

The Effect of Subgingival Irrigation
with Chlorhexidine in the
Treatment of Adult Periodontitis

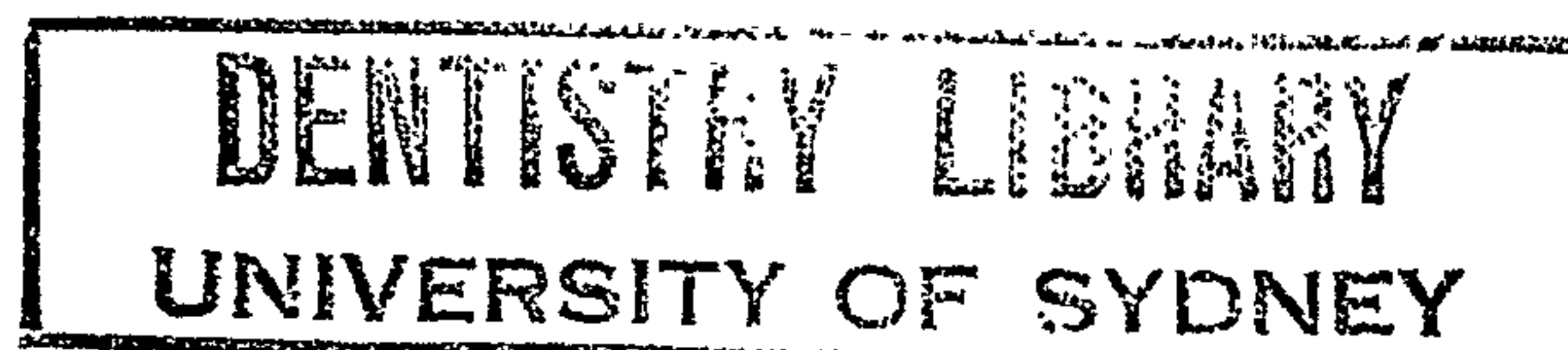
by

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SUMMARY

Studies indicate that subgingival irrigation with chlorhexidine has a beneficial effect in the treatment of periodontitis. Few studies have examined whether this has any additional benefit over that of mechanical debridement alone. The purpose of this study was to compare the effect of a single subgingival irrigation with chlorhexidine with that of scaling and root planing and then to determine if there was any additional benefit.

Thirteen adult human subjects who had at least one periodontal pocket ≥ 6 mm in depth in each quadrant were selected. Quadrants received different treatments. These were: scaling and root planing plus subgingival irrigation with chlorhexidine; scaling and root planing plus subgingival irrigation with saline, subgingival irrigation with chlorhexidine and no scaling or root planing; and subgingival irrigation with saline and no scaling or root planing (control).

Clinical and microbiological parameters were studied. The clinical variables were measured at baseline (week 0) and at weeks 1, 4, 8 and 12 after treatment. They consisted of Plaque, Gingival and Bleeding Indices, together with probing pocket depth and attachment level. The pocket flora was sampled at weeks 0, 1, 4 and 8 after treatment, with 7 subjects followed through to week 12. The main morphotypes (coccal forms, motile forms, spirochaetes, others) were identified and counted using differential dark field microscopy.

Treatment resulted in an improvement in periodontal health. Clinically, there was a reduction in inflammation and mean pocket probing depths, plus a mean gain of attachment, in all treatment groups. Microbiologically, treatment resulted in a reduction in the

mean proportion of spirochaetes and an increase in the mean proportion of coccal forms, followed by a return towards baseline values. The greatest clinical and microbiological improvement occurred in the group which received subgingival irrigation with chlorhexidine plus scaling and root planing. The results suggested that the effect of subgingival irrigation with chlorhexidine was to enhance the benefits of mechanical debridement in the short term.

The statistical significance of the results was determined. It was found that there were some statistically significant changes following treatment in some of the parameters studied. For example, probing pocket depth decreased and there was a gain of attachment in all groups over the study period. However, there was no statistically significant difference between the treatment groups. When the various indices and bacteriological variables were considered, isolated instances of significance were found amongst the many statistical tests that were done.

The statistical analysis indicated that chemical and mechanical therapy were of benefit in the treatment of moderate to severe adult periodontitis but that there was no additive effect of significance when scaling and root planing was combined with a single subgingival irrigation with 0.2% chlorhexidine.

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I can say to you quite honestly that at no point either before I came here, or since I have been here, has any calculus of possible benefit entered into our considerations.

R.J.L. Hawke
Prime Minister of Australia

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List of Abbreviations

PLI	Plaque Index
GI	Gingival Index
BI	Bleeding Index
Tx Group	Treatment Group
Wk	Week
Wk0	Baseline
Wkn	<i>n</i> th week after baseline

CHAPTER ONE

Introduction

Chronic inflammatory periodontal disease is a disease or, perhaps, a family of closely related diseases affecting the tissues supporting the teeth. Periodontitis was defined by *Page and Schroeder* as an inflammatory disease of the periodontium which is characterized by the presence of periodontal pockets colonized by bacteria with active bone resorption and acute inflammation (*Page and Schroeder, 1982, p.222*). They described five distinct forms of periodontitis: *prepubertal, juvenile, rapidly progressive, adult, and acute necrotizing ulcerative gingivo-periodontitis*. Other forms may exist but we do not have the knowledge to identify them.

Adult-type periodontitis is by far the most common of these conditions. The *World Health Organization* has stated that:

"Early signs of periodontal disease are frequently evident by the second decade of life and advanced destruction is commonly observed after the age of 40 years." (World Health Organization, 1978).

Since the 1960s, studies have established unequivocally that bacterial plaque plays the major role in the development of chronic inflammatory periodontal disease (*Loë et al., 1965; Theilade et al., 1966*). The establishment and development of periodontal disease can be avoided by the prevention of plaque accumulation (*Lovdal et al., 1961; Ramfjord et al. 1968, 1975; Suomi et al., 1971*). Mechanical or chemical means may be used to prevent plaque deposition or to remove it once it is *in situ*. The most commonly employed mechanical method is toothbrushing, usually supplemented by some form of interdental cleaning such as the use of dental floss, dental tape, wooden sticks and interproximal brushes.

Once periodontitis is established, active treatment consists of a sequence of phases aimed at teaching the patient to carry out satisfactory supragingival plaque control whilst the therapist controls subgingival plaque by scaling and root planing, sometimes requiring surgery, followed by maintenance. The mechanical removal of plaque and calculus in this manner results in healing of inflamed tissues.

Lindhe and Nyman demonstrated that

"... it is possible to treat periodontal disease successfully, even in advanced stages, in patients willing to carry out optimal plaque control programmes..."

Their clinical study of seventy five adult patients with advanced periodontitis used oral hygiene instruction, scaling, root planing and correction of restorations with ill-fitting margins in pre-surgical periodontal therapy (*Lindhe and Nyman, 1975*).

Chemical plaque control has been advocated as an adjunctive measure to mechanical plaque control. Methods that have been investigated include the use of enzymes (*Nyman et al., 1972*), antibiotics (*Ciancio, 1986*) and antiseptics (*Hull, 1980*). Various enzymes have been successfully tested *in vitro* but this has not translated into clinical success. Antibiotics such as tetracycline (*Listgarten et al., 1978*) and metronidazole (*Lindhe et al., 1983*) have also been used to advantage in plaque control. However, the side effects of long-term systemic antibiotics preclude their routine use. Other research has been into the use of hollow fibres impregnated with, for example, tetracycline, and placed in periodontal pockets (*Goodson et al., 1979*). Such local administration may alter the flora without some of the problems of cross-resistance, hypersensitivity and opportunistic infection that can arise from systemic use.

The biguanide, 1:6-di-4'-chlorophenyldiguanidohexane (chlorhexidine) has been studied extensively since 1954 when its *in vitro* anti bacterial effects were observed (Davies et al., 1954). Interest in its properties heightened when Loë and Schiott showed that twice-daily rinsing with an 0.2% solution of chlorhexidine gluconate over twenty-one days prevented the formation of dental plaque and the development of gingivitis in dental students (Loë and Schiott, 1970).

Chlorhexidine acts as an antimicrobial and can reduce the oral flora by up to 95% but the remaining bacteria are still enough to establish recolonization (Schiott et al., 1970). Its most beneficial effect lies in its adsorption onto hydroxyapatite, tooth surfaces, pellicle, bacterial plaque and mucous membranes, from where it is gradually released, leading to a local antibacterial action (Rolla et al., 1976). Since chlorhexidine is lipophilic, it is adsorbed onto cell walls, damaging permeability barriers. At high concentrations it may also cause coagulation and precipitation of cell contents.

There have been no reports of adverse systemic effects resulting from the systemic ingestion of chlorhexidine. Its regular use leads to brown discolouration of the teeth, the tongue and certain filling materials, parotitis and interference with taste sensation in some people (Flotra et al., 1971). Problems with staining can be controlled by mechanical cleaning and the use of solutions of lower concentrations.

The delivery of chlorhexidine has been via a mouthrinse in many studies. Since the penetration of rinses into periodontal pockets is limited (Pitcher et al., 1980) chlorhexidine is unlikely to have a predictable effect on the subgingival flora when used in this manner (Flotra 1971). Local subgingival application of chlorhexidine could

however be employed to alter the flora in periodontal pockets from a pathogenic flora, characterized by higher numbers of spirochaetes and motiles, to a healthier flora, characterized by higher numbers of coccal forms (*Mousques et al., 1980*). A recent study suggests that this beneficial effect of subgingival irrigation with chlorhexidine may be prolonged by up to several weeks in otherwise untreated periodontal lesions (*Lander et al., 1986*).

Scaling and root planing promote periodontal health resulting in reduced bleeding on probing, decreased pocket depth and resolution of gingival inflammation. It has also been shown that chlorhexidine is of benefit in the control of plaque through its antimicrobial effect, both supragingivally and subgingivally. Subgingival irrigation with chlorhexidine used jointly with scaling in therapy may have an additive or even synergistic, beneficial influence on periodontal health.

Thus, the aim of this study is to examine the effects of a once-only subgingival irrigation with chlorhexidine solution, with or without scaling and root planing, when used in the treatment of moderate to advanced adult periodontitis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical Perspective

Pathological change in the periodontium has been recognized and its treatment attempted since antiquity when ancient civilizations accurately observed the link between poor oral hygiene and gum disease. Theories of causation reflected magico-religious beliefs: disease was a result of offending the gods; malady, a deserved punishment. Toothcleaning with toothpicks and chewsticks featured in religious rituals as empirical methods were used to counter divine dismay.

A more rational approach to disease control was ushered in with the rise of Ancient Greece. *Hippocrates (460-377 BC)* recognized that illness developed from natural causes and could respond to specific treatments. Nonetheless, some remedies owed more to superstition than to science. Dental Science made progress in the Arabian school of the 9th to 12th centuries when, for example, *Albucasis (936-1031 AD)* described dental instruments such as scalers and surgical knives (*Gold, 1985*)a.

Europe floundered in ignorance throughout the Middle Ages with dental knowledge expanding little even through the Renaissance. It was not until the 17th century that an understanding of the microbial aetiology of periodontal disease began, with a Dutch merchant, *Antony van Leeuwenhoek (1632-1723)*. Here was an enthusiast, a skilled instrument maker who was driven by scientific curiosity to study a wide range of plant and animal structures through microscopes of his own construction. The most remarkable of his achievements was his discovery of the microbial world of "*animacules*". Leeuwenhoek, in his

many letters to the Royal Society in London, described protozoa, algae, yeasts and bacteria, emphasizing their variety and number. Untrammelled by formal education and with few learned contacts, Leeuwenhoek was the first to describe plaque bacteria:

"there are more animals living in the scum on the teeth in a man's mouth, than there are men in a whole kingdom"
(Stanier, 1977).

Pierre Fauchard (1678-1761) was born at about the time when Leeuwenhoek was observing animacules. This scholar laid the foundations for modern periodontics by categorizing the clinical signs and characteristics of periodontitis, together with its local treatment and aetiology (Gold, 1985)b. Fauchard's ideas lie at the heart of the fundamental issues faced in periodontal practice and research today, so many years later. An English anatomist, *John Hunter* (1728-1793), has been credited with establishing the scientific principles of dental anatomy and physiology during this period when the work of *Fauchard, Hunter* and others established dentistry as a separate field of endeavour.

Microbiological study during the intervening years focussed on fermentation and food preservation. Scientists were reluctant to believe that tiny microorganisms could cause disease.

In 1840 *Jacob Henle* laid down the criteria necessary to establish a causal relationship between a specific microorganism and a specific disease, stated today as:

- (i) the pathogenic organism is regularly found in the lesions of the disease;
- (ii) it can be isolated in pure culture on artificial media;
- (iii) inoculation of this culture produces a similar disease in experimental animals;
- (iv) the organism can be recovered from the lesions in the animal.

The next significant step came from *Joseph Lister* in about 1864. General anaesthesia had been introduced in 1842 and two dentists, *Horace Wells* and *William Morton*, figured prominently in its development (*Langa, 1976*). This encouraged the development of more sophisticated surgical techniques but an unfortunate corollary of this was surgical sepsis: patients often died from it. *Lister* hypothesized, correctly, that the prevention of bacterial contamination of surgical sites would decrease post-operative infection. Thus he provided the first, albeit indirect, evidence for the germ theory of disease.

The first person to experimentally apply the criteria laid down by Henle was *Robert Koch* who demonstrated the microbial aetiology of anthrax in 1876. Consequently, Henle's criteria became known as Koch's postulates. Support for a microbial aetiology for periodontal disease grew towards the end of the century. However *Willoughby Dayton Miller*, a dentist who worked with Koch, investigated the oral microbiota and periodontal disease and in 1890 concluded that periodontal disease did not fulfil any of Koch's postulates (*Gold, 1985*)c.

Almost a century later this dilemma still bedevils periodontal research:

"The problem is somewhat unique in that one attempts to establish etiologic significance of an organism in the

presence of continual infection by large numbers of possibly non-pathogenic or even beneficial species .. a bacterial physiologist's delight (or nightmare)." (Socransky, 1977).

Socransky was prompted to set up alternative weighted criteria for determination of a periodontal pathogen. The criteria are that there should be:

- (i) a quantitative association with disease;
- (ii) altered host immune response to a given species;
- (iii) animal pathogenicity;
- (iv) possession of virulence factors; and that
- (v) the disease should be cured by specific elimination of the suspected species without otherwise changing the plaque.

(Socransky, 1977).

No single pathogen has been identified that fulfils all of these criteria.

Concomitantly, two theories of the microbial aetiology of periodontal disease have evolved: the non-specific and the specific theories. The non-specific theory attributes causation to the proliferation of the total plaque flora above the threshold of host resistance and the specific theory attributes causation to a single pathogenic species *(Theilade, 1986).*

Neither a specific nor a non-specific microbial aetiology for periodontal disease has been definitely established but, starting in the early 1960's, various studies have demonstrated conclusively that there is a causal relationship between bacterial plaque and periodontal disease.

2.2 The Relationship of Plaque to Periodontal Disease

Dental plaque is the adherent bacterial mass that attaches to tooth surfaces. It tends to form in the cervical portion of the crown and, undisturbed, will extend into the gingival sulcus and occlusally.

The evidence implicating plaque as the major aetiological factor in periodontal disease comes from four main sources: *epidemiological, animal, post mortem and clinical studies.*

2.2.1 Epidemiological Studies

Epidemiology studies the occurrence and distribution of a disease or a condition as a mass phenomenon. Epidemiological studies that have examined the relationship between oral hygiene and the severity of periodontal disease have generally found a consistent correlation between poor oral hygiene and increasing incidence and severity of the disease.

In surveying disease it is often enough to know whether the condition is simply present or absent. Periodontitis differs in that the degree of involvement may vary considerably, between individuals and between different sites in the one individual. Periodontal indices have been developed to cope with this spectrum of significance but surveys may still yield quite disparate results, not necessarily reflecting true differences between populations. This may be because of methodological differences or because, historically, disease definitions were not clearly established.

Early epidemiological surveys such as that of *Marshall-Day et al. (1949)* did not examine the relationship of oral hygiene to periodontal disease as it was considered to be systemic in origin:

"... the common local etiologic factors, including mechanical irritation such as calculus formation and food impaction,

diminished masticatory function, infection and malocclusion are merely exciting causes superimposed upon a systemic condition which predisposes to a breakdown of the supporting structures of the teeth."

Later, in a survey of 935 people living in Boston and ranging in age from thirteen to sixty-five years of age, oral hygiene was examined in relation to the prevalence and incidence of periodontal disease (Marshall-Day et al., 1955). Assessment of oral hygiene was based on people's recall of the frequency of dental visits, prophylaxes and toothbrushing. Clinical observation will show though that most people brush their teeth regularly but the efficiency of plaque removal ranges from the scrupulous to the abysmal.

Females were found to have less gingival disease and, as a group, to tend to brush more often and attend for dental prophylaxes more frequently. It was commented that:

"The relative importance of these oral hygiene habits as factors in the demonstrated lower incidence of gingival disease in female subjects is a matter for speculation."

Other surveys (Russell 1957a, 1957b) linked age, racial grouping, and socioeconomic status to the prevalence of periodontal disease. Russell (1963) found that there was a strong linear correlation between increasing age, the Oral Hygiene Index (Greene and Vermillion, 1960) and the Periodontal Index (Russell, 1956); and stated that:

"less than 10 per cent of the variance in group PI scores remain to be explained after the combined influence of age and mouth cleanliness has been estimated. A residual factor wholly independent of age or hygiene, therefore, can have little effect on periodontal disease." (Russell, 1963).

The ongoing study of the natural history of periodontal disease in man by Loë and co-workers seeks to compare periodontal parameters in Sri Lanka and Norway (Loë et al., 1978)a. This study has found a direct relationship between increasing plaque levels and disease prevalence. The Norwegian population with good oral hygiene has a constant and slow loss of attachment with a low mean mortality rate for teeth (Loë et al., 1978b, 1978c). This contrasts with the Sri Lankan population which is characterized by poor oral hygiene, earlier and more rapidly progressive loss of attachment and an increased tooth mortality rate (Loë et al., 1978b, 1978c).

2.2.2. Post Mortem Studies

Waerhaug studied the location of microbial deposits in relation to the extent of the periodontal inflammatory lesion in autopsy specimens. It was found that if plaque was supragingival then the lesion was limited to the supraalveolar connective tissue. Where plaque was present on cementum, there were lesions in the bone and periodontal ligament. The distance from the periphery of plaque to the lateral and apical extension of soft tissue inflammation was usually less than 1 or 2 mm. There was usually a narrow zone (about 1 mm) of normal connective tissue between the inflammatory infiltrate and bone (Waerhaug, 1952).

2.2.3 Animal Studies

Inflammatory periodontal destruction has been found or can be induced in many mammalian species including dogs, monkeys, rodents and sheep (Page and Schroeder, 1982, p.229).

Definite evidence of the bacterial nature of periodontal disease came when Jordan and Keyes (1964) demonstrated that periodontitis in

hamsters is transmissible. *Rovin et al.* showed that the effect of increasing local accumulation of plaque with silk ligatures in rats was to markedly increase the severity of the gingival inflammatory response. The lack of response in gnotobiotic rats shows that this is related to plaque and not to the mechanical effect of the ligature. The placement of silk ligatures at the cervical margins of rats' teeth facilitated bacterial accumulation and gingival inflammation developed. The lack of response when these were placed in gnotobiotic animals would indicate a microbial aetiology for the condition (*Rovin, Costich and Gordon, 1966*). Rodents are not a good experimental model for periodontal pathology because of differences in anatomical form and pathogenic mechanisms (*Page and Schroeder, 1982, p.230-231*). This has led to the development of alternative animal model systems.

A number of researchers have demonstrated the relationship between plaque and periodontal disease in dogs, a species that develops periodontal disease naturally (*Saxe et al., 1965; Lindhe et al., 1973; Egelberg, 1965*). Chronic gingivitis can be experimentally induced in beagle dogs, by feeding them a plaque-inducing diet. This has been shown to proceed to a progressively destructive periodontitis with bone and attachment loss, but only in sites showing pre-existing gingival inflammation (*Lindhe et al., 1973*). *Gad* studied a group of sixty two mongrel dogs and concluded that there was a direct relationship between increasing age, plaque deposits and disease severity. Only two animals, aged three and five months, had clean teeth. These were the only periodontally healthy dogs (*Gad, 1968*).

Development of a phylogenetically closer disease model using monkeys has confirmed the role of bacteria in periodontal disease. Teeth in twenty six squirrel monkeys were ligated and observed over a period of

one year. Non-ligated teeth in the same animals served as controls. Bacteria accumulated on the silk and a gingivitis developed which progressed to an experimentally reproducible periodontitis with apical migration of the junctional epithelium and alveolar bone loss (*Kennedy and Polson, 1973*).

Periodontitis has many features which are common to all the species that have been studied. For example, the disease is infectious and caused by bacterial colonization and extension around the gingival margin. Associated pathogenic mechanisms are also probably very similar (*Page and Schroeder, 1982, p.231-231*). However, there is no true animal model of human periodontitis as:

"Anatomic, physiologic, genetic, dietary and environmental factors, and habits are of sufficient importance in the pathogenesis as to impose significant differences in the nature and manifestations of the disease, depending on the species affected." (Page and Schroeder, 1982, p.230).

Care should be taken in extrapolating data from one species to another as this may result in entirely erroneous conclusions (*Cotton, 1988*).

2.2.4 Clinical Trials

Clinical experience suggests that the incidence and severity of periodontal disease is related to deficient oral hygiene. Such observations are merely anecdotal and their interpretation is fraught with error because of a lack of standardized measures and systematic data collection. The advent of well-planned clinical trials of both long and short duration has established the microbial origin of periodontal disease.

(i) Short-term Trials

In their landmark paper *Loë* and co-workers studied experimental

gingivitis in twelve people who were initially periodontally healthy. With cessation of tooth cleaning, plaque accumulated and gingivitis developed. With resumption of tooth cleaning the gingivitis resolved in about a week. It was noted that the time taken for the development of gingivitis varied from ten to twenty-one days implying differences in host responsiveness (*Loë et al., 1965*). Concurrent microscopic evaluation showed qualitative and quantitative changes with time. That the observed effect was due to plaque accumulation (and removal) and not to the lack of mechanical stimulation by toothbrushing was demonstrated by *Loë and Schiott* who observed that chemical plaque control, using chlorhexidine in a similar experimental gingivitis study design, prevented the development of gingivitis (*Loë and Schiott, 1970*). Thus it could be seen that plaque bacteria, their components or their products induced gingival inflammation.

ii) Long-term Trials

Adult periodontitis produces loss of attachment that cannot yet be predictably reversed. As a result, a human experimental model producing disease is unacceptable for ethical reasons. Instead, some long-term studies have focussed on the effectiveness of programmes aimed at the prevention of the development or progression of disease (*Lövdal et al., 1961; Suomi et al., 1969, 1971, 1973; Axelsson and Lindhe, 1974, 1978, 1981; Lindhe et al., 1975*). Although one study was of schoolchildren who also received fluoride treatments, the principle remained the same: *oral hygiene measures controlled disease* (*Axelsson and Lindhe, 1978*). In a six-year study of the effect of controlled oral hygiene procedures on caries and periodontal disease in approximately four hundred and fifty individuals, *Axelsson and Lindhe* found that those who utilized proper oral hygiene measures prevented

the progression of periodontal disease, while the control group with less than optimal oral hygiene showed increasing probing depth and attachment loss (*Axelsson and Lindhe, 1978, 1981*).

Other clinical trials have studied the effects of meticulous plaque control in patients with established periodontitis (*Lindhe and Nyman, 1975, 1984; Lindhe et al., 1982, 1984; Nyman et al., 1977; Ramfjord et al., 1982*). These studies demonstrate that, even in cases of advanced disease, it is possible to obtain resolution of inflammation and prevention of further attachment loss using different treatment techniques so long as a careful oral hygiene programme is maintained by patients. Where subgingival plaque is allowed to recur, disease progression and further destruction may occur (*Lindhe et al., 1984; Ramfjord et al., 1982*).

2.3 Plaque Formation and Development

Plaque bacteria maintain a commensal relationship with their host. They are a normal finding in both health and disease so establishing the significance of a particular species presents difficulties. Additionally, the bacteria associated with periodontal disease have fastidious requirements which make difficult their study by the chief methods: cultural and morphological.

2.3.1 Ecology of the Oral Cavity

The oral cavity is an open growth system, that is, nutrients and bacteria are repeatedly introduced to and removed from it.

Microorganisms that can successfully colonize the mouth are those that can adhere to oral surfaces or be retained in some other way such as in pits and fissures. Once adherent bacteria have to be able to utilize available nutrients to effect further proliferation. Factors

that act against adherence include chewing, oral hygiene procedures, desquamation of cells, and the flow of saliva and gingival crevicular fluid.

If bacteria are retained then the mouth forms an ideal environment with an optimal temperature, high humidity, saliva to buffer pH and remove waste, and variable oxygen tension to support different species.

