodontal
Pockets Associated with Teeth
Numbers 3 and 19 of Experimental
Group B, Patient J.K.

TIME	GINGIVAL INDEX (GI)		PROBING DEPTH (PD)	
	$\frac{3}{2}$	$\frac{19}{2}$	$\frac{3MB}{5}$	$\frac{19DB}{4}$
Pre-scaling and root planing				
2-3 weeks post-scaling and root planing = Day 0	1.5	1.75	5	3
Week 1	1	1.25	3	3
Week 4	1	1.25	3	3
Week 8	2	2	4	5
Week 9	2	1.75	5	4

Table 5. GI and PD of Selected Periodontal
          Pockets Associated with Teeth
          Numbers 3 and 19 of Experimental
          Group B, Patient B.M.

TIME	GINGIVAL INDEX (GI)		PROBING DEPTH (PD)	
Pre-scaling and root planing	$\frac{3}{2}$	$\frac{19}{2}$	$\frac{3MB}{4}$	$\frac{19DB}{6}$
2-3 weeks post-scaling and root planing = Day 0	1.75	1.25	4	5
Week 1	1	1	3	5
Week 4	1	1	3	5
Week 8	1.5	1.25	3	5
Week 9	1.5	1.25	3	5

Table 6. GI and PD of Selected Periodontal
Pockets Associated with Teeth
Numbers 3 and 19 of Experimental
Group B, Patient B.P.

TIME	GINGIVAL INDEX (GI)		PROBING DEPTH (PD)	
Pre-scaling and root planing	$\frac{3}{2}$	$\frac{19}{2}$	$\frac{3DB}{7}$	$\frac{19MB}{4}$
2-3 weeks post-scaling and root planing = Day 0	1.75	2	5	4
Week 1	1.25	1.5	3	3
Week 4	1.5	1.5	4	3
Week 8	(missed)	(missed)	(missed)	(missed)
Week 9	1.75	1.5	4	3

Table 7. GI and PD of Selected Periodontal
Pockets Associated with Teeth
Numbers 3 and 19 of Experimental
Group C, Patient R.S.

TIME	GINGIVAL INDEX (GI)		PROBING DEPTH (PD)	
Pre-scaling and root planing	$\frac{3}{2}$	$\frac{19}{2}$	$\frac{3MB}{7}$	$\frac{19ML}{7}$
2-3 weeks post-scaling and root planing = Day 0	1.5	1.5	7	7
Week 1	1.25	1.25	5	6
Week 4	1.5	1.5	7	6
Week 8	1.75	1.75	7	7
Week 9	1.5	1.75	7	7

Table 8. Tooth Number 3 Counts/μl of Spirochetes,
Motile Rods and Non-motile Forms of
Control Group A, Patient B.F.

Time	Spirochetes	Motile Rods	Non-motile Forms
Pre-scaling and root planing	6.60×10^3	2.60×10^4	1.19×10^4
2-3 Weeks Post-scaling and root planing = Day 0	1.80×10^3	2.57×10^3	1.31×10^4
Week 1	0.00	1.54×10^3	2.08×10^4
Week 4	2.77×10^3	4.01×10^3	2.53×10^4
Week 8	1.54×10^3	1.85×10^4	6.08×10^4
Week 9	2.05×10^3	8.73×10^3	4.36×10^4

Table 9. Tooth Number 19 Counts/μl of Spirochetes,
Motile Rods and Non-motile Forms of
Control Group A, Patient B.F.

Time	Spirochetes	Motile Rods	Non-motile Forms
Pre-scaling and root planing	2.02×10^4	1.32×10^4	6.69×10^4
2-3 Weeks Post-scaling and root planing = Day 0	0.00	2.57×10^2	2.03×10^4
Week 1	0.00	2.05×10^3	1.41×10^4
Week 4	7.92×10^3	8.80×10^3	2.82×10^4
Week 8	0.00	4.11×10^3	4.16×10^4
Week 9	4.93×10^4	1.36×10^5	1.48×10^5

Table 10. Tooth Number 3 Counts/μl of Spirochetes, Motile Rods and Non-motile Forms of Experimental Group B, Patient J.K.

Time	Spirochetes	Motile Rods	Non-motile Forms
Pre-scaling and root planing	6.16×10^4	2.88×10^4	3.18×10^4
2-3 Weeks Post-scaling and root planing = Day 0	2.46×10^4	1.58×10^4	3.70×10^4
Week 1	2.57×10^2	2.57×10^3	1.44×10^4
Week 4	6.93×10^3	4.88×10^3	1.05×10^4
Week 8	1.23×10^5	1.17×10^5	3.08×10^4
Week 9	1.23×10^5	2.34×10^5	9.86×10^4

Table 11. Tooth Number 19 Counts/μl of Spirochetes,
Motile Rods and Non-motile Forms of
Experimental Group B, Patient J.K.

Time	Spirochetes	Motile Rods	Non-motile Forms
Pre-scaling and root planing	1.81×10^5	3.90×10^4	5.14×10^4
2-3 Weeks Post-scaling and root planing = Day 0	6.37×10^4	2.67×10^4	1.23×10^4
Week 1	7.70×10^2	4.88×10^3	1.34×10^4
Week 4	1.85×10^4	1.54×10^4	2.36×10^4
Week 8	5.39×10^4	5.55×10^4	4.47×10^4
Week 9	1.07×10^5	7.40×10^4	4.93×10^4

Table 12. Tooth Number 3 Counts/μl of Spirochetes,
Motile Rods and Non-motile Forms of
Experimental Group B, Patient B.M.

Time	Spirochetes	Motile Rods	Non-motile Forms
Pre-scaling and root planing	3.08×10^5	1.36×10^5	1.11×10^5
2-3 Weeks Post-scaling and root planing = Day 0	1.64×10^5	1.40×10^5	4.11×10^4
Week 1	8.80×10^2	2.86×10^4	1.72×10^4
Week 4	2.11×10^4	1.98×10^4	4.40×10^3
Week 8	1.05×10^5	9.86×10^4	2.46×10^4
Week 9	9.24×10^4	7.60×10^4	4.93×10^4

Table 13.   Tooth Number 19 Counts/µl of Spirochetes,
Motile Rods and Non-motile Forms of
Experimental Group B, Patient B.M.

Time	Spirochetes	Motile Rods	Non-motile Forms
Pre-scaling and root planing	2.46×10^5	1.36×10^5	6.16×10^4
2-3 Weeks Post-scaling and root planing = Day 0	1.23×10^5	9.86×10^4	4.93×10^4
Week 1	0.00	2.80×10^4	4.01×10^3
Week 4	1.28×10^4	2.57×10^3	2.46×10^4
Week 8	3.45×10^4	7.52×10^4	2.22×10^4
Week 9	3.70×10^4	2.67×10^4	8.22×10^3

Table 14. Tooth Number 3 Counts/μl of Spirochetes, Motile Rods and Non-motile Forms of Experimental Group B, Patient B.P.

Time	Spirochetes	Motile Rods	Non-motile Rods
Pre-scaling and root planing	2.71×10^5	8.63×10^4	4.93×10^4
2-3 Weeks Post-scaling and root planing = Day 0	6.68×10^4	3.90×10^4	4.72×10^4
Week 1	0.00	3.13×10^4	1.49×10^4
Week 4	9.45×10^4	9.66×10^4	2.05×10^4
Week 8	- unavailable for data collection -		
Week 9	7.64×10^4	4.07×10^4	1.36×10^4

Table 15. Tooth Number 19 Counts/μl of Spirochetes,
Motile Rods and Non-motile Forms of
Experimental Group B, Patient B.P.

Time	Spirochetes	Motile Rods	Non-motile Rods
Pre-scaling and root planing	3.47×10^4	2.39×10^4	2.08×10^4
2-3 Weeks Post-scaling and root planing = Day 0	2.77×10^4	5.96×10^4	3.39×10^4
Week 1	0.00	1.95×10^4	2.98×10^4
Week 4	7.70×10^3	1.06×10^5	7.24×10^4
Week 8	- unavailable for data collection -		
Week 9	1.27×10^5	3.70×10^4	4.52×10^4

Table 16. Tooth Number 3 Counts/μl of Spirochetes, Motile Rods and Non-motile Forms of Experimental Group C, Patient R.S.

Time	Spirochetes	Motile Rods	Non-motile Rods
Pre-scaling and root planing	7.09×10^4	2.65×10^5	8.01×10^4
2-3 Weeks Post-scaling and root planing = Day 0	5.87×10^2	1.70×10^4	4.28×10^4
Week 1	5.14×10^2	1.54×10^3	9.76×10^3
Week 4	3.39×10^4	1.23×10^4	4.40×10^2
Week 8	9.86×10^4	1.97×10^5	9.86×10^4
Week 9	3.47×10^4	3.78×10^4	7.70×10^3

Table 17. Tooth Number 19 Counts/µl of Spirochetes,
Motile Rods and Non-motile Forms of
Experimental Group C, Patient R.S.

Time	Spirochetes	Motile Rods	Non-motile Rods
Pre-scaling and root planing	3.08×10^5	2.65×10^5	1.36×10^5
2-3 Weeks Post-scaling and root planing = Day 0	1.03×10^3	7.19×10^3	2.93×10^4
Week 1	2.57×10^2	7.70×10^2	4.11×10^3
Week 4	1.09×10^5	4.31×10^4	3.08×10^3
Week 8	1.54×10^4	3.08×10^4	3.47×10^4
Week 9	5.18×10^4	3.20×10^4	5.05×10^4

Table 18. Neutrophil Counts from the Plaque Samples Obtained from the Selected
Periodontal Pockets Associated with Teeth Numbers 3 and 19.

Subject and Pocket Site	Pre-scaling	2-3 Weeks Post-scaling = Day 0	Week 1	Week 4	Week 8	Week 9
B.F. - 3 DP	2	14	1	0	2	0
B.F. - 19 ML	0	0	0	0	2	0
J.K. - 3 MB	0	0	0	0	0	0
J.K. - 19 DB	0	0	0	0	1	1
B.M. - 3 MB	0	0	0	0	0	0
B.M. - 19 DB	0	1	0	3	20	1
B.P. - 3 DB	0	0	1	0	missed	0
B.P. - 19 MB	1	0	0	0	missed	0
R.S. - 3 MB	0	0	3	0	0	1
R.S. - 19 ML	0	0	10	3	0	0

Figure 1. Sample of the informed consent form.

IRB NUMBER: 4/81-5b

LOYOLA UNIVERSITY MEDICAL CENTER
MAYWOOD, ILLINOIS

DEPARTMENT OF PERIODONTICS

INFORMED CONSENT

Patient's Name: _____   Date:_____

Project Title:___The Effect of Systemic Minocycline Therapy on the_____

_____Microflora in Moderate Periodontitis._____

Patient Information

Description and explanation of procedure: As a result of periodontal (gum) disease, you have many periodontal pockets that are filled with harmful bacteria that cause destruction of the gum and bone tissue which surround the teeth. Minocycline (Minocin) is a tetracycline antibiotic ordinarily used against bacterial infections including periodontal disease. You will be asked to take orally 150 mg of minocycline (Minocin) (50 mg in the morning and 100 mg in the evening) for seven days as protection against periodontal bacteria.

Risks and discomforts: Minocycline should not be given to pregnant women; patients with renal (kidney) impairment; or children up to eight years of age. Dizziness may occur with minocycline.

Potential benefits: The suppression of the injurious bacteria that are causing your periodontal disease and an improvement in the health of your gums. The possible regrowth of normal bone to tooth and tooth to gum attachment.

Treatment Alternatives: The surgical eradication of periodontal pockets via the separation of the gum tissue from the tooth and bone, and the recontouring of bone around the tooth (with surgical burs and instruments). The gum tissue is then replaced and sutured together. Alternatives to participation include total withdrawal from the study.

Figure 1. (continued)   63

Consent

I have fully explained to _____
the nature and purpose of the above-described procedure and the risks that
are involved in its performance. I have answered and will answer all ques-
tions to the best of my ability.

                                    _____
                                    Stephen A. Folson, D.D.S.

I have been fully informed of the above-described procedure with its pos-
sible benefits and risks. I give permission for my participation in this
study. I know that Dr. Stephen Folson or his associates will be available
to answer any questions I may have. If, at any time, I feel my questions
have not been adequately answered, I may request to speak with a member
of the Medical Center Institutional Review Board. I understand that I am
free to withdraw this consent and discontinue participation in this pro-
ject at any time without prejudice to my medical care. I have received a
copy of this informed consent document.

