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The Effects of Achromycin V (Tetracycline) on the Percentage Anaerobic, Facultative and Tetracycline-Resistant Organisms, and on Selected Clinical Parameters in Advanced Periodontal Disease

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THE EFFECTS OF ACHROMYCIN V (TETRACYCLINE) ON THE PERCENTAGE ANAEROBIC, FACULTATIVE AND TETRACYCLINE - RESISTANT ORGANISMS, AND ON SELECTED CLINICAL PARAMETERS IN ADVANCED PERIODONTAL DISEASE

by

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To Dr. Anthony Gargiulo, I thank you for the tools with which I might attain excellence in the field of Periodontics.

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VITA

Eric J. Coontz was born March 22, 1949 in Worcester, Massachusetts. He is the third son of six children of Gus and Clare Coontz.

He grew up in Worcester, Massachusetts, receiving his elementary and secondary education there. He graduated from North High School in June, 1967, and went on to study at Assumption College in Worcester. On June 6, 1971, he graduated cum laude and received a B.A. in Biology with a minor in Natural Sciences.

From August 1971 to March 1972, he studied human anatomy-histology at Saint Louis University Medical School Department of Anatomy. He entered Loyola University School of Dentistry in September, 1972, and graduated with his D.D.S. Degree on June 12, 1976. While he was a dental student, he held various offices including Student Congress Representative, President of Beta Chapter, Delta Sigma Delta and Vice-President, Saint Appolonia Guild.

In March, 1973, he was commissioned Second Lieutenant in the United States Army as a result of being a recipient of the Army Health Professions Scholarship. Upon graduating from dental school, he was stationed for three years at the United States Army Hospital, Nurnberg, Germany. As a result of his accomplishments while a general dental officer, he was awarded the Army Commendation Medal for Meritous Service.

In September, 1979, he entered the Periodontics Graduate Residency at Loyola University School of Dentistry from which he graduated in May, 1981.

DEDICATION

To my mother and father, Clare E. and Gus Coontz, for their love, encouragement, guidance and faith in me, without whose unending support would have made my professional career an impossible dream.

To Esther, whose constant looking over my shoulder has instilled in me the Christian ideals so necessary to have, as a scientist and as a clinician.

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CHAPTER I

INTRODUCTION

The presence of a certain microbial population adjacent to, and within, the gingival sulcus is now considered to be the primary etiologic factor in the pathogenesis of most forms of periodontal disease.[1,2,3] The rationale for conventional therapy, (e.g. periodontal scaling and root planing and patient oral hygiene procedures), is based on studies of experimental gingivitis.[4,5] When oral hygiene measures are stopped, there is a shift in the microbial content of plaque from predominantly Gram-positive and coccoid, to one that becomes increasingly filamentous with various Gram-negative species.

Actinomyces species has been found both in conjunction with gingivitis[6,7] and advanced periodontal disease.[8] Potentially important pathogens have been identified by Socransky[9] and include spirochetes and various Gram-negative bacteria, such as Fusobacterium nucleatum, capnophilic fusiform rods known as "Capnocytophaga", corroding species of Vibrio, Bacteroides and Eikenella, Bacteroides melaninogenicus ss. asaccharolyticus as well as many unidentified Gram-negative organisms.

Traditionally, the periodic removal of microbial plaque from the tooth surfaces has been a benchmark for the long term success of periodontal treatment,[10,11, 12,13] It would appear that removing the microbes, or changing the microbial content to that associated with health, is indeed necessary. The amount of time and energy expended by the patient and clinician is extraordinary in accomplishing such a

result, and it is because of this that chemotherapeutic agents, especially the tetracyclines[14,15,16,17,18] have come to the forefront of periodontal therapy.

CHAPTER II

REVIEW OF THE LITERATURE

A. The Microbiology of Periodontal Disease

In 1958, Arnim recognized that the primary etiologic factor in periodontal disease was "the adherent colonized microbial mass found on the tooth surface at the gingival crest, in the sulcus and in the periodontal pocket with its associated bordering communications of motile and nonmotile bacteria, protozoa, leukocytes, drifting desquamated cells as well as other varieties of microscopic life, living or dead."[21] More recently, there have been a number of longitudinal studies that have correlated the existence of bacterial plaque with the subsequent development of gingival inflammation, In these studies, Loe, et al.[22,23] showed that a group of 12 dental students developed accumulations of soft debris (plaque) and clinically observable inflammation of the gingiva when oral hygiene measures were stopped. When oral hygiene measures were resumed, the plaque was removed and gingivitis abated. Lindhe and Nyman[24] and Rosling et al.[25] showed that the progress of advanced periodontal disease could be halted or partially reversed by surgical procedures when accompanied by twice monthly professional tooth cleaning. These studies suggest that suppression of the total microbiota could be effective in controlling both gingivitis and destructive periodontitis in humans. The investigations described above deomstrated that a wide variety of microorganisms have been observed in plaque associated with periodontal disease states in man. However, until recently, only

a few species have been shown to be capable of inducing similar disease in experimental animals or of satisfying Koch's postulates.[26]

Various studies have implicated certain species of the genus Actinomyces as major etiologic agents of gingivitis and periodontitis.[27,28] These studies have also shown that periodontal disease is transmissible in rodent model systems. Hamsters monoinfected with A. viscosus develop signs of periodontal disease. Certain other species of the genus Actinomyces, such as A. naeslundii and A. israelii, which are found in plaque of patients with advanced periodontal disease, have been shown to cause destructive periodontal disease when transferred to gnotobiotic rats.[29,30]

Specific Gram-negative anaerobic or capnophilic organisms are capable of inducing severe periodontal breakdown with rapid alveolar bone loss in laboratory animals.[31,32] It has also been shown that both juvenile and adult patients with aggressively osteolytic and with advanced periodontal disease have shown a predominance of Gram-negative anaerobic or capnophilic flora associated with the region of greatest pocket depth.[33,34,35,36,37,38] These organisms include Bacteroides corrodens, Eikenella corrodens, vibrio corroders and Campytobacter sp., fusiforms including Fusobacterium nucleatum and F. polymorphum, Selenomonas sputigena and Bacteroides melaninogenicus. Spirochetes are also found to be abundant in the advanced periodontal lesion. Slots[38] found that B. melaninogenicus ss. asaccharolyticus is the predominant Gram-negative rod isolated from deep pockets in adult advanced periodontitis.

Newman, et al.[34,35,36] found a group of Gram-negative anaerobic

capnophilic rods described as the "Periodontosis Group" and grouped I through V. Some of these have been named "Capnocytophaga" and Bacteroides ochraceus.[39,40] Unlike the adult form of periodontal disease, the microflora of the juvenile form is saccharolytic. At least some of the microflora of the adult form of the aggressive disease are found in the juvenile disease and vice versa. In addition, these same microorganisms can be found in relatively normal sulci of patients with and without gingival disease, but in lower numbers.[41] Finally, Actinobacillus actinomycetecomitans (strain Y4), an anaerobic gram-negative microorganism, has been isolated from the subgingival plaque of patients with juvenile periodontitis.[42] Refer to Table 1-2 for the organisms found in health and periodontal disease.

B. The Microflora's Relationship to the Pathogenesis of Periodontal Disease

An important feature in periodontal disease is the predominance of increasing numbers of white blood cells, specifically neutrophils, in the junctional epithelium and gingival sulcus as gingivitis is initiated and progresses to an established lesion. It has been demonstrated in vitro that dental plaque and sertain oral bacteria contain or produce neutrophil chemotactic factors.[43,44,45] Enzymes within the neutrophil lysosomes are capable of hydrolyzing certain tissue constituents.[46]

The bacteria themselves have a direct effect on gingival inflammation by virtue of the production of enzymes or cytotoxic materials. Hyaluronidase is one such enzyme produced by oral bacteria, and its activity has been detected in dental plaque.[48,49,50] It may be

a factor in the breakdown of intercellular substances and thus facilitate the penetration of other substances.

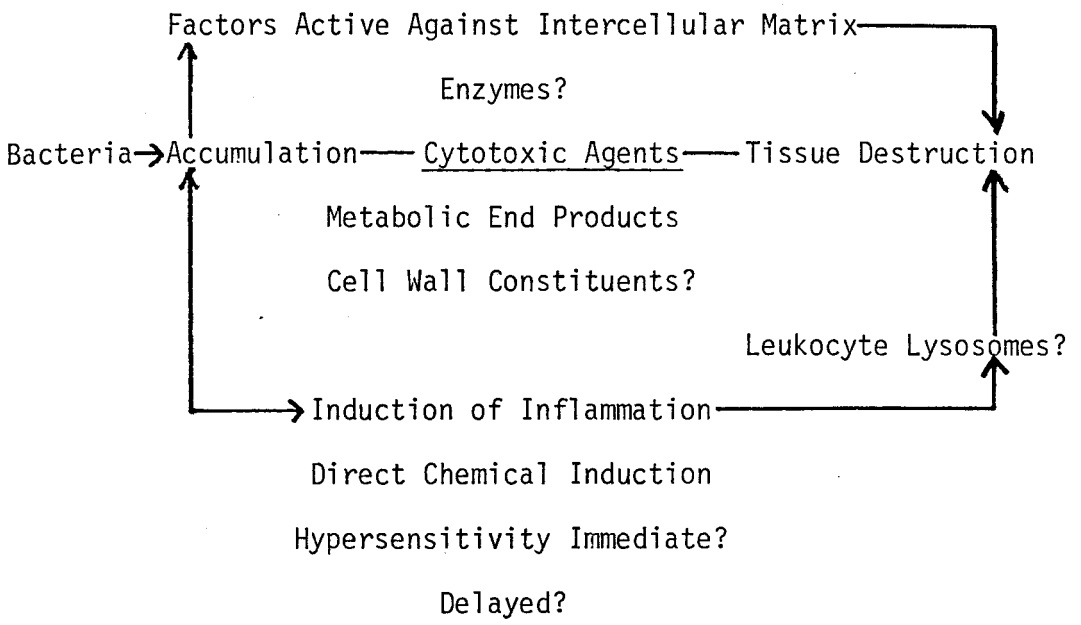
Certain substances produced by the oral bacteria have toxic effects on cells, which could be a mechanism causing some cellular destruction. Their exotoxins may destroy erythrocytes (hemolysins), fibrin (fibrinolysins), and tissue (necrotoxins). Bacterial endotoxins (lipopolysaccharides) from the cell membrane (gram-negative organisms), may cause: a pyrogenic action resulting in an increase in the host's temperature; a transient leucopenia followed by leucocytosis; hypoglycemia; a wide variety of circulatory disturbances, especially hemorrhage; altered resistance to bacterial infection; and a vascular hyperactivity to adrenergic drugs.[51]

In various studies, stimulation of peripheral lymphocytes with antigen preparations from plaque or selected oral microroganisms induced a greater degree of blastogenesis in patients with some degree of periodontal disease than in healthy individuals.[52,53,54,55,56,57] The production of lymphokines, biologically active effector substances produced by sensitized lymphocytes, also has been reported when circulating lymphocytes are stimulated by antigens from plaque or oral microorganisms.[56,58,59,60] These studies give evidence that the bacteria in plaque stimulate the host's cellular immunity, and therefore, are involved in the pathogenesis of periodontal disease.

Various studies have shown serum antibody reaction with oral organisms, and thus there is stimulation of the host's humoral immunity. Antibody has been detected to antigens from Fusobacterium,[61,62]

Actinomyces naeslundii[63,64] and Bacteroides melaninogenicus.[65,66] Other organisms may also react with serum antibody. When bacterial antigens to which there is an antibody titer enter the gingiva, antigen-antibody complexes might form and cause extensive tissue damage.

An overall view of the hypothesized role of bacteria in the pathogenesis of periodontal tissue destruction is seen below.[67]



C. Tetracycline and Periodontal Disease

It is a logical hypothesis that antibodies could effect a change in the composition of subgingival plaque if adequate levels are achieved in gingival fluid and saliva. Tetracycline, a broad-spectrum bacteriostatic agent used in the treatment of long-term bacterial diseases such as acne vulgaris, has been used for years as an adjunct in the treatment of periodontal disease. The basis for its selection has been its broad spectrum, low incidence of sensitivity in patients, and favorable safety record.[78]

In addition, the tetracyclines have, in the past, been used for the following oral conditions:

1. Recurrent aphthous stomatitis.[74]
2. Acute (primary) herpetic gingivostomatitis.[75]
3. To improve periodontal surgical results.[76]
4. As an initial therapy in acute necrotizing ulcerative gingivitis.[77]
5. Periodontal abscess.[77]

Some have used tetracycline as an adjunct in the treatment of juvenile periodontitis and agressive forms of the disease in adults. Tetracycline has been used at high doses (1 gm/day) for short periods (10 to 30 days) and at lower doses (250 mg to 500 mg/day) for extended periods in the management of periodontitis.