2.3.2 Plaque Formation

Plaque formation has been divided into two phases comprising:

- (i) formation of an initial non-bacterial matrix, the acquired pellicle;
- (ii) the adherence and proliferation of microorganisms to develop a well-defined structure as in mature plaque.

(i) Acquired Pellicle

Oral surfaces are covered by a film of precipitated salivary mucins. The mucins collect on the teeth to form the acquired pellicle which provides a suitable surface for colonization (*Gibbons and van Houte, 1973*).

(ii) Adherence and Proliferation

Several factors act to modulate the initial adherence and subsequent growth of bacteria, including:

- * their innate ability to initially adhere;
- * the potential for interspecies and intraspecies binding;
- * the number and relative proportions of species present; and
- * relative rates of growth and available nutritional factors.

Initial adherence is the result of a number of physicochemical influences such as electrostatic forces and cell surface characteristics. Most bacteria are surrounded by polysaccharide matrices called glycocalyxes which form coherent or loosely associated capsules (*Costerton et al., 1981*). Other bacteria may have appendages at their surfaces, called pili or fimbriae. Since cells have forces acting upon them which result in a balance between attraction and repulsion, these cell surface structures are important if they bridge the resulting gaps between bacteria or between teeth and bacteria.

Once initial adherence occurs the bacteria may start to proliferate.

The resultant mass is held together by bacterial glycocalyces, salivary glycoproteins and other specific and non-specific binding mechanisms. For example, *Streptococcus mutans* and some *Actinomyces* species synthesize polymers that bind similar species together. Salivary mucins called agglutinins and secretory immunoglobulin A may also act against adherence (Lindhe, 1989, p.145).

The host or other bacteria may act as sources of nutrition. Supra-gingivally, nutrients are present in the saliva but in low concentrations. Contrast this with the abundance of the subgingival environment where gingival crevicular fluid contains tissue fluid and cellular breakdown products. Exchange between species may also lead to the formation of food webs. For example, *Bacteroides* species can produce carbon dioxide which is a requirement of *Capnocytophaga* species.

Bacteria sometimes lack the enzymes that help to synthesize their constituents. Instead they may breakdown macromolecules in their immediate environment to obtain essential nutrients, thus contributing to the pathogenesis of periodontal disease.

2.3.3 Morphological Studies of Plaque

When Loë and coworkers studied experimental gingivitis in man they examined its bacteriology as well as its clinical characteristics (Loë et al., 1965). Three main phases in plaque accumulation were noted.

During the first phase, which followed almost immediately upon tooth cleaning and pellicle formation, single bacteria adhered to irregularities in the pellicle. Mostly cocci, the bacteria increased in number, forming colonies which eventually coalesced to form

adherent plaque. Desquamated cells and some polymorphonuclear leukocytes were observed.

The second phase which occurred after two to four days' plaque accumulation was marked by an increase in the number of fusiform and filamentous bacteria, slender rods and polymorphonuclear cells.

Between the fifth and ninth days of plaque accumulation there was an increase in the numbers of spirochaetes and spirilla with rods, cocci and filaments still present. Whereas the microflora was ninety per cent gram-positive cocci during the first phase, it now contained a higher number of gram-negative forms, which accounted for fifty per cent of the bacteria (*Loë et al., 1965*).

Further morphological information on plaque formation was provided by an elegant study by *Listgarten* who allowed plaque to form on twenty eight epoxy resin crowns in six subjects (*Listgarten et al., 1975*). This allowed removal, embedding, sectioning and microscopic examination of the crowns at various time intervals with the bacteria kept in their original orientation to the surface. Early plaque consisted mostly of cocci which grew in columnar colonies and then coalesced. At one week filamentous forms made their appearance, colonizing the surface of the coccal plaque and growing into it. A more mature plaque was observed near the gingival crevice. Subgingivally, plaque had formed by two months. It contained many spirochaetes, filaments, flagellated forms and some small rods (*Listgarten et al., 1975*). Plainly, little more could be learnt from looking at plaque structure. Researchers embarked on cultural studies to try to identify the species in plaque.

2.3.4 Cultural Studies of Plaque

The composition of plaque varies between individuals, between one site and another, and from time to time in the same individual (*Moore et al., 1984*). The study of the subgingival microflora, that is, the microflora of periodontal disease, demands sophisticated techniques in order to obtain viable, representative and uncontaminated samples and then to culture and identify the isolates obtained.

The heterogeneity of the species that are involved, together with their often fastidious requirements, makes it difficult to culture them. *Moore* states that:

"no one medium or method grows all of the bacterial types that are present. Therefore laboratories that work with different media work with different parts of the flora, even though these parts may overlap" (Moore, 1987).

As supragingival plaque matures there is a successional acquisition of new bacterial forms and with progressive extension a subgingival microflora develops. Approaches to studying the subgingival bacteria have included:

- (a) animal studies of bacterial pathogenicity;
- (b) detection of noxious products from periodontal bacteria;
- (c) analysis of the microflora associated with periodontal health and disease.

One result of these studies is that microbial profiles have been established for health, gingivitis, acute necrotizing ulcerative gingivitis (*ANUG*), localized juvenile periodontitis (*LJP*) and adult periodontitis (for review see *Socransky, 1977; van Palenstein Helder, 1981; Moore, 1987*).

It is normal for the healthy gingival sulcus to contain microorganisms. Slots cultured plaque samples from seven periodontists and found that healthy sites contained 85% gram-positive bacteria mainly consisting of rods and coccal forms. Typically these are members of the *Streptococcus* and *Actinomyces* genera including *Streptococcus mitis*, *Streptococcus sanguis*, *Actinomyces viscosus* and *Actinomyces naeslundii*. Obligate anaerobes formed 24.3% of the cultivable flora in health but 89.5% in deep pockets (Slots, 1977)a. Dark field microscopy has shown that the healthy sulcus harbours 74.3% coccal forms with spirochaetes forming only 1.8% of the sample (Listgarten et al., 1978).

The development of periodontal disease is associated with quantitative and qualitative changes in the microflora. Thus, in early gingivitis the proportion of *Actinomyces viscosus* increases to about 50% but in longstanding gingivitis the gram-negative forms such as *Veillonella* and *Fusobacteria* may increase to form 25% of the total cultivable flora.

The microflora of diseased sites in LJP has been described as being dominated by gram-negative anerobic rods. However, whether they initiate the condition or whether they preferentially colonize diseased sites has not been unequivocally demonstrated (Newman and Socransky, 1977). Impaired neutrophil chemotaxis has also been found in some cases of LJP (Lavine et al., 1979; van Dyke et al., 1985). This has been disputed by researchers who point out that neutrophil chemotaxis may be elevated or vary from time to time in patients (Page et al., 1984; Kinane et al., 1989). Others have suggested that the bacteria themselves are infected by bacteriophages (Preus et al., 1987).

Despite these findings, *Actinobacillus actinomycetemcomitans* and *Capnocytophaga* species have been implicated as probable pathogens in *LJP*. Depending on the source cited their proportion at diseased sites may vary from 0.1% to 90% (Moore, 1987). The same species have also been cultured from prepubertal periodontitis (Sweeney et al., 1987).

Müller and Flores-de-Jacoby studied relatively old (17-24 years of age) *LJP* patients and control patients with rapidly progressive periodontitis (Müller and Flores-de-Jacoby, 1985). They found, using dark field microscopy, that disease control sites had a higher ratio of motile to non-motile forms than either diseased *LJP* sites or healthy control sites, the respective ratios being 1:1, 1:4 and 1:27.

Acute necrotizing ulcerative gingivitis (*ANUG*) is characterized by its rapid onset and painful, necrotic and ulcerative gingival lesions. It typically occurs in individuals under physiological or psychological stress (Cohen-Cole et al., 1983). The bacteria that have been associated with *ANUG* are *Bacteroides intermedius* and an unnamed medium-sized spirochaete (Newman and Nisengard, 1988). Their exact role is unclear although it is known that fusobacteria and spirochaetes can release endotoxins into the connective tissue, necrosis being characteristic of the "*Schwartzman reaction*" that could possibly be produced. It is likely that such direct bacterial effects, in conjunction with indirect host responses are responsible for the clinical presentation seen in *ANUG*.

Untreated gingivitis may progress to adult periodontitis and with this progression there is the development of a complex and highly variable flora in the pocket and, in advanced disease, probably the adjacent tissues (Saglie et al., 1982). Bacterial invasion has been reported in

LJP, ANUG and advanced periodontitis (Gillett and Johnson, 1982; Allenspach-Petrzilka and Guggenheim, 1983).

The microflora of adult periodontitis is predominantly composed of gram-negative rods (Slots, 1977)b. Amongst the species that are often mentioned as being present are *Bacteroides gingivalis*, *Bacteroides intermedius*, *Fusobacterium nucleatum*, *Eikenella corrodens* and various *Treponema* species (for review see Socransky, 1977; Slots, 1979; van Palenstein Helderma, 1981; Moore, 1987). Other species such as *Actinobacillus actinomycetemcomitans*, *Wolinella recta*, *Candida albicans*, the *Enterobacteriaceae* and pseudomonads have also been identified in culture (Slots et al., 1988).

Immunological studies showing an enhanced cell-mediated immune response to certain species indicates that *Bacteroides intermedius* and *gingivalis* are the major pathogens associated with adult periodontitis. A direct relationship between *Bacteroides gingivalis* and periodontitis seemed to be demonstrated recently when implantation of a rifampin-resistant strain into *Macaca fascicularis* resulted in elevated antibody titres to *Bacteroides gingivalis* and rapid bone loss (Holt et al., 1988).

The relative proportions of certain bacterial morphotypes may correlate with either health or disease. Thus, dark field microscopy can be used to differentiate forms such as cocci, spirochaetes and motiles but not to identify species (Listgarten et al., 1978). In Listgarten, Lindhe and Hellden's study, samples of the subgingival microflora were taken from two healthy and two diseased sites in each of the twelve patients. The samples were placed in saline containing 1% gelatin and between one hundred and two hundred bacteria were morphologically categorized within one hour using dark field

microscopy. It was found that the microflora of periodontal disease contained a higher proportion of motile rods (12.7%), curved rods (1.7%) and spirochaetes (37.7%) than healthy sites where their proportions were 0.3%, nil and 1.8% respectively. The proportion of coccal forms was lower in diseased sites (22.3%) than in healthy sites (74.3%) (*Listgarten et al., 1978*). In a further study using dark field microscopy it was found that the proportion of spirochaetes with or without motile rods could also be used as a predictor of periodontal destruction (*Listgarten and Levin, 1981*) although the validity of this concept has since been questioned (*Listgarten et al., 1984*).

It can be seen from these studies that dark field microscopy can be used to morphologically categorize periodontal bacteria with a predominance of coccal forms indicating relative health, and a predominance of spirochaetes and motile forms indicating a relative lack of health.

2.4 Treatment of Periodontal Disease

2.4.1 Evolution of Concepts

Since the microbial aetiology of periodontal disease was not clearly understood until recently, its treatment was originally empirical and directed at correction of supposed systemic causes. Later on treatment revolved around symptoms such as the pocket *per se*. Current concepts derive a rational basis from our increased knowledge. Thus, we aim to prevent new disease and arrest disease progression by using plaque control to prevent or heal inflammatory lesions.

Plaque control involves a combination of routine supragingival oral hygiene which is the patient's responsibility and subgingival control of the microflora which is usually the therapist's province.

2.4.2. Overview of Treatment

The treatment of periodontal disease consists of a sequence of phases which have been described by *Lindhe (1989, p.323-325)*. The first of these is known as initial, preparatory or cause-related therapy and its objective is the removal or control of infection. It involves teaching the patient to practise good oral hygiene - a process that needs continual reinforcement - and meticulous instrumentation, by the therapist, of root surfaces where

"by reason of the accretions and concretions" (Riggs quoted in Gold, 1985)b.

rapid bacterial recolonization could occur. Additional tasks at this stage might involve the removal of other plaque retention factors such as overhanging margins of restorations, occlusal adjustment, the treatment of carious and non-vital teeth, and the extraction of teeth with hopeless prognoses.

The second phase of treatment is known as corrective therapy and its course is determined by the degree of success of the initial therapy. Periodontal surgery, prosthetic, orthodontic and restorative treatment may be considered but only after taking into account the patient's needs, ability to cooperate and previous tissue response. Health is relative: although it is not merely the absence of disease, there is a whole spectrum of different treatment outcomes that may equate with functional and aesthetic health.

The third phase of periodontal treatment is termed maintenance therapy or care and, sometimes, supportive therapy. Since plaque bacteria

cannot realistically be eliminated from the mouth, regular reexamination and professional assistance is necessary, focussing on the prevention of disease recurrence and further attachment loss.

Health exists where the host's immune response copes with the presence of microbial pathogens without resultant inflammation. This equilibrium is disturbed in periodontal disease. Since current knowledge does not allow sufficiently subtle intervention in the immune response, the prevention and treatment of periodontal disease centres on the control of bacterial plaque. Logically, this may be either mechanical or chemical. Mechanical plaque removal is exacting for both patient and dentist: Thus *Socransky* wrote:

"Therapy generally lacks a defined end point and imposes an exceptional demand on patient motivation" (Socransky et al., 1982).

Partly as a result of this thinking, there has been much interest in chemical plaque control both independently and as an adjunct to mechanical debridement.

2.4.3. Mechanical Methods of Plaque Control

Mechanical plaque control falls into two categories: that which is carried out by the patient ("home care") and that which is professionally delivered.

(i) Home Care

The usual means by which plaque is removed from tooth surfaces by patients are: toothbrushing with a toothbrush and toothpaste; use of dental floss; use of wooden sticks; and use of other interdental cleaning aids such as interproximal brushes.

The efficacy of a person's toothbrushing depends firstly upon their

manual mechanical skill and application (*Hansen and Gjermo, 1971*). It may also depend upon the size, design, quality, and wear of the toothbrush as well as frequency and duration of brushing (*Bergenholtz et al., 1984*). Even with good manual dexterity and frequent brushing of an appropriate duration with a well-designed toothbrush in good condition, it is impossible to remove all plaque by toothbrushing alone (*Hill et al., 1973*). In particular, plaque located between the teeth and at the gingival margins will not normally be removed by brushing. Tooth cleaning may be facilitated by the use of dental floss and other interdental cleaning aids such as interdental brushes which are specially designed to brush between the teeth and may assist in removing more plaque than would conventional brushing alone (*Vogel et al., 1975, Wolffe, 1976*). *Wolffe* emphasized the need to suit the method to the individual (*Wolffe, 1976*). It is clear that these methods of home care are demanding of time and ability. Further, their teaching on a one-to-one basis is inefficient in terms of delivering preventive care to the wider community.

(ii) Rationale for Scaling and Root Planing

Plaque control in the dental office involves supragingival and subgingival instrumentation to remove plaque and calculus and to render the root surface biologically acceptable, facilitating healing and reducing the likelihood of rapid recolonization by bacteria (*Lindhe, 1989, p.323-325*).

Historically, periodontitis-affected cementum was likened to "dead bone" and its removal was advocated as part of therapy (*Riffle, 1956*). A rational basis for this was lacking: some said diseased cementum was 'softened' and, therefore, almost inherently pathological. Others said that it was the opposite: hypermineralized, possibly from exposure to

the high ion content of gingival fluid (*Selvig and Hals, 1977*).

Microscopic observations of the structure of periodontally diseased cementum disclose changes both on and below the surface. Bass described 'pathogenic granules' in cementum exposed by disease (*Bass, 1951*). These were further investigated by *Armitage and Christie* (*Armitage and Christie, 1973a, 1973b*). Using light and electron microscopy, they postulated that the granules represented areas of collagen fibril degradation. Their role remains unclear today.

Surface cemental changes in periodontitis include roughening, and the presence of resorption lacunae and areas of matrix dissolution and fibril detachment. The surface itself is littered with calculus and plaque bacteria and their remnants. Apical to the base of a periodontal pocket, microscopy reveals epithelially-lined cementum, and zones of partial and complete collagen fibre destruction and a zone of intact attachment (*Selvig, 1966*).

The finding that the transition from periodontal health to disease is accompanied by the development of a predominantly gram-negative microbial flora led to the suggestion that a component of those bacteria could be responsible for some of the observed tissue changes (*Aleo et al., 1974*). This seemed a promising line of investigation as it had already been observed that epithelial monolayers underwent progressive degeneration when incubated *in vitro* with periodontitis-affected roots (*Hatfield and Baumhammers, 1971*).

The factor thought to be responsible was endotoxin, a highly toxic lipopolysaccharide component of gram-negative cell walls, which may be released during life or upon cell disintegration. Endotoxins can cause direct tissue damage and activate host responses, thus causing

indirect damage (*Newman and Nisengard, 1988*).

A number of studies indicate that periodontitis-affected cementum contains high levels of endotoxin (*Aleo et al., 1974; Jones and O'Leary, 1978*). However, the most commonly used assay, the limulus amoebocyte lysate assay, is not specific and may be activated by thrombin, thromboplastin, ribonuclease and RNA, amongst others (*Elin and Wolff, 1973*). Thus, the amount of endotoxin may have been overestimated but these studies do indicate the presence of cytotoxic substances in these root surfaces.

A number of studies examine the role of endotoxin in periodontitis (for review see *Daly et al., 1980*). Its association with the disease seems to indicate that affected root surfaces should be root planed as thoroughly as is necessary to remove contaminated cementum (*Jones and O'Leary, 1978*).

It has recently been suggested that endotoxin is associated with, rather than bound to, periodontally involved root surfaces (*Moore et al., 1986*). In this study it was found that 99% of the assayed lipopolysaccharide could be removed by washing or brushing, leaving a residual 1%. This is in contrast to studies endorsing extensive removal of cementum (*Daly et al., 1982, Jones and O'Leary, 1978*).

Further experimentation in dogs supports the contention that healing occurs equally well whether or not previously exposed root cementum is removed (*Nyman et al., 1986*). Similarly, Nyman's study in humans, using different parameters, found that polishing the cementum surface to remove soft microbial deposits resulted in the same degree of improvement as scaling and root planing (*Nyman et al., 1988*).

To summarize, it appears that instrumentation sufficient to allow removal of calculus and plaque and smoothing of surface roughness should be enough to allow healing. Such debridement will almost inevitably involve removal of cementum but this need not be as extensive as has been recommended in the past.

(iii) Method of Instrumentation

Instrumentation may involve the use of hand, ultrasonic or rotary instruments, singly or in combination. It would seem that their effectiveness, like that of toothbrushing, may be more dependent on the operator's manual dexterity and application than on the instrument itself.

Lie and Meyer examined calculus removal and loss of tooth substance when using different instruments. A diamond instrument removed the most calculus and also the most tooth substance. An ultrasonic curette was less effective than hand instruments in calculus removal in the same study (*Lie and Meyer, 1977*).

Nishimine and O'Leary assessed the effectiveness of hand versus ultrasonic instrumentation in endotoxin removal (*Nishimine and O'Leary, 1979*). They concluded that hand instrumentation was more effective than ultrasonic instrumentation but both methods effected a considerable reduction in the assay used.

Dark field microscopy has also been used to compare the effect of hand scaling with that of ultrasonic debridement in thirty three furcated molars. Although the two methods were equally effective in changing bacterial proportions to those of a healthy state in Class I furcations, in Class II and III furcations the ultrasonic method was significantly more effective, possibly because of a physical irrigating effect (*Leon and Vogel, 1981*).

(iv) The Effect of Scaling and Root Planing

Oral hygiene measures alone have little effect on the subgingival microflora and the associated inflammation in deep periodontal pockets (*Listgarten et al., 1978, Cercek et al., 1983*).

Subgingival scaling and root planing is a highly effective method of treating periodontitis at most of the sites in most patients (*Lövdal et al., 1961; Hirschfeld and Wasserman, 1978; Badersten et al., 1981; Axelsson and Lindhe, 1978, 1981*).

Mechanical debridement changes the characteristics of the subgingival microflora from one dominated by gram-negative microorganisms to one with a high proportion of, for example, *Streptococcus sanguis* and *Actinomyces viscosus* and a low proportion of *Bacteroides gingivalis* (*Loesche et al., 1985*). The subgingival microflora is not static and pockets that have been instrumented tend to recolonize. Thus, *Mousques et al., (1980)* found that coccoid forms returned to baseline numbers in twenty-one days and spirochaetes returned to baseline numbers in forty two days. *Magnusson et al., (1984)* studied recolonization and found that the subgingival microbiota returned to baseline characteristics approximately four to eight weeks after instrumentation. This indicates the importance of maintenance treatment for adequate continued control of subgingival microorganisms and has been confirmed by a number of studies (*Nyman et al., 1977; Ramfjord et al., 1982; Lindhe et al., 1984*).

Since mechanical debridement may be of limited benefit in certain sites, (*Leon and Vogel, 1981; Bower, 1978*) and is time-consuming, researchers have turned their attention to chemical therapeutic alternatives.

2.4.4. Chemical Methods of Plaque Control

(i) Evaluation of Chemotherapeutic Agents

Chemical agents can be used in plaque control in either of two ways: preventive or therapeutic. Agents which retard or prevent supra-gingival plaque accumulation should be mainly active against gram-positive bacteria. Therapeutic agents act to reduce the amount of plaque when it has already formed and is likely to contain a high proportion of gram-negative forms.

The most common antimicrobial agents are antiseptics, which work in one of two ways. Either an antiseptic kills bacteria directly, that is, it is bactericidal, or it interferes with the biological processes of the living bacteria and prevents them from multiplying, that is, it is bacteriostatic.

There are two stages involved in considering the efficacy of a chemotherapeutic in plaque control:

- (a) firstly, one must evaluate the effectiveness of the agent on bacteria. This testing can be *in vitro* and is a matter of assessing the effect of the chemical on plaque bacteria over clinically relevant time periods; and
- (b) secondly, one must consider the efficacy of the chemical agent in the mouth - *in vivo*.

In the laboratory a chemotherapeutic agent may be demonstrated to be effective against plaque bacteria. However, the same efficacy may not be demonstrated *in vivo*. This finding is not uncommon because the oral environment is different from the laboratory environment. In particular, the existence of food debris, blood and its products,

cellular remnants and other organic and inorganic materials make the mouth a complex environment.

There are two criteria by which a chemotherapeutic agent which is safe and stable should be judged for its efficiency in attacking plaque bacteria:

- (a) substantivity is the quality that some agents may have of physically or chemically attaching to plaque and oral tissues. This substantivity is measured by the rate of release of the active agent which has been bound to plaque or oral tissues. The longer the period required for release the more effective the agent is likely to be; and
- (b) efficacy is the efficiency of the antimicrobial agent in either reducing bacterial numbers or altering their environment to their detriment. Each of these factors will vary depending on the characteristics of the particular chemical agent that is used.

Some of the difficulties that are encountered in assessing the efficacy of antimicrobial agents are that:

- (a) there are few comparative studies between different agents;
- (b) delivery systems are widely variable;
- (c) concentration and dose responses have not been evaluated for many substances and, therefore, optimal dosage regimens are unknown;
- (d) there are no uniform methods of evaluation, either quantitative or qualitative (Addy, 1986).

The ideal chemotherapeutic agent would be:

- (a) clinically effective in plaque control;
- (b) clinically effective in control of inflammation;
- (c) specific for periodontopathogens;
- (d) highly substantive;
- (e) safe;
- (f) stable (*Ciancio and Nisengard, 1988*).

(ii) Delivery of Chemotherapeutic Agents

Chemotherapeutic agents may be delivered either systemically or locally. Although systemic use of antibiotics overcomes the problem of inaccessibility to the subgingival microbiota by supragingival agents, there is a high incidence of side effects by this route, including the development of resistance, superinfection or hypersensitivity (*Addy, 1986; Slots et al., 1988*).

There is a far lower incidence of side effects with local delivery of chemotherapeutics for plaque control. The most common topical application is via mouthrinsing which is convenient and easy. However mouthrinses are unlikely to affect directly the subgingival microflora. *Pitcher et al. (1980)* examined this using periodontally involved teeth that were scheduled for extraction. Reference grooves were cut on the proximal surfaces of the tooth at the gingival margin and a disclosing agent was applied as a mouthrinse or by direct irrigation. After the teeth were extracted, the depth of penetration of the solution was measured from the reference point to the apical plaque border and the coronal level of the remaining periodontal ligament. Neither rinsing nor direct irrigation reached the apical

plaque border and mouthrinsing did not achieve any significant penetration of pockets.

Consequently, alternative topical delivery systems have been developed and studied. These include:

- (a) chemically impregnated acrylic strips (*Addy et al., 1984, Addy et al., 1985*);
- (b) hollow fibres containing antimicrobial agents (*Goodson et al., 1979; Coventry et al., 1982*);
- (c) subgingival irrigation using needles or cannulas (*Braatz et al., 1985; Ciancio et al., 1989; Lander et al., 1986; Listgarten et al., 1989; Southard et al., 1989*).

These systems apply antimicrobials directly to the subgingival microflora and are likely to be of therapeutic benefit.

(iii) Chemotherapeutic Agents

(a) Antibiotics

There are disadvantages in using systemic antibiotics to control plaque. Firstly, the problem of developing bacterial resistance would indicate that such drugs should be reserved for more serious infections.