I understand that biomedical or behavioral research such as that in which
I have agreed to participate, by its nature, involves risk of injury. In
the event of physical injury resulting from these research procedures,
emergency medical treatment will be provided at no cost, in accordance
with the policy of Loyola University Medical Center. No additional free
medical treatment or compensation will be provided except as required by
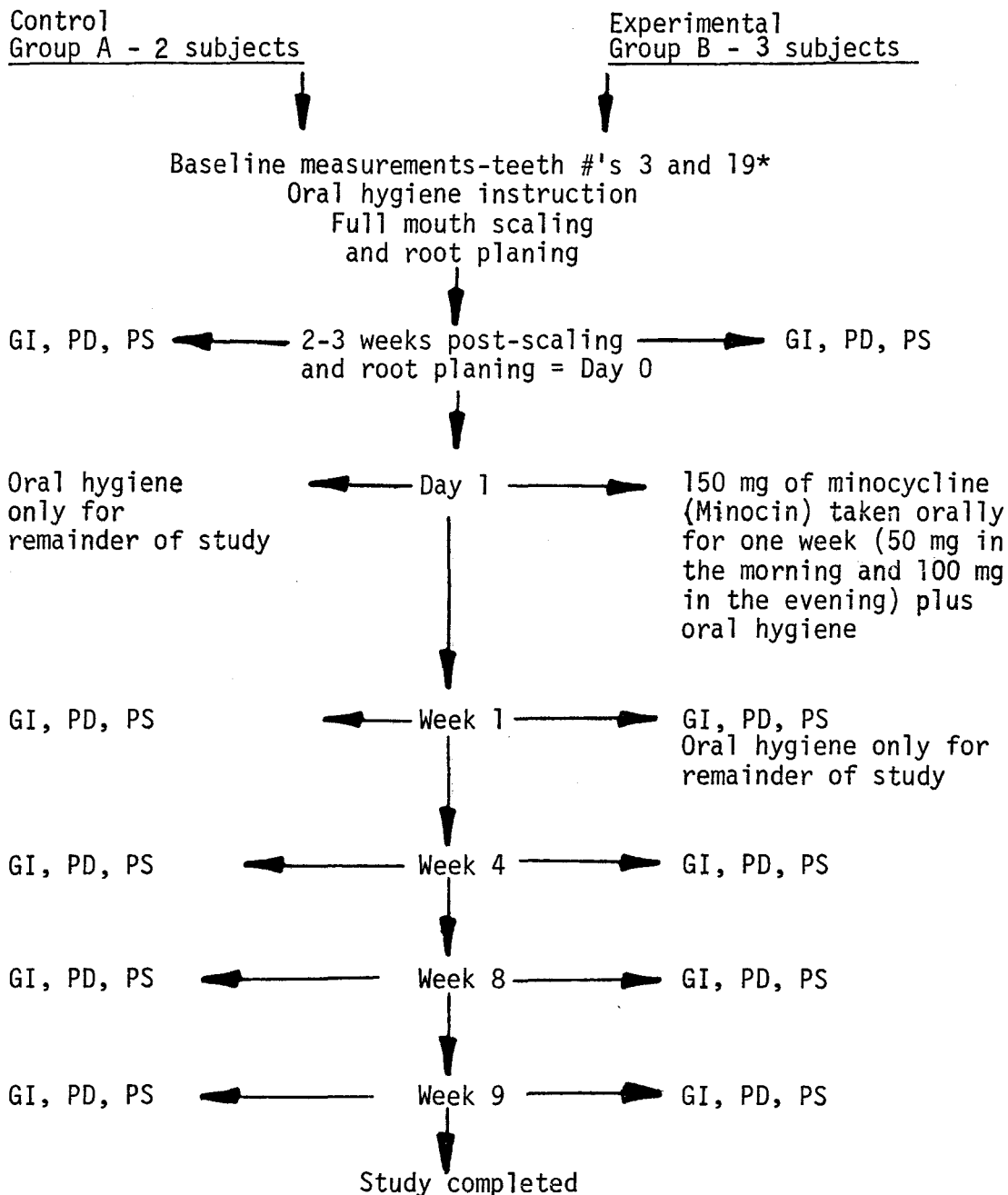Illinois law.

In the event I believe that I have suffered any physical injury as the
result of participation in the research program, I may contact, Dr. S.
Aladjem, Chairman, Institutional Review Board for the Protection of Human
Subjects at the Medical Center, telephone (312) 531-3380.

I agree to allow my name and medical records to be available to other
authorized physicians and researchers for the purpose of evaluating the
results of this study. I consent to the publication of any data which
may result from these investigations for the purpose of advancing medical
knowledge, providing my name is not used in conjunction with such publi-
cation. All precautions to maintain confidentiality of the medical
records will be taken.

                                    _____
                                    (signature: patient

_____
(signature: witness to signature)

Figure 2. Experimental design schematically summarized.

Control
Group A - 2 subjects

Experimental
Group B - 3 subjects

Baseline measurements-teeth #'s 3 and 19*
Oral hygiene instruction
Full mouth scaling
and root planing

GI, PD, PS ◄——— 2-3 weeks post-scaling ———► GI, PD, PS
and root planing = Day 0

Oral hygiene
only for
remainder of study
◄——— Day 1 ———►
150 mg of minocycline
(Minocin) taken orally
for one week (50 mg in
the morning and 100 mg
in the evening) plus
oral hygiene

GI, PD, PS ◄———Week 1———► GI, PD, PS
Oral hygiene only for
remainder of study

GI, PD, PS ◄——— Week 4 ———► GI, PD, PS

GI, PD, PS ◄——— Week 8 ———► GI, PD, PS

GI, PD, PS ◄——— Week 9 ———► GI, PD, PS

Study completed

* Gingival Index (GI), probing depth (PD), plaque sample (PS)

Figure 3.

Sample of a darkfield microscopic microbial and neutrophil counting form.

LOYOLA UNIVERSITY SCHOOL OF DENTISTRY - DEPT. OF PREVENTIVE DENTISTRY

DARKFIELD MICROBIOLOGY

ORAL BIOLOGY RESERACH PROJECT - Stephen A. Folson, D.D.S.

| PATIENT'S NAME: | TYPE OF TREATMENT: |
| OBSERVATION DAY: | DATE (MO/DAY/YR): |

TYPE	WBC	Spirochetes			Motile Rods			Non-Motile Organism			
Tooth Number and Field Number	Neutrophil	Small	Medium	Large	Small	Large	Curved	Cocci	Fusiform	Filament	Short Rods

SUMMARY: Tooth number   microliters/plaque sample   Total Fields   Total Cells

Figure 4.   Darkfield microscopic calculations.

1.   Area under cover slip (5 μl sample):

   22 mm x 22 mm = 4.84 cm^2 / sample

2.   Area of one field on oil immersion:

   $\pi \cdot r^2 = \pi \, (.01\text{cm})^2 = \pi \cdot 10^{-4} \text{ cm}^2/\text{field}$

3.   Constant K = $\dfrac{\dfrac{5\mu l}{\text{sample}} \times \dfrac{\pi \cdot 10^{-4}\text{cm}^2}{\text{field}}}{4.84 \text{ cm}^2/\text{sample}}$