Recent studies have evaluated the effectiveness of both short-term and long-term use of tetracycline in the treatment of periodontal disease. In the studies by Hellden et al.[79] and Listgarten, et al.[80] tetracycline administered orally for two weeks was compared with scaling and root planing in the treatment of advanced periodontal disease. Their results showed that the greatest reduction in pocket depth and clinical indexes of inflammation occured in the group that was treated by scaling and root planing. The addition of tetracycline to the group that received scaling did not improve the results. Tetracycline administered without conventional treatment gave results comparable with those from a control group who received only oral hygiene instruction. When sub-gingival samples were examined the tetracycline alone group showed a

return of high numbers of spirochetes and gram-negative rods occuring between 8 and 25 weeks. In contrast, the microlfora of the scaled patients remained consistent with gingival health through the 25th week.

In the Slots[81] study, the clinical and microbiological effects of adjunctive tetracycline were not very different from the effects of periodontal scaling and root planing. However, in this study, two patients who did not respond well to scaling did show significant improvement in parameters examined after a two week course of the antibiotic.

There have been very few studies on the effect of long-term tetracycline therapy in periodontal disease. The studies that have been done suggest that long-term therapy (250 mg/day) is not more advantageous than a two week course of 1000 mg/day.[82,83]

Lindhe, et al.[84] found that tetracycline administered via a hollow fiber device markedly changed the composition of the subgingival flora of initially diseased periodontal sites. This method proved effective in reducing or eliminating the clinical symptoms of periodontal pathology. Goodson, et al.[85] found that the hollow fiber filled with tetracycline virtually eliminated spirochetes after only a single application. Once eliminated, they do not recolonize even though there is a persistence of viable organisms elsewhere in the mouth.

The side effects and toxicities of the tetracyclines are listed in Table 4.

CHAPTER III

MATERIALS AND METHODS

A. Experimental Design

Five patients, three females (PL,BR,SS) and two males (JC, JK) from Loyola University School of Dentistry, Department of Periodontics (Loyola University Medical Center Institutional Review Board #3/80-5a), were selected on the basis of the severity of their periodontitis. The age of the patients varied from 25 to 31 years with a mean of 27.2 years. In order to ascertain and determine the systemic health of the participants, a sequential multiple analysis (SMA-24) and a complete blood count was ordered from the clinical laboratory at Loyola University Medical Center.

Each patient had at least one pair of contralateral teeth, with corresponding diseased surfaces, and where pockets could be probed to 5mm or more. Radiographically, on the average, one half of the original alveolar bone had been lost. The participants had not received any antibiotics in the past 12 months, nor did they have any history of allergy, diabetes, blood dyscrasias, or chronic liver or kidney disease. None of the women were aware of being pregnant.

Following the baseline examinations, the patients were randomly distributed into two groups, T_1 and P_1. The patients in group T_1 received 250 mg Achromycin v* (tetracycline hydrochloride), four times a

*Lederle Laboratory

day, for a 2 week period. The patients in group P_1 received a placebo resembling the Achromycin capsule. Prior to the study, a code was assigned to both the Achromycin and the placebo capsules so that the examiner was unaware as to which patients were in which group. Therefore, the study was double-blind.

All patients received a scaling on the contralateral tooth and received oral hygiene instructions after the second baseline examination. One week separated the first and second baseline examinations. Further examinations were conducted at the 3rd, 12th and 24th week of the study. This experimental design provided complete (i.e. 0-, 1-, 3-, 12- and 24- week) data on diseases test sites which, had been left untreated (P_1S_0) scaled only (P_1S_1), tetracycline administration only (T_1S_0) and sites that received both scaling and tetracycline (T_1S_1).

B. Parameters Tested

The examination of sites listed in Table 5 involved as assessment of the following parameters:

1. Gingival index (See Table 6) score was estimated on a scale of 0-3, according to the method of Loe and Silness.[19] Six areas were examined, i.e. on both the vestibular and oral surfaces, the mesial, distal and an area midway between these were examined.

2. Plaque index (See Table 7) score was estimated on a scale of 0-3, according to the method of Silness and Loe.[20]

3. Probing depth was recorded with a color-coded periodontal probe* to the nearest mm. measurement. Measurements were made from both the vestibular and oral surfaces at the mesial, distal and a point

midway between these.

4. Microbial content (percentage anaerobes, facultative and tetracycline resistnat organisms) at sites. (See Table 5) Prior to the sampling, supragingival deposits were removed from the isolated teeth with sterile gauze. Bacterial samples were obtained from subgingival sites with a sterile paper point (Coarse)** placed as far subgingivally as possible into the periodontal pocket, until resistance was met. The paper point was left in place for 10 seconds, at the end of which time, it was immediately placed in 1 ml of sterile thioglycolate. This was repeated with another paper point.

The subgingival deposits were displaced from the points and dispersed via vortex mixing for one minute. The bacterial suspension was serially diluted in 10-fold steps in dilution blanks with sterile saline. Three series of duplicate plates at the three dilutions were prepared for each sample. In addition to enriched blood agar, one of the series was supplemented with 1.0 ug/ml tetracycline hydrochloride. Aliquots of 0.1 ml from dilutions of 10^{-3}, 10^{-4} and 10^{-5} were placed on duplicate sets of growth media with sterile pipets. Plating was accomplished using a sterile bent rod. The plates were immediately placed in an anaerobic jar*** after which a vacuum was applied and an anaerobic grade gas was introduced. The vacuuming of the jar, followed by introduction of the anaerobic gas was done a minimum of three times (evacuation - replacement method). The anaerobic gas contained a mixture

*Nordent Mfg. Corp.
**Johnson & Johnson
***BBL

of 5% carbon dioxide, 10% hydrogen and 85% nitrogen (Union Carbide, New York). The plates were incubated for at least 72 hours at 37°C. The presence of an anaerobic environment was monitored with oxygen-sensitive indicator strips (BBL). The third group of plates was incubated under aerobic conditions at least 72 hours at 37° C. After 72 hour growth period, viable counts were recorded from plates containing between 30 and 300 colonies. These colonies were counted using a counting chamber with transmitted light and a magnifying lens.

The growth media used for isolation of bacterial colonies was prepared by combining 3% Todd Hewitt Broth (BBL), 0.5% Yeast extract (BBL), and 1.5% granulated agar (BBL). After autoclaving, the growth media was allowed to cool to 60°C in a water bath after which time 5% defibrinated sheep's blood*, 5.0 ug/ml hemin** and 0.5 ug/ml of menadione** was added.

*Ovine Laboratories, Chicago
**Syma Chemical Co., Mo.

CHAPTER IV

RESULTS

Table 5 shows the distribution of patients used in this study and the treatment regimen received. The mean pocket depths ranged from 5 mm to 9 mm. The gingival index ranged from 1.30 to 2.66 and the plaque index ranged from 0 to 2. Therefore, according to Table 8, all the test subjects had at least moderate inflammation or plaque accumulation. Table 5 shows three sites received placebo plus scaling, three sites received placebo only, two sites received tetracycline plus scaling and two sites received tetracycline only.

A. Clinical Parameters

The effect of the various treatment modalities on the gingival index is shown in Table 9. All patients with the exception of BR showed a reduction in the gingival index from the pretreatment period to Week 3 (two weeks post treatment). In the placebo group, the mean gingival index reduction was greater at the site that was scaled, from Day 0 to Week 3. In the tetracycline group, the mean gingival index reduction was also greater in the sites that received tetracycline in addition to scaling. From Week 3 to Week 12, there continued a reduction in the gingival index scores. A significant reduction is seen with patient PL receiving placebo plus scaling and patient SS receiving the same regimen. However, patient JK receiving placebo only, had a significant reduction from 2.66 to 0.50. Patient JC showed a greater reduction in the gingival index after tetracycline therapy, than after tetracycline plus scaling.

Patient BR showed an equal reduction regardless of the treatment rendered. When the mean scores for all patients are compared, there is a greater reduction in the gingival index of the sites treated with placebo alone compared to treatment with palcebo plus scaling from Week 3 to Week 12. There is also a greater reduction in the gingival index from Week 3 ro Week 12 in the sites receiving tetracycline alone when compared to the sites receiving both tetracycline and scaling. The mean gingival index scores increased from Week 12 to Week 24, for all sites except for the sites receiving tetracycline plus scaling. These sites showed a continued reduction. No return to the pretreatment gingival index scores was noted in any case.

Table 10 shows the changes in the plaque index scores over a 24 week period. All sites showed a reduction in that score from Day 0 to Week 3, with the exception of patient BR who was relatively constant throughout the 24 week period. When the mean scores are compared for all sites, the placebo group showed a similar reduction regardless if the site had been scaled or not and the tetracycline group showed a similar reduction from Day 0 to Week 3 regardless if the site had been scaled or not. From Week 3 to Week 12, there continued to be a reduction in the plaque index score; however, there was an increase in the plaque index score from Week 12 to Week 24 for most of the patients although it was still less than the pretreatment level.

Table 11 shows the changes in the pocket depths over a 24 week period. The sites receiving placebo plus scaling showed a sustained reduction over the 24 week period. The sites receiving placebo alone

either remained relatively the same or increased in depth. The sites receiving tetracycline plus scaling and those receiving tetracycline alone showed a sustained reduction in pocket depth throughout the 24 week period.

B. Microbiological Parameters

Table 12 shows the baseline values for the anaerobic, facultative and tetracycline resistant organisms. All patients and all sites showed a predominance of anaerobic organisms, when compared to a healthy individual (Table 13). The tetracycline-resistant-organisms varied from patient to patient and site to site. Table 14 shows the percentage of anaerobic organisms over the test period. The greatest reduction from Day 0 to Week 3 was seen in the sites receiving scaling as a treatment modality. The sites receiving placebo only showed an increase in the anaerobes, with the exception of patient JK, who showed 13.5% decrease in these organisms. Patient JC showed a slightly larger decrease (35% compared to 33.5%) in the percentage anaerobes when scaling was coupled with tetracycline therapy. All sites showed an increase in the percentage of anaerobes from Week 3 to Week 12 and there was a continued rise through the rest of the 24 week period. Table 15 shows the concomitant changes of the facultative organisms which inversely proportional to the anaerobes in Table 14.

Table 16 shows the changes in percentages of the tetracycline-resistant-organisms. The percentage increased significantly in three sites receiving tetracycline therapy but remained relatively constant in one site receiving tetracycline alone. When the means for all sites

are compared, the greatest increase in tetracycline resistant organisms occured in the sites treated by scaling and tetracycline therapy. There was a reduction in these organisms from Week 12 to Week 24 when comparing the means for all the sites.

Figures 4 - 13 were developed for each patient to help demonstrate the changes in the clinical and microbiological parameters over the 24 week period. The dramatic effect of scaling and/or tetracycline on these parameters can be seen from the baseline period to the third week.

Figures 14 - 17 are a composite according to the treatment rendered. It can be seen that regardless of treatment, there is an improvement in the clinical parameters when there is a reduction in the anaerobic bacteria.

CHAPTER V

DISCUSSION

From the results presented, it is apparent that at the treated diseased sites, the gingival index scores, the plaque index scores and the rpobing measurements were reduced from baseline values at the 3-, 12- and 24-week examination periods as a result of the treatment conditions, i.e. scaling, scaling plus tetracycline and tetracycline alone. When oral hygiene was the only treatment, as was the case with the sites treated by placebo only, there was a clinical improvement with regard to the gingival index and plaque index, but the pocket depths remained about the same or actually increased. The only time the pocket depths increased was when no treatment was rendered other than oral hygiene. Therefore, scaling and/or tetracycline therapy were probably responsible for the improvement in the clinical parameters.

The main improvement in gingival index and plaque index scores occures in the first 12 weeks. No significant clinical improvement was noted after the 12 week period in the sites receiving sclaing plus a placebo. The sites receiving scaling plus tetracycline showed a continued clinical improvement past the 12 week period. The sites receiving tetracycline therapy alone showed no clinical improvement past the 12 week period.

The changes in the relative percentages of the anaerobes and facultative is dramatic in those sites receiving scaling and/or tetracycline therapy, with the percentage of anaerobes varying inversely to

the percentage of facultative organisms. The facultative organisms increased with scaling alone or in combination with tetracycline. These organisms also increased when tetracycline was administered but not as dramatically as when tetracycline was combined with scaling.