Secondly, adult periodontitis is not a classical infectious disease. In a classical infectious disease a specifically directed bacteriostatic is usually given which allows the host to destroy the pathogens and the normal indigenous flora to re-establish itself. However, the pathogens that have been implicated in adult periodontitis are not foreign, they are normal commensals, and the success of treatment is

dependent on their suppression followed by selective regrowth (*van Palenstein Helderma, 1986*).

Clinical studies conducted to ascertain whether there is benefit to be gained from using, for example, tetracycline, indicate that scaling and root planing is of greater long-term benefit as tetracycline is a bacteriostatic that only suppresses the flora. There does not seem to be any gain from joint therapy (*Listgarten et al., 1978; Hellden et al., 1979*).

It has recently been suggested that tetracycline may inhibit tissue collagenase and slow the breakdown of collagen. If this is so, then the reduction in chemotactic collagen fragments would decrease the number of polymorphonuclear cells attracted to the site and, therefore, inflammation (*Golub et al., 1984*).

(b) Quaternary ammonium compounds

These compounds are cationic surfactants which are capable of reducing surface tension and disrupting cell membranes. This decreases the ability of bacteria to reproduce and adhere to tooth surfaces as well as causing cell lysis through increased cell membrane permeability.

In vitro, members of this group such as cetylpyridinium chloride exhibit antimicrobial characteristics that are, broadly, on a par with chlorhexidine. However, that parity with chlorhexidine is not matched *in vitro* in the presence of anionic substances, or by its *in vivo* performance. The quaternary ammonium compounds have moderate antiplaque activity, being rapidly adsorbed to the tooth surface at high concentrations but they are also rapidly released as they lack substantivity (*Kornman, 1986*). Clinical trials with cetylpyridinium chloride suggest that it has some antiplaque activity when used often

enough with minimal extraneous anionic matter present (*Ashley et al., 1984a; Ashley et al., 1984b*).

(c) Enzymes

It has been proposed that enzymes could specifically or non-specifically work against the formation of plaque, pellicle or their components by interfering with bacterial attachment, initial tooth colonization and plaque succession. At present there is little conclusive data available (*Kornman, 1986*).

(d) Phenolic Compounds

The phenolic formulation that is used most often is a mixture of chemicals, each of which may or may not contribute to efficacy and substantivity. At the concentrations that are used in the mouth it is probable that their mechanism of action is to disrupt essential bacterial enzyme systems.

Clinical trials using these agents are inconclusive. Phenols appear to be more effective in reducing the rate of plaque accumulation than in reducing pre-existing plaque (*Kornman, 1986*). Thus, a recent study by *Ciancio et al., (1989)* found that oral irrigation with a phenolic compound reduced plaque accumulation and decreased bacterial counts in generally healthy subjects in comparison with a matched control group. However, the types of bacteria present in healthy people are likely to be different from those in periodontitis or gingivitis.

(e) Oxygenating agents

These agents are used to raise the oxygen tension and act against obligate anaerobes. However, the question of safety has been raised as there is the possibility, with long-term use, of mucosal irritation, opportunistic infection and histological changes associated with

cancer (*Rees and Orth, 1986*). These agents also lack substantivity. Hydrogen peroxide, for example, acts on direct contact but is cleared rapidly.

A recent study by *Listgarten et al., (1989)* looked at the effect of subgingival irrigation with tetrapotassium peroxydiphosphate (*PDP*) on scaled and untreated periodontal pockets. Clinical parameters were assessed and the microflora examined using dark field microscopy. It was found that there was no significant difference in therapeutic effect between *PDP* and placebo although there was a trend towards a greater proportion of coccal forms and a smaller proportion of motiles and spirochaetes in sites irrigated with *PDP*.

(f) Heavy metal salts

Miller tested silver, mercury and zinc salts for their antibacterial activity as far back as 1884 (*Mandel, 1988*). Salts such as zinc chloride, zinc citrate and copper sulphate may inhibit plaque growth, especially in the early stages of its formation (*Addy et al., 1979; Harrap et al., 1982; Waerhaug et al., 1984*).

The evidence for the effect of fluoride on the reduction of dental caries is overwhelming but the mechanism of action is not well understood. Research into this led to the realization that fluorides might be useful in plaque control, possibly by blocking enzyme systems involved in bacterial metabolism and the synthesis of cell components. Stannous fluoride appears to be a promising antibacterial agent *in vitro* (*Hedberg, 1987*).

Problems of staining and unpleasant taste would have to be overcome for clinical usage.

(g) Sanguinarine

Sanguinarine is a benzophenanthradine alkaloid derived from the blood root plant, *Sanguinaria Canadensis*. It has been incorporated into a rinse containing zinc ions, and a dentifrice.

In vitro studies suggest that sanguinarine is effective against oral isolates (Dzink and Socransky, 1985) but *in vivo* studies have had variable results (Wennstron and Lindhe, 1985; Etemadzadeh and Ainamo, 1987), possibly because the subjects in the studies varied in their state of health and the location and type of microflora could differ.

A recent paper compared the effects of chlorhexidine, phenolic and sanguinarine mouthrinses on plaque and gingivitis in a placebo-controlled double blind study of six months' duration (Grossman et al., 1989). Compared to placebo, the group rinsing with 0.12% chlorhexidine had significantly less gingival inflammation, bleeding and plaque. Both sanguinarine and phenolic mouthrinses decreased plaque but were significantly less effective than chlorhexidine. They were not significantly different from placebo in their effect on gingivitis and gingival bleeding.

This underscores the importance of assessing the effect of anti-microbials on the associated disease rather than plaque alone: plaque is not the disease. Agents may reduce plaque but be more effective against a certain part of the flora which may not be the main causative agent in, for example, gingivitis or adult periodontitis.

(h) Others

Other agents that have been studied for their potential antiplaque activity include triclosan, sodium benzoate and sodium lauryl sulphate.

Triclosan is a nonionic antibacterial agent that is active against both gram-positive and gram-negative forms. It has no adverse side effects when used in a dentifrice and is compatible with anionic substances, unlike cationic antibacterials such as cetylpyridinium chloride and chlorhexidine which may be neutralized by anionic substances. Triclosan incorporated with a copolymer has enhanced substantivity and this form may be effective in the reduction of plaque and gingivitis (*Palomo et al., 1989; Saxton et al., 1988*).

Sodium lauryl sulphate is the most commonly used detergent in toothpaste. Both sodium lauryl sulphate and sodium benzoate have some antibacterial activity *in vitro* (*Pader, 1987*). It has been suggested that their incorporation in a pre-brushing rinse could loosen plaque, possibly by disrupting cohesive bonds in the plaque mass (*Bailey, 1989*). However, the few clinical trials that have been carried out yield inconclusive results.

To summarize, much research has gone into the evaluation of chemotherapeutics for their potential in plaque control. However, none of the agents discussed so far successfully meets as many of the criteria for an ideal chemotherapeutic agent as does the bisbiguanide, chlorhexidine.

2.5 Chlorhexidine

Chlorhexidine is the most effective antiplaque and antigingivitis chemotherapeutic that is currently available. It was first synthesized by ICI in England during the search for an antimalarial drug with a proguanil base. It was observed that polyguanide compounds had antibacterial properties and that one in particular, a dimer of proguanil, 1:6-di-4'-chlorophenyldiguanidohexane, was especially

active (*Davies et al.*, 1954). Since then the action of that chemical, chlorhexidine, has been extensively researched. Its use in periodontics dates from research in the late 1960's and early 1970's (*Loe and Schiott*, 1970) and has been reviewed by several authors (*Addy*, 1986; *Fardal and Turnbull*, 1986; *Gjerme*, 1974; *Hirst*, 1972; *Tonelli et al.*, 1983).

2.5.1. Chemistry of Chlorhexidine

A cationic bisbiguanide, chlorhexidine consists of two 4-chlorophenyl rings and two biguanide groups connected by a hexamethylene chain (Figure 1). It is most stable as its salts. The hydrochloride and acetate salts have low solubility but the gluconate is freely soluble and forms micelles in solution.

As a cationic agent chlorhexidine is easily inactivated by inorganic anions such as chlorides, nitrates, phosphates and carbonates as well as organic anionic soaps and detergents such as sodium lauryl sulphate (*Gardner*, 1983). Many ingredients in pharmaceutical formulations, such as alginate, carboxymethylcellulose and tragacanth, will inactivate chlorhexidine by adsorption. Most non-ionic surfactants will also reduce its antimicrobial activity (*Gardner*, 1983).

Chlorhexidine is strongly basic, the optimal pH for its activity is 5.5 to 7.0 but even within this range its microbicidal activity against different species varies widely.

Animal toxicity tests and long clinical experience indicate that chlorhexidine has unusually low toxicity, possibly due to poor ingestion. Very few cases of sensitization have been reported (*Foulkes*, 1973).

2.5.2. Mechanism of Action

Chlorhexidine is active against a wide range of microorganisms including gram-positive and gram-negative bacteria, fungi such as *Candida albicans* and yeasts. It is not effective against viruses or spores.

The antibacterial activity of chlorhexidine is derived from its adsorption onto bacterial cell walls allowing leakage of intracellular components. At low concentrations (i.e. 0.11 ug/ml) it is bacteriostatic and allows small molecular weight substances such as potassium and phosphate ions to leach out. However, at high concentrations (i.e. more than 100 ug/ml) chlorhexidine is bactericidal as it causes precipitation and coagulation of the cytoplasm, possibly through protein cross-linkage.

Bacteria vary in their susceptibility to chlorhexidine. Gram-positive forms may be inhibited at concentrations less than 10 ug/ml whereas concentrations of 50 ug/ml may be required to inhibit gram-negative forms. Chlorhexidine has antibacterial activity against oral species such as *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, *Actinomyces naeslundii* and *Streptococcus salivarius* (Fardal and Turnbull, 1986). Rogers et al., (1987) have shown that chlorhexidine appears to inhibit glycolysis and arginine synthesis in *Streptococcus sanguis*, an additional mechanism of action to those already discussed.

Schiott et al. (1970) tested the effect of mouthrinsing with 0.2% chlorhexidine and found that there was an eighty five to ninety percent reduction in the total number of bacteria in saliva over forty days. Bacterial numbers returned to baseline levels by forty eight hours after ceasing rinsing. After two years' use of chlorhexidine mouthrinses, the same group found that the number of bacteria was only

reduced by thirty to fifty per cent (*Schiott et al., 1973*). This study raised the spectre of bacterial resistance, as did a study in beagle dogs (*Hamp et al., 1973*) when plaque and gingivitis reappeared after six months' use of chlorhexidine mouthrinses.

There would appear to be no theoretical basis for resistance to a non-specific antibacterial, although a chlorhexidine-resistant strain of *Pseudomonas* has been described which may have a decreased ability to adsorb chlorhexidine as a result of having little lipopolysaccharide in its cell wall (*Gardner, 1983*).

Other antimicrobials that have similar or better *in vitro* activity than chlorhexidine do not have its *in vivo* properties (*Roberts and Addy, 1981*). This is because chlorhexidine has greater substantivity, binding reversibly to acidic macromolecules, hydroxyapatite, pellicle, salivary proteins, bacteria, extracellular polysaccharides of microbial origin and mucous membranes (*Rolla et al., 1970*). It is likely that chlorhexidine binds to phosphate groups in lipopolysaccharides and carboxyl groups in proteins (*Kornman, 1986*). The adsorbed chlorhexidine is released back into the mouth over about twenty four hours as the concentration drops, resulting in a type of timed release (*Bonesvoll et al., 1974*).

Jenkins et al. (1988) have questioned whether or not this 'oral reservoir' is responsible for the greater antibacterial activity seen with chlorhexidine. Removable acrylic appliances with enamel inserts were worn by volunteers and the appliances were treated with chlorhexidine applied either topically or by rinsing. There was no difference in plaque accumulation which led to the suggestion that chlorhexidine has an immediate antibacterial action plus a

bacteriostatic action from adsorption onto pellicle-coated enamel (*Jenkins et al., 1988*).

It has recently been suggested that chlorhexidine may alter host defence mechanisms by modifying superoxide generation in induced human neutrophils (*Gabler et al., 1987*). If it reduced responsiveness of neutrophils to chemotactic factors then this would provide an anti-inflammatory role for chlorhexidine.

2.5.3. Side Effects

The use and acceptance of chlorhexidine has been limited by its side effects. The main ones are its bitter taste, staining of teeth and restorations, and reversible selective interference with taste sensation (*Flotra et al., 1971; Lang et al., 1988*). Mucosal irritation and parotitis have been less commonly reported and have been associated with the use of high concentration solutions. It is possible that precipitation of proteins in the mucin layer may reduce the lubricating effect on the mucous membrane and interfere with anti-virus mechanisms including secretory IgA (*Gjerme, 1974*).

Addy and Roberts (1981) developed a polymethylmethacrylate model to compare the adsorption and staining of cationic antiseptics. Findings suggested that the antiseptics bound in a stable adsorbed monolayer to acrylic. Desorption behaviour was consistent with that seen *in vivo*.

The characteristic yellow-brown staining from chlorhexidine usage was also examined and observations supported a dietary aetiology through the precipitation of chromogenic compounds. Quantity alone did not account for differences in staining although this had already been proposed as a mechanism (*Heyden, 1973*). Alexidine stained more than chlorhexidine and this stained more than cetylpyridinium chloride.

This is probably related to the first two being dicationic and more reactive with acid moieties than cetylpyridinium chloride which is monocationic.

2.5.4. Clinical Trials

Chlorhexidine is often used where conventional oral hygiene is difficult. Such dental applications may be post-surgical (*Bakaeen and Strahan, 1980; Westfelt et al., 1983*), during intermaxillary fixation, during orthodontic treatment and in the management of medically compromised patients. It has also been reported that chlorhexidine may be of value in the treatment of recurrent oral aphthous ulceration (*Addy, 1987*). However, most research has been into its use in the prevention and treatment of gingivitis and periodontitis.

(i) Short-term Trials

Several investigators have shown that, in the absence of any mechanical oral hygiene measures, mouthrinsing on five, two or one occasions daily with 10 mls of 0.2% chlorhexidine will prevent plaque accumulation and the development of gingivitis (*Löe and Schiött, 1970; Schiött, 1973*).

Using the experimental gingivitis model, *Löe and Schiött, (1970)* showed that twice daily rinsing with chlorhexidine prevented plaque accumulation and gingivitis developing over twenty-one days. It may also prevent calculus formation (*Loe et al., 1976*). *Schiött (1973)* reported that there was an 85% to 90% reduction in the total number of bacteria present in saliva after forty days of daily rinsing with 10 mls of 0.2% chlorhexidine.

These findings have been confirmed by others in man and various animal models (*Hamp et al., 1973; Addy and Moran, 1983*).

(ii) Long-term trials

The results from long-term trials have been much less conclusive. This is partly because the chemotherapeutic effect is often assessed in relation to mechanical debridement which may be either the control or an independent variable. Mechanical plaque control results in a substantial reduction in plaque and gingivitis when used alone so the superimposition of chemical measures often produces only a minimal effect.

Flötra et al. (1972) reported on a four month clinical trial on fifty soldiers, finding that twice daily rinses with 0.2% or 0.1% chlorhexidine resulted in lower Plaque and Gingival Index Scores.

A two year study of the effects of mouthrinsing on students had a flawed experimental design (*Loe et al., 1976*). Students brushed their teeth once daily with 0.2% chlorhexidine or a placebo but the clinical gain over brushing alone was very small. Subjects' teeth were given a prophylaxis after nine months and from then on they were assigned to either the active rinse or a placebo rinse and allowed to brush their teeth at other times. The difference between the effect of the placebo and the active rinses was small. It was later found that the flavouring in the active rinse reduced its efficacy by 75% (*Kornman, 1986*). In addition it is possible that anionic surfactants were introduced if the students used toothpaste and this would be detrimental to the anti-plaque effect of chlorhexidine (*Addy et al., 1989*).

Aziz-Gandour and Newman (1986) reported on a clinical trial of eighty four days' duration involving twenty two patients. After scaling and

root planing, patients were instructed in the Bass toothbrushing technique and use of a pulsating water jet for supragingival irrigation. Irrigation with 0.02% chlorhexidine was carried out each day for twenty eight days and compared with the effect of a placebo rinse. Chlorhexidine reduced plaque and gingival inflammation up to the fifty sixth day but, although differences were statistically significant, the clinical difference was slight.

These studies did not evaluate the effect of chlorhexidine on parameters characterizing adult periodontitis. Consequently, the same centre carried out another clinical trial using the same mechanical plaque control regime - scaling and root planing followed by Bass toothbrushing - plus supragingival irrigation with chlorhexidine to study its effect on the subgingival microflora. Subjects irrigated supragingivally with either the test solution or a control solution, once daily for twenty eight days. Molar teeth were not irrigated, they were only mechanically debrided and served as controls. Samples were taken from test and control sites at days 0, 7, 28, 56 and 84. These were monitored using dark field microscopy on the basis of morphological characteristics as described by *Listgarten, Lindhe and Hellden (1978)*. It was found that supragingival irrigation appeared to be of additional clinical benefit, especially during the first four weeks. However, the benefits were not statistically significant and findings indicated that supragingival irrigation with chlorhexidine did not affect the subgingival microbiota.

(iii) Trials Using Subgingival Irrigation

Subgingival irrigation would seem to be difficult for patients to accomplish as clinical experience indicates that many find effective toothbrushing an exacting task.

Soh et al., (1982) investigated the effect of chlorhexidine on the subgingival microflora when periodontal pockets were irrigated by patients themselves. The eighteen people in this study used a blunt needle and disposable syringe to irrigate approximal molar and premolar sites over a twenty eight day period. It was found that there was a highly significant reduction in periodontal inflammation to below baseline levels for a further twenty eight days. The authors considered that subgingival irrigation could be a useful home care oral hygiene method. In a study from the same school (*Khoo and Newman, 1983*) eight subjects subgingivally irrigated sites according to the same protocol. Subgingival bacterial samples from chemically treated and non-chemically treated sites were collected at days 7,14,21,28,56 and 84 from baseline. It was found, using dark field microscopy, that the proportion of coccal forms increased greatly and the proportions of spirochaetes, motile forms and others reduced.

Irrigation did not seem to have any effect beyond that of mechanical debridement alone in another study (*MacAlpine et al., 1985*). Here periodontal pockets were irrigated professionally every two weeks for twenty four weeks after one episode of oral hygiene instruction and root planing. The number of bleeding sites and spirochaetes decreased, probing pocket depths decreased and there was some gain in attachment irrespective of subgingival irrigation with chlorhexidine, tetracycline, saline or no irrigation at all.

Using a similar protocol but with the patients irrigating their pockets daily for the twenty four weeks, *Braatz et al., (1985)* found the same results and concluded that subgingival irrigation with chlorhexidine did not augment the effect of non-surgical periodontal treatment.

Phase contrast microscopy was used in another clinical trial where the clinical and microbiological effects of once daily subgingival irrigation with chlorhexidine were evaluated (*Haskel et al., 1986*). The Gingival Index reduced and there was a significant increase in the proportions of coccal forms and decrease in the proportion of spirochaetes. No subgingival instrumentation was carried out and the authors concluded that subgingival irrigation alone was of limited clinical benefit.

Watts and Newman (1986) examined the effects of Bass toothbrushing, scaling and root planing, and self-administered subgingival pulsed jet irrigation in eleven patients with periodontitis. Patients irrigated once daily for twenty eight days with either 0.02% chlorhexidine or a placebo rinse. Periodontal inflammation and pocket depths were decreased but there was no added benefit in using 0.02% chlorhexidine. The authors concluded that further study was warranted using stronger solutions of chlorhexidine.

Lander studied sixteen subjects with at least four moderate to advanced periodontal lesions. These were irrigated once with either chlorhexidine solution, chlorhexidine gel or saline, whilst the other site was left as an untreated control. Changes from baseline values were monitored clinically and bacteriologically over ten weeks. It was found that there was a shift in bacterial colonization patterns and clinical variables in certain sites such that:

"CH (chlorhexidine) solution and gel sites were demonstrably healthier than control or saline-irrigated sites" (Lander et al., 1986).

Thus clinical trials evaluating the effects of subgingival irrigation with chlorhexidine in adult periodontitis have been inconclusive. In

particular, no attempt has been made to rank mechanical debridement, subgingival irrigation with chlorhexidine or combined treatment using both modalities.

CHAPTER THREE

Materials and Methods

3.1 Subjects

Thirteen subjects were randomly selected from the waiting list of the United Dental Hospital to form the sample population. The general population was defined, for the purpose of this clinical trial, as all individuals who:

- (i) were on the hospital waiting list;
- (ii) were above the age of thirty years;
- (iii) had periodontitis;
- (iv) had at least one periodontal pocket > 6 mm in probing depth in each quadrant;
- (v) had no systemic illnesses;
- (vii) had not taken antibiotics for three months.

Individuals' informed consent to participation was obtained prior to their inclusion in the study. Subjects were then examined and given oral hygiene instruction in the Modified Bass technique of tooth-brushing.

3.2 Treatment Groups

The main variables in this study were:

- (i) scaling and root planing (S1) or no mechanical debridement (S0); and
- (ii) subgingival irrigation with 0.2% chlorhexidine gluconate solution (C1) or subgingival irrigation with saline (C0).

Each quadrant of a subject's mouth received different treatments at the outset, resulting in four matched treatment groups and thus allowing analysis of the interaction between mechanical debridement

and chemical therapy. The four treatment groups were: COS0; COS1; C1S0 and C1S1. The treatments were randomly assigned.

3.3. Treatment Schedule

At baseline (week 0), two quadrants were scaled in each subject's mouth, using local anaesthesia if required. One designated pocket in one scaled and one unscaled quadrant was then subgingivally irrigated with 0.2% chlorhexidine solution delivered via an oral irrigator* fitted with a cannula adapted for subgingival irrigation. Designated pockets in each of the remaining quadrants were subgingivally irrigated with saline. Thus, the physical effect, but not the chemical effect, of chlorhexidine irrigation was common. All pockets were irrigated for twenty seconds, delivering 10 mls of solution, and overflow was unavoidable. To make the effect of the overflow uniform, subjects were instructed to rinse once with 15 mls of 0.2% chlorhexidine solution following irrigation and then to spit the solution out immediately.

The unscaled quadrants remained so for the duration of the trial unless clinical signs and symptoms supervened to contraindicate this. After the trial these quadrants were treated.

Clinical variables were measured and bacterial samples collected at baseline (prior to treatment) and then at weeks 1,4,8 and 12 after treatment.

*Water-Pik, Prevident

3.4. Clinical Variables

The clinical variables that were assessed were the Plaque Index (*Silness and Loe, 1964*), the Gingival Index (*Loe, 1967*), a bleeding index, probing pocket depth and attachment level. The bleeding index used was a dichotomous index and not one of the ordinal bleeding

indices used occasionally (e.g. the Muhlemann Bleeding Index, the Papillary Bleeding Index).

Measurements of probing pocket depth and attachment level were made using a sterile designated periodontal probe assigned to each subject in the study. The same probe was used for individual subjects throughout the study. The pattern used was the University of Michigan "0" probe with Williams markings and the probes were selected to ensure minimal variation in the actual lengths marked on them. Prior to starting the trial the operator was calibrated against a senior member of the University staff to ensure reproducibility of measurements.

3.5 Microbiological Variables

At each examination a sample of the subgingival microflora was collected from the designated sites using a Gracey curette. The sample was placed in a sterile vial containing 0.5 ml of normal saline, dispensed in a vortex mixer for twenty seconds and 15 ul micropipetted onto a slide which was then coverslipped and sealed.

Bacterial samples were examined under dark field microscopy at 800x magnification within one hour of collection. The bacteria present were morphologically categorized as coccal forms, motile forms, spirochaetes or others, according to the method described by *Listgarten, Lindhe and Hellden (1978)*.

Twenty fields were counted from each sample except in two subjects where ten fields were counted. A set number of fields was counted rather than a certain number of bacteria because of time constraints and because treatment was likely to produce both qualitative and quantitative changes in the subgingival flora.

3.6 Statistical Analysis

There were three assumptions made in this study. They were:

- (i) that the subjects were a random sample;
- (ii) that there was an equal correlation between measurements made at different intervals because the measurements were made on the same people;
- (iii) that there was a constant standard deviation in all subgroups.

The ordinal variables, that is, the Plaque, Gingival and Bleeding Indices were analysed non-parametrically using Cochran's Q-test to compare proportions.

The other variables, probing pocket depth, attachment level and proportions of bacterial morphological types, were analysed parametrically using a three-way repeated measures analysis of variance.

Appropriate transformations, together with analyses of error and the validity of baseline assumptions were also made.

The statistical analysis was carried out using Minitab Statistical Software run on a Hewlett-Packard microcomputer.

CHAPTER FOUR

RESULTS

4.1.1 Plaque Index

At baseline the percentage frequency of sites scoring PlI(0) ranged from 0% (COS0) to 30.77% (C1S0), that of sites scoring PlI(1) ranged from 23.08% (C1S0) to 46.15% (COS0, C1S1) and that of those scoring PlI(2+3) ranged from 30.77% (COS1) to 53.85% (COS0, C1S1) (Table 3).