   $= \dfrac{5}{4.84} \, \pi \cdot 10^{-4} \, \mu l/\text{field}$

4.   Microbial counts*/total fields converted to microbial counts/μl:

   counts/fields x 1/K = counts/μl

* spirochetes, motile rods, and the combined non-motile organisms

Figure 5. Gingival Index (GI) and probing depth (PD) of the selected
periodontal pocket associated with tooth number 3 in the
control group A, patient B.F.
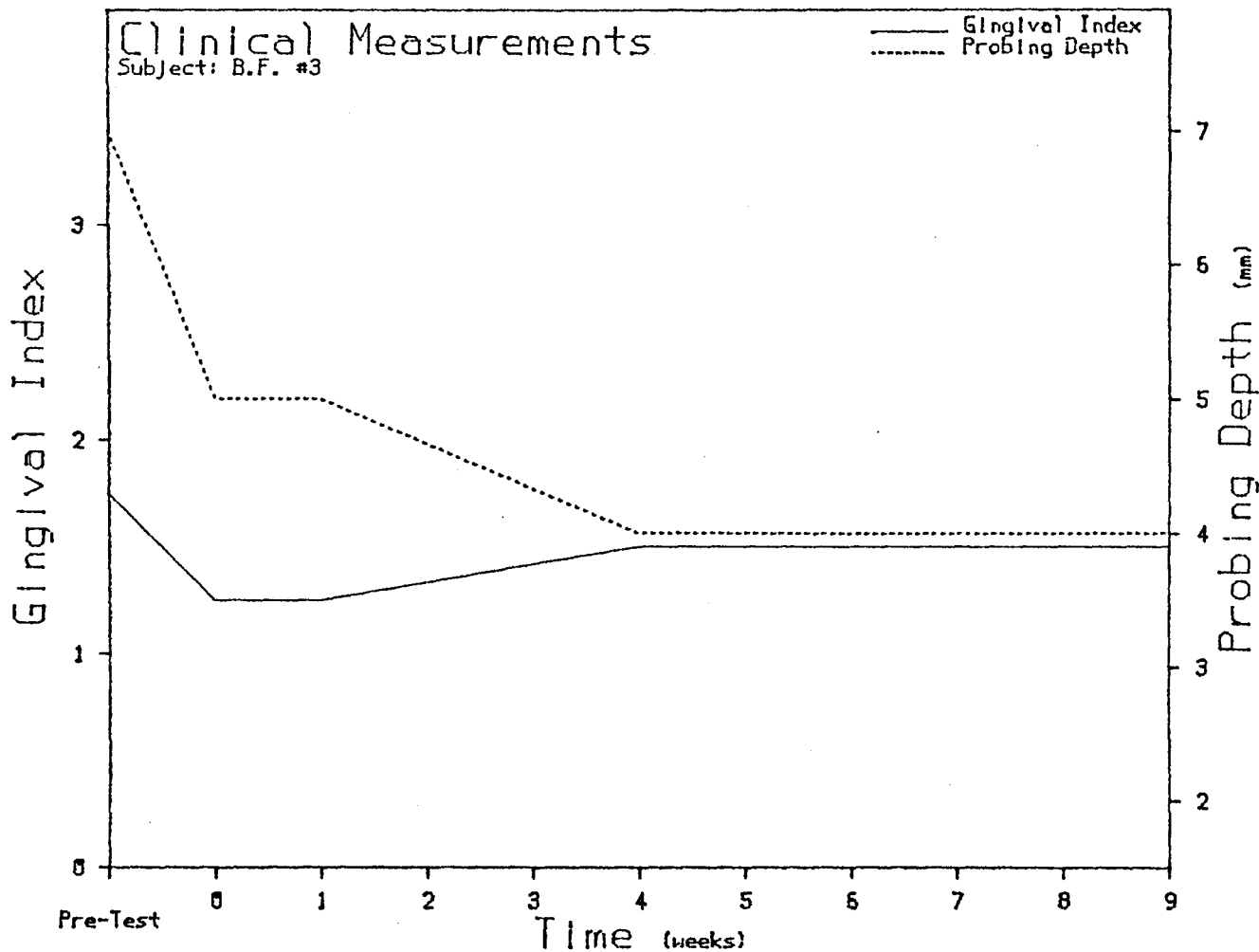
Figure 6. GI and PD of the selected periodontal pocket associated with tooth number 19 in the control group A, patient B.F.
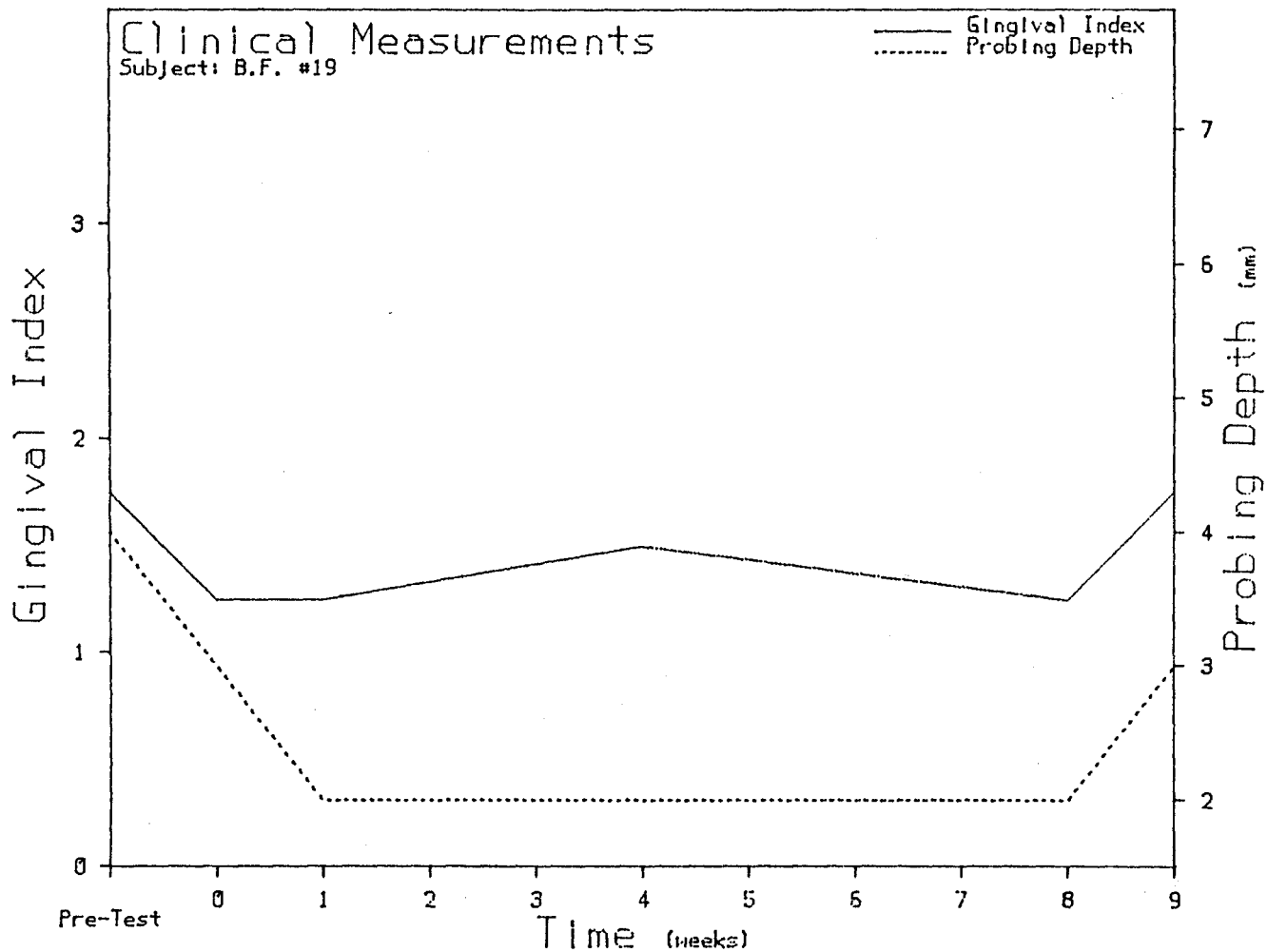
Figure 7. GI and PD of the selected periodontal pocket associated
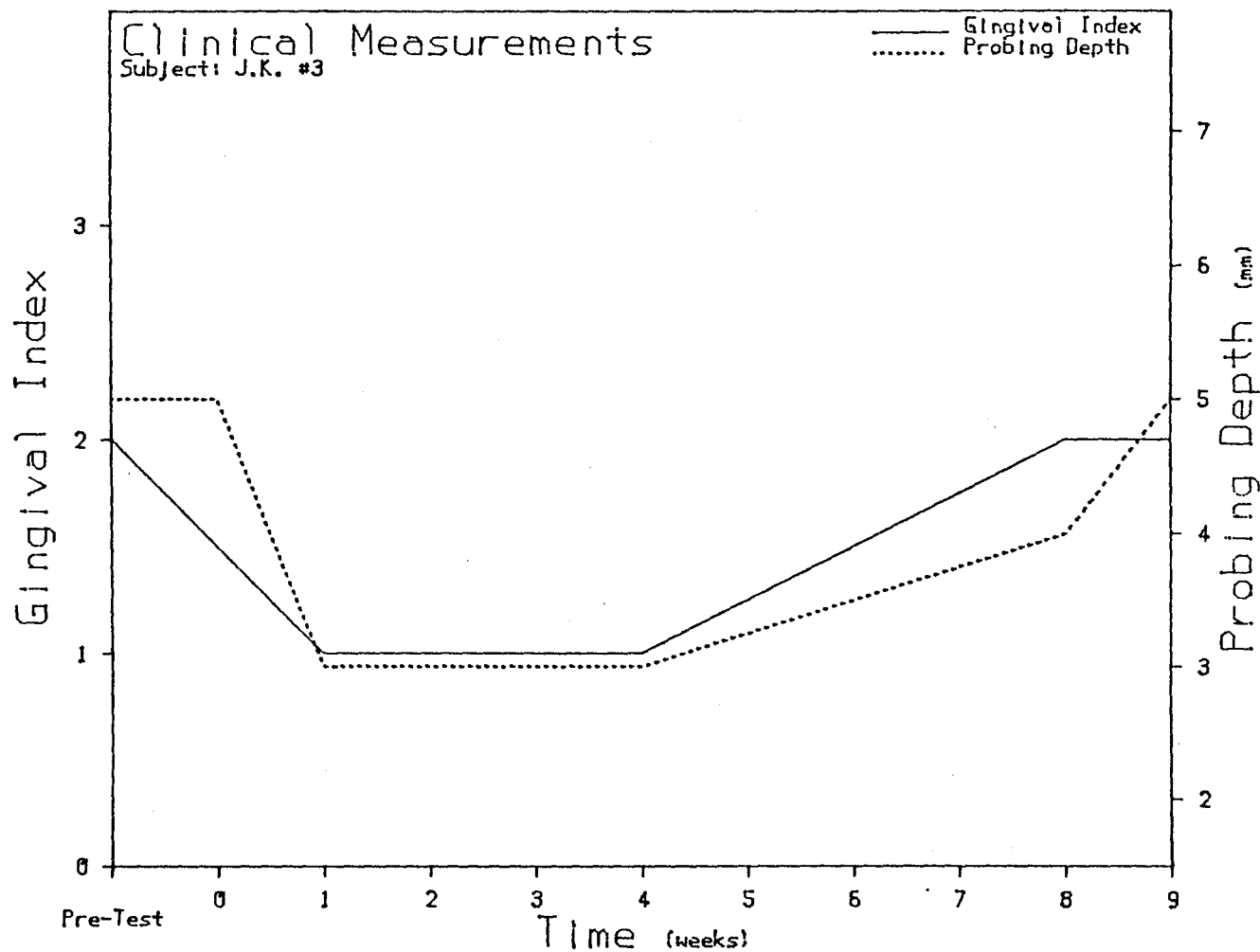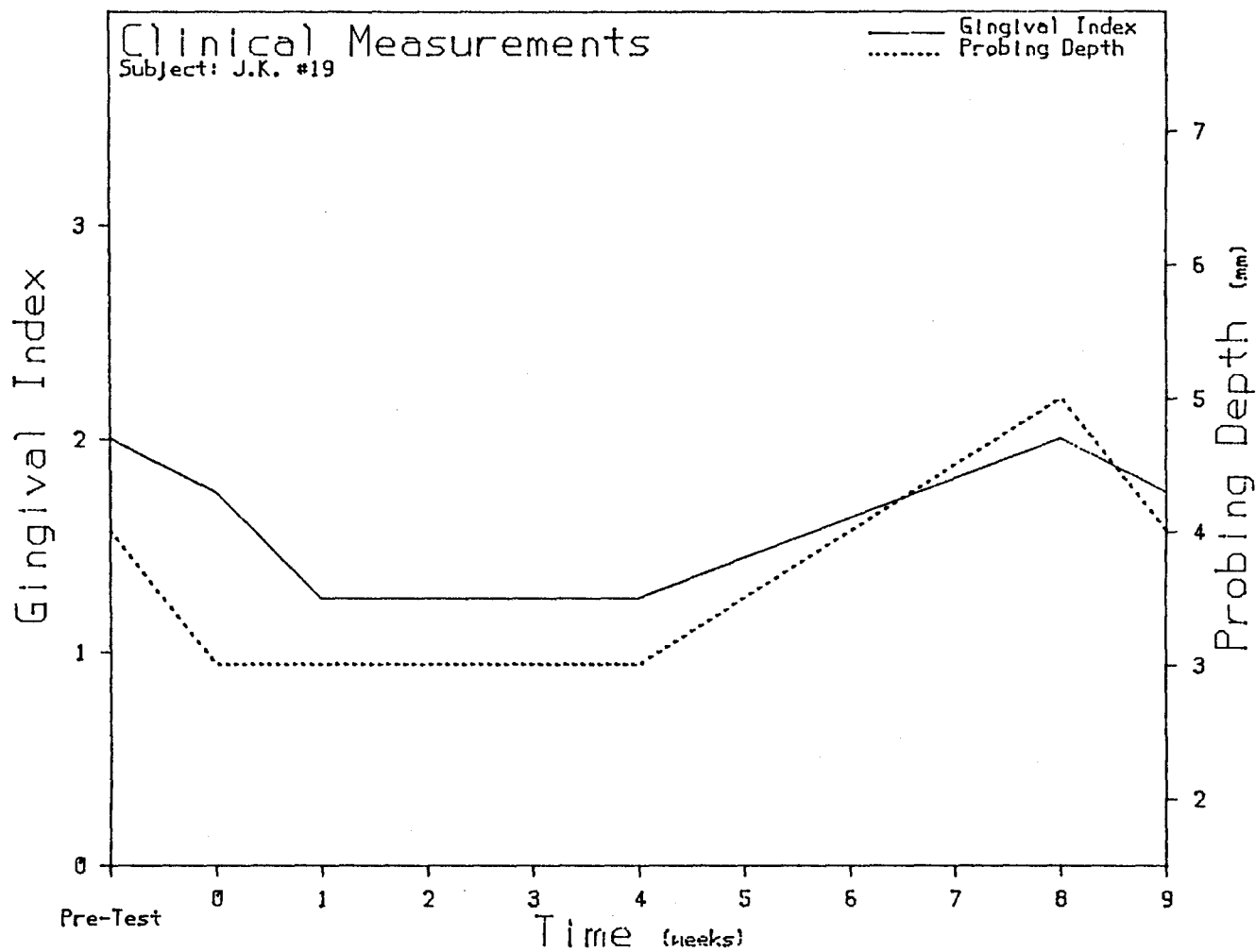   with tooth number 3 in the experimental group B, patient J.K.

Figure 8. GI and PD of the selected periodontal pocket associated
with tooth number 19 in the experimental group B, patient J.K.