An identical but reverse pattern was detected with respect to changes in the proportion of anaerobes, the proportions of which markedly reduced by scaling and/or tetracycline therapy. However, there was a rebound effect after 3 weeks where the anaerobes gradually returned to theri baseline values.

The results suggest that a microbial flora consistent with that observed at periodontally diseased sites can be shifted through treatment to one more typical of the flora observed at healthy sites.[87] One would expect to see changes in the microflora with a broad spectrum antibiotic, but it is interesting to note that similar but less dramatic changes can be seen with conventional therapy. This effect on the microflora lasted for nearly 12 weeks at which time the microflora approximated pretreatment levels.

It would appear that the microbial shifts precede the clinical alterations and in fact may cause the clinical alterations. As the microflora returns to the baseline values at three months, the clinical parameters also return toward baseline values. Both scaling and tetracycline, therefore, have a transient effect on the microflora and the clinical parameters described.

Tetracycline-resistant organisms were found in all the subjects prior to therapy, and the proportions increased immediately after

tetracycline therapy. Sites treated with placebo only showed tetracycline-resistant organisms and this would suggest a naturally occuring tetracycline-resistant population in the subgingival microflora.

A variable which must be taken into account when reviewing the microbiological data is the effect of the sampling on the populations of the microflora. Mousques, et al. showed that sampling has a minor effect on the microbial content of the pockets. In that experiment, a periodontal curette was used. However, in sampling the periodontal pocket, various potential problems arise. The sample must be removed so that it is not contaminated with microorganisms in the vicinity of the sample site. Perhaps it is necessary to quantify the amount of plaque removed.

Waerhaug in 1952, sterilized the tooth and gingival surfaces with glycerol and ethanol prior to sampling the contents of the gingival sulcus. He found that the sulcus was sterile when free of debris. Boyd[90] and Gavin[91] found that the glycerol-ethanol solution used by Waerhaug sterilized the gingival crevice and the solution penetrated into the crevice by capillary action. As a means to prevent contamination of the sample, Slots[92] exposed the sample site via a mucoperiosteal flap. The impracticality of this technique is obvious. Listgarten and Hellden[87] used a sterile curette to retrieve microbial samples and this became a popular technique but with the inherent problem that primarily adherent plaque was sampled and this may not be entirely representative. Finally, Slots, et al.[81] used sterile paper points to sample pockets. The authors believed that this technique allowed for standardization of

the microbial flora from the entire pocket. The use of sterile paper points was used in this study for that reason.

The use of tetracycline in periodontitis was discussed in Chapter 2. Chow, et al.[93] showed that tetracycline inhibited 36% Bacteroides fragilis, 57% Bacteroides melaninogenicus, 60% Bacteroides corrodens, 68% Fusobacterium, 67% Peptostreptococcus, 67% Actinomyces at 6.25 ug/ml (blood concentration after therapeutic dose). It is because the tetracyclines are more effective against anaerobic bacteria that they have a real value in periodontal infections.

A problem that arises with the tetracyclines is that resistant strains do develop and it was seen in this study that all the patient's studied had resistant strains. There are three principal means by which bacteria may be resistant to antimicrobial agents:[72]

1. Alternation of the target (ribobome) so that it is no longer susceptible to the action of the drug.
2. Production of an enzyme which degrades the antibiotic.
3. A change in the permeability of the bacterium to the agent.

Acquired resistance to the tetracycline appears to be due to the second or third of the above mechanisms.[94]

Tetracycline resistance has been wide-spread among the Bacteroides fragilis group. It has been shown that its resistance is transferable to susceptible strains. In gram-negative facultative anaerobes, tetracycline resistance is generally an inducible property.[95] Therefore, care must be taken when using the tetracyclines.

Therefore, does tetracycline have a value in treating periodon-

tal disease considering the potential problems associated with this drug? This study showed that scaling alone, scaling in combination with tetracycline and tetracycline alone changed the microflora to that associated with health, but this was only a transient effect. Slots, et al.[81] reported that two of the patients studied did not respond to conventional therapy, in that there was little or only moderate changes in the subgingival flora and in the clinical measurement. There was, however, marked changes when these patients were administered tetracycline. This may be the real value of the antimicrobial agents.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Five patients were randomly assigned to two groups. One group received tetracycline and the other group received a placebo, both for a 14 day period. The tetracycline (Achromycin V) was administered orally in the dosage of 250mg four times a day. Within each group, the patient had one tooth scaled while the contralateral tooth was left untouched. After two baseline examinations of Gingival Index, Plaque Index, pocket depth and the microflora, examinations were made at week 3, week 12 and week 24.

As a result of this study, the following conclusions can be made:

1. After one session of scaling and root planing alone, there was an improvement in the clinical parameters over a 12-week period. This trend reversed this time and continued through the 24th week. The percent microorganisms changed to that more compatible with health, but after the three week period, the composition reversed to baseline values.

2. After one session of scaling and root planing plus tetracycline administration, there was an improvement in the clinical parameters and this was sustained throughout the 24-week period. The percent microorganism change was similar to scaling and root planing alone, but the percent tetracycline-resistant organisms increased to a greater extent.

3. Sites receiving tetracycline alone showed a deterioration of the clinical parameters from the 12- through 24th week. The percent change in microorganisms was not as great as the sites that also received

scaling and root planing.

4. The site receiving placebo alone showed an improvement in the clinical parameters which may be due to the improvement in oral hygiene technique. The anaerobic microorganisms remained predominant and their percentage increased over the 24-week period.

Table 1. Organisms of the Human Gingival Crevice Region*

Group	Approximate Percentage of Cultivalbe Microbiota	Genera and/or Species Commonly Found in this Site
Gram-positive facultative cocci	28.8	Staphylococci Enterococci *S. mutans* *S. sanguis* "*S. mitis*"
Gram-positive anaerobic cocci	7.4	*Peptostreptococcus*
Gram-positive facultative rods	15.3	*Corynebacterium* *Lactobacillus* *Nocardia* *O. viscosus* *B. matruchotti*
Gram-positive anaerobic rods	20.2	*A. bifidus* *A. israelii* *A. naeslundii* *A. odontolyticus* *P. acnes* *L. buccalis* Corynebacterium
Gram-negative facultative cocci	0.4	Neisseria
Gram-negative anaerobic cocci	10.7	*V. alcalescens* *V. parvula*
Gram-negative facultative rods	1.2	
Gram-negative anaerobic rods	16.1	*B. melaninogenicus* *B. oralis* *V. sputorum* *F. nucleatum* *S. sputigenum*
Spiral organisms	1 to 3	*T. denticola* *T. oralis* *T. macrodentium* *B. vincentii*

*Adpated from Socransky, J. Dent. Res., 1970

Table 2. Distribution of Subgingival Organisms (X) in Health and Various Forms of Periodontal Disease*

	HEALTHY		GINGIVITIS		PERIODONTITIS				
Gram-positive organisms:	Aged	Healthy	Gingivitis	ANUG	Incipient	Advanced	Rapid	Abscess	Juvenile
Streptococcus	X	X	X		X	X	X	X	X
Staphylococcus									
Actinomyces	X	X	X		X	X	X	X	X
Rothia, Arachnia									
Peptococcus	X	X	X		X	X	X	X	X
Peptostreptococcus	X	X	X		X	X	X	X	X
Propionibacterium	X	X	X		X	X	X	X	X
Other	X	X	X	X	X	X	X	X	X
Gram-negative organisms:									
Veillonella	X	X	X		X	X	X	X	X
Eikenella	X	X	X				X	X	X
Capnocytophaga	X	X	X		X	X	X	X	X
Bacteroides Melaninogenicus	X		X	X	X	X	X	X	X
Bacteroides	X	X	X	X	X	X	X	X	X
Fusobacterium	X	X	X	X	X	X	X	X	X
Leptotrichia					X				X
Selenomonas			X	X		X	X	X	X
Campylobacter			X	X	X	X	X	X	X
"Vibrio-corroding"	X	X	X		X	X	X	X	X
Unidentified	X	X	X	X	X	X	X	X	X
Spirochetes	X	X	X	X	X	X	X	X	X

*Adapted from Grant, D.A.; Stern, I.B.; Everett, F.G. Periodontics, The C.V. Mosby Co., St. Louis, 1979.

Table 3. Side Effects and Toxicities of Tetracycline*

Teeth	Permanent discoloration, dysgenesis due to administration of tetracycline during last half of pregnancy or first 6 years of life.
Bone	Possible retardation of growth and development - may be transient.
Gastrointestinal Tract	Overgrowth with monilial microorganisms has been reported on a number of occasions in conjunction with tetracycline therapy. However, some articles question this statement. Alteration in absorption of vitamin K may occur leading to inadequate formation of prothrombin-bleeding problems may follow.
Liver	Lethal hepatic toxicity has been reported in conjunction with use in pregnancy, shock and sepsis. Abnormal liver function tests have been reported.
Blood Urea Nitrogen	Elevation of blood urea nitrogen has been reported and appears to occur mainly in patients taking diuretics or presenting initially with a high blood urea nitrogen. Nausea, vomiting and the sequelae are associated with this rise.
Renal	Azotemia. Also, renal disorders have been reported following administration during pregnancy. A Fanconi type syndrome has been associated with the use of outdated or degraded tetracycline. Therefore, they should be stored, until their expiration date, away from UV light sources, moisture and in a sealed container. Nephrogenic diabetes insipidus has been reported in conjunction with administration of demethylchlortetracycline.
Vertigo	Reported with the use of minocycline.
Teratogenesis	The literature suggests that these agents are potential teratogens, and have resulted in amlformed hands and limbs. Do not use in females in the childbearing age range who have missed one or more menstrual periods.
Skin	Photosensitivity (especially with demethylchlortetracycline), rash, oncholysis.

* Adapted from Ciancio, Journal of Periodontol., 1976.

Table 4. Description of Patients and Selected Sites

Patient	Age	Race	Sex	Periodontal Pocket Site	Probing Depth (mm)	GI	PI	Therapy
JC	25	C	M	19 MB*	5	1.60	1.25	Scaling and root planing & TTC **
				30 BF*	9	1.30	1.40	TTC only
PL	25	C	F	21 MB	8	2.00	1.75	Scaling and root planing & placebo
				28 MB	7	2.00	1.88	Placebo only
JK	26	C	M	19 MB	7	1.83	1.75	Scaling and root planing & placebo
				30 MB	6	2.66	1.75	Placebo only
BR	29	C	F	19 MB	5	1.33	0.00	Scaling and root planing & TTC
				30 DB*	6	1.50	0.00	TTC only
SS	31	C	F	3 MB	9	2.00	2.00	Scaling and root planing & placebo
				14 MB	9	2.00	2.00	Placebo only

*MB refers to mesial buccal; BF refers to buccal furcation; DB refers to distal buccal
**TTC refers to tetracycline

Table 5. Criteria for the Gingival Index*

Score	Description
0	Absence of inflammation
1	Mild inflammation; slight change in color and little change in texture.
2	Moderate inflammation; moderate galzing, redness, edema and hypertrophy. Bleeding on pressure.
3	Severe inflammation; marked redness and hypertrophy. Tendency to spontaneous bleeding. Ulceration

*Adapted from Loe and Silness, Acta Odontol. Scand., 1963.

Table 6. Criteria for the Plaque Index**

Score	Description
0	No plaque.
1	A film of plaque adhering to the free gingival margin and adjacent areas of the tooth. The plaque may be seen in site only after the application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket or on the tooth and gingival margin which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and/or the tooth and gingival margin.

**Adapted from Silness and Loe, Acta Odontol. Scand., 1964.

Table 7. Correlation of Mouth Scores to Degree of Inflammation and Plaque Accumulation*

Mouth Scores (GI or PI)	Description
0.1 - 1.0	Mild inflammation or plaque accumulation
1.1 - 2.0	Moderate inflammation or plaque accumulation
2.1 - 3.0	Severe inflammation or plaque accumulation

*Adapted from Silness and Loe, Acta Odontol. Scand., 1964.