Treatment resulted in decreased levels of plaque in all groups. In the COS0 treatment group, the percentage of sites scoring PlI(2+3) remained fairly constant, as did the frequency of sites scoring PlI(0) and PlI(1) when considered together (Figure 2).

There was an increase in the percentage of sites with scores of PlI(0), from 23.08% at week 0 to 46.15% at week 1 in the COS1 group (Figure 3). This was maintained for the remainder of the trial. At the same time, the percentage of sites scoring PlI(2+3) decreased from 30.77% at week 0 to 18.18% at week 12.

Similar changes were seen in the C1S0 treatment group although these were not as pronounced as 30.77% of sites already scored PlI(0) at week 0 (Figure 4).

At week 0 7.69% of sites scored PlI(0) in the C1S1 group but this increased to 30.77% at week 1 and 63.64% by week 12. However, 38.46% of sites scored PlI(0) at week 0 but none did at week 12. The percentage of sites scoring PlI(2+3) decreased from 53.85% at week 0 to 15.38% at week 1 but returned to 38.46% at week 4, this was then maintained through to week 12 (Figure 5).

Overall, the percentage of sites with scores of PlI(0) increased in all groups with the least improvement in the control group (COS0)

(Figure 6). The increase was mostly at the expense of the percentage of sites with scores of PlI(1) which decreased across all treatment groups with time (Figure 7). The percentage of sites scoring PlI(2+3) also tended to decrease at week 1, especially in the COS1 and C1S1 treatment groups. However, the percentage of sites scoring (2+3) then increased at week 4, except in the C1S0 group, and tended to return toward baseline values by week 12 (Figure 8).

Statistical analysis using Cochran's Q test to compare proportions showed that there was a statistically significant increase in the proportion of sites scoring PlI(0) over weeks 0 to 8 in the COS1 and C1S1 treatment groups at the 0.05 level of significance. When changes from weeks 0 through to 12 were analysed, with subjects 5 and 8 omitted because they did not complete the trial, significance only persisted for the C1S1 treatment group, at the 0.05 confidence level. However, the C1S1 group had a lower proportion of PlI(0) at baseline than did the COS1 group.

4.1.2. Gingival Index

At baseline the percentage frequency of sites scoring GI(0) ranged from 0% (COS0) to 15.38% (COS1, C1S1) (Table 5) and that of sites scoring GI(2+3) ranged from 23.08% (COS1) to 53.84% (COS0) (Table 6). The Gingival Index scores improved during the trial in all treatment groups. At week 12 the percentage frequency of sites scoring GI(0) ranged from 18.18% (COS0) to 54.55% (C1S1) and that of sites scoring GI(2+3) ranged from 9.09% (COS1) to 27.27% (COS0) (Table 6).

At baseline no sites scored GI(0) in the COS0 group (Figure 9). With treatment this increased to 15.38% at week 1 and was maintained to week 12. There was a concomitant decrease in the proportion of sites

scoring GI(2+3) from 53.84% at week 0 to 38.46% at week 1. After this the distribution of scores in the COSO treatment group did not show much variation.

In the COS1 treatment group at baseline, 15.38% of sites had a score of GI(0). This increased to a maximum of 38.46% at week 4 with a corresponding fall in the proportion of sites scoring GI(1) (Figure 10).

Subgingival irrigation with chlorhexidine (C1S0) resulted in an increase in the proportion of sites scoring GI(0) from 15.38% at baseline to 38.46% at week 4 corresponding to a decrease in the proportion of sites scoring GI(1). At week 12 there was an increase in the proportion of sites scoring GI(1) to 54.55% and a decrease in the proportion scoring GI(2+3) to 18.18% in the C1S0 group (Figure 11).

The C1S1 treatment group showed an increase in the proportion of sites scoring GI(0), from 7.69% at baseline to 54.55% at week 12 (Figure 12). The proportion of sites scoring GI(1) decreased from 61.54% at baseline to 27.27% at week 12 and the proportion of sites scoring GI(2+3) after treatment was lower in this than in any other treatment group.

The percentage of sites scoring GI(0) increased in all groups during the trial but more so with C1S1 than with the COS1 or C1S0 treatments where there was a return towards baseline values by week 12. The control group showed the least change (Figure 13).

The changes in proportions of sites scoring GI(1) in each treatment group are shown in Figure 14. The C1S1 treatment group showed a greater decrease in the proportion of sites scoring GI(1) than either the COS1 or C1S0 groups which showed some decrease from baseline

values but returned towards baseline by week 12. The control group showed little change.

The proportion of sites with scores of GI(2+3) decreased from baseline to week 12 when 27.27% of the control sites scored GI(2+3), 18.1% of sites in the C1S0 and C1S1 treatment groups scored GI(2+3) and 9.09% of the COS1 sites scored GI(2+3) (Figure 15).

Statistical analysis using Cochran's Q test to compare the proportions of Gingival Index scores in the four treatment groups indicated that there was a significant ($p < 0.05$) increase from week 0 to week 8 in the proportion of sites scoring GI(0) in comparison with those scoring GI(1), GI(2) or GI(3) in the COS1 and C1S1 treatment groups. When the analysis was applied to changes from week 0 to 12, with the omission of subjects 5 and 8 because they did not complete the trial, this significance was only sustained for the C1S1 group. However, the C1S1 group had a lower proportion of GI(0) at baseline than did the COS1 group.

4.1.3. Bleeding Index

There was a trend towards a reduction in the proportion of sites that bled on probing and, therefore, an increase in the proportion of sites that did not bleed on probing, across all treatment groups from baseline to week 12. However, consistent change in the same direction at each examination was only approached in the C1S0 treatment group (Figures 16, 17, 18, 19).

There was no change in the percentage of sites that bled on probing in the COS0 and COS1 treatment groups from week 0 to week 1 whereas there was a reduction in the C1S0 and C1S1 treatment groups. Between week 1 and week 4 there followed a reduction in all except the C1S0 group so

that by week 4 the C1S1 and COS1 treatment groups showed the most pronounced reduction in proportions of bleeding sites. Following this there was some rebound in all treatment groups except C1S0 at week 8, but by week 12 the proportion of bleeding sites in all treatment groups was still less than it was at baseline (Figure 20).

Statistical analysis using Cochran's Q test to compare proportions showed that there was a statistically significant reduction ($p < 0.025$) in the proportion of bleeding sites in the COSO group during the study. However, the control group had the highest percentage of bleeding sites (84.62%) at baseline and this reduced to a level similar to that of the other treatment groups by week 12.

4.1.4. Probing Pocket Depths

Mean probing pocket depths at baseline ranged from 6.15mm in the C1S0 group to 6.53 mm in the C1S1 group (Table 9). There was a decrease in the mean probing depth in all four groups at week 1 with the greatest reduction being 0.77 mm in the C1S1 group (Table 10). The control group showed a comparable reduction of 0.76 mm during the same period. The mean probing depths tended to plateau from week 1 to week 8 and then decrease again to week 12 in all groups. At the end of the study the mean pocket probing depths were 5.00 mm in the C1S1 and C1S0 groups and 5.18 in the COSO and COS1 groups (Table 9).

There was a decrease in mean probing pocket depth in all four treatment groups over the study period (Figure 21). The greatest reduction was seen in the C1S1 group (1.53 mm) with the least reduction occurring in the C1S0 group (1.15 mm) (Figure 22).

Analysis of variance showed that there was a statistically significant ($p < 0.01$) reduction in mean probing pocket depths in all four treatment

groups from baseline to week 12 but, in this sample, there was no statistically significant difference between the effect of the treatments on mean probing pocket depths. The significant effect of time remained after p-values were adjusted to allow for assumptions not being met (Greenhouse Geisser $p < 0.0005$; Huynh Feldt $p < 0.0002$).

4.1.5. Attachment Levels

The mean attachment levels in the four treatment groups were similar at baseline and varied from 7.23 mm (COS1, C1SO groups) to 7.46 mm (C1S1 group) (Table 11).

At week 1 the COS1 group had a mean loss of attachment (0.08 mm) in comparison with the other groups which gained attachment. Between week 1 and week 8 there was a tendency towards a further mean gain in attachment in all groups but the control (Figure 23). By week 12 the mean gain in attachment varied from 1.04 mm in the COSO group to 1.64 mm in the C1S1 group (Table 12, Figure 24).

During the twelve weeks of the clinical trial there was a mean gain in attachment in all groups (Figure 23) so that by week 12 the mean attachment level varied from 5.64 mm in the C1SO group to 6.27 mm in the COSO group (Table 11, Figure 23).

Analysis of variance indicated that there was a statistically significant ($p < 0.0001$) mean gain in attachment in all groups over the period of study. When p-values were adjusted to allow for assumptions not met, significance remained (Greenhouse Geisser $p < 0.0004$; Huynh Feldt $p < 0.0001$). There was no statistically significant interaction of treatment with weeks in terms of changes in mean attachment levels within or between the four treatment groups.

4.2 Microbiological Variables

4.2.1. Bacterial numbers

The mean number of bacteria counted per sample varied from 201.00 (COS0) to 216.23 (C1S0) at baseline (Table 14).

At week 1 the mean number of bacteria counted in each treatment group decreased, the greatest change being a reduction to 111.77 in the C1S1 group (Table 14). There was a rebound in the mean number counted in all groups at week 4 and following this there was a further reduction in the mean number counted in all except the scaling group (Figure 25).

The greatest change was seen in the C1S1 group followed in order by the C1S0, COS0 and COS1 groups (Figure 25).

The number of fields that were counted in each sample varied (Table 13). The mean number of bacteria counted per high power field (H.P.F.) was calculated to give a more accurate representation of trends (Table 15). Similar trends were observed for each treatment when the mean number of bacteria per H.P.F. were considered (Figure 26).

4.2.2. Coccal forms

The mean total number of coccal forms counted at baseline ranged from 67.54 (C1S0) to 78.31 (COS1) (Table 16). Following treatment at week 0, the mean total number counted in each treatment group varied. At weeks 1 and 4 it decreased in the COS1 and C1S1 groups whereas it increased in the C1S0 and COS0 groups. By week 12 the mean numbers counted had decreased in all groups to range from 33.29 (C1S1) to 45.14 (COS1) (Table 16).

The mean number of coccal forms counted per H.P.F. also decreased from baseline to week 12 (Figure 27). At baseline the mean number counted

per H.P.F. ranged from 3.93 (COSO, C1S0) to 4.53 (COS1). The rate of decrease was not constant with all groups showing some rebound at week 4. However, the net change with time was similar in all groups (Figure 27) so that at the conclusion of the study the range was from a minimum of 2.06 (C1S1) to a maximum of 2.86 (COS1) (Table 17).

The mean proportions of coccal forms counted in samples were also calculated, (Table 18) At baseline the mean percentage proportions varied from 32.29% (C1S0) to 38.72% (COS1). At week 1 following treatment, the mean proportion of coccal forms increased in all groups, most markedly in the C1S1 group followed by the COS1 and C1S0 groups, the control group showing the least change. In the C1S0 group the mean proportion of coccal forms increased from week 0 to week 4 while in the other treatment groups the mean percentage proportion of coccal forms increased from week 0 to week 1 but showed a decrease from weeks 1 to 4. There was a trend to a return to baseline values by week 12 in all groups albeit with a rebound at week 8 in the C1S1 group (Figure 28).

The mean percentage proportion of coccal forms counted in the treatment groups irrigated with chlorhexidine (C1S0, C1S1) were compared with those in the treatment groups irrigated with saline (COS1, COS0). The mean percentage proportion of the CO groups was 44.35% at baseline which was greater than that of the C1 groups (36.75%) (Table 19). Following irrigation with chlorhexidine, the mean proportion of coccal forms climbed to 54.17% at week 1 which was a greater increase in the mean proportion of coccal forms than that which occurred in the CO group where the mean proportion was 49.52%. From week 1 through to week 12 the mean proportion of coccal forms in

the C1 group remained higher than that of the C0 group or that at baseline in either group (Figure 29).

The mean percentage proportions of coccal forms counted were subjected to analysis of variance within and between groups.

The mean percentage proportion of coccal forms showed a statistically significant change ($p=0.0003$) from weeks 0 to 8 in the groups that received subgingival irrigation with chlorhexidine (C1S0, C1S1). When p -values were adjusted to allow for assumptions not being met, significance remained (Greenhouse Geisser $p=0.0008$, Huynh Feldt $p=0.0003$). When the analysis was performed for changes from weeks 0 to 12, with the exclusion of subjects who did not complete the trial, significance was not maintained. The analyses were confirmed by reanalysis in a rank scale.

A diagnostic plot was made to determine whether there was a relationship between cell means and cell standard deviations.

This indicated that the standard deviation increased with an increase in the mean and that a log transformation was necessary to permit appropriate analyses.

The mean percentage proportions of coccal forms were subjected to a log transformation followed by analysis of variance. It was found that the change in the mean percentage proportion of coccal forms counted from weeks 0 to 8 was statistically significant in the groups that received subgingival irrigation with chlorhexidine ($p=0.0020$). This significance was maintained when adjustments were made to allow for baseline assumptions not being met (Greenhouse Geisser $p=0.0047$; Huynh Feldt $p=0.0020$). When the analysis was continued to week 12 with the

omission of subjects who did not complete the trial, significance was not found.

When subgingival irrigation with chlorhexidine and scaling were considered it could be seen that there was little difference between the effect in the COS1 and C1S1 groups. That is, the effect of subgingival irrigation with chlorhexidine on coccal forms was less for teeth that were scaled than for those that were not scaled.

4.2.3. Motiles

The mean number of motile forms counted varied from 33.08 (COS0) to 44.46 (C1S0) at baseline. At week 1 the mean number of motile forms dropped in all except the control group where mean numbers remained relatively constant during the study. There was a trend over the next eleven weeks for the mean numbers to return to baseline values in the COS1 and C1S0 groups. In comparison, mean numbers tended to remain depressed in the C1S1 group (Table 20).

When the mean numbers of motile forms per H.P.F. were considered, the same patterns emerged (Figure 30). Baseline values ranged from 1.98 (COS0) to 2.53 (C1S0) and dropped to between 0.70 (C1S1) and 1.51 (COS0) at week 1. At week 12 the range was from 0.97 (C1S1) to 2.78 (COS1) (Table 21).

The mean proportion of motile forms ranged from 17.35% (COS0) to 20.86% (COS1) at baseline and dropped in all treatment groups at week 1 (Table 22). During the remaining study period the mean percentage proportions tended to return to or exceed baseline values in all treatment groups (Figure 31).

An analysis of variance was performed on the mean proportions of motile forms counted in order to determine if there were any

significant changes between or within treatment groups during the study. The effect of scaling was found to be significant ($p=0.0324$) over the twelve weeks but when adjustments were made to allow for assumptions not being met, there was a reduction in the level of significance (Greenhouse Geisser $p=0.0785$; Huynh-Feldt $p=0.0490$).

4.2.4. Spirochaetes

At baseline the mean number of spirochaetes that were counted ranged from 42.92 (COS1) to 55.77 (C1S0) (Table 23). At week 1 the mean number counted had dropped in all treatment groups but less markedly so in the control group. Over the remainder of the study there was a tendency towards a return to baseline values. However, numbers were still depressed at week 12 when the range was from 11.14 (C1S0) to 26.00 (COS1) (Table 23).

The mean number of spirochaetes counted per H.P.F. showed similar trends. At baseline the range was from 2.38 (COS1) to 3.12 (C1S1) (Table 24). The mean number per H.P.F. decreased at week 1 in all groups. There was variation in all groups over the study period but the week 12 levels were still lower than at baseline, ranging from 0.66 (C1S0, C1S1) to 2.15 (COS1) (Figure 32).

The mean percentage proportions of spirochaetes ranged from 20.36% (COS1) up to 26.22% (C1S1) at baseline. There was a decrease in the proportion of spirochaetes in all groups at week 1 (Table 25).

Between weeks 1 and 12 there was little change in the control group. In the COS1 treatment group the 9.91% drop between week 0 and week 1 was followed by a steady return towards baseline values through to week 12 when the proportion was 18.94%. The decrease in proportions from week 0 to week 1 was most pronounced in the C1S1 group (18.04%)

but in both this and the C1S0 group there was considerable variation in the mean proportions before reaching, at week 12, levels that were lower than those at baseline (C1S0 = 9.72%, C1S1 = 12.07%). (Table 25, Figure 33).

Changes in the mean proportions of spirochaetes, both within and between groups, were subjected to an analysis of variance procedure to determine statistical significance. It was found that there was a statistically significant interaction between subgingival irrigation with chlorhexidine and scaling over weeks 0 to 12 ($p=0.0210$). The level of significance was reduced when allowance was made for baseline assumptions not being met (Greenhouse Geisser, $p=0.0570$; Huynh Feldt, $p=0.0289$).

4.2.5. Others

At baseline the mean number of others ranged from 39.77 (COS1) to 51.23 (COS0) (Table 26). At week 1 following treatment the mean number had dropped in all four groups, most markedly in the C1S1 group (22.85). At week 12 the mean numbers counted were still lower than at baseline in all except the scaling group where the mean number counted was 55.14 (Table 26).

The mean count of others per H.P.F. ranged from 2.35 (COS1) to 2.93 (COS0) at baseline (Table 27). Following treatment there was a sharp decrease in the mean number per H.P.F. at week 1 in all groups. At week 4 the mean number per H.P.F. of others had risen again in all groups (Figure 34). A further decrease resulted in the mean number per H.P.F. at week 12 being lower than at baseline in all groups except the COS1 where the mean number of others per H.P.F. was 3.56 (Table 27).

When the mean proportions of others were considered it could be seen that baseline values ranged from 20.10% (COS1) to 25.66% (COS0) (Table 28). After treatment the mean proportion of others decreased in all but the COS1 group where there was an increase of 3.56% (Figure 35). During the remainder of the trial there was an overall tendency for the mean proportion of others to rise so that by week 12 the mean proportion in all four treatment groups was higher than it had been at baseline (Figure 35). Thus, at week 12 the mean proportion of others ranged from 28.10% (COS0, C1S0) to 31.99% (C1S1).

The changes in the mean proportions of others were subjected to an analysis of variance. No significant changes were found either within or between groups over the study period.

4.2.6. Trends Within Treatment Groups

In the COS0 group at baseline the mean proportion of coccal forms was 34.41%, that of motiles was 17.35%, that of spirochaetes was 22.53% and that of other forms was 25.66% (Table 29). There was a small increase of 4.82% in the mean proportion of coccal forms concurrent with a decrease in the other bacterial groups (Figure 36). The mean proportions of the four morphotypes remained fairly constant for the remainder of the study.

Scaling and subgingival irrigation with saline (COS1) produced an increase in the mean proportion of coccal forms, from 38.72% at baseline to 51.60% at week 12 (Table 30). In contrast, spirochaetes and motile forms showed a reduction from baseline to week 1 before approaching baseline values at week 12 (Figure 37). Others showed a relatively steady increase from 20.10% as baseline to 31.37% at week 12 (Table 30).

In the C1S0 group the mean proportion of coccal forms increased from 32.29% at baseline to 48.03% at week 4 and from then on it approximated baseline levels (Table 31). The mean proportions of motile forms and others at week 0 were 20.97% and 22.43% respectively. These parameters showed a small reduction between week 0 and week 1 but then showed a steady increase to week 12 when the mean proportion of others was 28.10% and that of motile forms was 25.59% (Figure 38).

The C1S1 group showed wide variation in the mean proportions of certain bacterial types, overlaid on distinct trends consequent upon treatment. At baseline the mean proportion of coccal forms was 34.03%. This rose to a maximum of 61% at week 1 (Table 32). All other forms decreased their mean proportion between week 0 and week 1 (Figure 39). The mean proportion of motile forms approached baseline levels of 18.66% by week 12 when the mean proportion of motile forms was 17.83% whereas the mean proportion of others rose from 21.09% at week 0 to 31.99% at week 12. In contrast, spirochaetes reduced their mean proportion from 26.22% at week 1 to 12.07% at week 12 (Table 32). That said, all except motile forms showed pronounced variation in mean proportions between weeks 1 and 12.

Table 1

Plaque Index: Frequency of sites scoring PlI(0),PlI(1),PlI(2), and PlI(3) in each Tx group at each examination.

Tx Group	PlI	Week0	Week1	Week4	Week8	Week12
COS0	0	0	3	4	3	4
	1	6	5	4	4	2
	2	6	4	5	6	5
	3	1	1	0	0	0
COS1	0	3	6	7	5	6
	1	6	5	2	6	3
	2	3	2	4	1	2
	3	1	0	0	1	0
C1S0	0	4	5	7	6	5
	1	3	3	3	4	2
	2	4	5	1	2	4
	3	2	0	2	1	0
C1S1	0	1	4	5	4	7
	1	5	7	3	4	0
	2	7	1	4	4	3
	3	0	1	1	1	1

Table 2

Plaque Index: Percentage of sites scoring PlI(0),PlI(1),PlI(2) and PlI(3) in each Tx group at each examination.

Tx Group	PlI	Week0	Week1	Week4	Week8	Week12
COS0	0	0	23.08	30.77	23.08	36.36
	1	46.15	38.46	30.77	30.77	18.18
	2	46.15	30.77	38.46	46.15	45.45
	3	7.69	7.69	0	0	0
COS1	0	23.08	46.15	53.85	38.46	54.55
	1	46.15	38.46	15.38	46.15	27.27
	2	23.08	15.38	30.77	7.69	18.18
	3	7.69	0	0	7.69	0
C1S0	0	30.77	38.46	53.85	46.15	45.45
	1	23.08	23.08	23.08	30.77	18.18
	2	30.77	38.46	7.69	15.38	36.36
	3	15.38	0	15.38	7.69	0
C1S1	0	7.69	30.77	38.46	30.77	63.64
	1	38.46	53.85	23.08	30.77	0
	2	53.85	7.69	30.77	30.77	27.27
	3	0	7.69	7.69	7.69	9.09

Table 3

Plaque Index: Percentage of sites scoring PlI(0), PlI(1) and PlI(2,3) in each Tx group at each examination.

TxGroup	PlI	Week0	Week1	Week4	Week8	Week12
C0S0	0	0	23.08	30.77	23.08	36.36
	1	46.15	38.46	30.77	30.77	18.18
	2,3	53.84	38.46	38.46	46.15	45.45
C0S1	0	23.08	46.15	53.85	38.46	54.55
	1	46.15	38.46	15.38	46.15	27.27
	2,3	30.77	15.38	30.77	15.38	18.18
C1S0	0	30.77	38.46	53.85	46.15	45.45
	1	23.08	23.08	23.08	30.77	18.18
	2,3	46.15	38.46	23.08	23.08	36.36
C1S1	0	7.69	30.77	38.46	30.77	63.64
	1	38.46	53.85	23.08	30.77	0
	2,3	53.85	15.38	38.46	38.46	36.36

Table 4

Gingival Index Scores: Frequency of sites scoring GI(0), GI(1), GI(2) and GI(3) in each Tx group at each examination.

Tx Group	GI	Week0	Week1	Week4	Week8	Week12
C0S0	0	0	2	2	3	2
	1	6	6	6	5	6
	2	6	5	5	4	3
	3	1	0	0	1	0
C0S1	0	2	4	5	4	3
	1	8	6	5	6	7
	2	2	2	3	2	1
	3	1	1	0	1	0
C1S0	0	2	4	5	6	3
	1	6	4	3	3	6
	2	4	4	5	3	2
	3	1	1	0	1	0
C1S1	0	1	4	6	6	6
	1	8	8	5	5	3
	2	3	1	1	1	2
	3	1	0	1	1	0

Table 5

Gingival Index Scores: Percentage of sites scoring GI(0), GI(1), GI(2) and GI(3) in each Tx group at each examination.

Tx Group	GI	Week0	Week1	Week4	Week8	Week12
COS0	0	0	15.38	15.38	23.08	18.18
	1	46.15	46.15	46.15	38.46	54.55
	2	46.15	38.46	38.46	30.77	27.27
	3	7.69	0	0	7.69	0
COS1	0	15.38	30.77	38.46	30.77	27.27
	1	61.54	46.15	38.46	46.15	63.64
	2	15.38	15.38	23.08	15.38	9.09
	3	7.69	7.69	0	7.69	0
CIS0	0	15.38	30.77	38.46	46.15	27.27
	1	46.15	30.77	23.08	23.08	54.55
	2	30.77	30.77	38.46	23.08	18.18
	3	7.69	7.69	0	7.69	0
CIS1	0	7.69	30.77	46.15	46.15	54.55
	1	61.54	61.54	38.46	38.46	27.27
	2	23.08	7.69	7.69	7.69	18.18
	3	7.69	0	7.69	7.69	0

Table 6

Gingival Index Scores: Percentage of sites scoring GI(0), GI(1) and GI(2,3) in each Tx group at each examination.