Figure 9. GI and PD of the selected periodontal pocket associated with tooth number 3 in the experimental group B, patient B.M.
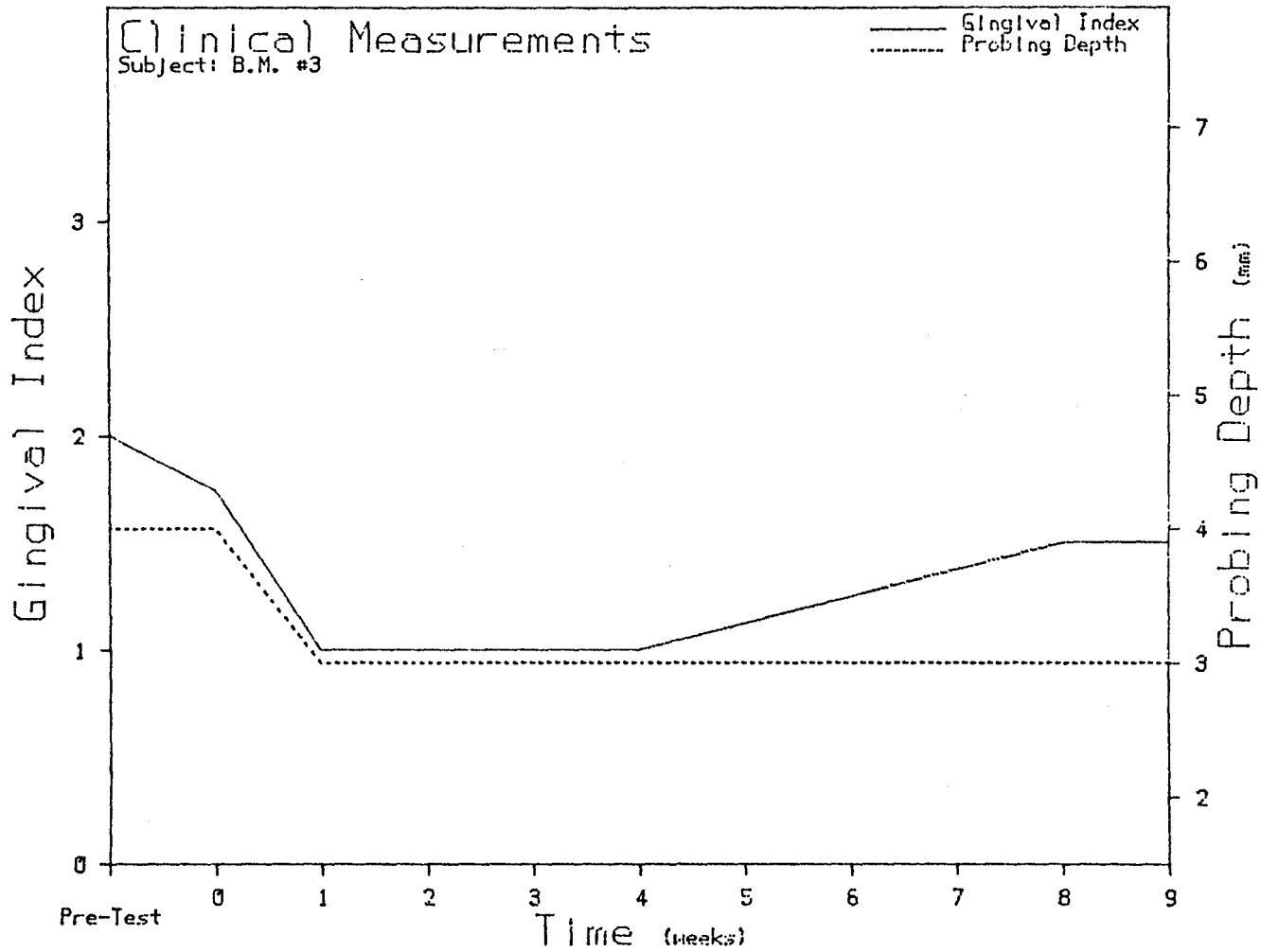
Figure 10. GI and PD of the selected periodontal pocket associated with tooth number 19 in the experimental group B, patient B.M.
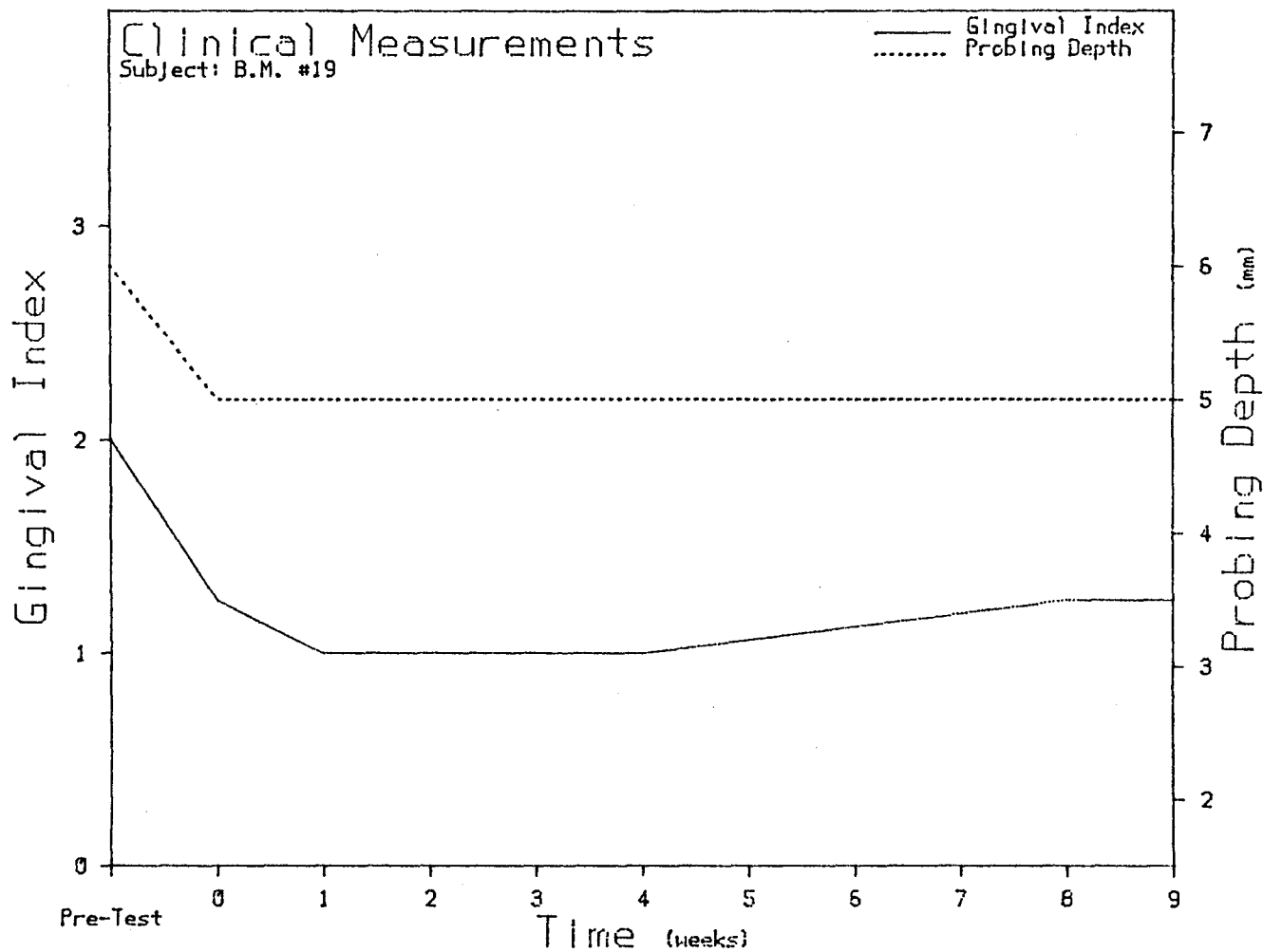
Figure 11. GI and PD of the selected periodontal pocket associated with tooth number 3 in the experimental group B, patient B.P.
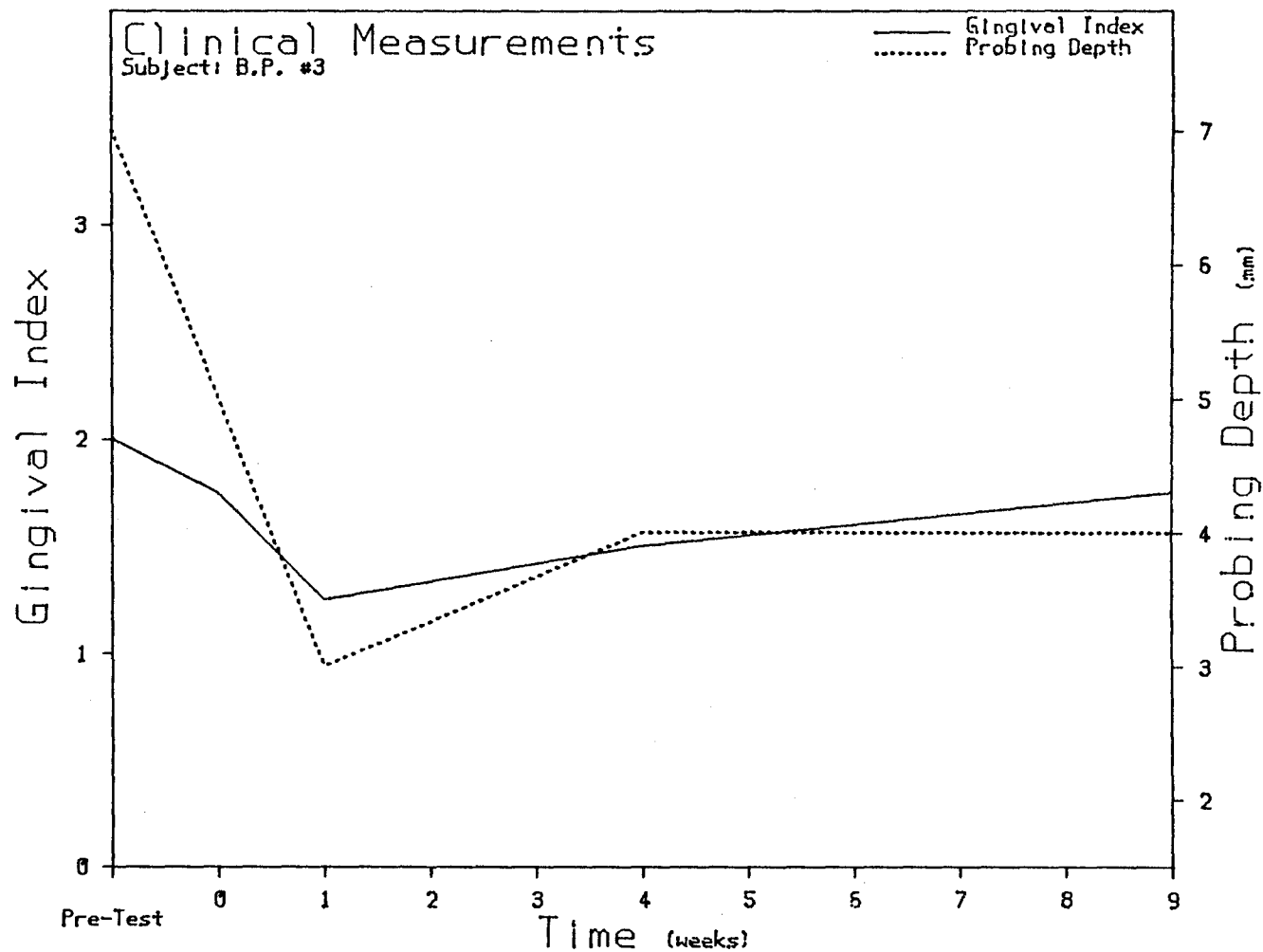