Table 8. Gingival Index Scores Over a 24 Week Period

Subject & Treatment	Day 0*	Week 3	Week 12	Week 24
PL				
1. P_1S_1	2.00	1.00	0.16	1.16
2. P_1S_0	2.00	1.00	o.60	1.60
JK				
1. P_1S_1	1.83	0.66	0.50	0.66
2. P_1S_0	2.66	2.66	0.50	0.66
SS				
1. P_1S_1	2.00	1.00	0.00	1.00
2. P_1S_0	2.00	1.33	0.50	1.00
JC				
1. T_1S_1	1.60	1.00	0.60	1.00
2. T_1S_0	1.30	1.00	0.20	1.00
BR				
1. T_1S_1	1.33	1.00	0.50	0.00
2. T_1S_0	1.50	1.50	1.00	1.16
$\overline{X}$ P_1S_1	1.94	0.88	0.22	0.94
$\overline{X}$ P_1S_0	2.22	1.66	0.53	1.08
$\overline{X}$ T_1S_1	1.46	1.00	0.55	0.50
$\overline{X}$ T_1S_0	1.40	1.25	0.60	1.30

* mean of two scores taken at one week intervals

$\overline{X}$ mean

P_1 Placebo

S_1 Scaled

T_1 Tetracycline

Table 9. Plaque Index Scores Over a 24 Week Period

Subject & Treatment	Day 0*	Week 3	Week 12	Week 24
PL				
1. P_1S_1	1.75	1.00	0.00	1.00
2. P_1S_0	1.87	1.00	0.00	1.00
JK				
1. P_1S_1	1.75	1.00	0.00	1.00
2. P_1S_0	1.75	1.16	0.00	1.00
SS				
1. P_1S_1	2.00	1.00	0.25	1.00
2. P_1S_0	2.00	1.00	0.00	0.50
JC				
1. T_1S_1	1.25	1.00	0.25	0.00
2. T_1S_0	1.37	1.00	0.00	0.25
BR				
1. T_1S_1	0.00	0.00	0.00	0.00
2. T_1S_0	0.00	0.00	0.00	0.25
$\bar{X}$ P_1S_1	1.83	1.00	0.08	1.00
$\bar{X}$ P_1S_0	1.87	1.04	0.00	0.83
$\bar{X}$ T_1S_1	0.62	0.50	0.12	0.00
$\bar{X}$ T_1S_0	0.68	0.50	0.00	0.12

* mean of two scores taken at one week intervals
$\bar{X}$ mean
P_1 Placebo
S_1 Scaled
T_1 Tetracycline

Table 10. Pocket Depths Over a 24 Week Period

Subject & Treatment	Day 0*	Week 3	Week 12	Week 24
PL				
1. P_1S_1	7.00	5.30	4.60	5.50
2. P_1S_0	6.30	5.60	5.60	6.80
JK				
1. P_1S_1	5.00	3.16	3.00	3.66
2. P_1S_0	4.83	4.50	4.00	4.50
SS				
1. P_1S_1	7.33	5.50	4.66	5.50
2. P_1S_0	7.33	7.33	7.66	7.83
JC				
1. T_1S_1	4.60	3.50	3.60	3.30
2. T_1S_0	6.50	5.00	5.20	5.20
BR				
1. T_1S_1	4.16	3.16	3.16	2.66
2. T_1S_0	5.00	4.16	4.16	4.33
$\bar{X}$ P_1S_1	6.44	4.65	4.08	4.88
$\bar{X}$ P_1S_0	6.15	5.81	5.75	6.37
$\bar{X}$ T_1S_1	4.30	3.33	3.38	2.98
$\bar{X}$ T_1S_0	5.75	4.58	4.68	4.76

* mean of two scores taken at one week intervals
$\bar{X}$ mean
P_1 Placebo
S_1 Scaled
T_1 Tetracycline

Table 11. Distribution of Subgingival Microflora In 5 Patients with Advanced Periodontal Disease. Baseline Values Obtained at One Week Intervals. (Percentage)

Subject		Anaerobes		Facultative		Tetracycline Resistant	
	Site:	#1	#2	#1	#2	#1	#2
JC	Sample 1	73	65	27	35	31	23
	Sample 2	65	66	35	34	27	47
PL	Sample 1	80	68	20	32	35	38
	Sample 2	73	63	27	37	30	49
JK	Sample 1	52	73	48	27	16	14
	Sample 2	60	64	40	36	26	20
BR	Sample 1	79	72	21	28	4	43
	Sample 2	68	69	32	31	5	50
SS	Sample 1	99	68	1	32	3	36
	Sample 2	99	58	1	42	2	46
$\overline{X}$		70.7		29.3		27.2	

$\overline{X}$ mean

Table 12. Distribution of the Subgingival Microflora In a Healthy Patient. (Percentage)

		Anaerobes		Facultative		Tetracycline Resistant	
Sample	Site	#1	#2	#1	#2	#1	#2
#1		25	15	75	85	14	5
#2		31	17	69	83	5	7
$\overline{X}$		22.0		78.0		7.75	

$\overline{X}$ mean

Table 13. Percentage of Anaerobic Bacteria Over a 24 Week Period

Subject & Treatment	Day 0*	Week 3	Week 12	Week 24
PL				
1. P_1S_1	76.5	34.0	71.0	76.0
2. P_1S_0	65.5	70.0	77.0	75.0
JK				
1. P_1S_1	56.0	30.0	56.0	84.0
2. P_1S_0	68.5	55.0	58.0	74.0
SS				
1. P_1S_1	99.0	90.0	92.0	96.0
2. P_1S_0	63.0	84.0	86.0	99.0
JC				
1. T_1S_1	69.0	34.0	73.0	78.0
2. T_1S_0	65.5	32.0	78.0	72.0
BR				
1. T_1S_1	73.5	30.0	45.0	62.0
2. T_1S_0	70.5	55.0	57.0	74.0
$\overline{X}$ P_1S_1	77.1	51.3	73.0	85.3
$\overline{X}$ P_1S_0	65.6	69.6	73.6	82.6
$\overline{X}$ T_1S_1	71.2	32.0	59.0	70.0
$\overline{X}$ T_1S_0	68.0	43.5	67.5	73.0

* mean of two scores taken at one week intervals
$\overline{X}$ mean
P_1 Placebo
S_1 Scaled
T_1 Tetracycline

Table 14. Percentage of Facultative Bacteria Over a 24 Week Period

Subject & Treatment	Day 0*	Week 3	Week 12	Week 24
PL				
1. P_1S_1	23.5	66.0	29.0	24.0
2. P_1S_0	34.5	30.0	23.0	25.0
JK				
1. P_1S_1	44.0	70.0	44.0	16.0
2. P_1S_0	31.5	45.0	42.0	26.0
SS				
1. P_1S_1	1.00	10.0	8.00	4.00
2. P_1S_0	37.0	16.0	14.0	1.00
JC				
1. T_1S_1	31.0	66.0	26.0	22.0
2. T_1S_0	34.5	68.0	27.0	28.0
BR				
1. T_1S_1	26.5	70.0	55.0	38.0
2. T_1S_0	29.5	45.0	43.0	26.0
$\bar{X}$ P_1S_1	22.8	48.6	27.0	14.6
$\bar{X}$ P_1S_0	34.3	30.3	26.3	17.3
$\bar{X}$ T_1S_1	28.7	68.0	40.5	30.0
$\bar{X}$ T_1S_0	32.0	56.5	32.5	32.0

* mean of two scores taken at one week intervals
$\bar{X}$ mean
P_1 Placebo
S_1 Scaled
T_1 Tetracycline

Table 15. Percentage of Tetracycline - Resistant Bacteria Over a 24 Week Period

Subject & Treatment	Day 0*	Week 3	Week 12	Week 24
PL				
1. P_1S_1	32.5	22.0	27.0	18.0
2. P_1S_0	43.5	46.0	49.0	37.0
JK				
1. P_1S_1	21.0	5.00	21.0	16.0
2. P_1S_0	17.0	49.0	16.0	16.0
SS				
1. P_1S_1	2.50	15.0	8.00	10.0
2. P_1S_0	41.0	35.0	28.0	1.0
JC				
1. T_1S_1	29.0	64.0	59.0	25.0
2. T_1S_0	35.0	64.0	47.0	31.0
BR				
1. T_1S_1	4.50	23.0	23.0	31.0
2. T_1S_0	46.5	47.0	51.0	42.0
$\overline{X}$ P_1S_1	18.6	14.0	18.6	14.6
$\overline{X}$ P_1S_0	33.8	43.3	31.0	18.0
$\overline{X}$ T_1S_1	16.7	43.5	41.0	28.0
$\overline{X}$ T_1S_0	40.7	55.5	49.0	36.5

* mean of two scores taken at one week intervals
$\overline{X}$ mean
P_1 Placebo
S_1 Scaled
T_1 Tetracycline

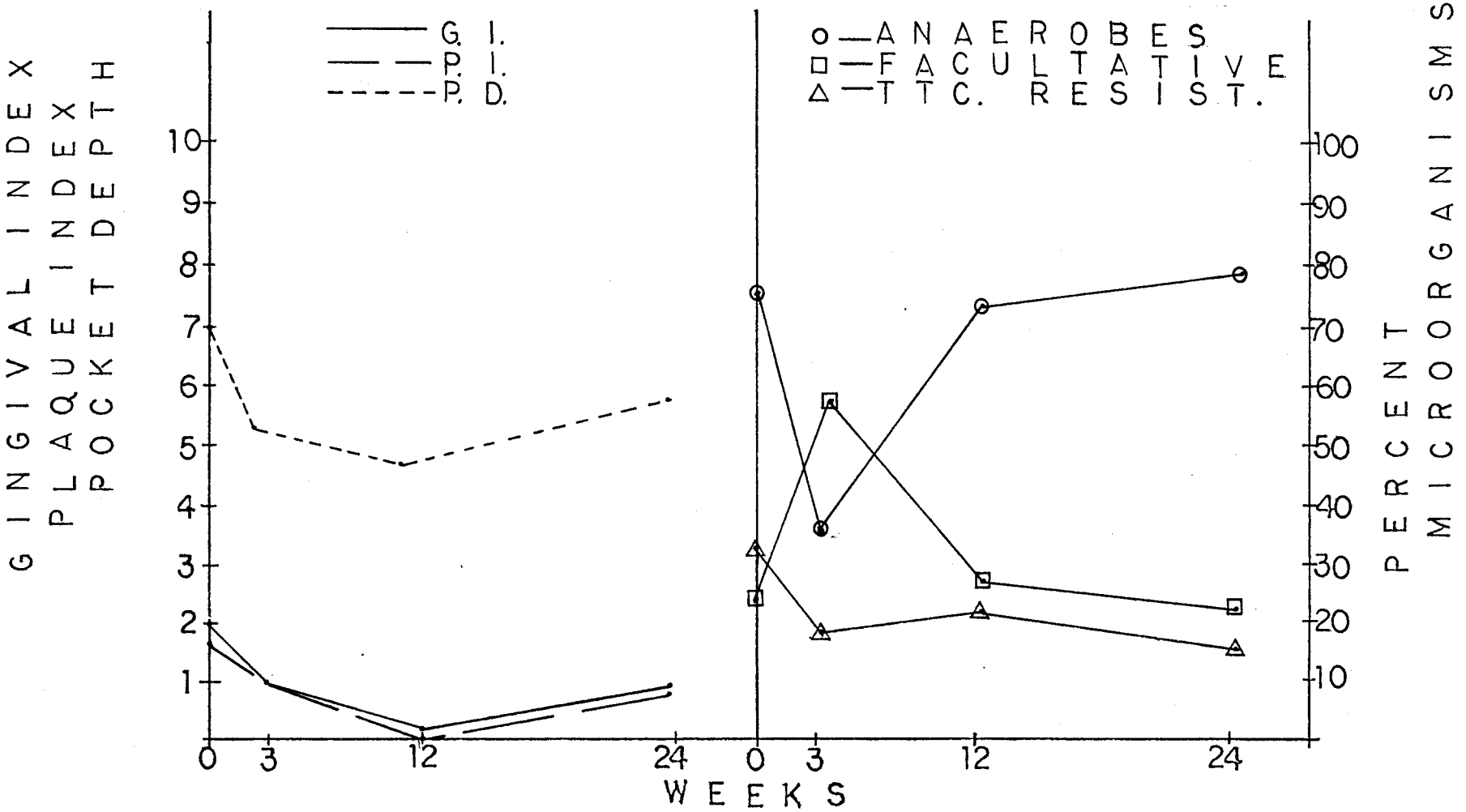


Figure 1. Patient PL. The effect of scaling and root planing plus a placebo on various clinical parameters and on the subgingival microflora over a 24 week period.