TxGroup	GI	Week0	Week1	Week4	Week8	Week12
COS0	0	0	15.38	15.38	23.08	18.18
	1	46.15	46.15	46.15	38.46	54.55
	2,3	53.84	38.46	38.46	38.46	27.27
COS1	0	15.38	30.77	38.46	30.77	27.27
	1	61.54	46.15	38.46	46.15	63.64
	2,3	23.08	23.08	23.08	23.08	9.09
CIS0	0	15.38	30.77	38.46	46.15	27.27
	1	46.15	30.77	23.08	23.08	54.55
	2,3	38.46	38.46	38.46	30.77	18.18
CIS1	0	7.69	30.77	46.15	46.15	54.55
	1	61.54	61.54	38.46	38.46	27.27
	2,3	30.77	7.69	15.38	15.38	18.18

Table 7

Bleeding Index Scores: Frequency of sites scoring 1 or 0 in each Tx group at each examination.

Tx Group	BI	Week0	Week1	Week4	Week8	Week12
C0S0	1	11	11	7	10	5
	0	2	2	6	3	6
C0S1	1	8	8	5	7	5
	0	5	5	8	6	6
C1S0	1	10	8	8	6	4
	0	3	5	5	7	7
C1S1	1	9	6	5	8	4
	0	4	7	8	5	7

Table 8

Bleeding Index Scores: Percentage of sites scoring 1 or 0 in each Tx group at each examination.

Tx Group	BI	Week0	Week1	Week4	Week8	Week12
C0S0	1	84.62	84.62	53.85	76.92	45.45
	0	15.38	15.38	46.15	23.08	54.55
C0S1	1	61.54	61.54	38.46	53.85	45.45
	0	38.46	38.46	61.54	46.15	54.55
C1S0	1	76.92	61.54	61.54	46.15	36.36
	0	23.08	38.46	38.46	53.85	63.64
C1S1	1	69.23	46.15	38.46	61.54	36.36
	0	30.77	30.77	61.54	38.46	63.64

1=bleeding on probing to base of pocket
0=no bleeding on probing to base of pocket

Table 9

Mean probing pocket depths (mm) in each Tx group at each examination.

TxGroup		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	6.38	5.62	5.77	5.46	5.18
	S.D.	0.84	0.62	1.42	1.08	0.94
C0S1	Mean	6.38	5.92	5.84	5.84	5.18
	S.D.	0.83	1.32	1.56	1.23	1.11
C1S0	Mean	6.15	5.61	5.30	5.38	5.00
	S.D.	0.53	0.83	1.13	1.21	0.95
C1S1	Mean	6.53	5.76	5.61	5.76	5.00
	S.D.	1.00	1.30	1.68	1.24	1.34

Table 10

Mean decrease in probing pocket depth from week 0 to weeks 1,4,8 & 12 in each Tx group.

TxGroup	Wk1	Wk4	Wk8	Wk12
C0S0	0.76	0.61	0.92	1.20
C0S1	0.46	0.54	0.54	1.20
C1S0	0.54	0.85	0.77	1.15
C1S1	0.77	0.92	0.77	1.53

Table 11

Mean attachment level (mm) in each Tx group at each examination.

TxGroup		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	7.31	6.62	6.69	6.62	6.27
	S.D.	1.07	0.84	1.38	1.15	0.86
C0S1	Mean	7.23	7.31	6.85	7.08	6.09
	S.D.	1.37	1.59	2.18	1.73	1.44
C1S0	Mean	7.23	6.61	6.23	6.38	5.64
	S.D.	1.48	0.74	0.89	1.39	1.15
C1S1	Mean	7.46	6.77	6.38	6.62	5.82
	S.D.	1.45	1.72	1.73	1.55	1.4

Table 12

Mean gain in attachment from week 0 to weeks 1,4,8 and 12 in all treatment groups.

TxGroup	Wk1	Wk4	Wk8	Wk12
C0S0	0.69	0.62	0.69	1.04
C0S1	-0.08	0.38	0.15	1.14
C1S0	0.62	1.00	0.85	1.59
C1S1	0.69	1.08	0.84	1.64

Table 13

Number of fields counted in each bacterial sample from each subject at each time interval.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	10	10	10	10	10
2	20	20	20	20	20
3	20	20	20	20	20
4	20	20	20	20	20
5	10	20	10	10	0
6	20	20	20	0	0
7	20	20	20	20	0
8	20	20	20	20	0
9	20	20	20	20	0
10	10	20	10	10	10
11	20	20	20	20	20
12	20	20	20	20	20
13	20	20	20	0	0

Table 14

Mean number of bacteria counted in each Tx group
at weeks 0,1,4,8 and 12.

Tx Group		Week0	Week1	Week4	Week8	Week12
C0S0	Mean	201.00	184.85	219.54	164.18	135.29
	S.D.	163.18	127.28	110.31	109.69	52.39
C0S1	Mean	202.00	121.15	142.69	101.64	165.00
	S.D.	173.77	50.69	69.53	42.25	88.41
C1S0	Mean	216.23	156.46	171.54	146.73	113.86
	S.D.	154.46	76.75	82.88	146.02	48.28
C1S1	Mean	204.69	111.77	158.31	91.36	90.57
	S.D.	123.16	67.25	62.43	28.06	46.89

Table 15

Mean number of bacteria counted per H.P.F. in each Tx group
at weeks 0,1,4,8 & 12.

Tx Group		Week0	Week1	Week4	Week8	Week12
C0S0	Mean	11.42	9.52	12.83	9.44	8.15
	S.D.	8.15	6.19	6.37	5.31	2.77
C0S1	Mean	11.69	6.55	8.95	6.95	11.35
	S.D.	9.02	3.09	5.67	5.11	8.96
C1S0	Mean	12.17	8.28	10.38	7.98	6.79
	S.D.	7.21	3.94	5.54	7.00	2.67
C1S1	Mean	11.90	5.99	9.99	5.46	5.47
	S.D.	6.35	3.59	6.99	1.48	2.38

Table 16

Mean number of coccal forms per sample in each Tx group at each examination.

TxGroup		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	69	71.54	71.00	48.45	42.57
	S.D.	31.08	47.57	34.79	26.86	18.05
C0S1	Mean	78.31	64.31	65.92	42.09	45.14
	S.D.	43.59	21.53	37.17	13.05	18.73
C1S0	Mean	67.54	68.31	80.46	42.55	39.71
	S.D.	36.58	31.42	39.51	20.7	14.56
C1S1	Mean	69.85	65.77	65.92	48.36	33.29
	S.D.	32.53	44.11	29.21	26.63	7.17

Table 17

Mean number of coccal forms per H.P.F. in each Tx group at each examination.

Tx Group		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	3.93	3.75	3.97	2.77	2.64
	S.D.	1.44	2.36	1.58	1.21	1.18
C0S1	Mean	4.53	3.38	3.75	2.61	2.86
	S.D.	2.17	1.05	1.79	1.17	1.59
C1S0	Mean	3.93	3.55	4.99	2.48	2.43
	S.D.	1.93	1.50	3.62	0.92	0.84
C1S1	Mean	4.05	3.66	3.71	2.77	2.06
	S.D.	1.62	2.73	1.22	1.19	0.54

Table 18

Cocci: Proportion of cocci in samples from each treatment group at each time interval, expressed as a percentage of the mean total bacteria per H.P.F.

Tx Group	Wk0	Wk1	Wk4	Wk8	Wk12
COS0	34.41	39.23	30.92	29.34	32.39
COS1	38.72	51.60	41.90	37.55	25.20
CIS0	32.29	42.87	48.03	31.08	35.74
CIS1	34.03	61.00	37.14	50.64	37.86

Table 19

Mean proportions of coccal forms counted at each examination in Tx groups irrigated with chlorhexidine (C1) or irrigated with saline (C0).

TxGroup	Week 0	Week 1	Week 4	Week 8
C0	44.35	49.52	41.27	39.93
C1	36.75	54.17	49.09	47.96

Table 20

Mean number of motiles in each group at each examination.

TxGroup		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	33.08	29.62	48.15	47.18	30.86
	S.D.	36.57	29.91	42.18	42.05	16.18
C0S1	Mean	41.00	14.77	20.92	16.27	38.71
	S.D.	57.31	19.00	20.54	17.38	25.98
C1S0	Mean	44.46	24.62	29.92	29.91	29.14
	S.D.	42.66	22.57	26.81	40.62	19.95
C1S1	Mean	36.23	13.31	25.23	9.09	15.86
	S.D.	38.14	12.78	24.05	7.66	20.04

Table 21

Mean numbers of motiles per H.P.F. at weeks 0,1,4,8 and 12 in each treatment group.

Tx Group		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	1.98	1.51	2.97	2.80	1.85
	S.D.	2.08	1.48	2.53	2.39	0.83
C0S1	Mean	2.44	0.87	1.62	1.30	2.78
	S.D.	3.15	1.15	2.07	2.07	2.61
C1S0	Mean	2.53	1.39	1.86	1.65	1.74
	S.D.	2.10	1.35	1.71	2.04	1.32
C1S1	Mean	2.22	0.70	1.81	0.67	0.97
	S.D.	2.23	0.64	2.23	0.69	1.01

Table 22

Motiles: Proportion of motiles in samples from each treatment group at each time interval, expressed as a percentage of the mean total bacteria per H.P.F.

Tx Group	Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	17.35	15.80	23.13	29.66	22.69
C0S1	20.86	13.28	18.10	18.71	24.49
C1S0	20.79	16.79	17.90	20.68	25.59
C1S1	18.66	11.66	18.12	12.25	17.83

Table 23

Mean number of spirochaetes in each Tx group at each examination.

TxGroup		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	47.69	38.23	43.77	29.82	23.86
	S.D.	62.32	45.27	49.69	26.17	21.01
C0S1	Mean	42.92	12.38	22.23	16.18	26.00
	S.D.	66.93	14.01	22.23	21.89	22.34
C1S0	Mean	55.77	31.46	26.38	39.91	11.14
	S.D.	54.67	35.44	36.19	66.97	7.49
C1S1	Mean	55.62	9.85	27.08	9.36	11.86
	S.D.	58.08	22.33	31.88	10.26	18.13

Table 24

Mean numbers of spirochaetes per H.P.F. in each treatment group at each examination.

Tx Group		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	2.57	1.93	2.48	1.63	1.37
	S.D.	3.07	2.25	2.58	1.31	1.00
C0S1	Mean	2.38	0.75	1.47	1.27	2.15
	S.D.	3.32	1.01	1.74	2.12	2.47
C1S0	Mean	2.98	1.62	1.47	2.00	0.66
	S.D.	2.65	1.75	1.80	3.32	0.38
C1S1	Mean	3.12	0.49	1.91	0.53	0.66
	S.D.	2.84	1.12	3.11	0.51	0.89

Table 25

Spirochaetes: Proportion of spirochaetes in samples from each treatment group at each time interval, expressed as a percentage of the mean total bacteria per H.P.F.

Tx Group	Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	22.53	20.18	19.32	17.27	16.81
C0S1	20.36	11.45	16.42	18.27	18.94
C1S0	24.49	19.57	14.16	25.06	9.72
C1S1	26.22	8.18	19.12	9.71	12.07

Table 26

Mean number of bacteria that were not cocci, motiles or spirochaetes in each Tx group at each examination.

TxGroup		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	51.23	46.23	56.62	35.50	38.00
	S.D.	45.65	30.49	29.83	24.10	13.06
C0S1	Mean	39.77	29.69	33.62	27.09	55.14
	S.D.	31.56	20.36	22.59	13.46	37.90
C1S0	Mean	48.46	32.08	34.77	34.36	33.86
	S.D.	32.83	17.81	19.78	29.01	17.24
C1S1	Mean	43.00	22.85	40.08	24.55	29.57
	S.D.	27.63	16.16	28.50	9.11	15.66

Table 27

Mean numbers of others per H.P.F. at weeks 0,1,4,8 and 12 in samples from each Tx group.

Tx Group		Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	Mean	2.93	2.37	3.42	2.24	2.29
	S.D.	2.29	1.48	2.01	1.30	0.67
C0S1	Mean	2.35	1.55	2.11	1.77	3.56
	S.D.	1.77	1.00	1.53	1.10	2.95
C1S0	Mean	2.73	1.72	2.07	1.85	1.97
	S.D.	1.55	0.96	1.10	1.39	1.06
C1S1	Mean	2.51	1.15	2.56	1.50	1.78
	S.D.	1.58	0.80	2.21	0.71	0.89

Table 28

Others: Proportion of others in samples from each treatment group at each time interval, expressed as a percentage of the mean total bacteria per H.P.F.

Tx. Group	Wk0	Wk1	Wk4	Wk8	Wk12
C0S0	25.66	24.89	26.65	23.73	28.10
C0S1	20.10	23.66	23.58	25.47	31.37
C1S0	22.43	20.77	19.92	23.73	28.10
C1S1	21.09	19.20	25.63	27.47	31.99

Table 29

Proportions of bacterial types counted per H.P.F. in the COS0 treatment group at each examination.

Bacteria	Wk0	Wk1	Wk4	Wk8	Wk12
Cocci	34.41	39.23	30.92	29.34	32.39
Motiles	17.35	15.80	23.13	29.66	22.69
Spirochaetes	22.53	20.18	19.32	17.27	16.81
Others	25.66	24.89	26.65	23.73	28.10
Total	99.95	100.1	100.02	100	99.99

Table 30

Proportions of bacterial types counted per H.P.F. in the COS1 treatment group at each examination.

Bacteria	Wk0	Wk1	Wk4	Wk8	Wk12
Cocci	38.72	51.60	41.90	37.55	25.20
Motiles	20.86	13.28	18.10	18.71	24.49
Spirochaetes	20.36	11.45	16.42	18.27	18.94
Others	20.10	23.66	23.58	25.47	31.37
Total	100.04	99.99	100	100	100

Table 31

Proportions of bacterial types counted per H.P.F. in the C1S0 treatment group at each examination.

Bacteria	Wk0	Wk1	Wk4	Wk8	Wk12
Cocci	32.29	42.87	48.03	31.08	35.74
Motiles	20.79	16.79	17.90	20.68	25.59
Spirochaetes	24.49	19.57	14.16	25.06	9.72
Others	22.43	20.77	19.92	23.73	28.10
Total	100	100	100.01	100.55	99.15

Table 32

Proportions of bacterial types counted per H.P.F. in the C1S1 treatment group at each examination.

Bacteria	Wk0	Wk1	Wk4	Wk8	Wk12
Cocci	34.03	61.00	37.14	50.64	37.86
Motiles	18.66	11.66	18.12	12.25	17.83
Spirochaetes	26.22	8.18	19.12	9.71	12.07
Others	21.09	19.20	25.63	27.47	31.99
Total	100	100.04	100.01	100.07	99.75

Figure 1

Chemical Structure: Chlorhexidine

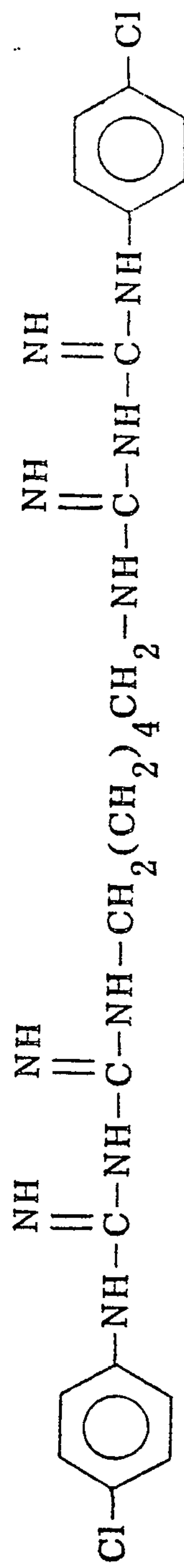


Figure 2

Plaque Index Scores

in COSQ Tx group at all time intervals.

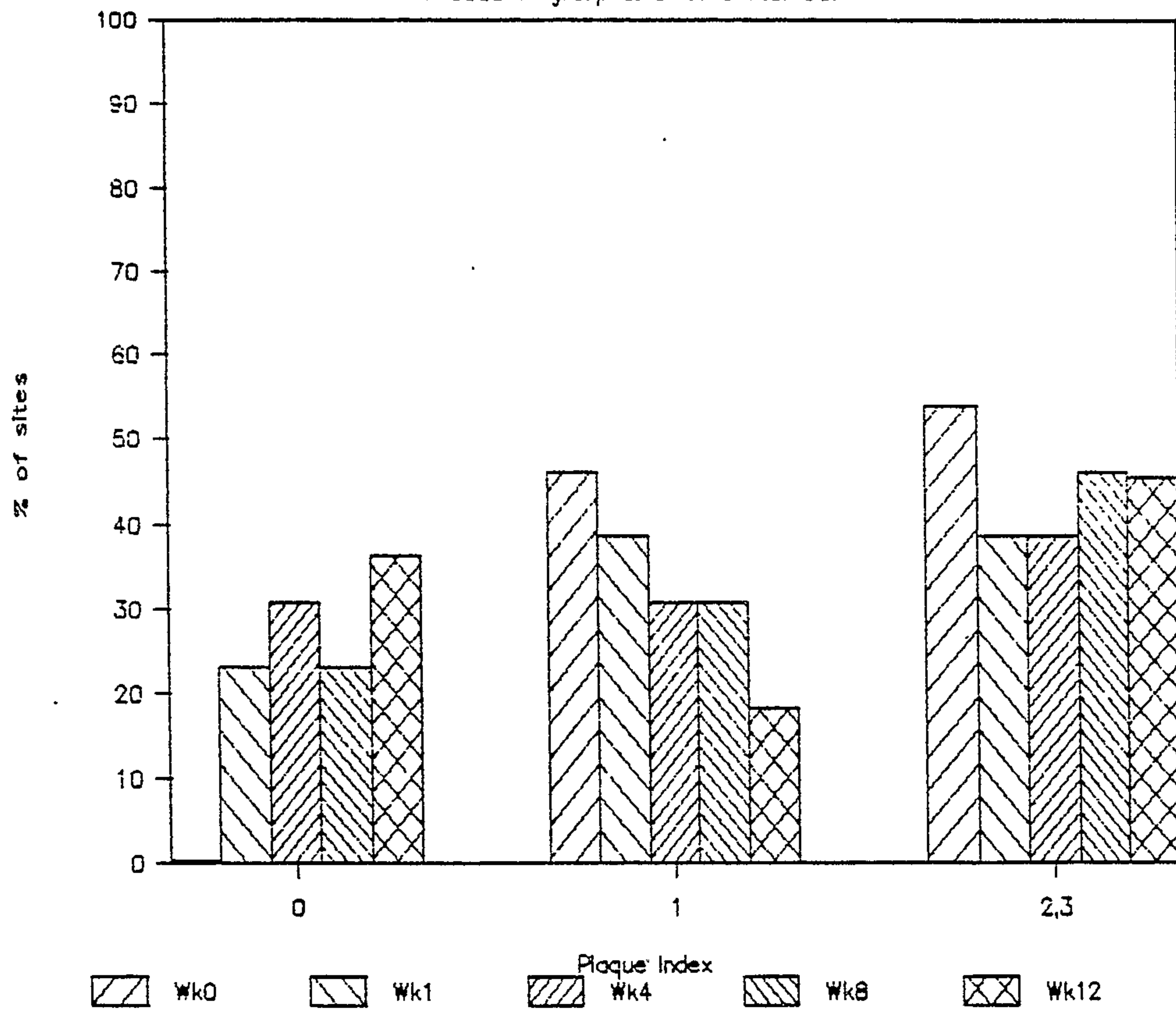


Figure 3

Plaque Index Scores

in COSI Tx group at all time intervals.