Figure 12. GI and PD of the selected periodontal pocket associated with tooth number 19 in the experimental group B, patient B.P.
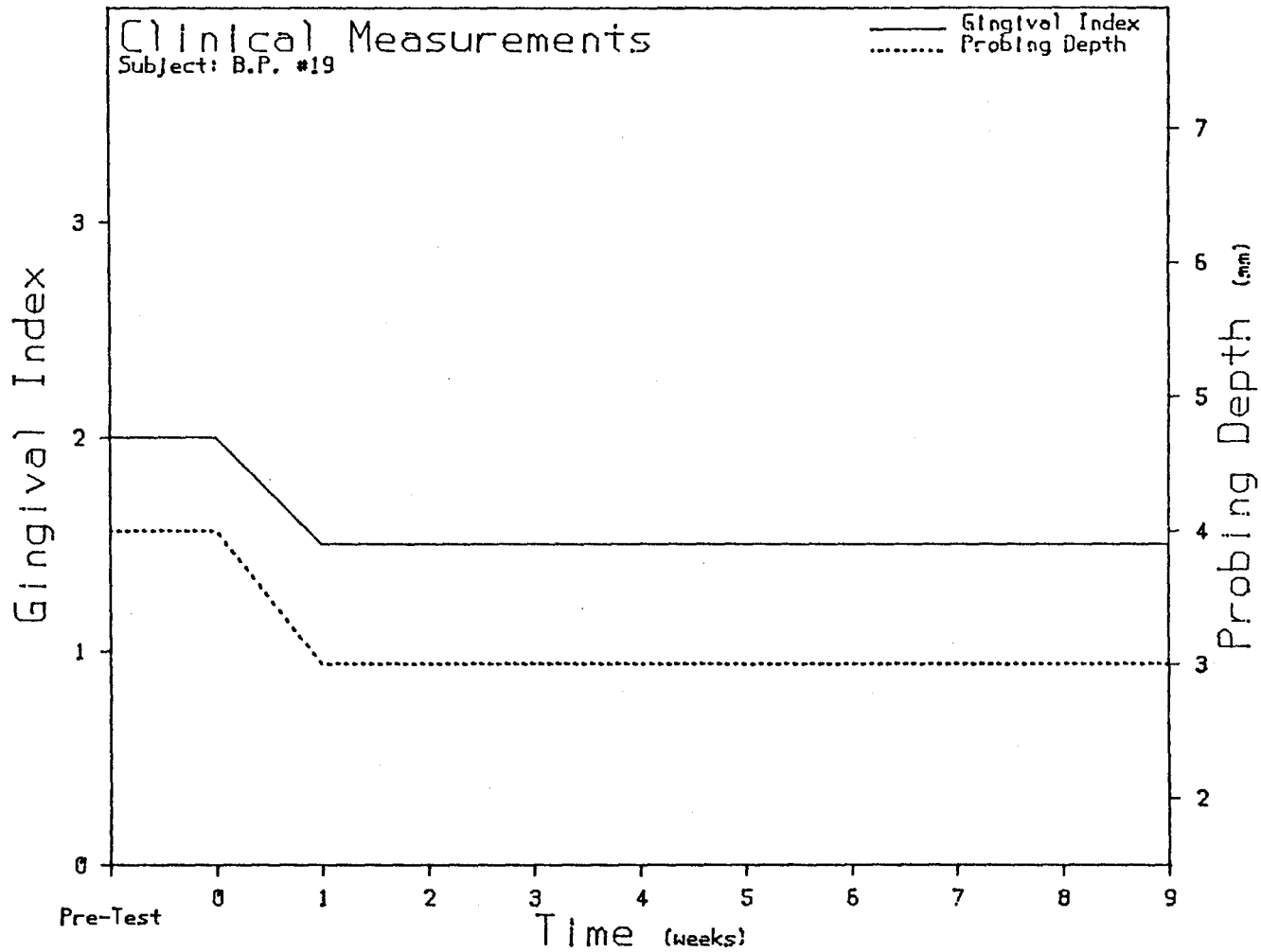
Figure 13. GI and PD of the selected periodontal pocket associated with tooth number 3 in the experimental group C, patient R.S.
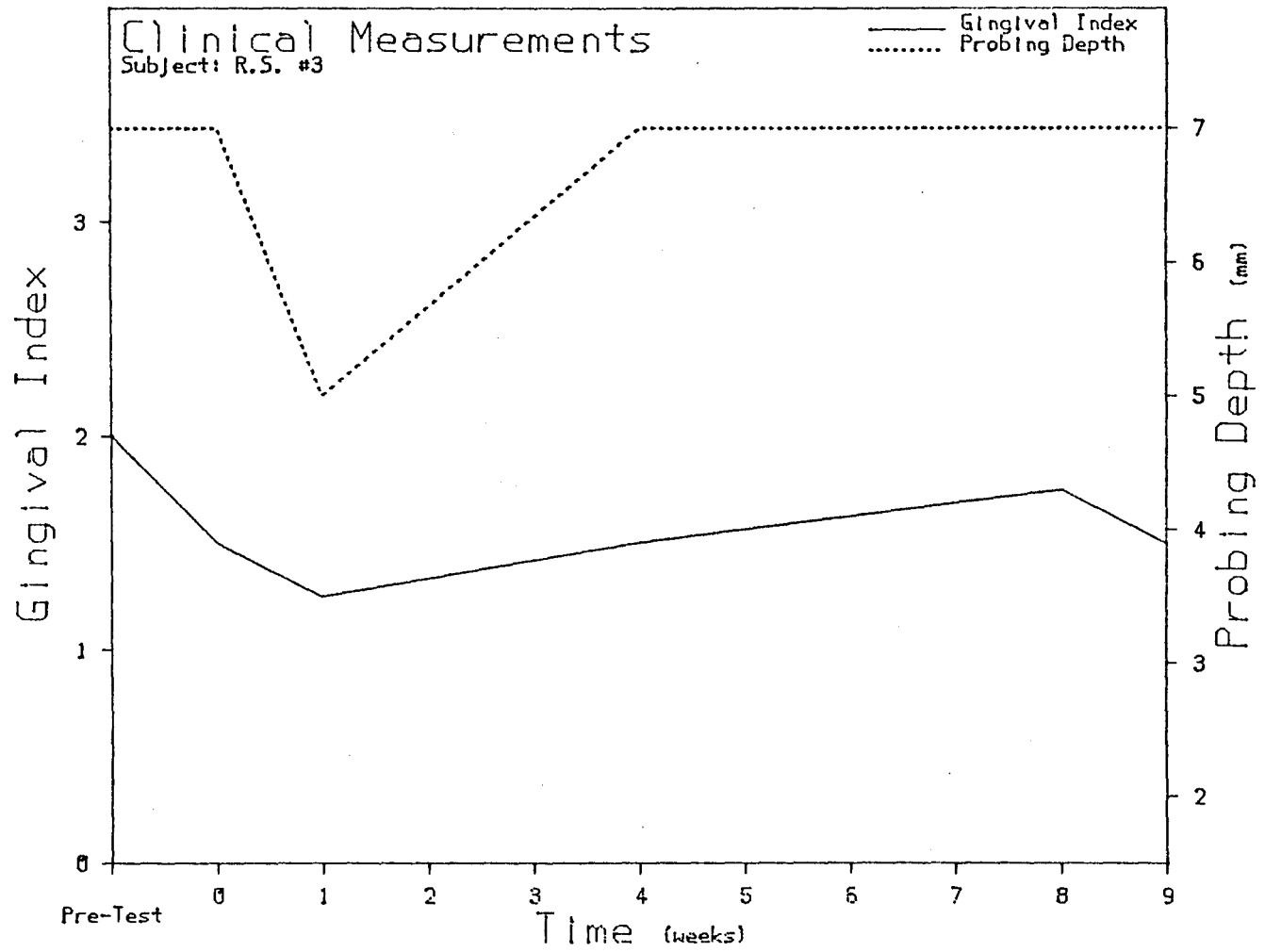
Figure 14. GI and PD of the selected periodontal pocket associated with tooth number 19 in the experimental group C, patient R.S.
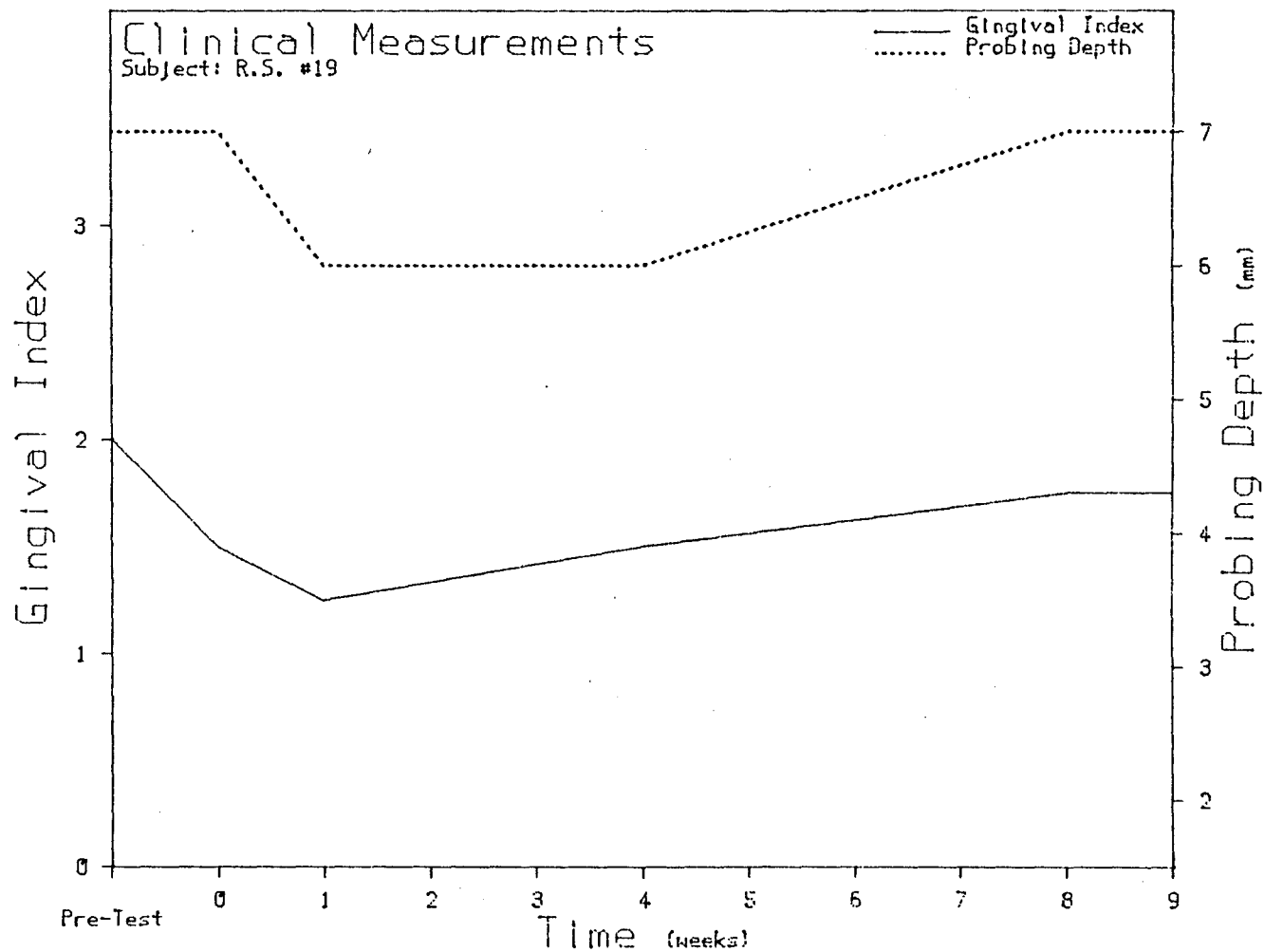