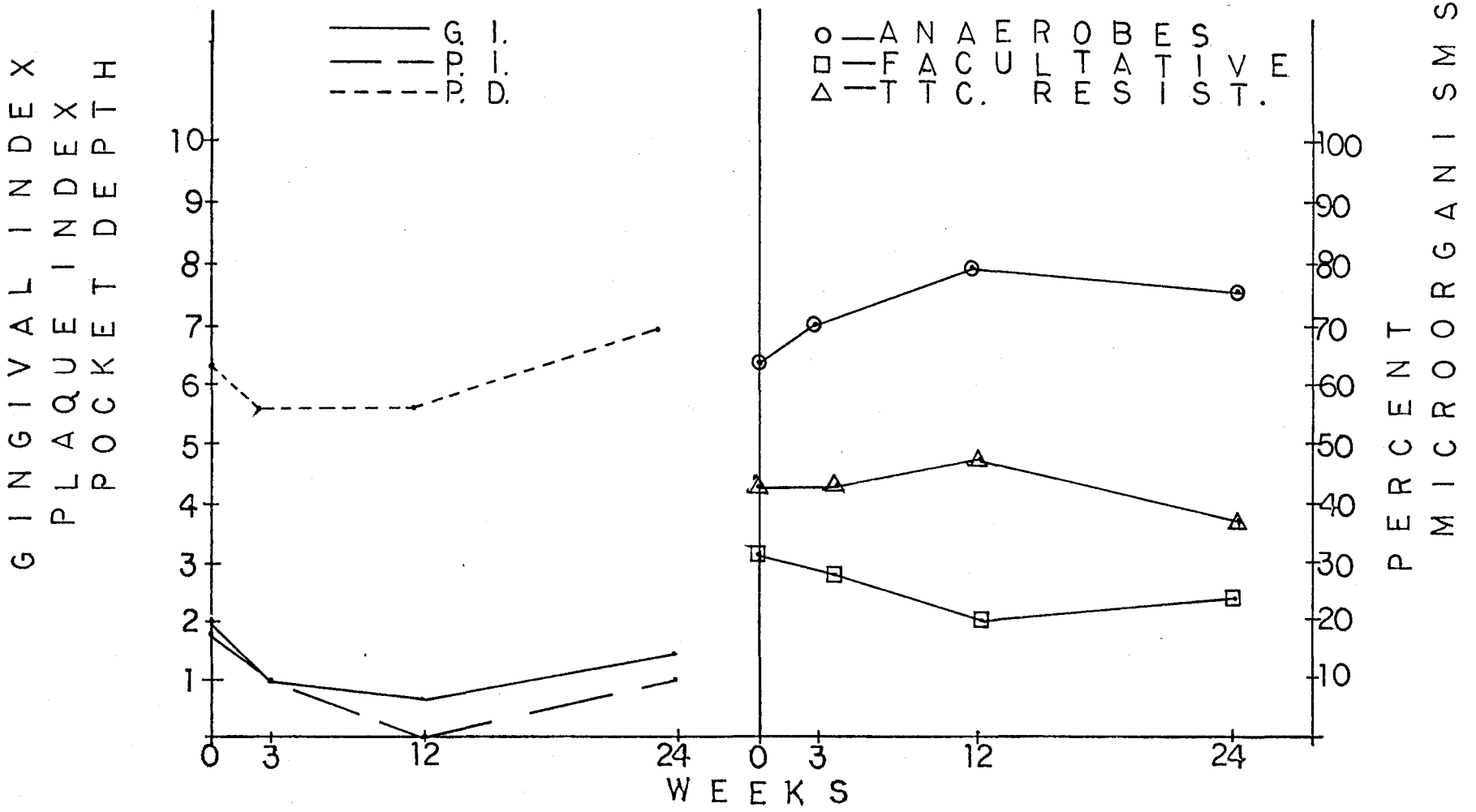


Figure 2. Patient PL. The effect of a placebo only on various clinical parameters and on the subgingival microflora over a 24 week period.

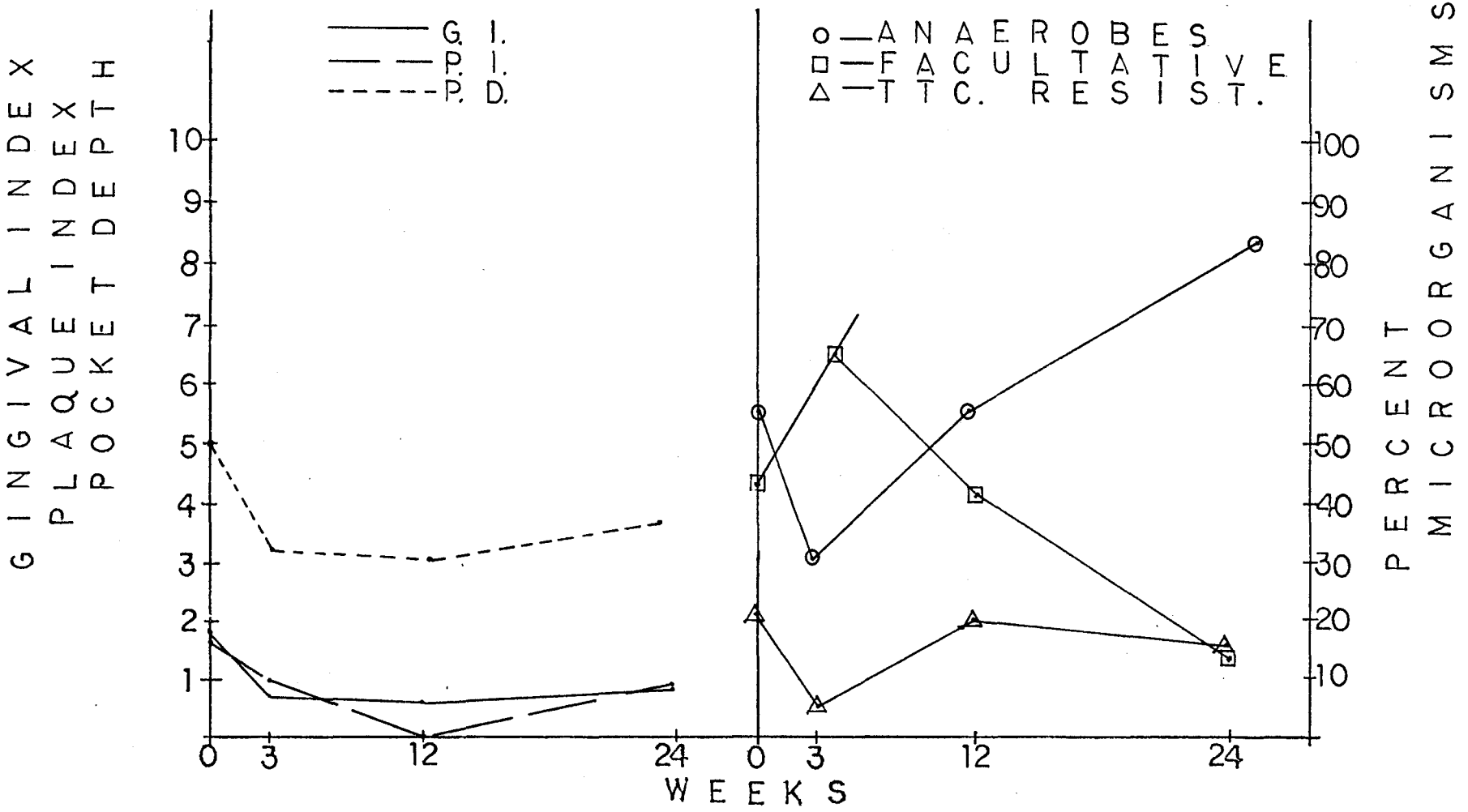


Figure 3. Patient JK. The effect of scaling and root planing plus a placebo on various clinical parameters and on the subgingival microflora over a 24 week period.

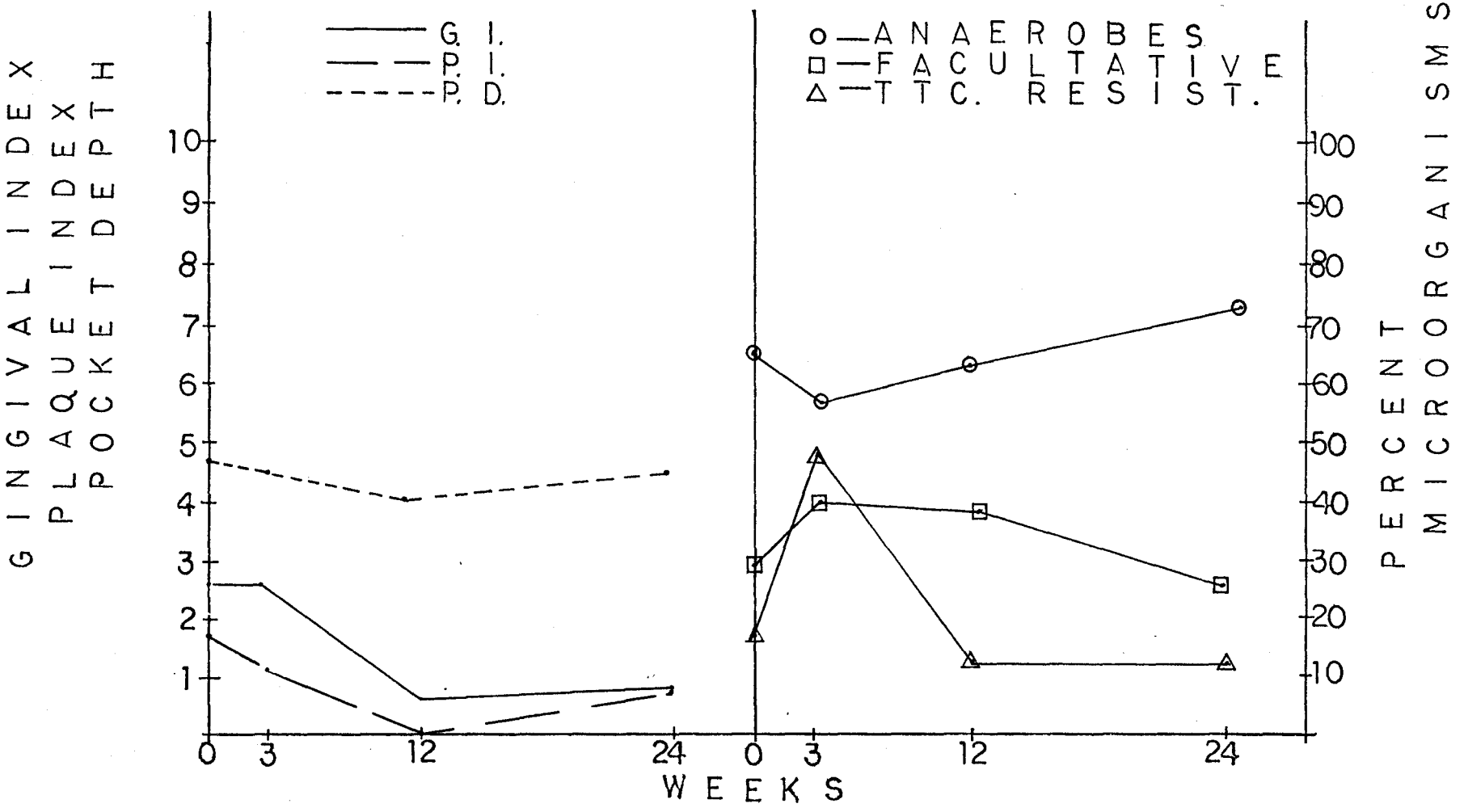


Figure 4. Patient JK. The effect of a placebo only on various clinical parameters and on the subgingival microflora over a 24 week period.