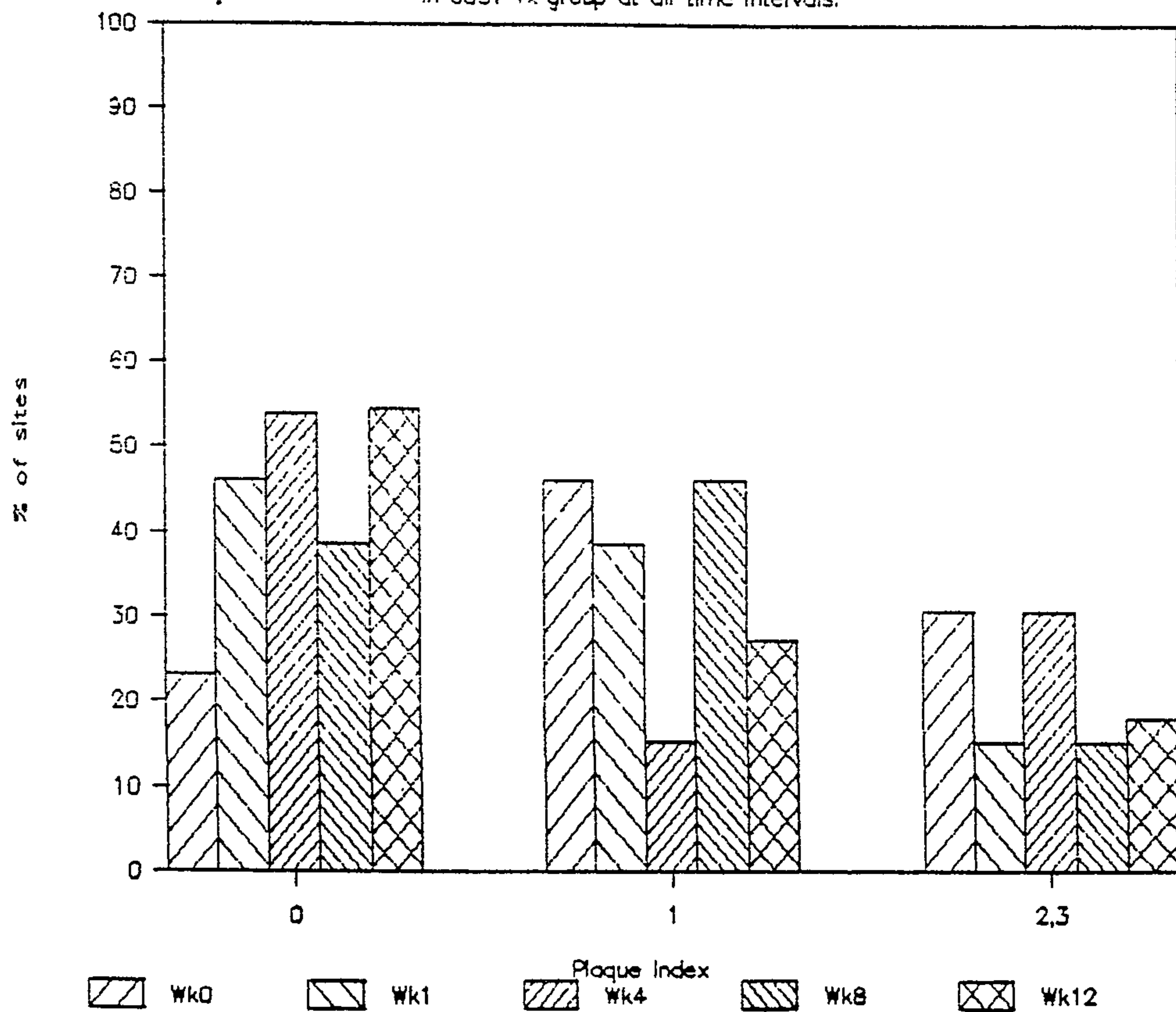


Figure 4

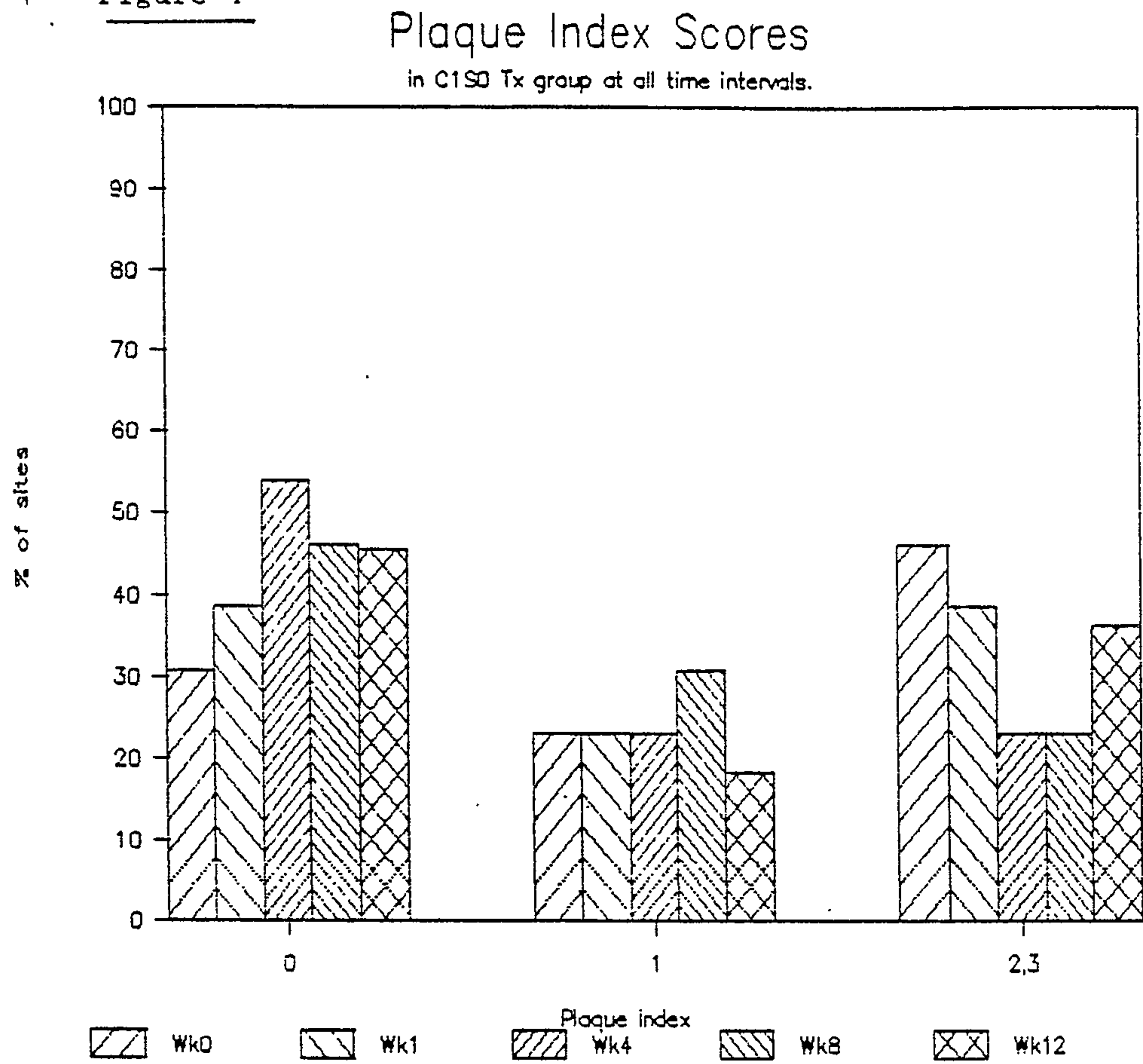


Figure 5

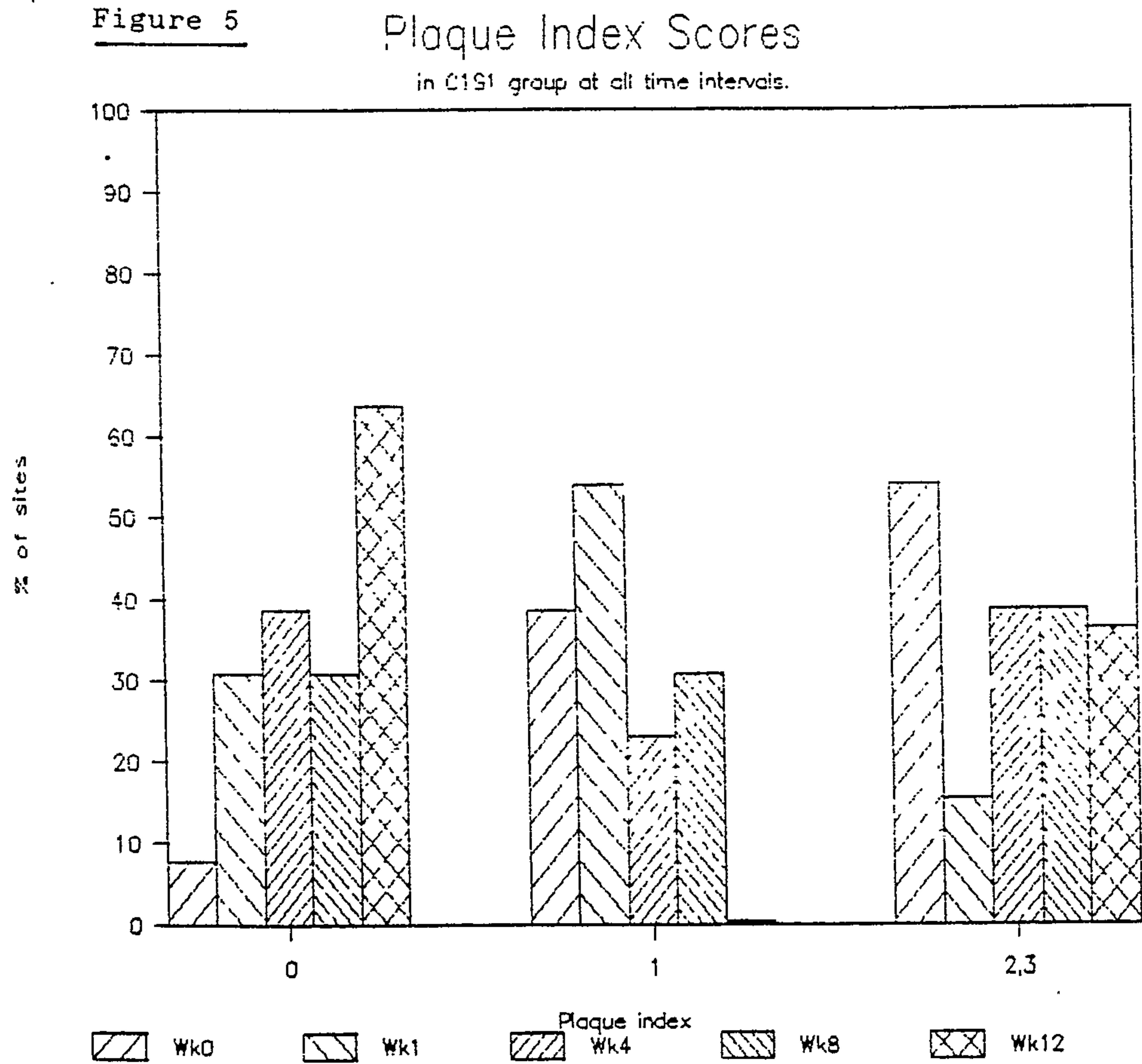


Figure 6

Plaque Index Scores of 0

In each Tx group at each examination.

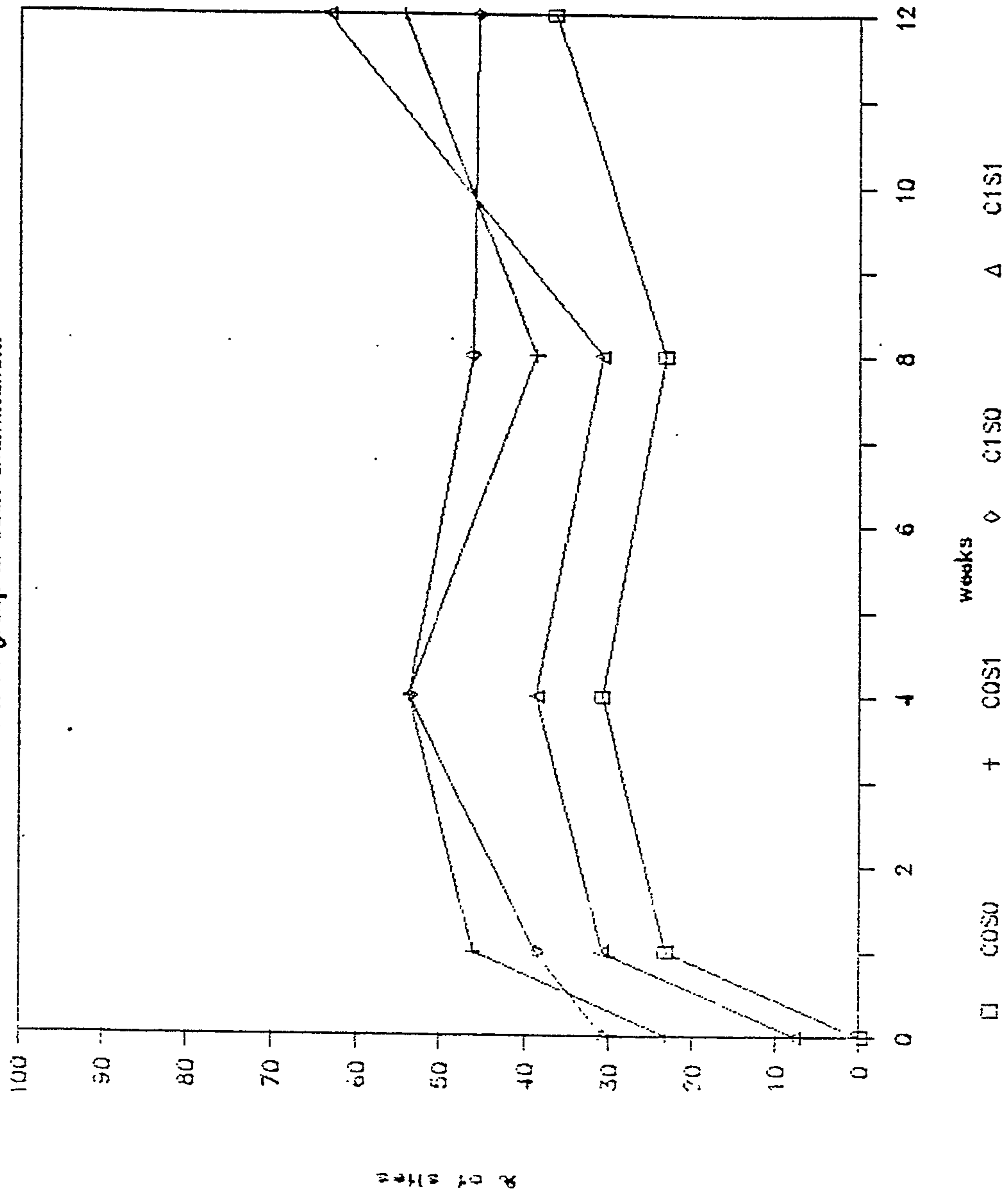


Figure 7

Plaque Index Scores of 1 In each Tx group at each examination.

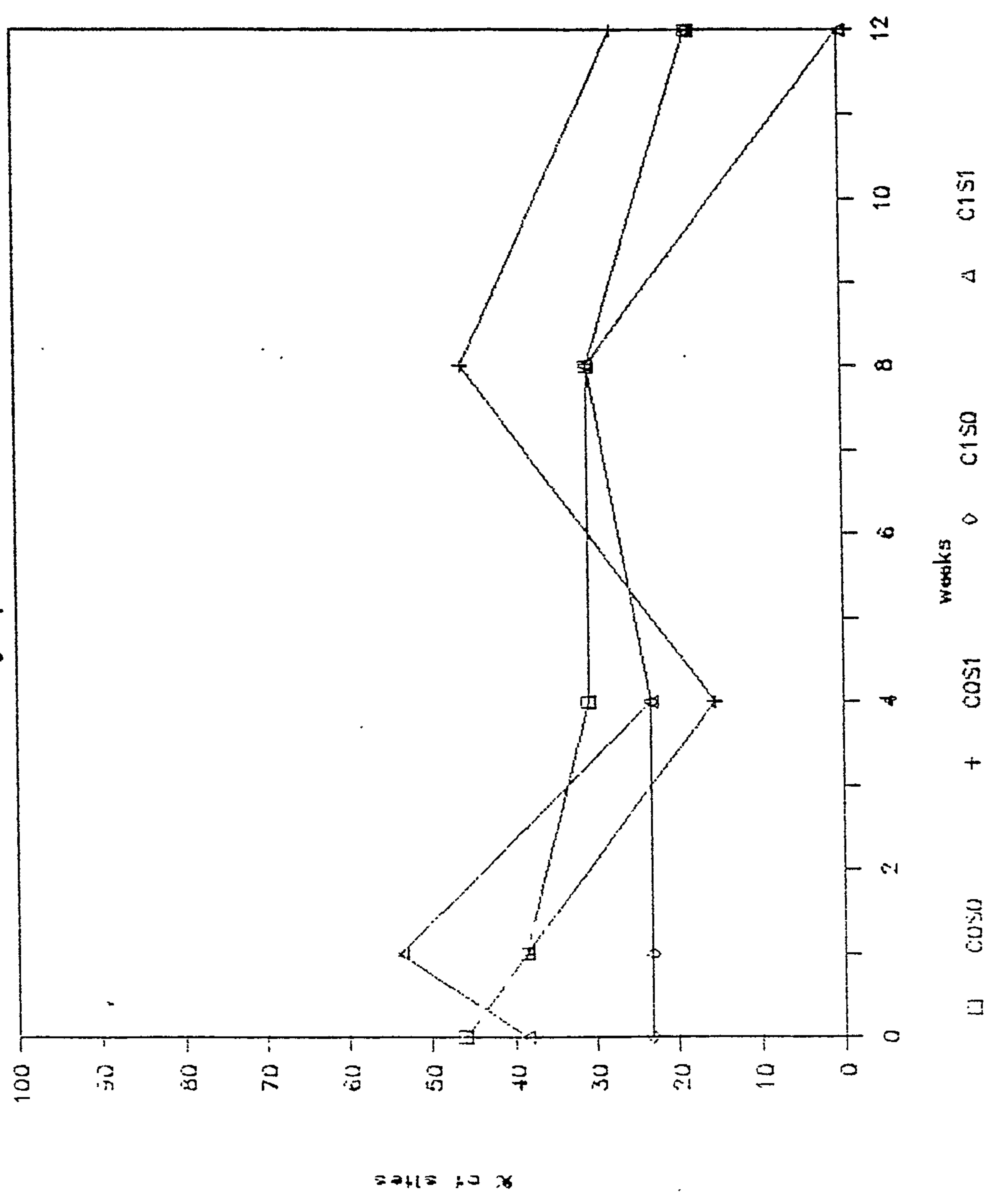


Figure 8 Plaque Index Scores of 2 or 3
in each Tx group at each examination.

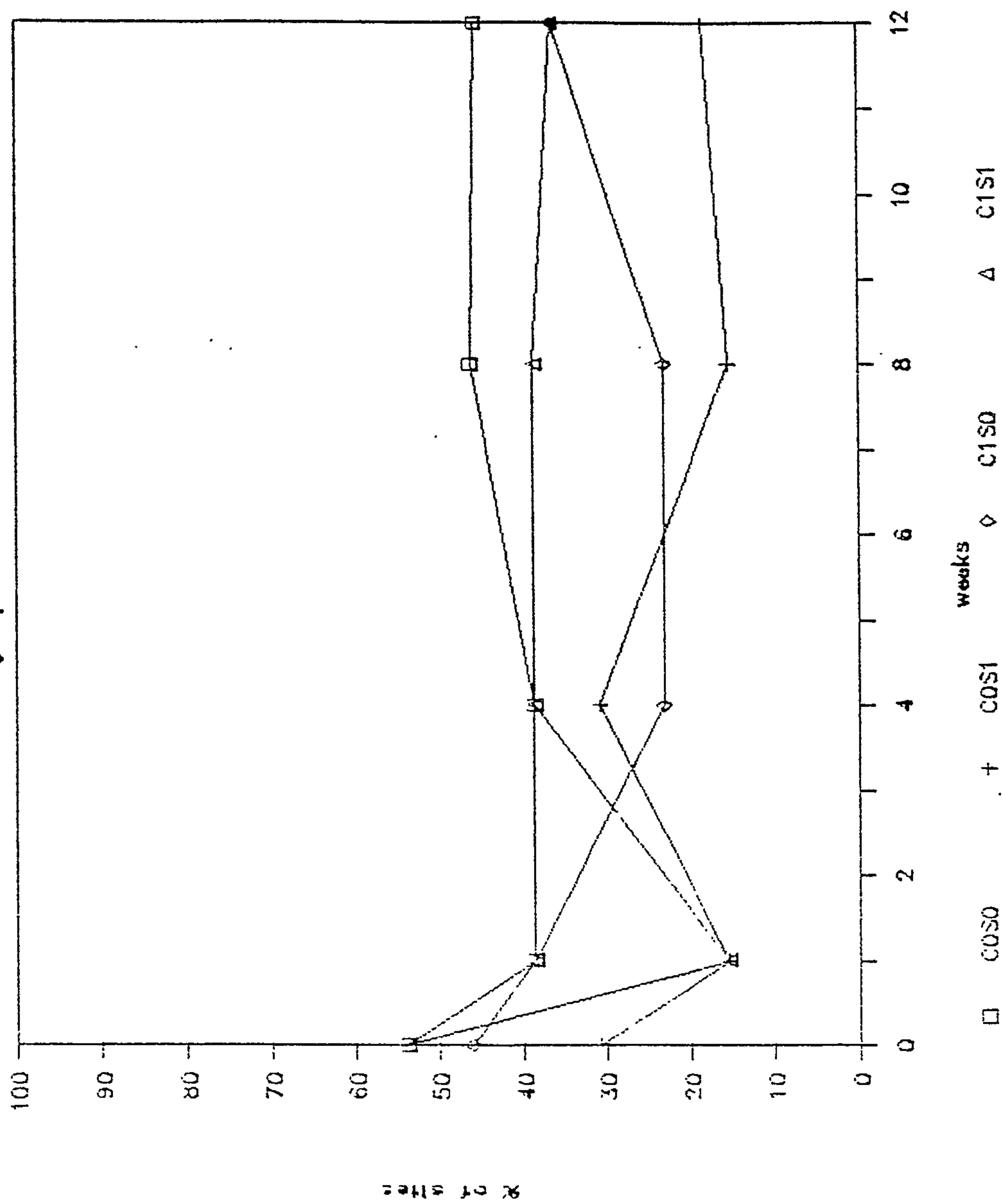


Figure 9

Gingival Index Scores

in COSO Tx group at each time interval.

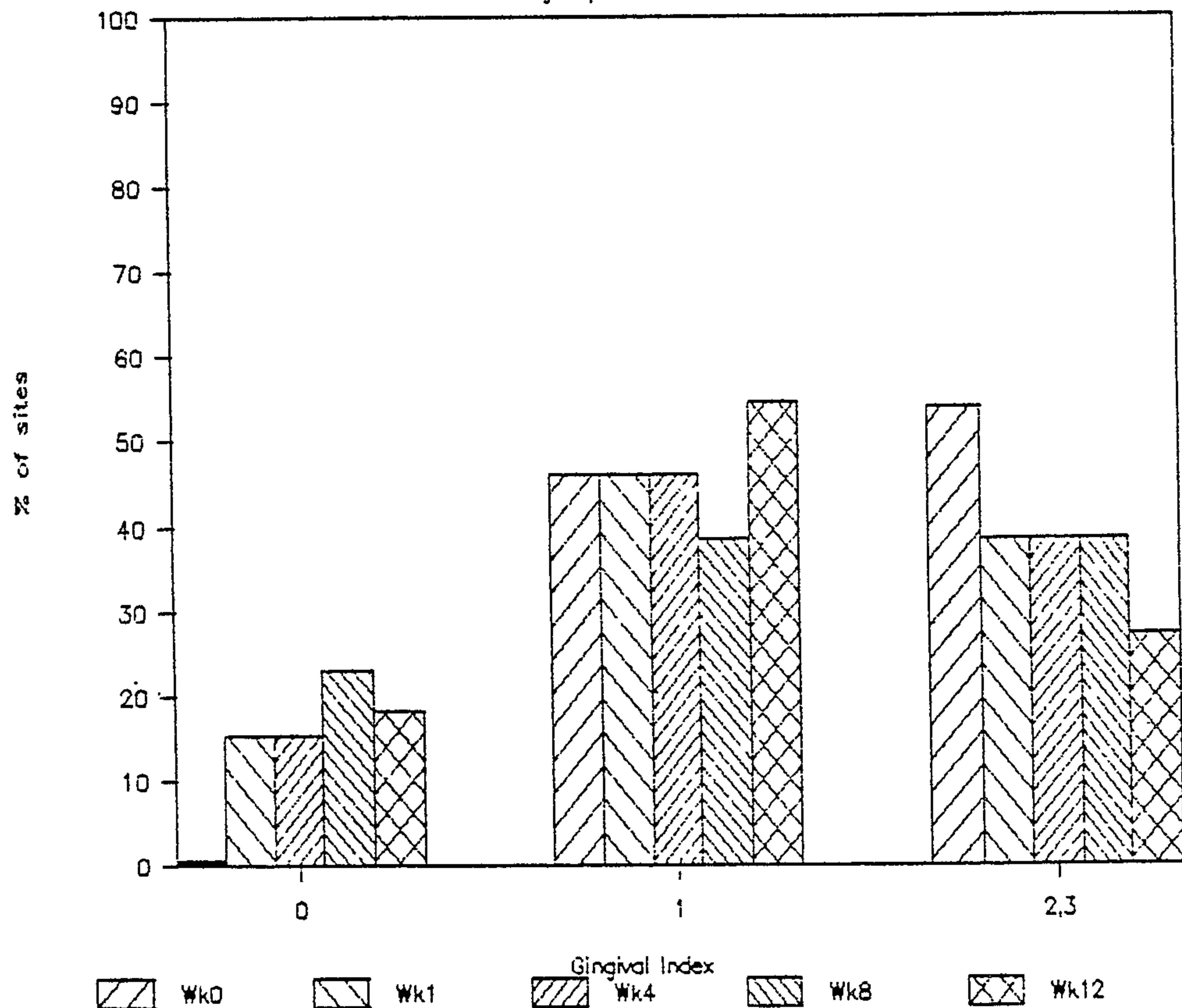


Figure 10

Gingival Index Scores

in COSI Tx group at each time interval.

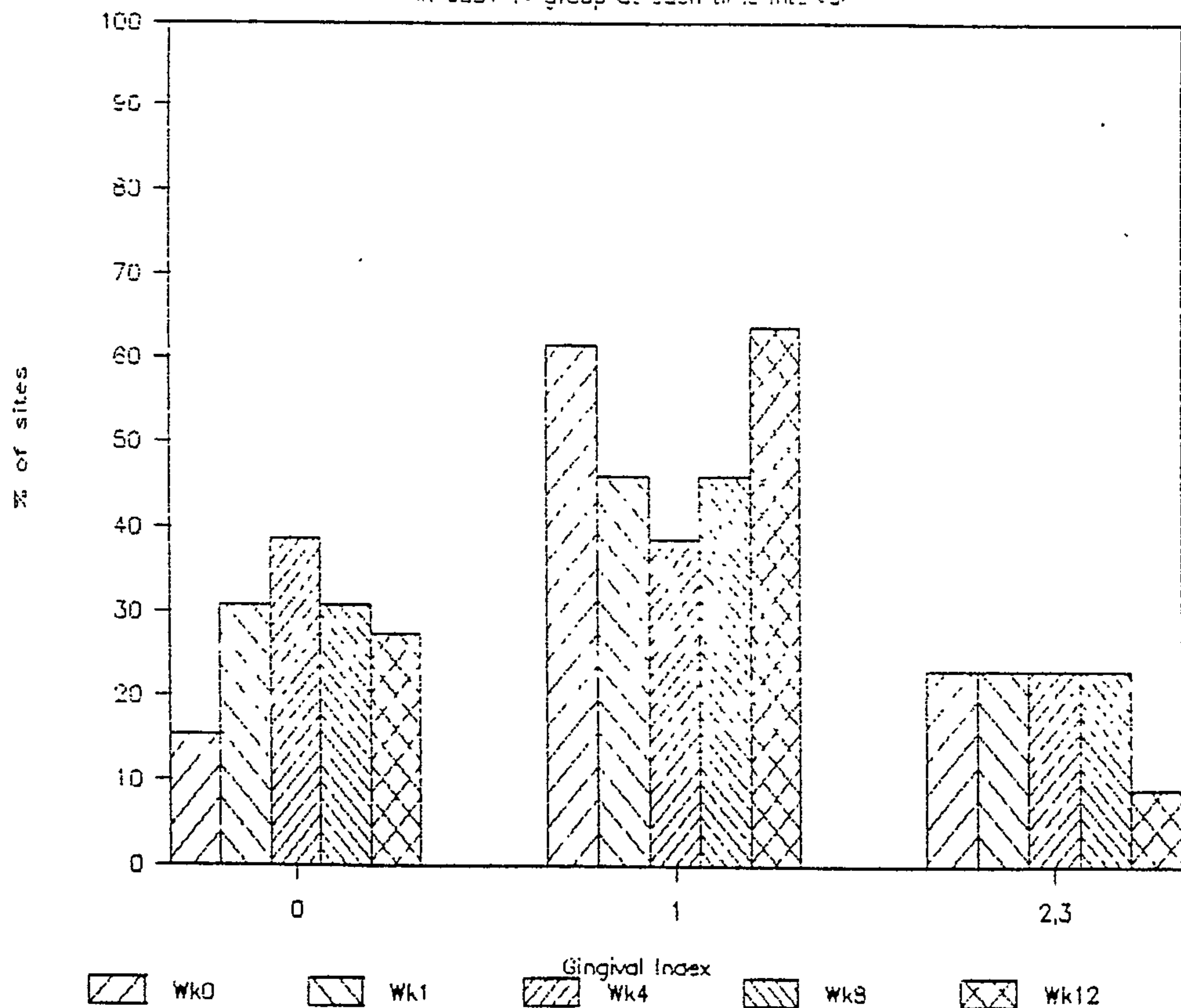


Figure 11

Gingival Index Scores

in C1S0 Tx group at each time interval.