Clinical Measurements
Subject: R.S. #19

——— Gingival Index
.... Probing Depth

Gingival Index

Probing Depth (mm)

Time (weeks)

Pre-Test

Figure 15. Counts/µl of spirochetes, motile rods and non-motile forms
of the selected periodontal pocket associated with tooth
number 3 in the control group A, patient B.F.

Figure 16. Counts/µl of spirochetes, motile rods and non-motile forms of the selected periodontal pocket associated with tooth number 19 in the control group A, patient B.F.
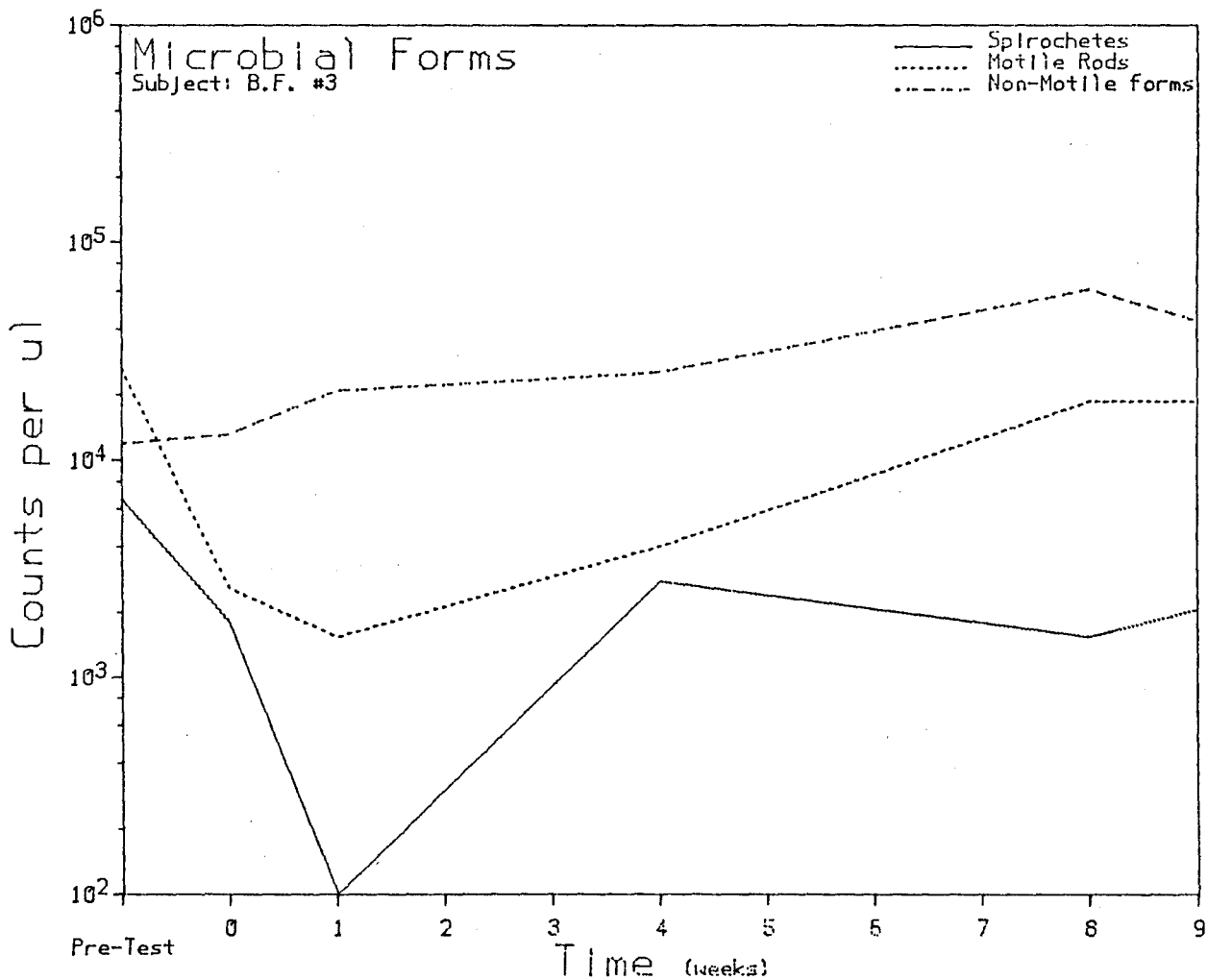
Figure 17. Counts/μl of spirochetes, motile rods and non-motile forms of the selected periodontal pocket associated with tooth number 3 in the experimental group B, patient J.K.