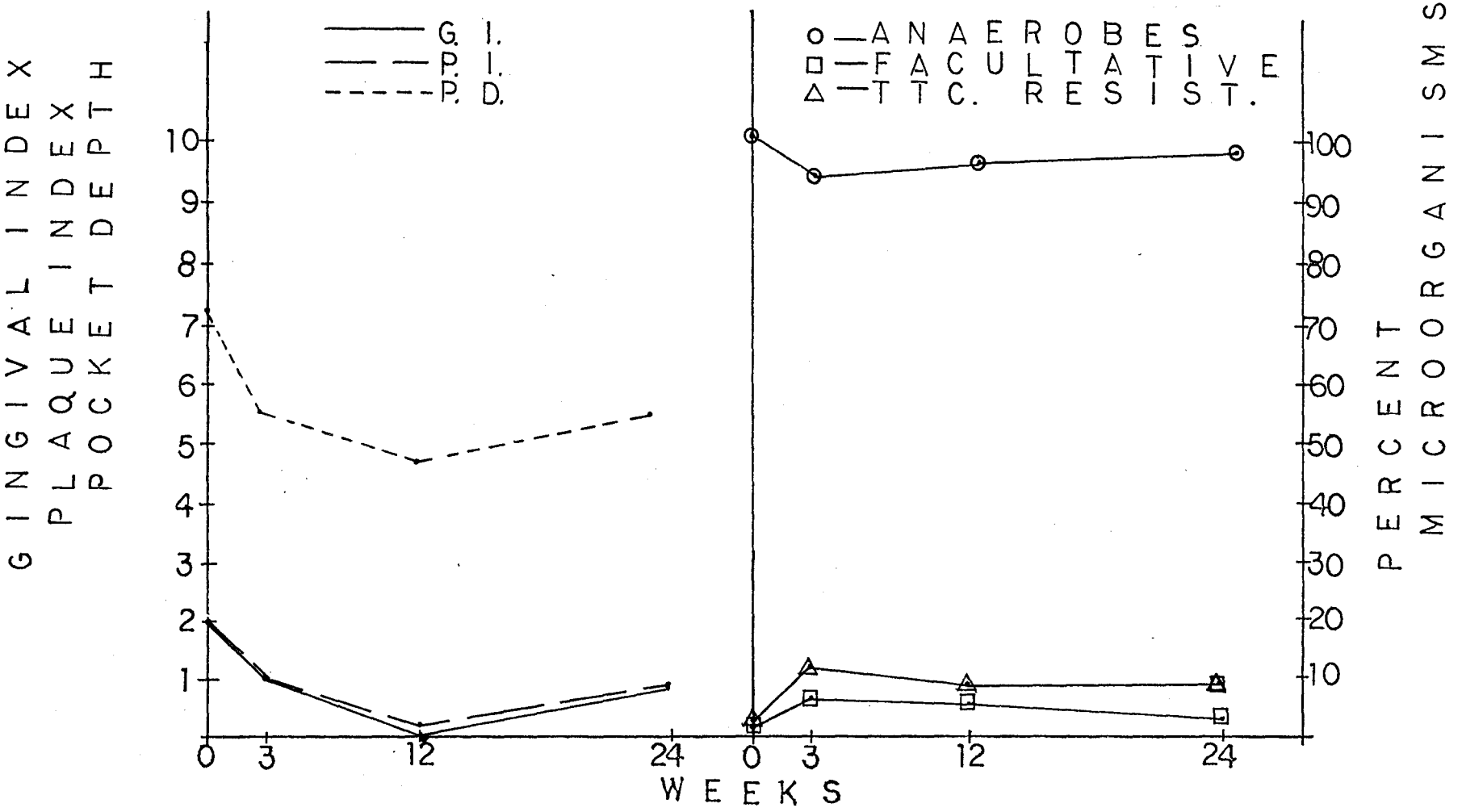


Figure 5. Patient SS. The effect of scaling and root planing plus a placebo on various clinical parameters and on the subgingival microflora over a 24 week period.

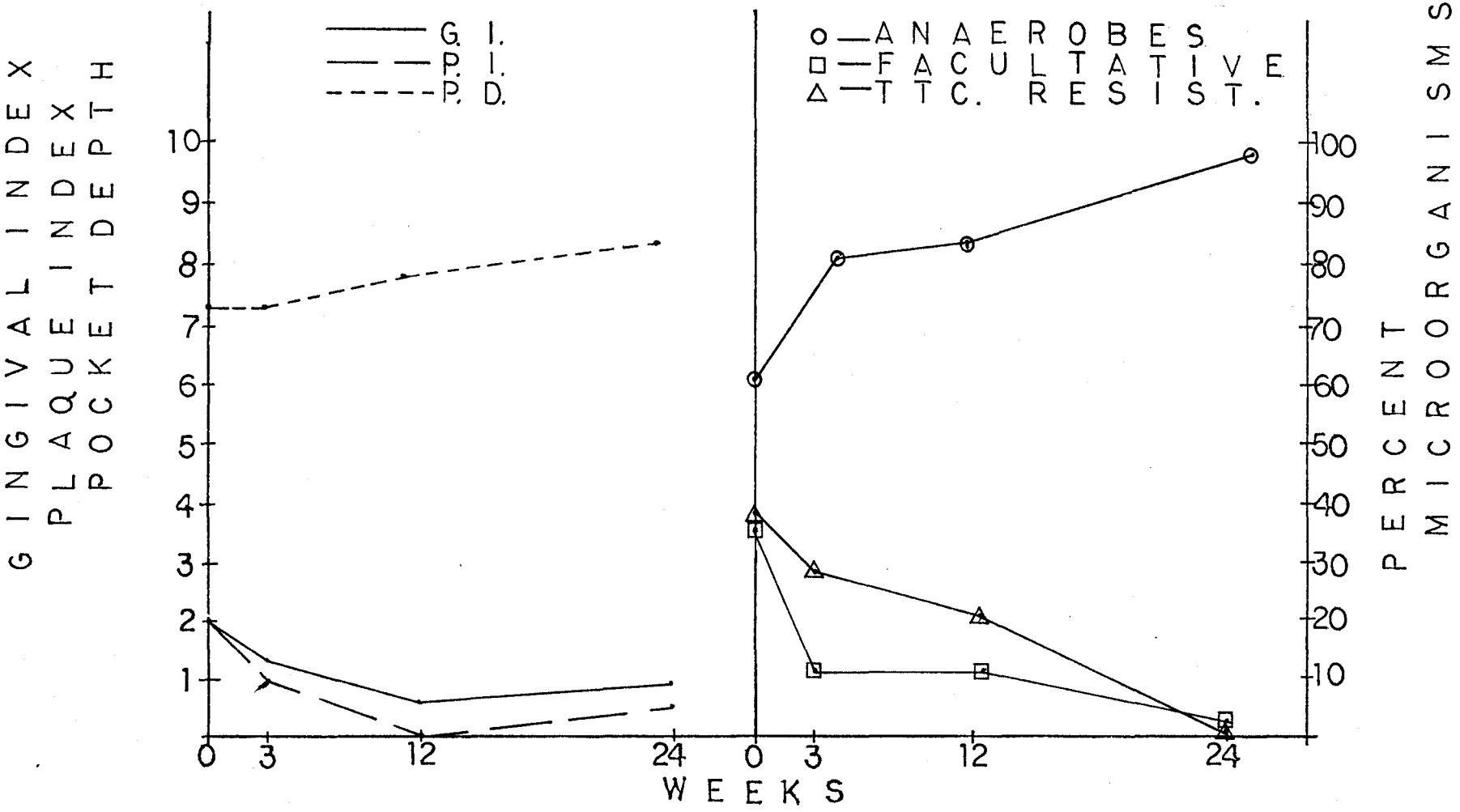


Figure 6. Patient SS. The effect of a placebo only on various clinical parameters and on the subgingival microflora over a 24 week period.

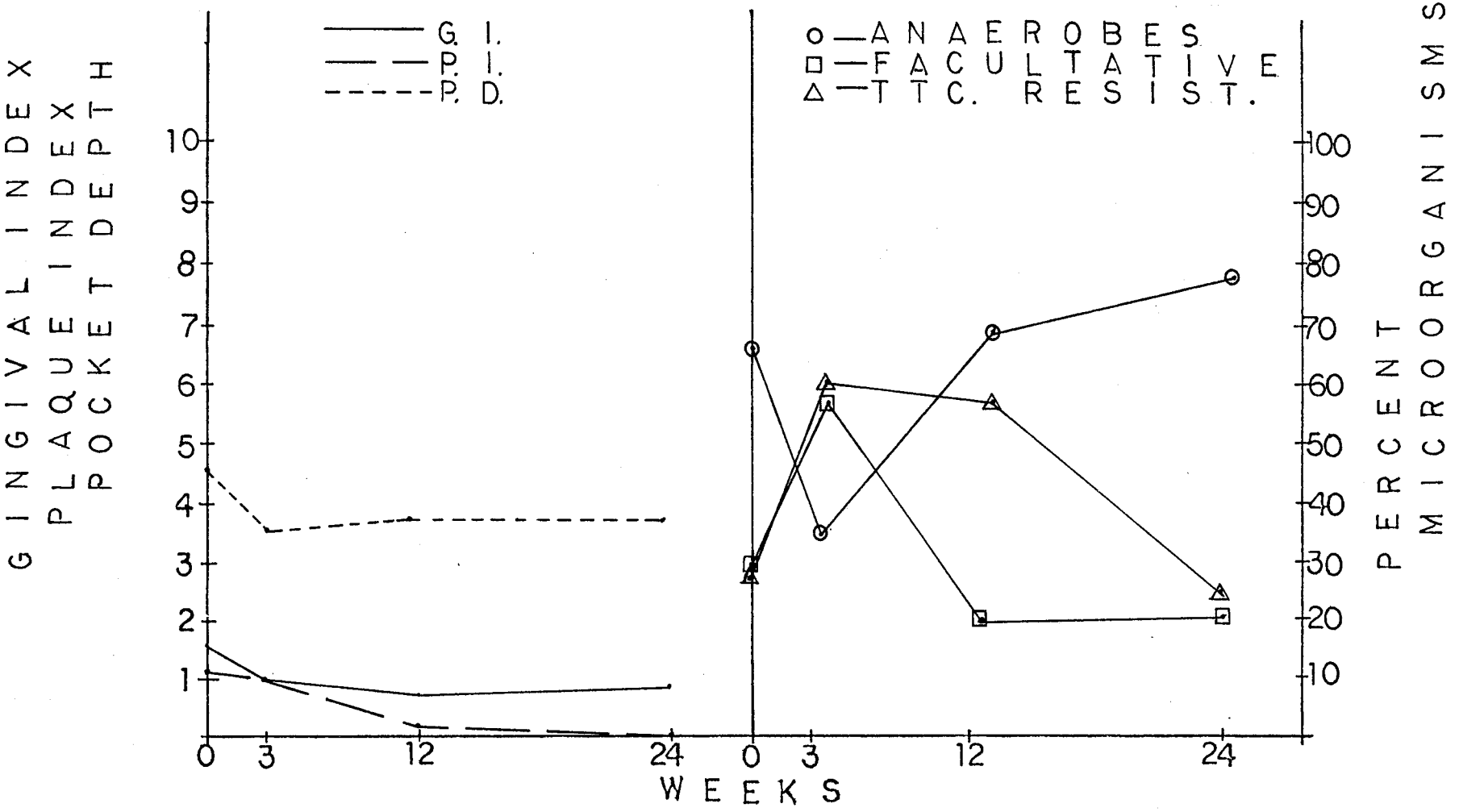


Figure 7. Patient JC. The effect of scaling and root planing plus tetracycline on various clinical parameters and on the subgingival microflora over a 24 week period.

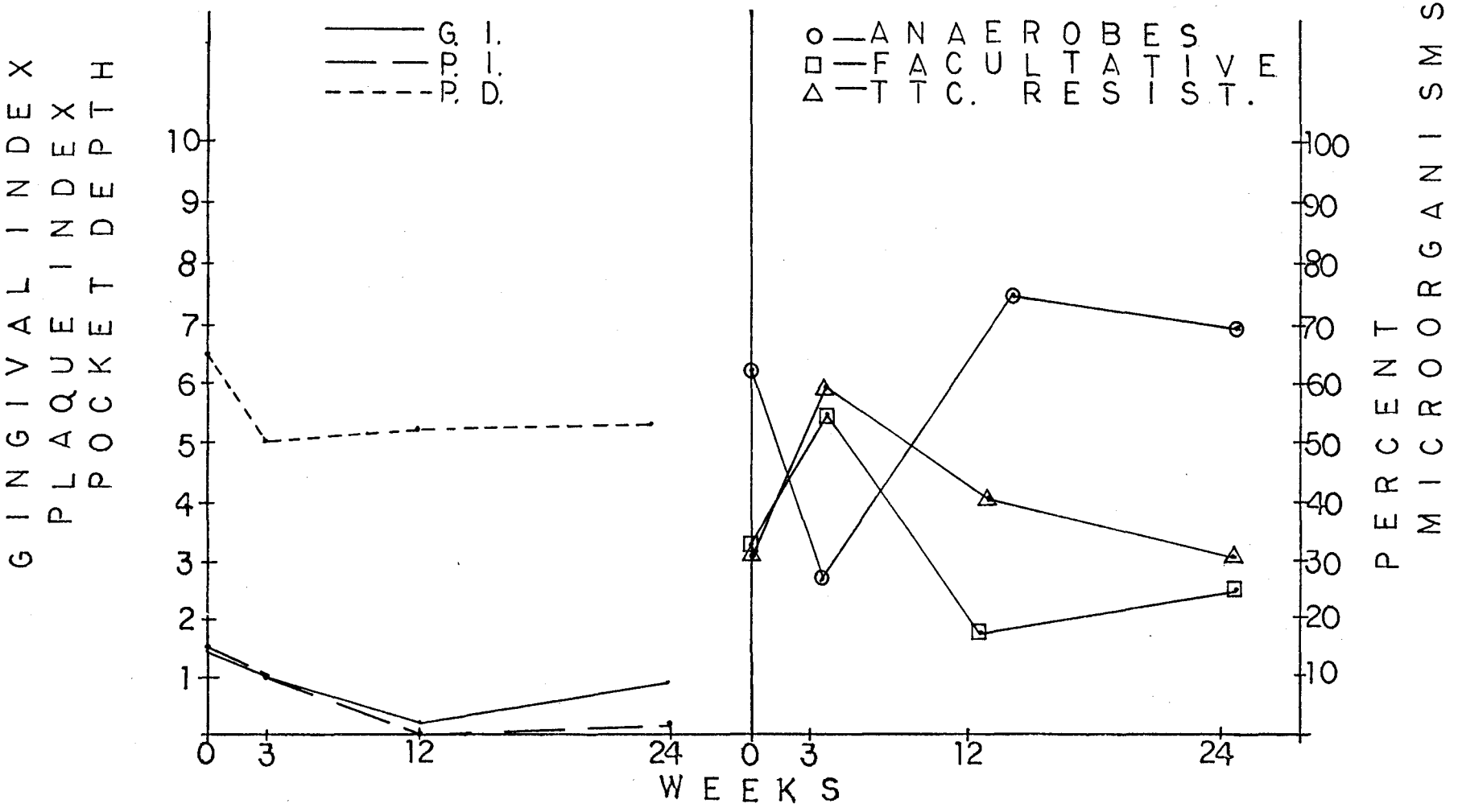


Figure 8. Patient JC. The effect of tetracycline on various clinical parameters and on the subgingival microflora over a 24 week period.