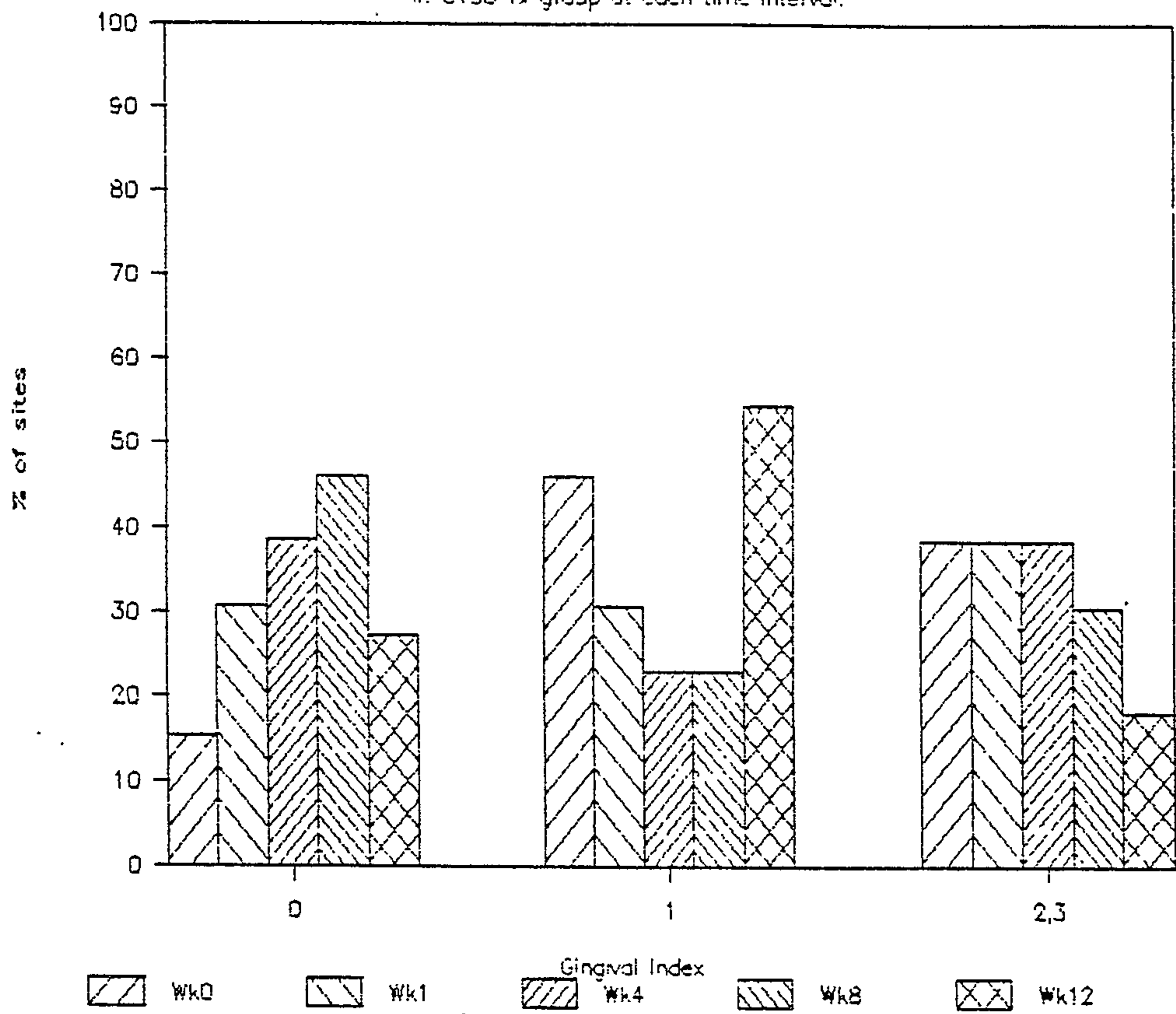


Figure 12

Gingival Index Scores

in C1S1 Tx group at each time interval.

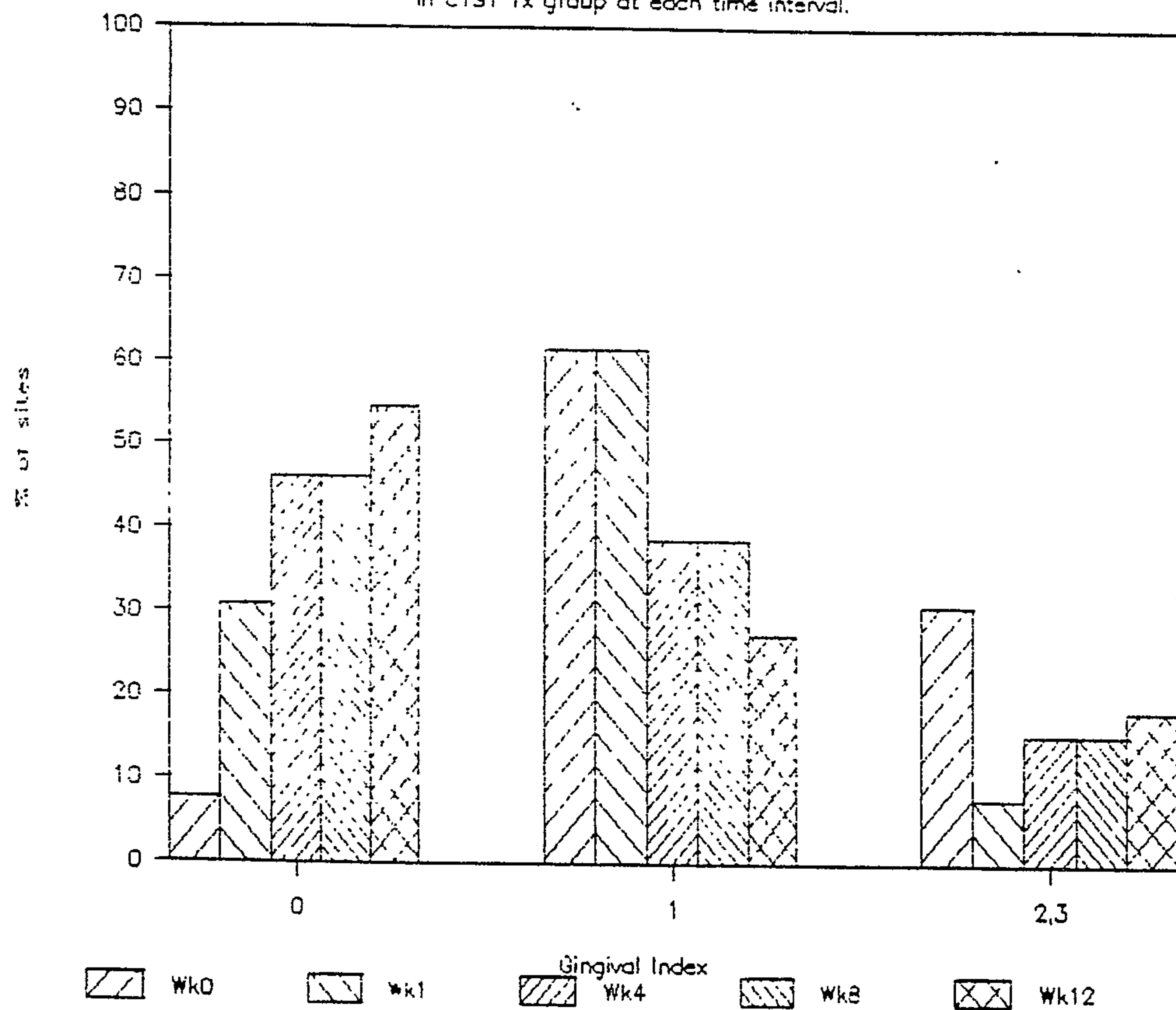


Figure 13 Gingival Index Scores of 0
in each Tx group at each examination.

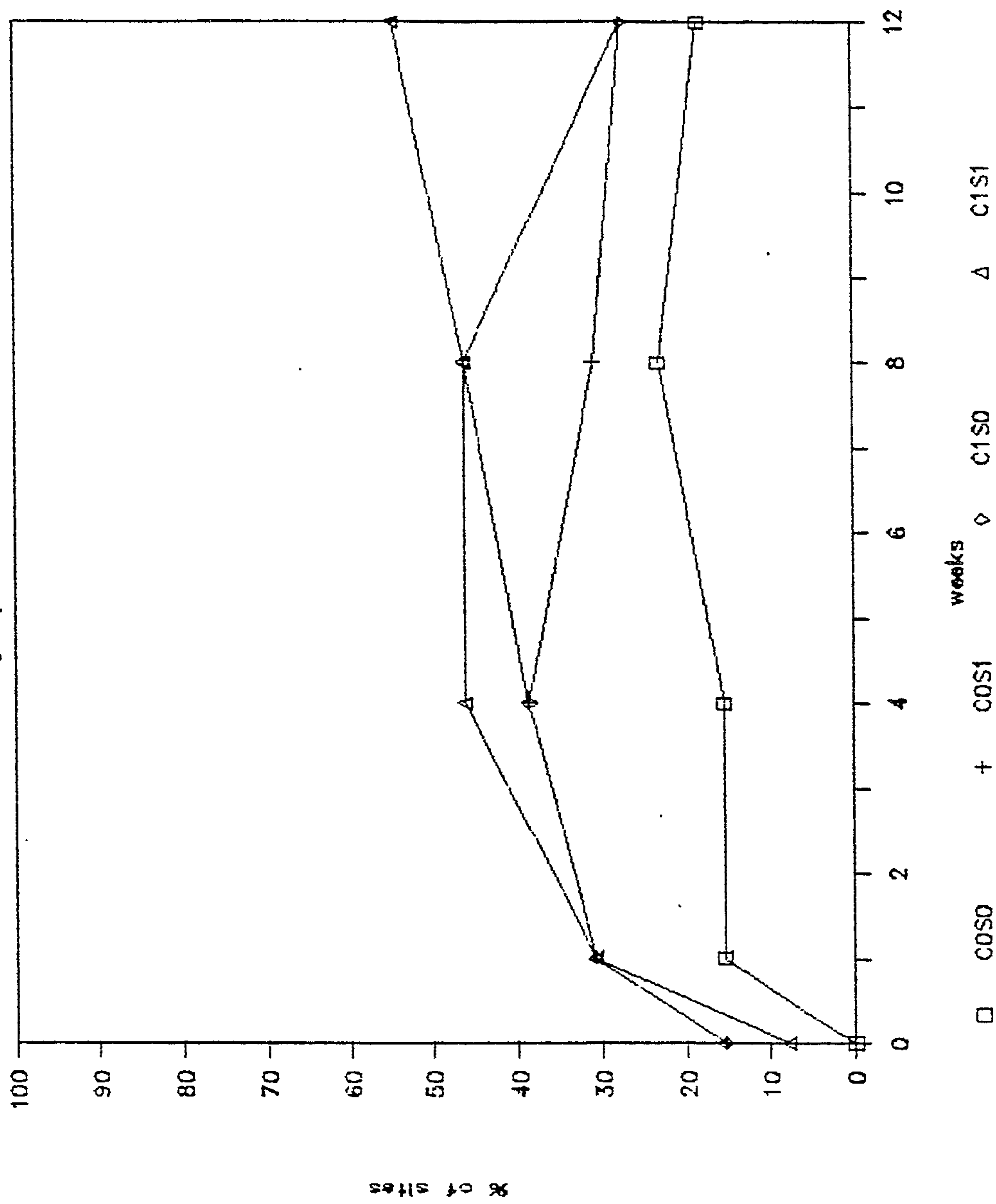


Figure 14
Gingival Index Scores of 1
 In each Tx group at each examination.

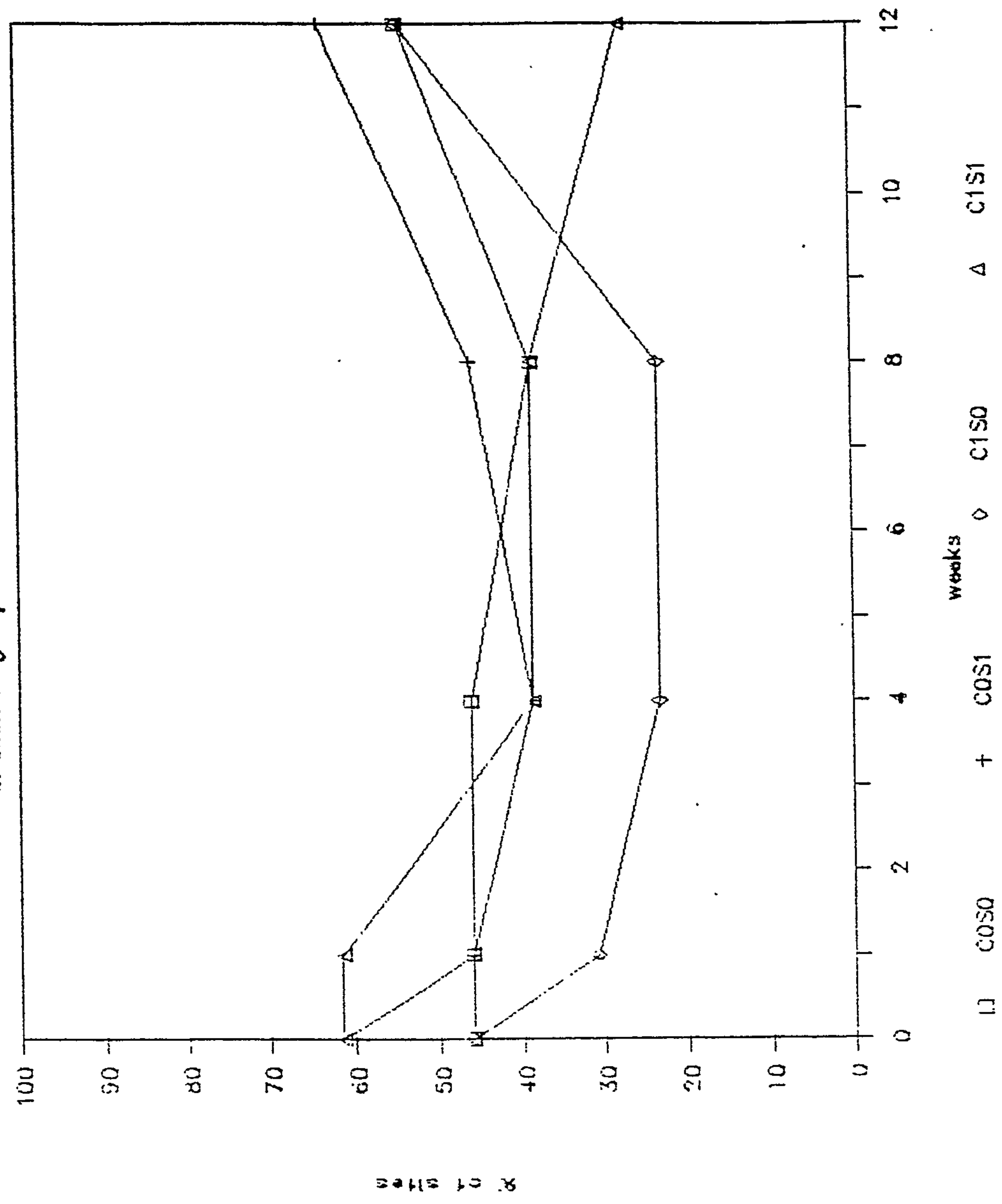


Figure 15
Gingival Index Scores of 2 or 3
in each Tx group at each examination.

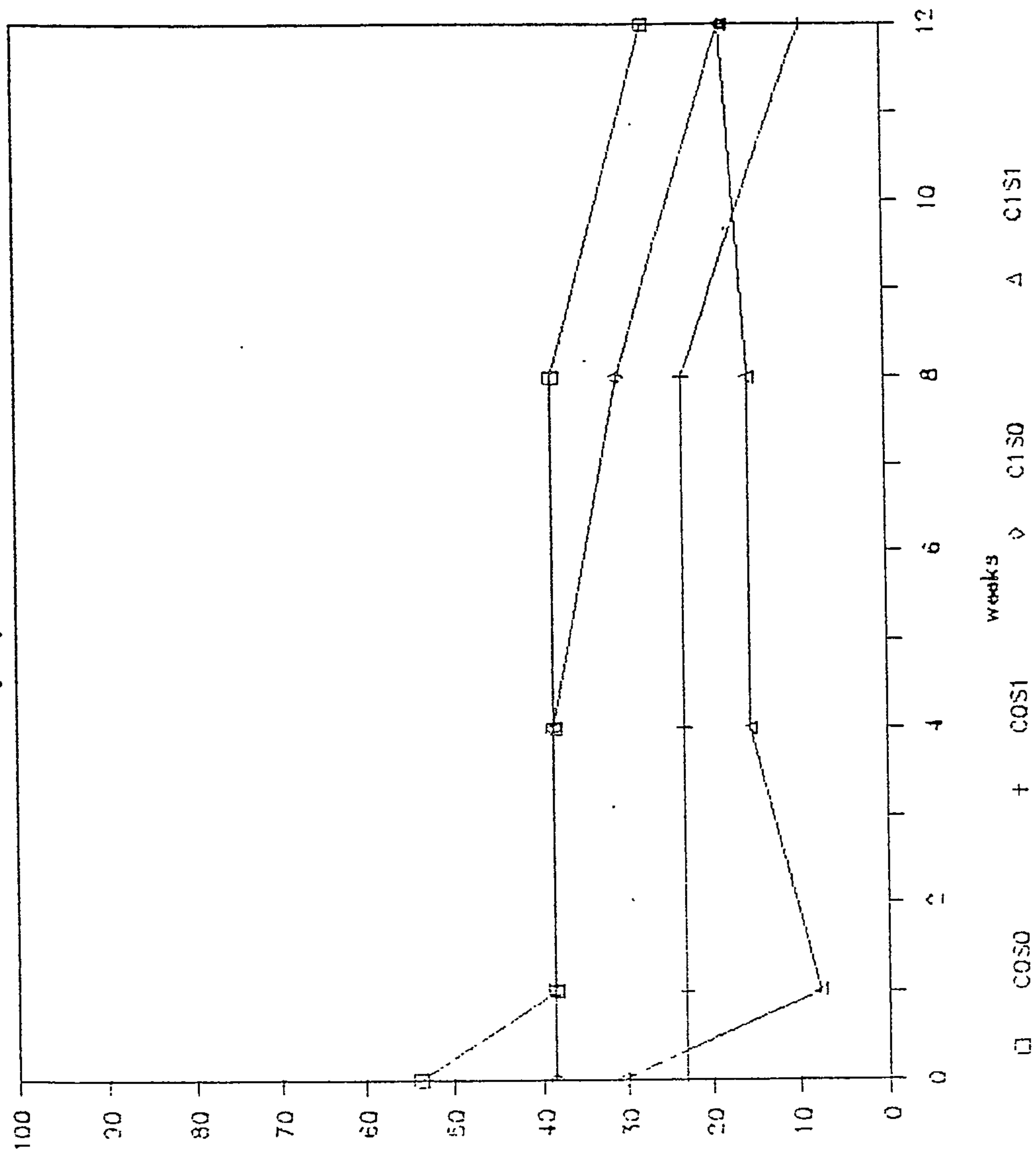


Figure 16

Bleeding on Probing

in CGSO Tx group at all time intervals

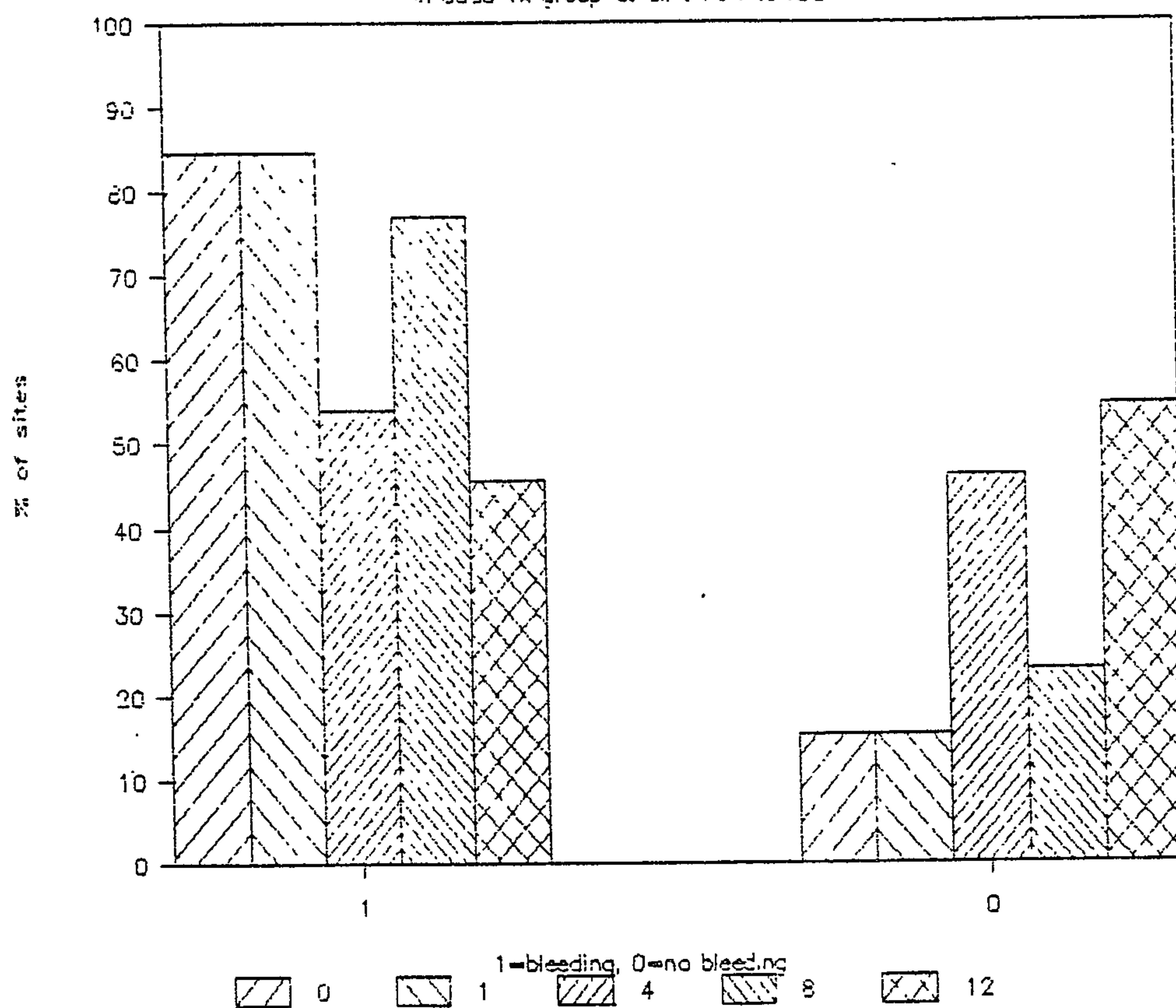


Figure 17

Bleeding on Probing

in CGS1 Tx group at all time intervals.