Figure 18. Counts/µl of spirochetes, motile rods and non-motile forms
of the selected periodontal pocket associated with tooth
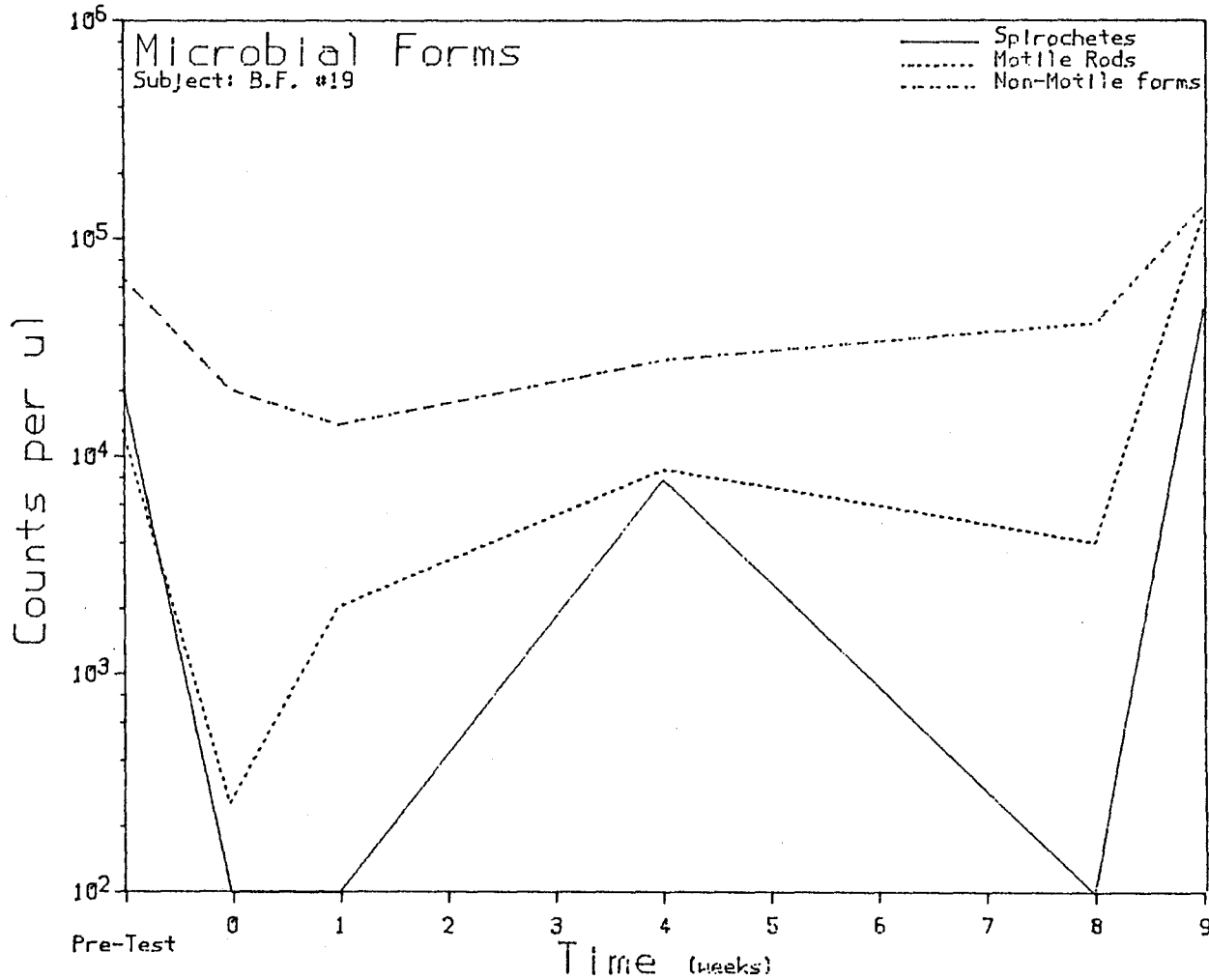number 19 in the experimental group B, patient J.K.

Figure 19. Counts/µl of spirochetes, motile rods and non-motile forms of the selected periodontal pocket associated with tooth number 3 in the experimental group B, patient B.M.

Microbial Forms
Subject: B.M. #3

——— Spirochetes
·········· Motile Rods
– – – – Non-Motile forms

Counts per ul

Time (weeks)

Pre-Test

Figure 20. Counts/µl of spirochetes, motile rods and non-motile forms
of the selected periodontal pocket associated with tooth
number 19 in the experimental group B, patient B.M.
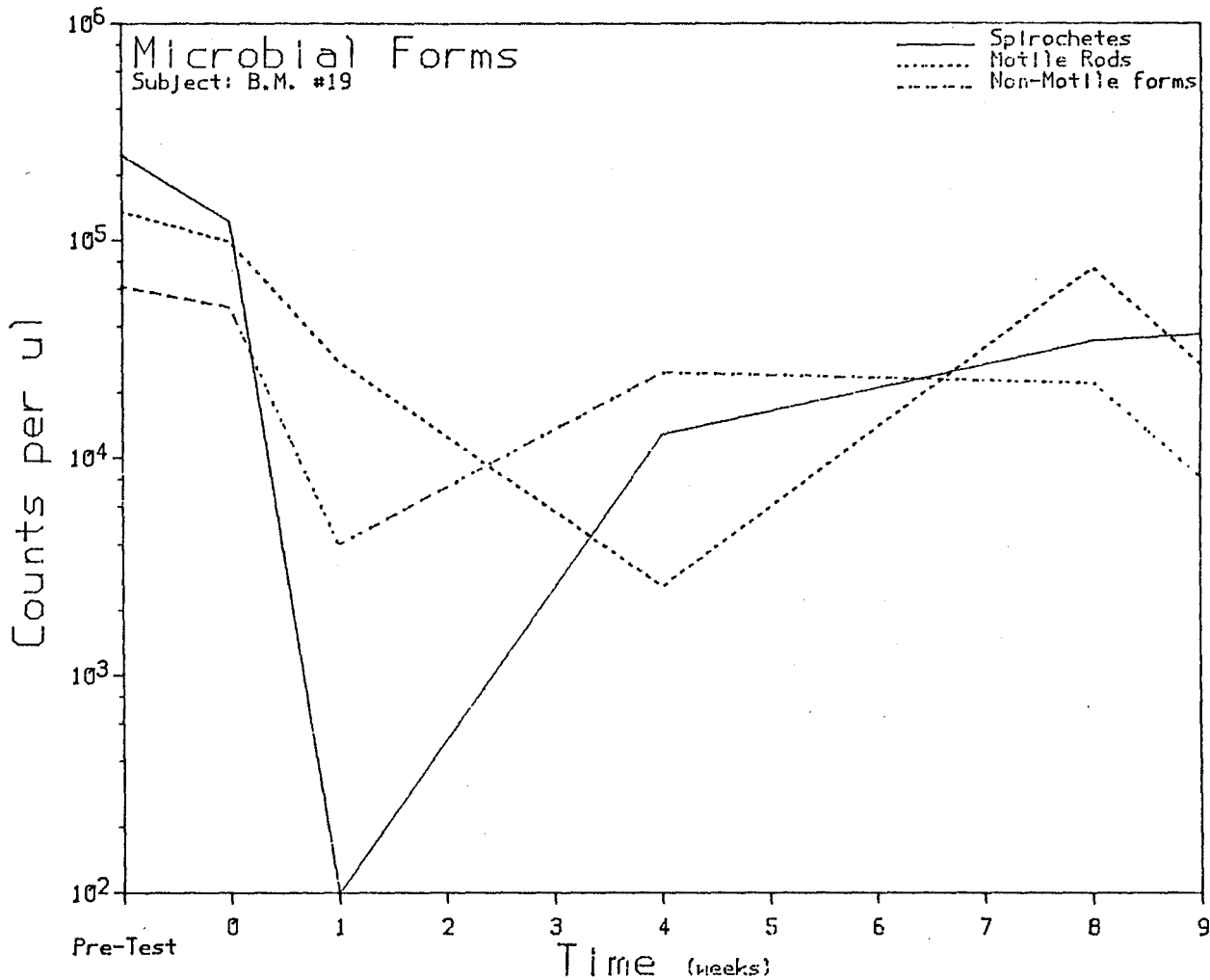
Figure 21. Counts/µl of spirochetes, motile rods and non-motile forms
of the selected periodontal pocket associated with tooth
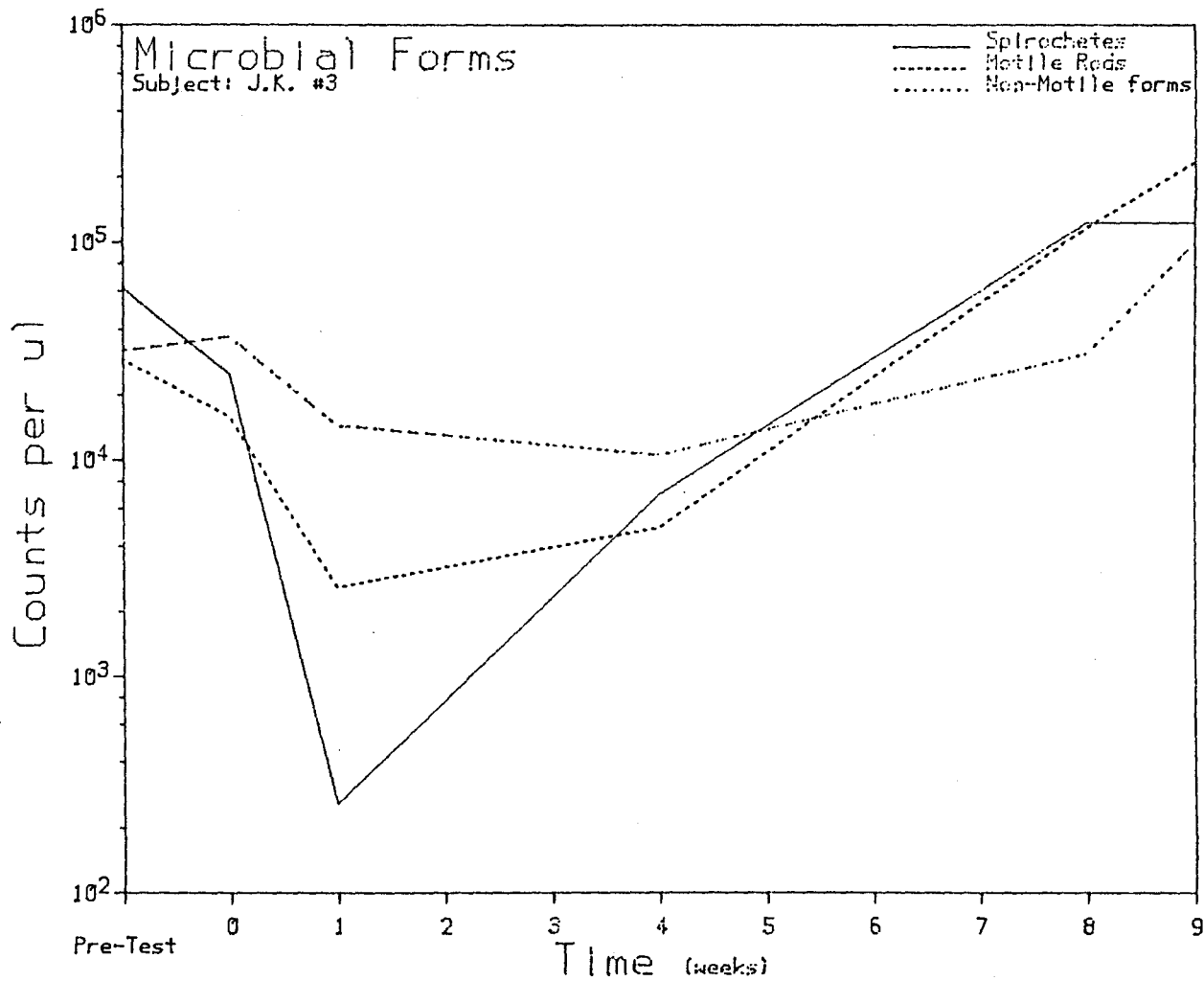number 3 in the experimental group B, patient B.P.