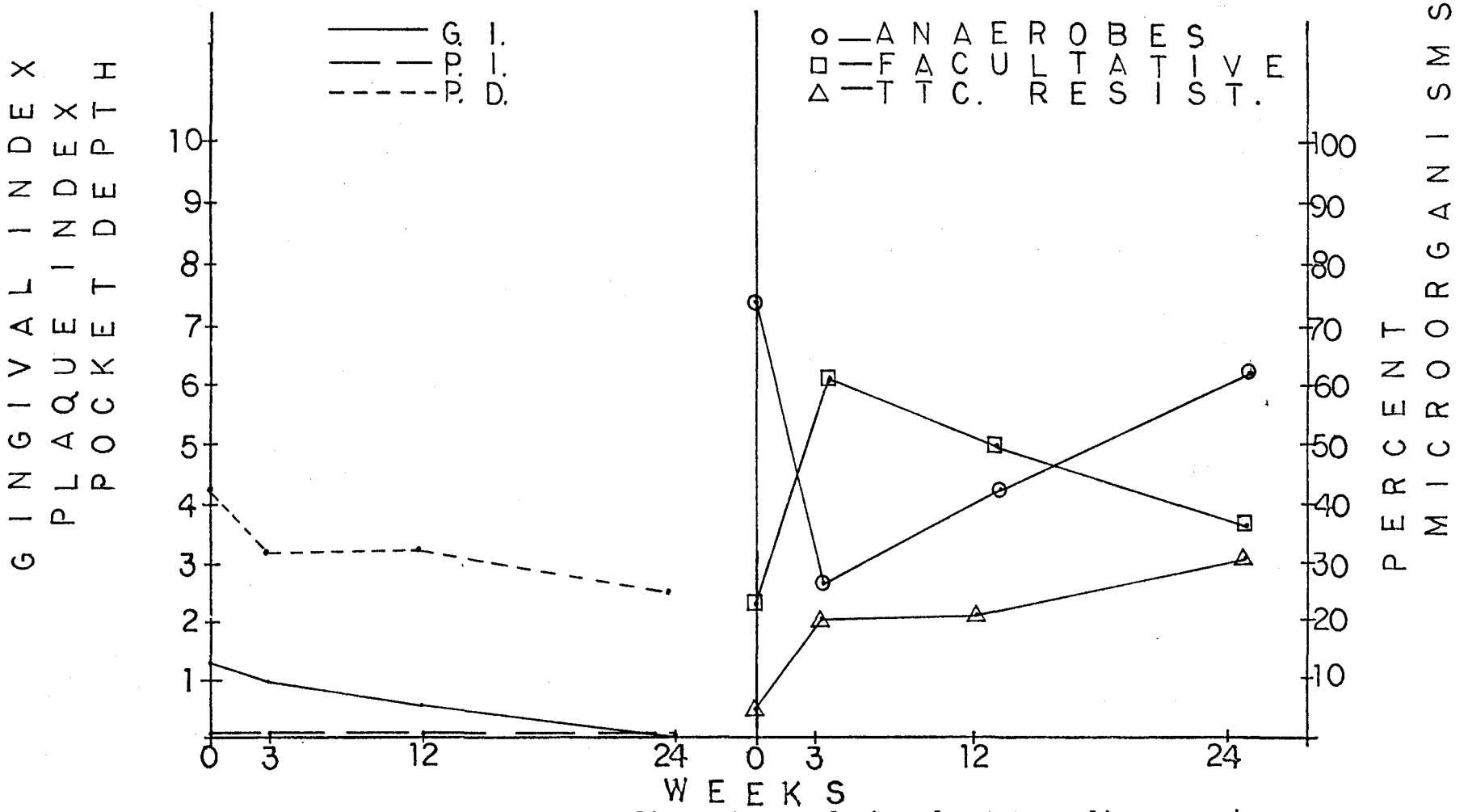


Figure 9. Patient BR. The effect of scaling and root planing plus tetracycline on various clinical parameters and on the subgingival microflora over a 24 week period.

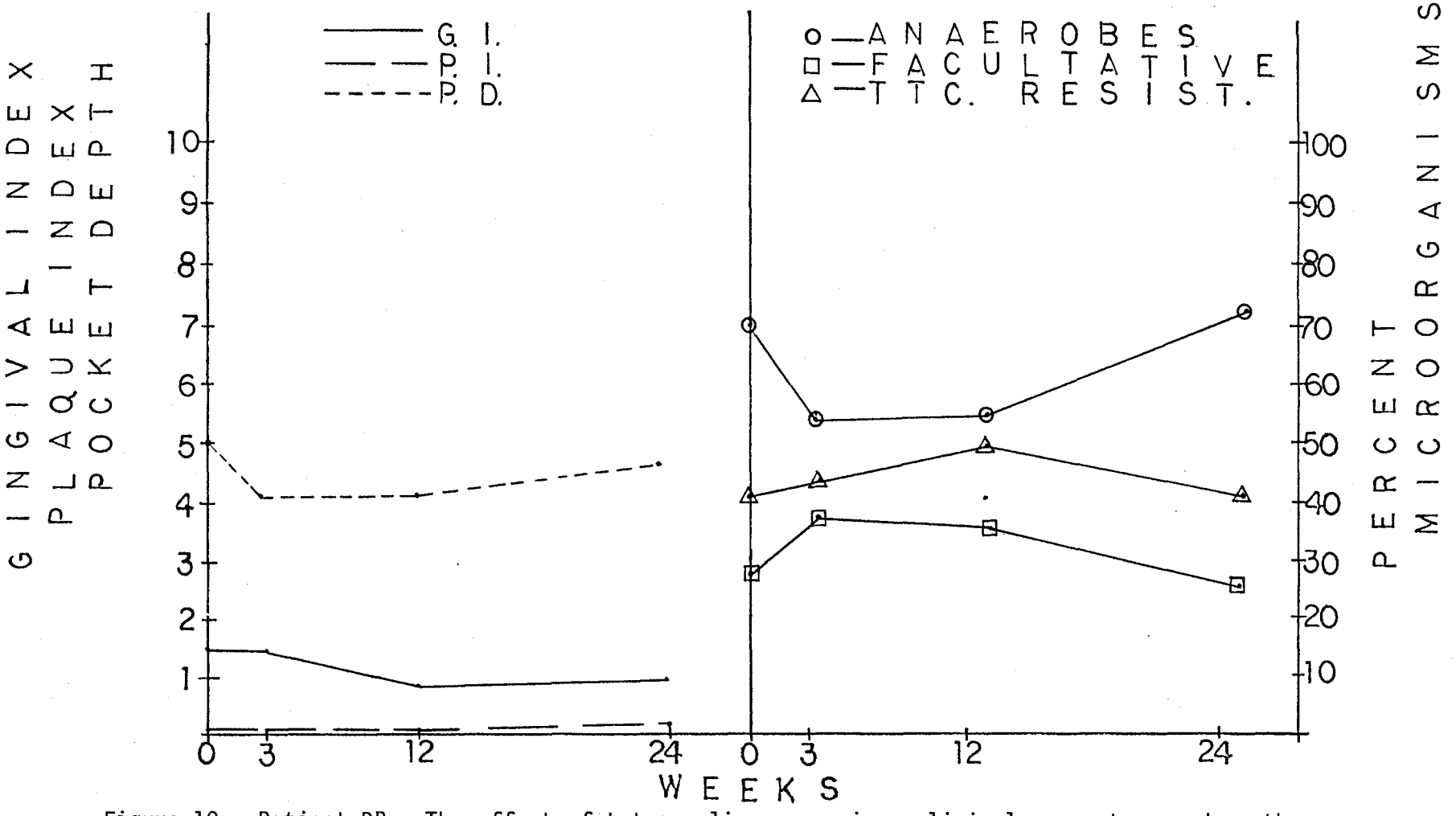


Figure 10. Patient BR. The effect of tetracycline on various clinical parameters and on the subgingival microflora over a 24 week period.

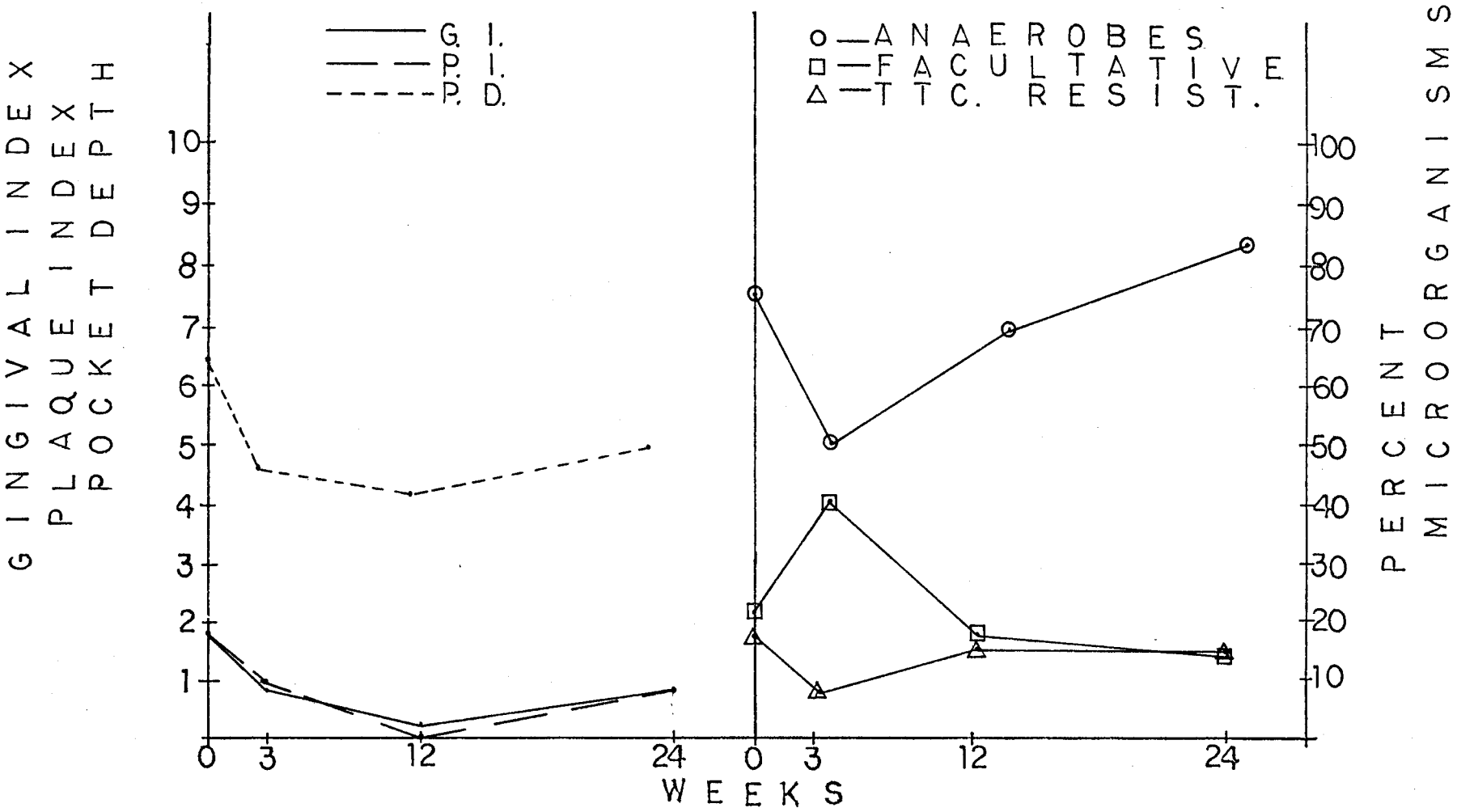


Figure 11. Composite graph of patients PL, JK and SS. Comparison of the effect of scaling and root planing plus a placebo on various clinical parameters and on the subgingival microflora over a 24 week period.