Figure 22. Counts/μl of spirochetes, motile rods and non-motile forms of the selected periodontal pocket associated with tooth number 19 in the experimental group B, patient B.P.
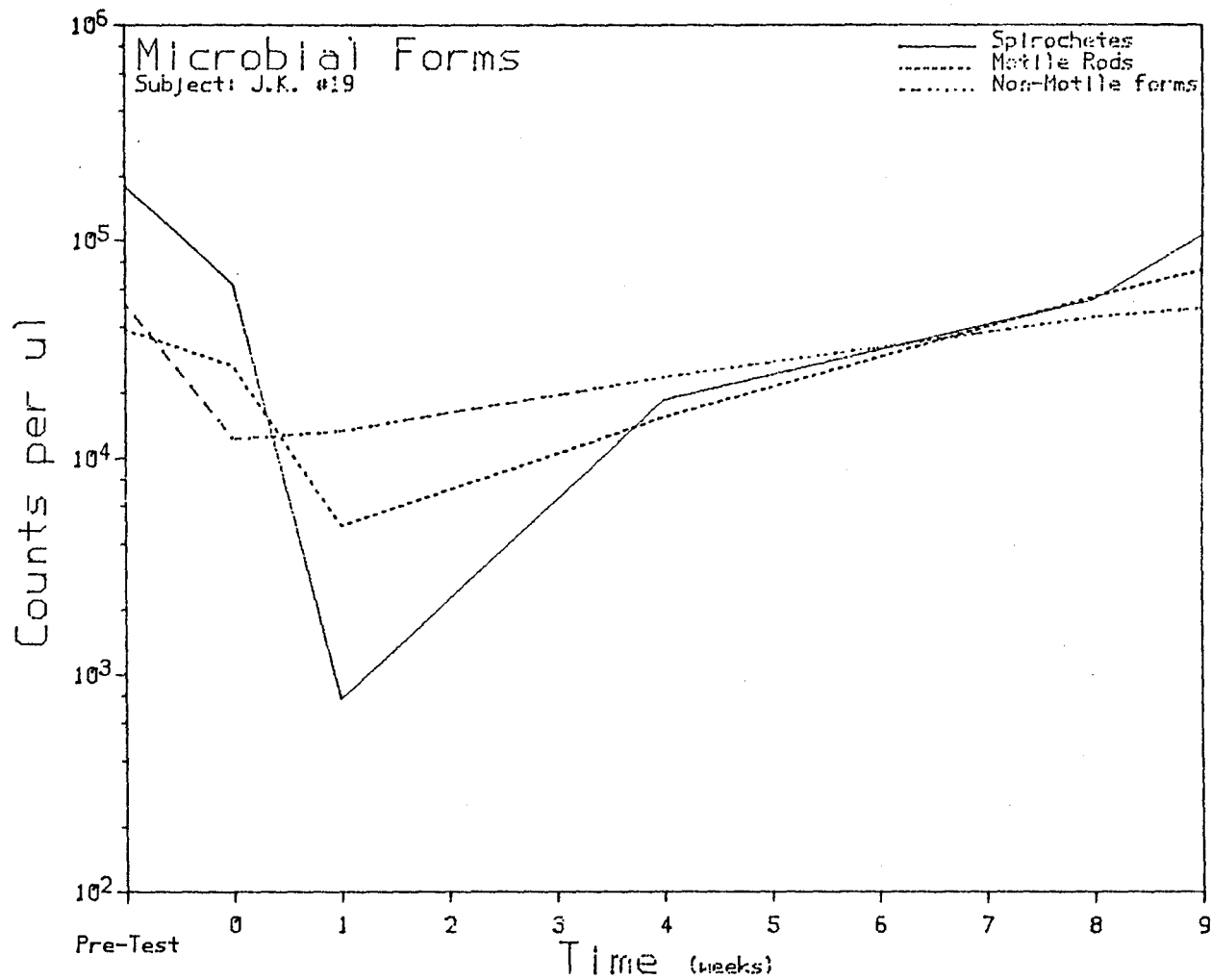
Figure 23. Counts/µl of spirochetes, motile rods and non-motile forms of the selected periodontal pocket associated with tooth number 3 in the experimental group C, patient R.S.
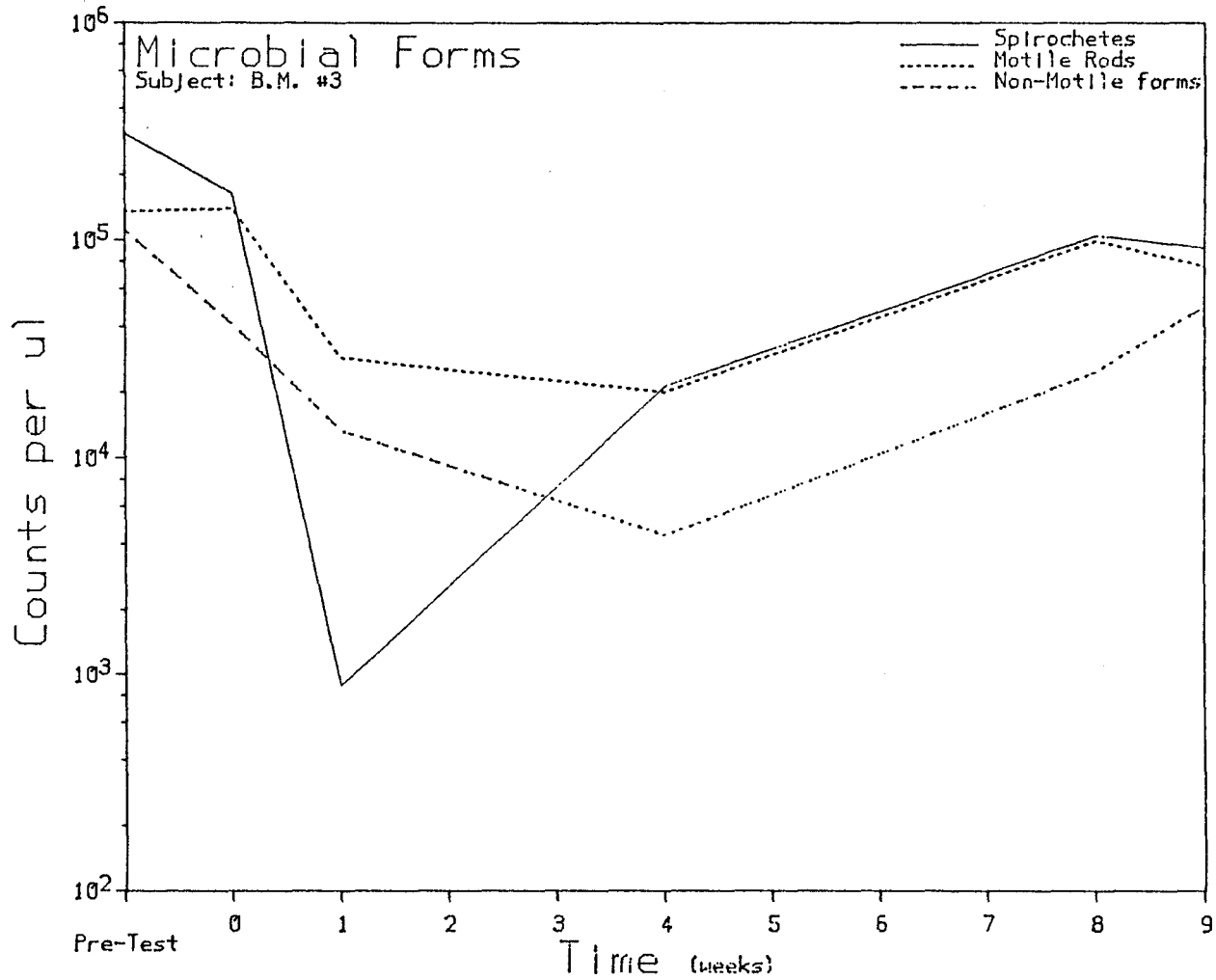
Figure 24. Counts/µl of spirochetes, motile rods and non-motile forms of the selected periodontal pocket associated with tooth number 19 in the experimental group C, patient R.S.
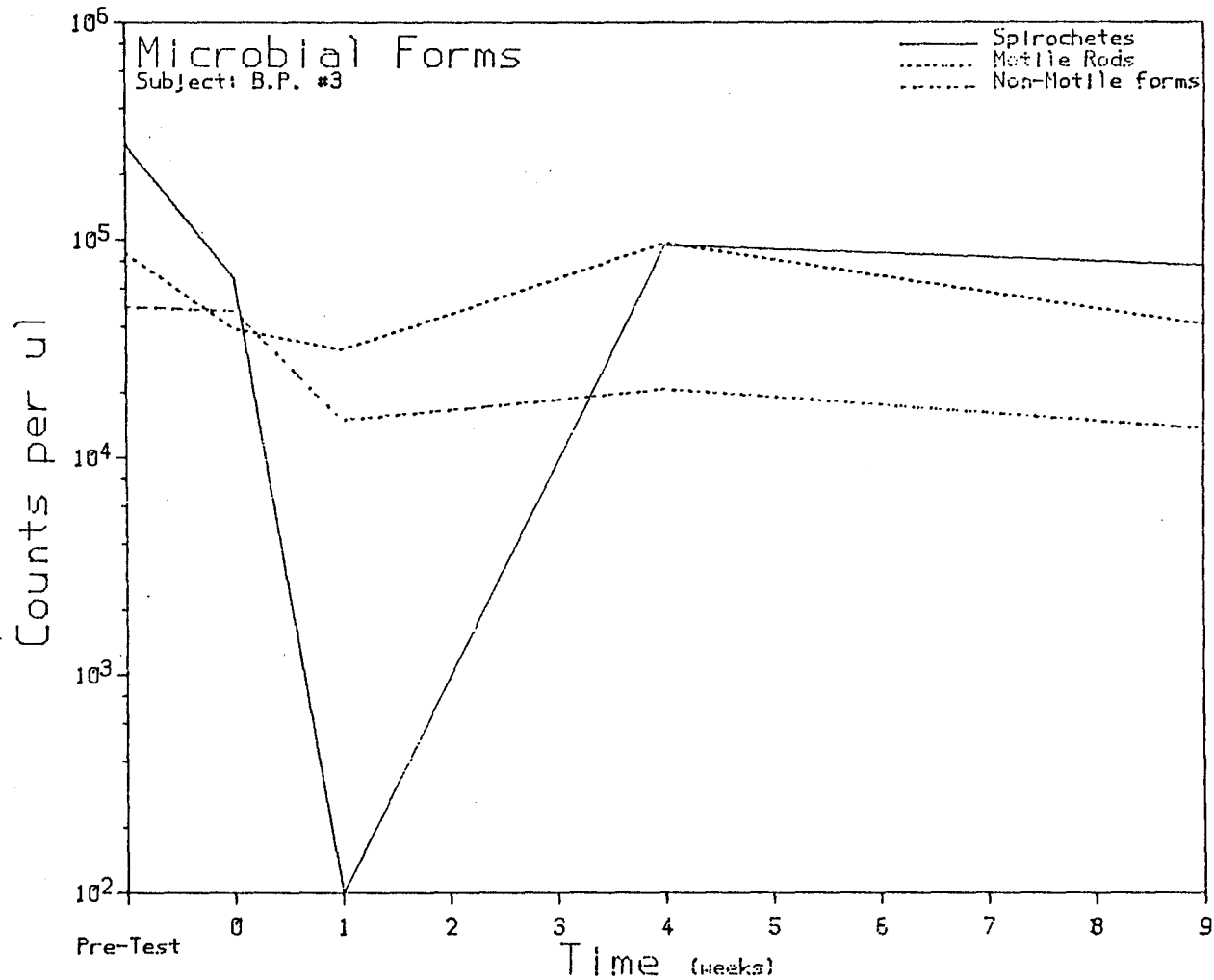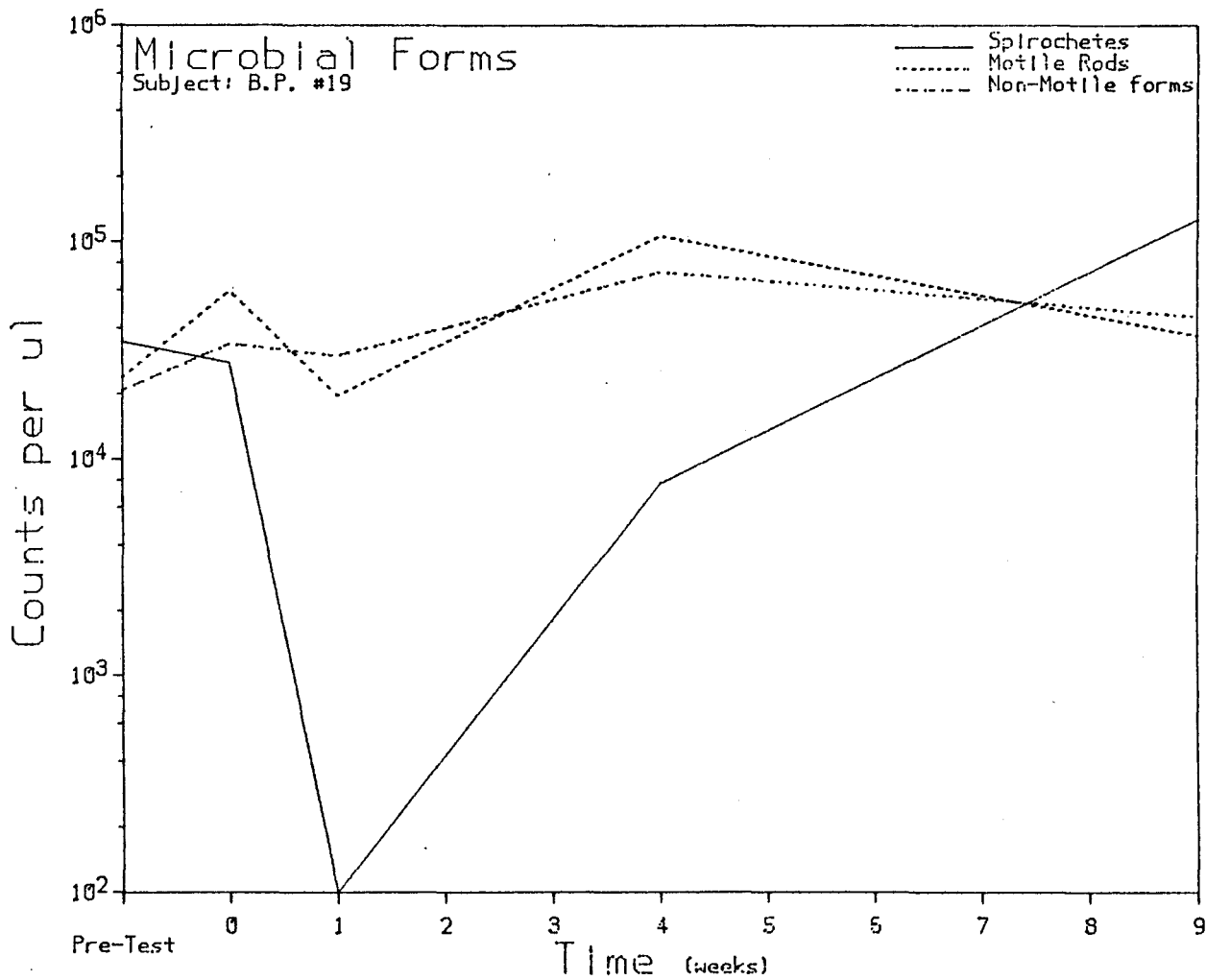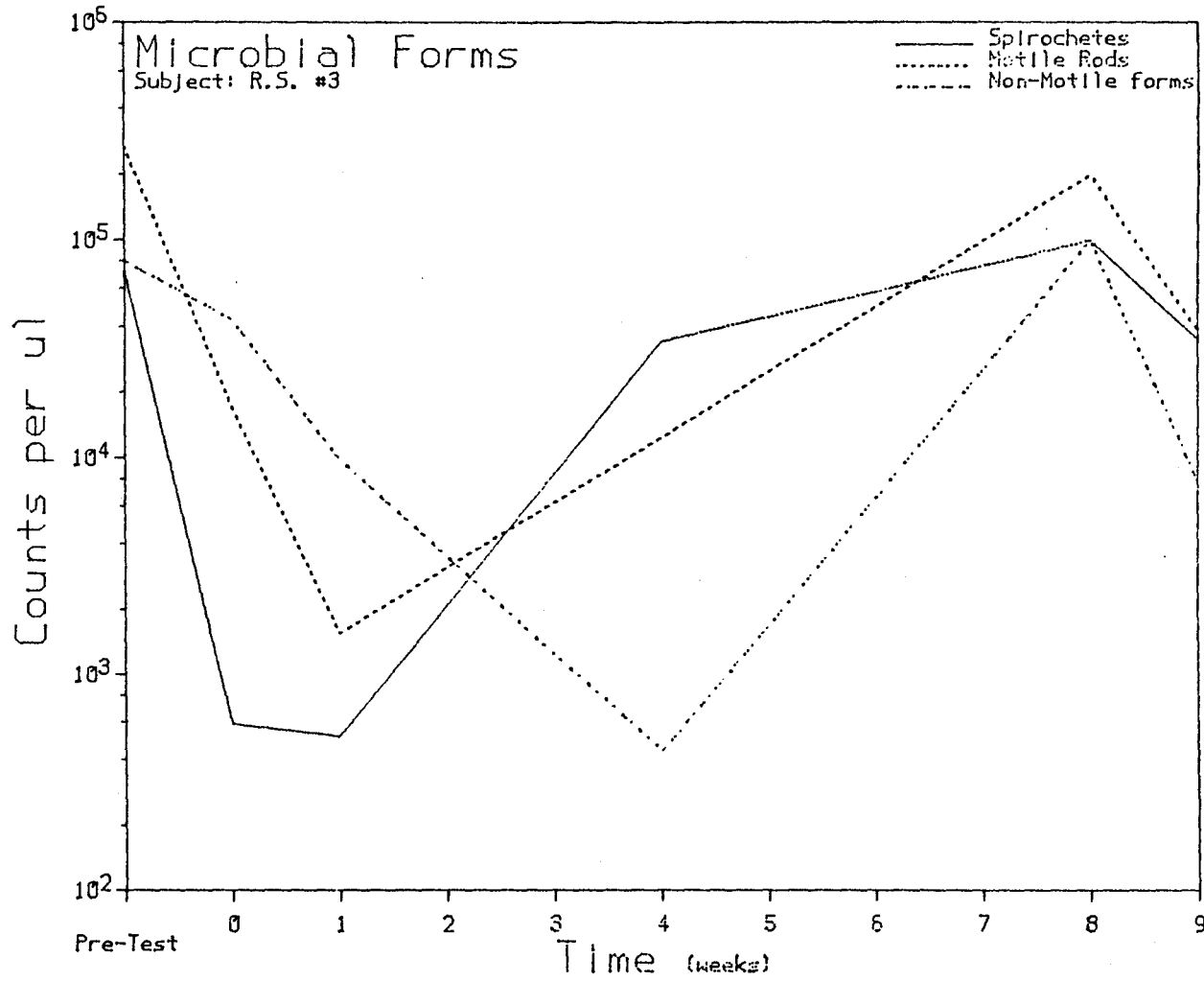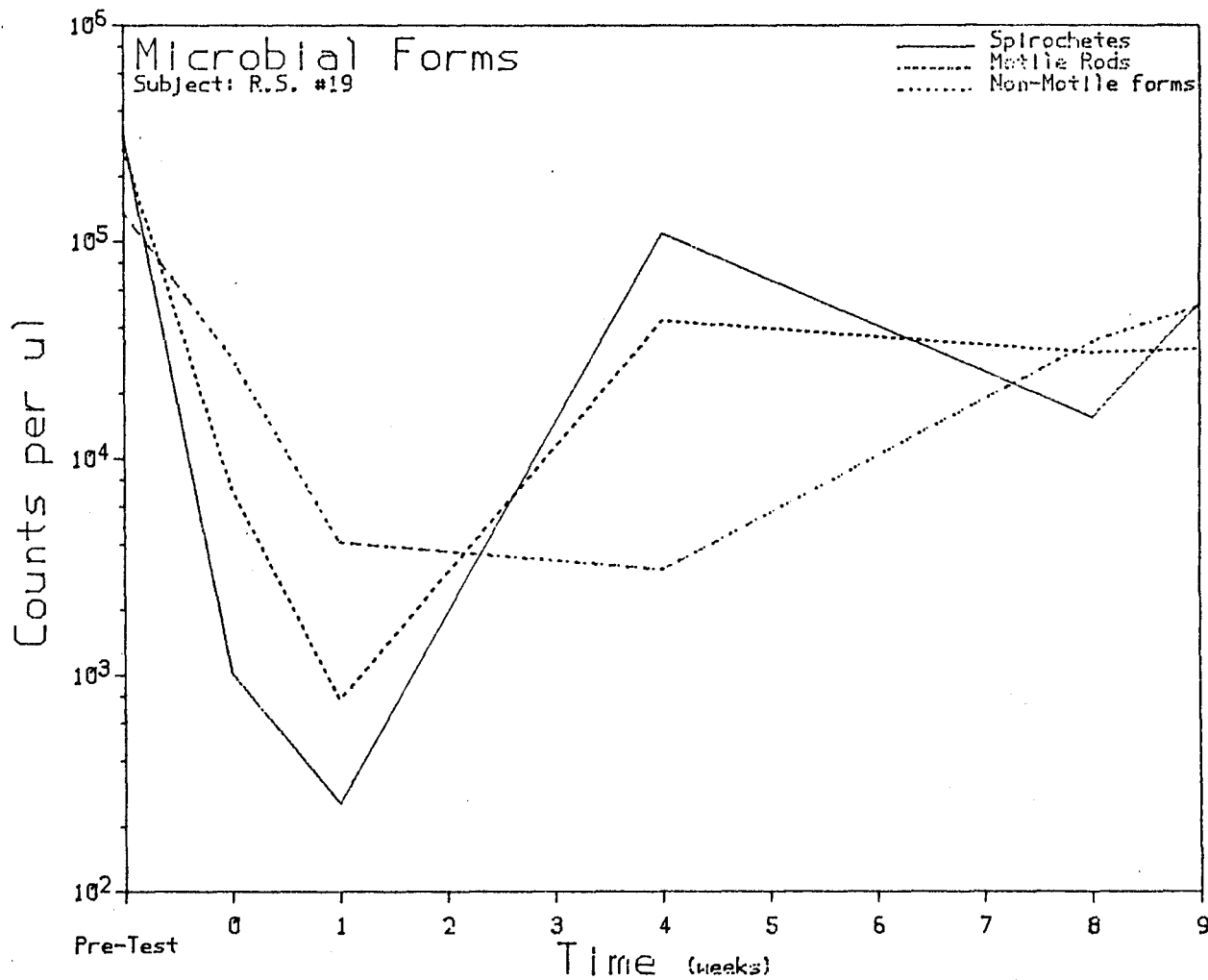
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