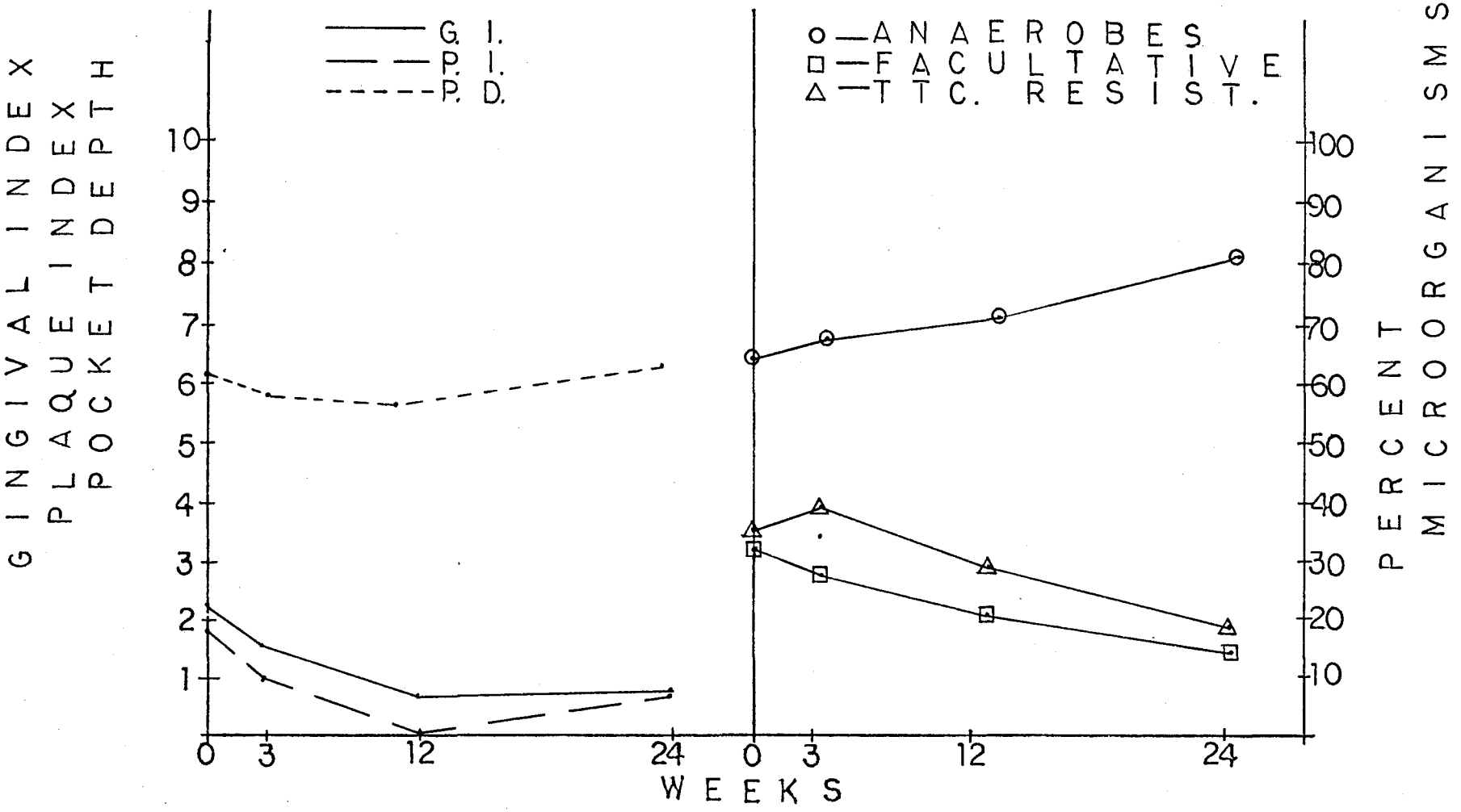


Figure 12. Composite graph of patients PL, JK and SS. Comparison of the effect of a placebo on various clinical parameters and the subgingival microflora over a 24 week period.

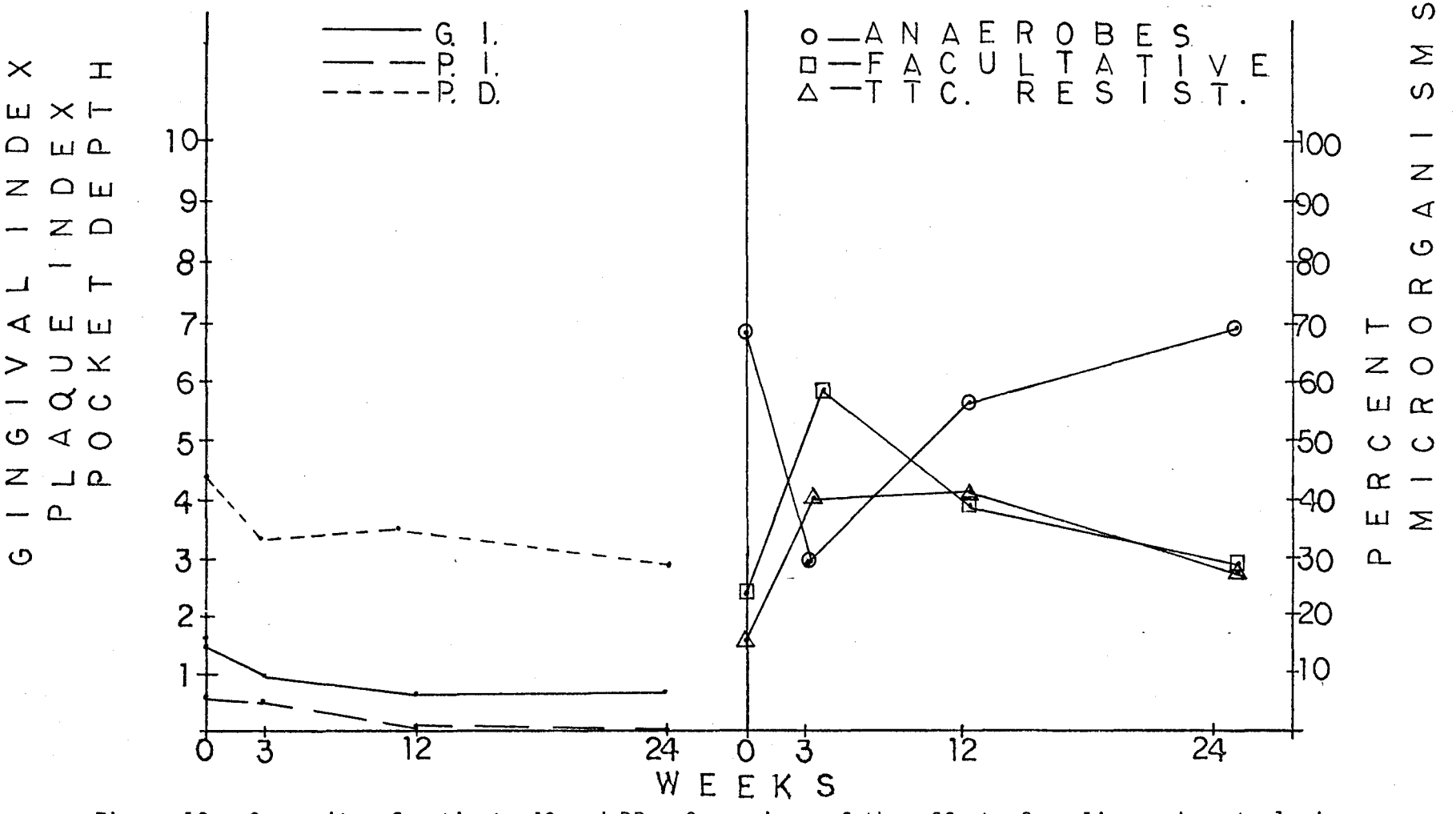


Figure 13. Composite of patients JC and BR. Comparison of the effect of scaling and root planing plus tetracycline on various clinical parameters and on the subgingival microflora over a 24 week period.

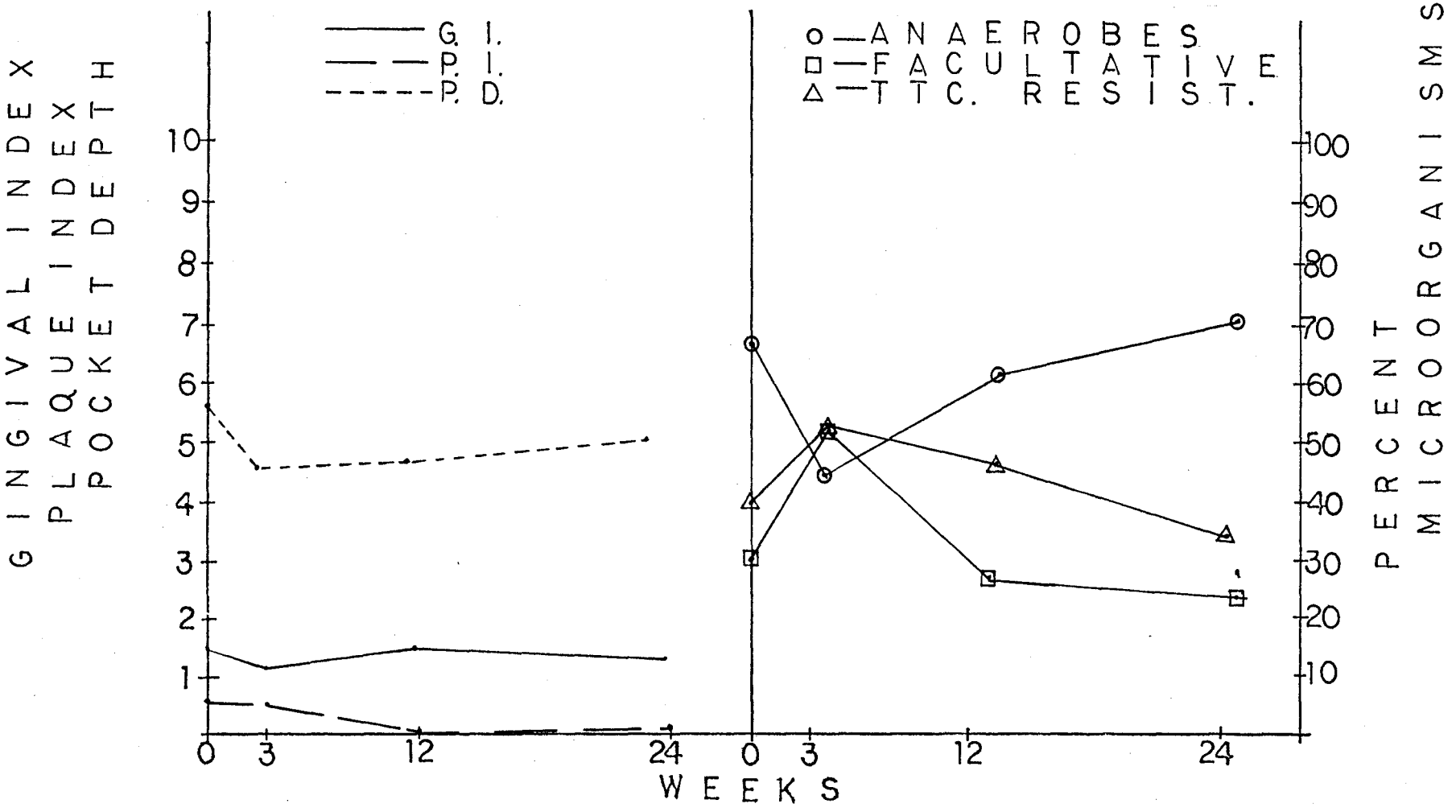


Figure 14. Composite graph of patients JC and BR. Comparison of the effect of tetracycline on various clinical parameters and on the subgingival microflora over a 24 week period.

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The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science in Oral Biology.

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