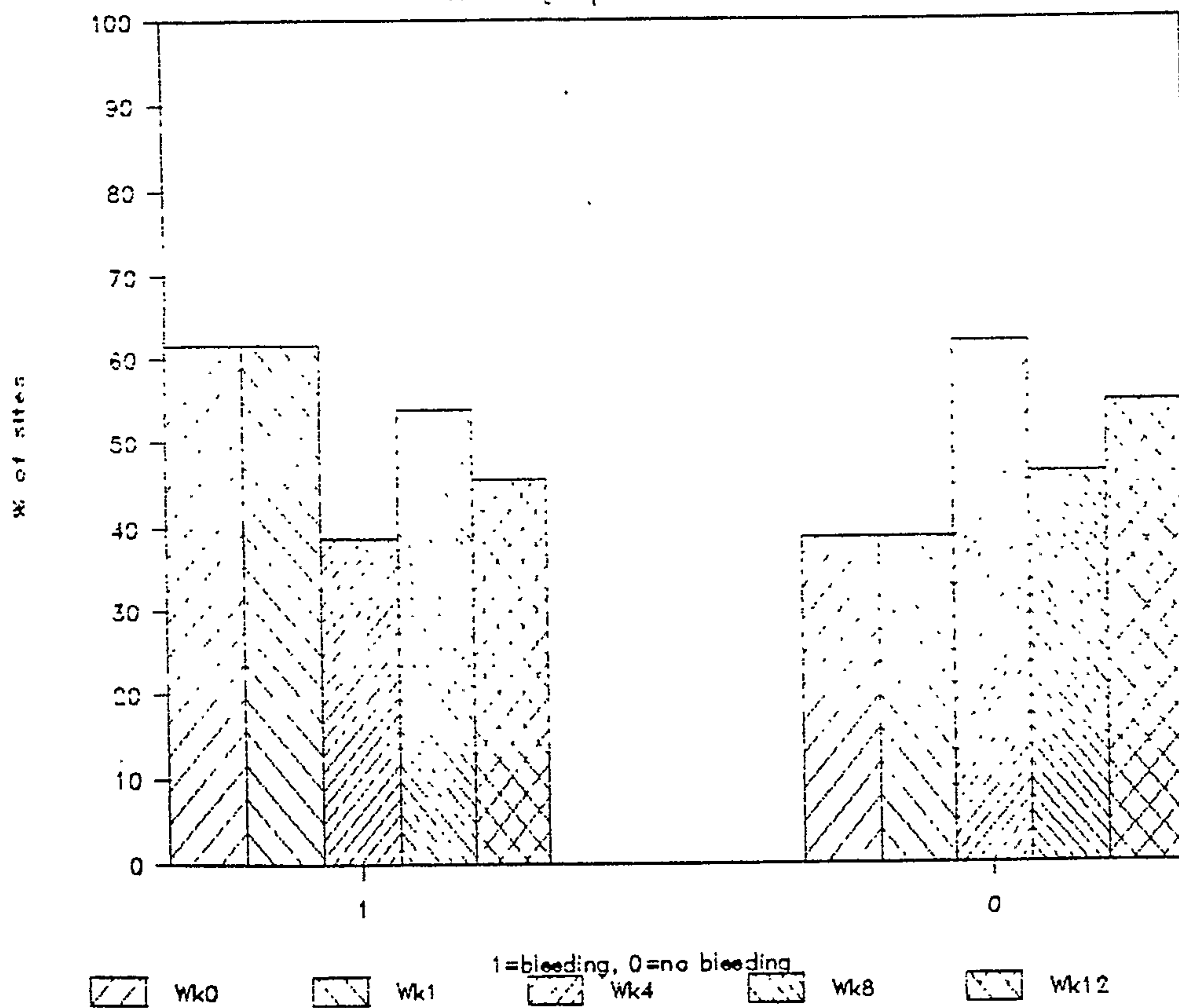


Figure 18

Bleeding on Probing

in C1S0 Tx group at all time intervals.

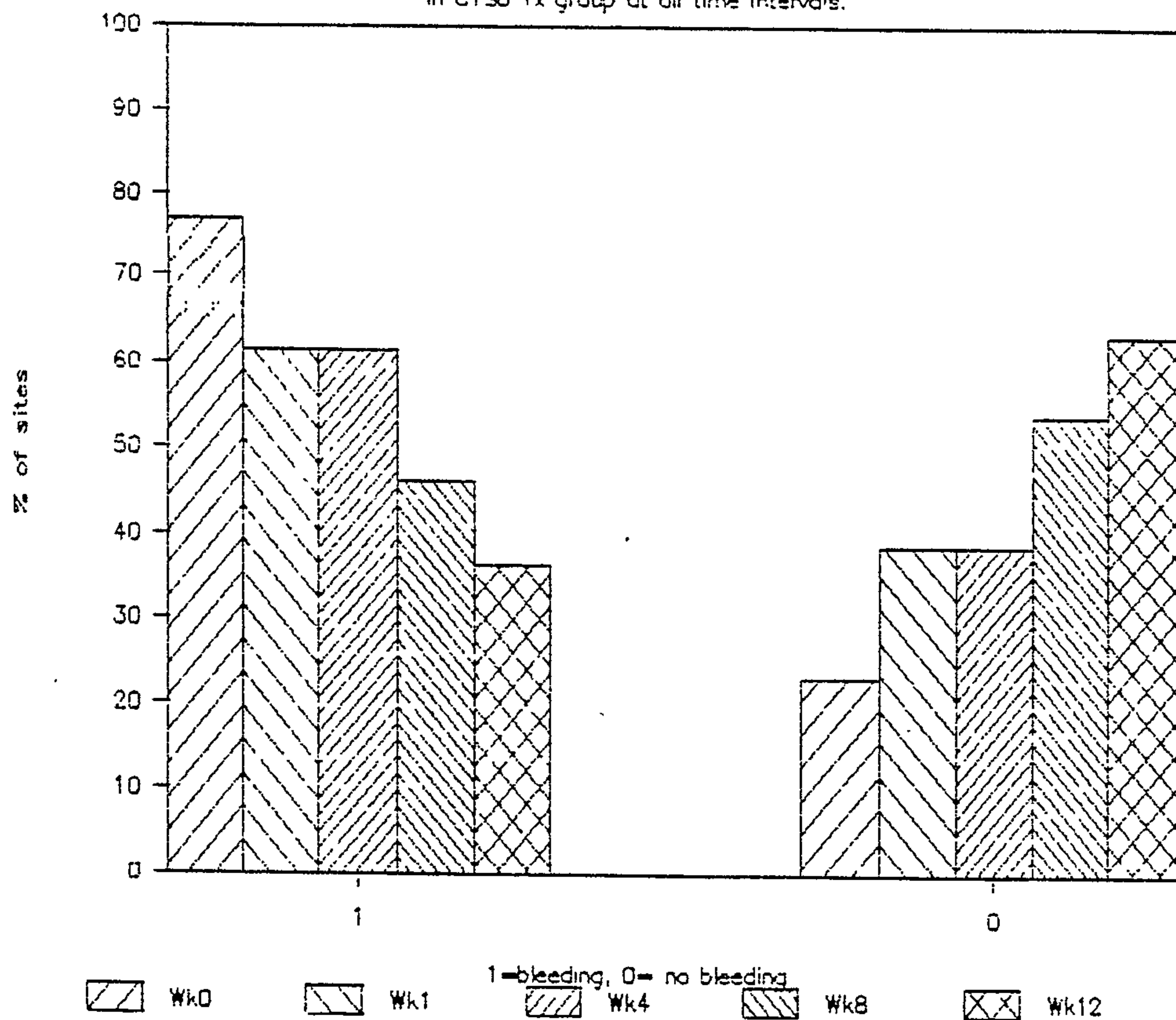


Figure 19

Bleeding on Probing

in C1S1 Tx group at all time intervals.

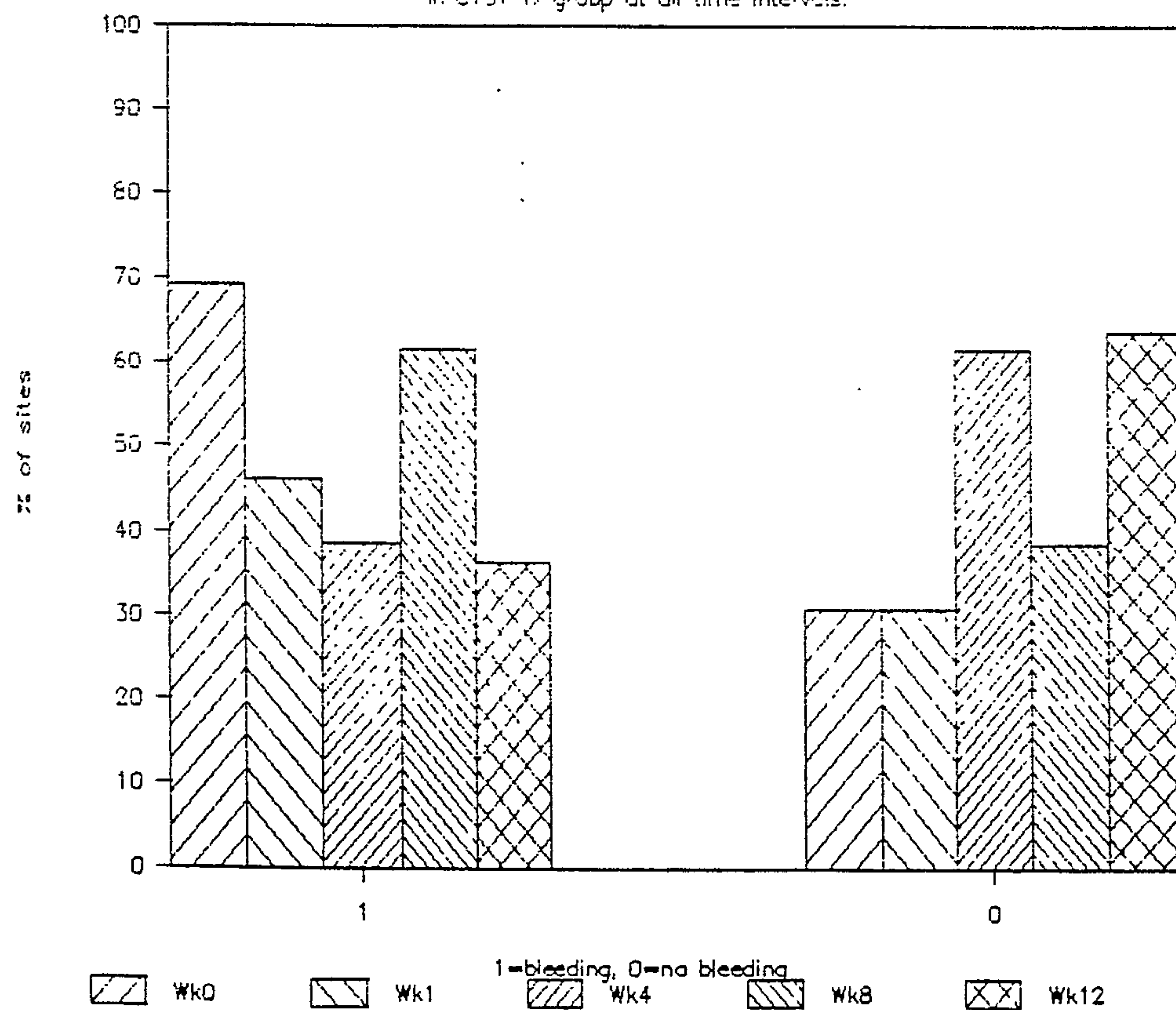


Figure 20

Bleeding on probing
in each Tx group at each examination.

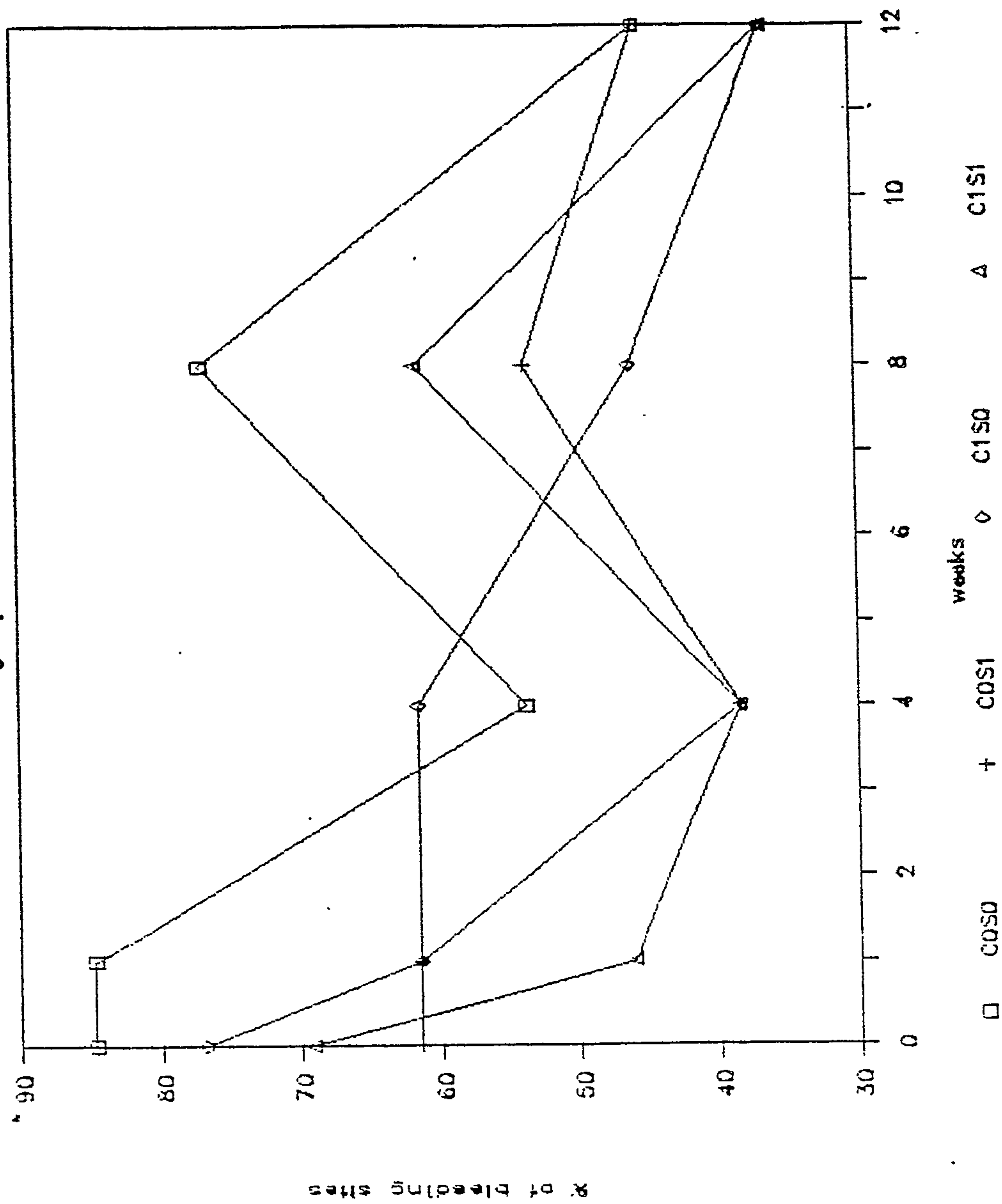


Figure 21
Mean probing pocket depths
 in each Tx group at each examination.

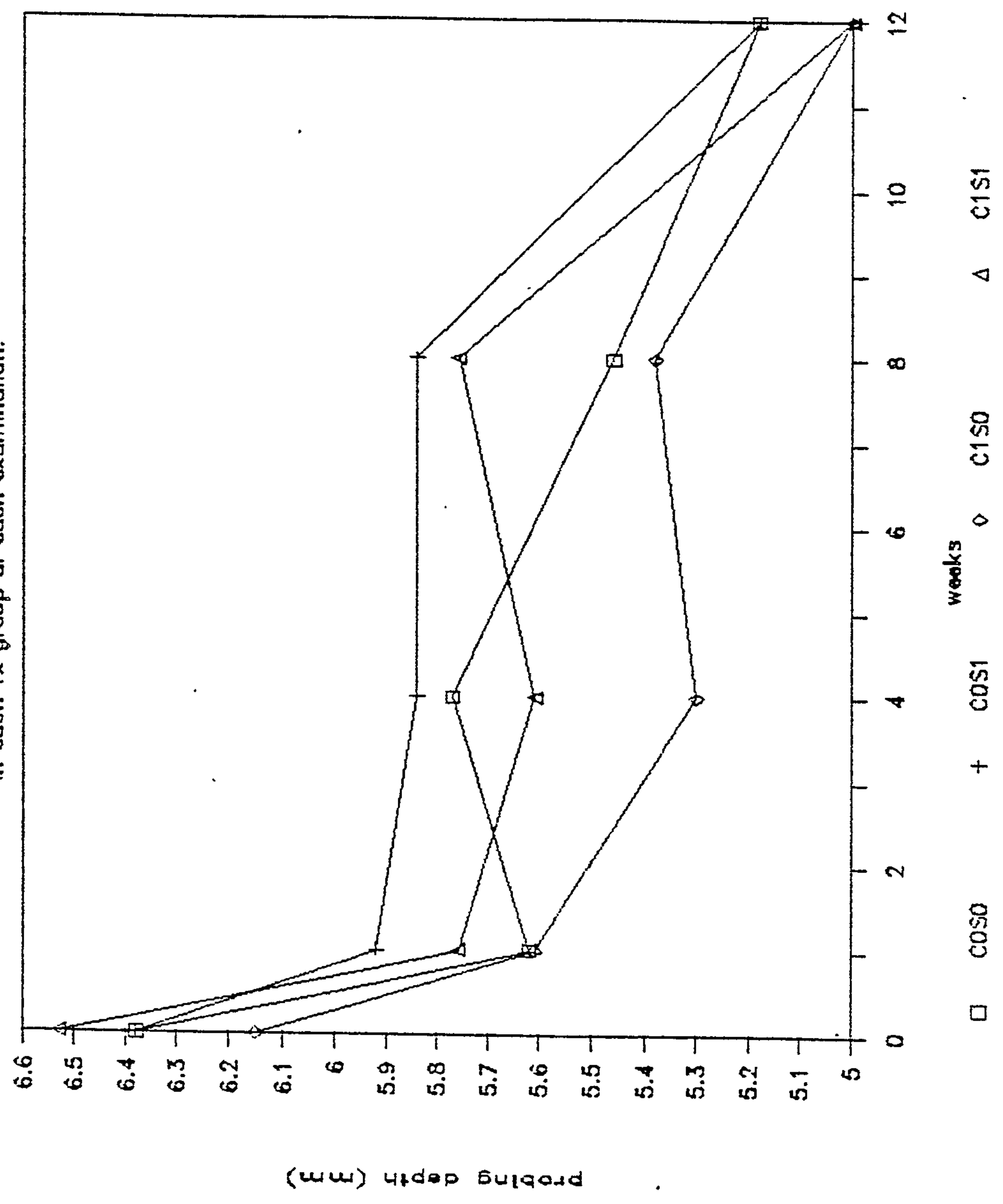


Figure 22

Decrease in probing pocket depth
from week 0 to weeks 1,4,8 & 12.

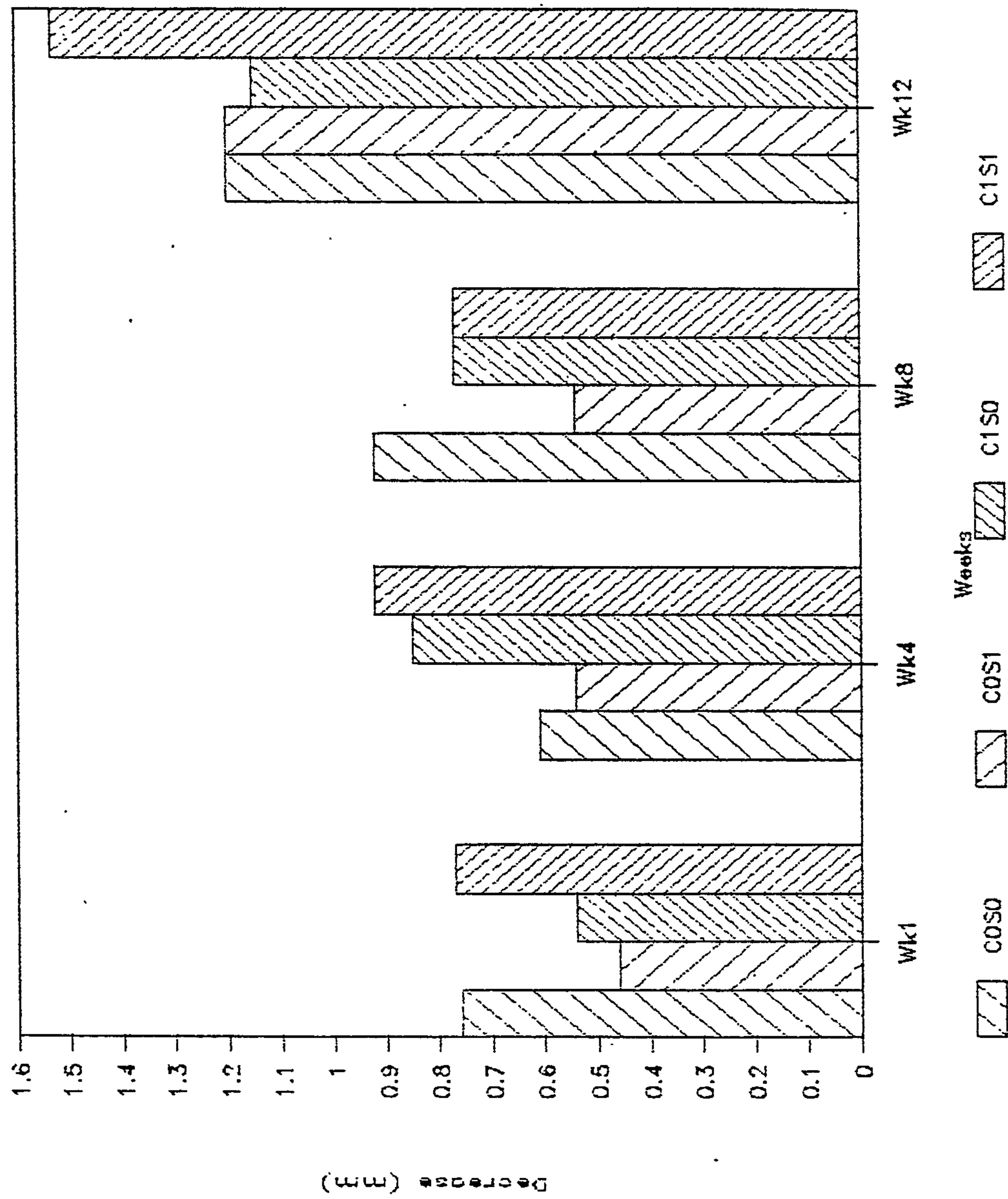


Figure 23

Mean attachment level
in each Tx group at each examination.

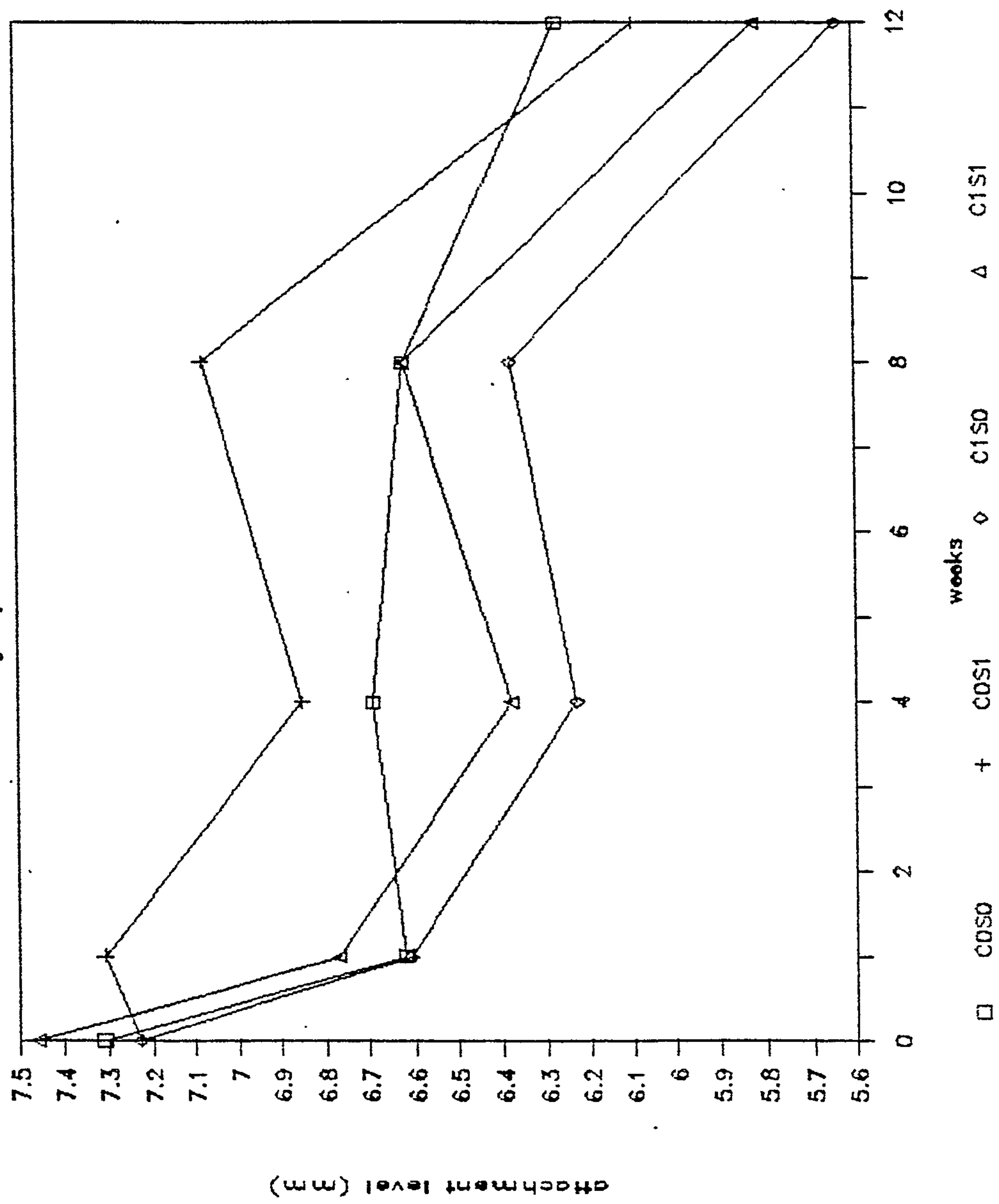


Figure 24

Mean gain in attachment
from week 0 to weeks 1,4,8 & 12.

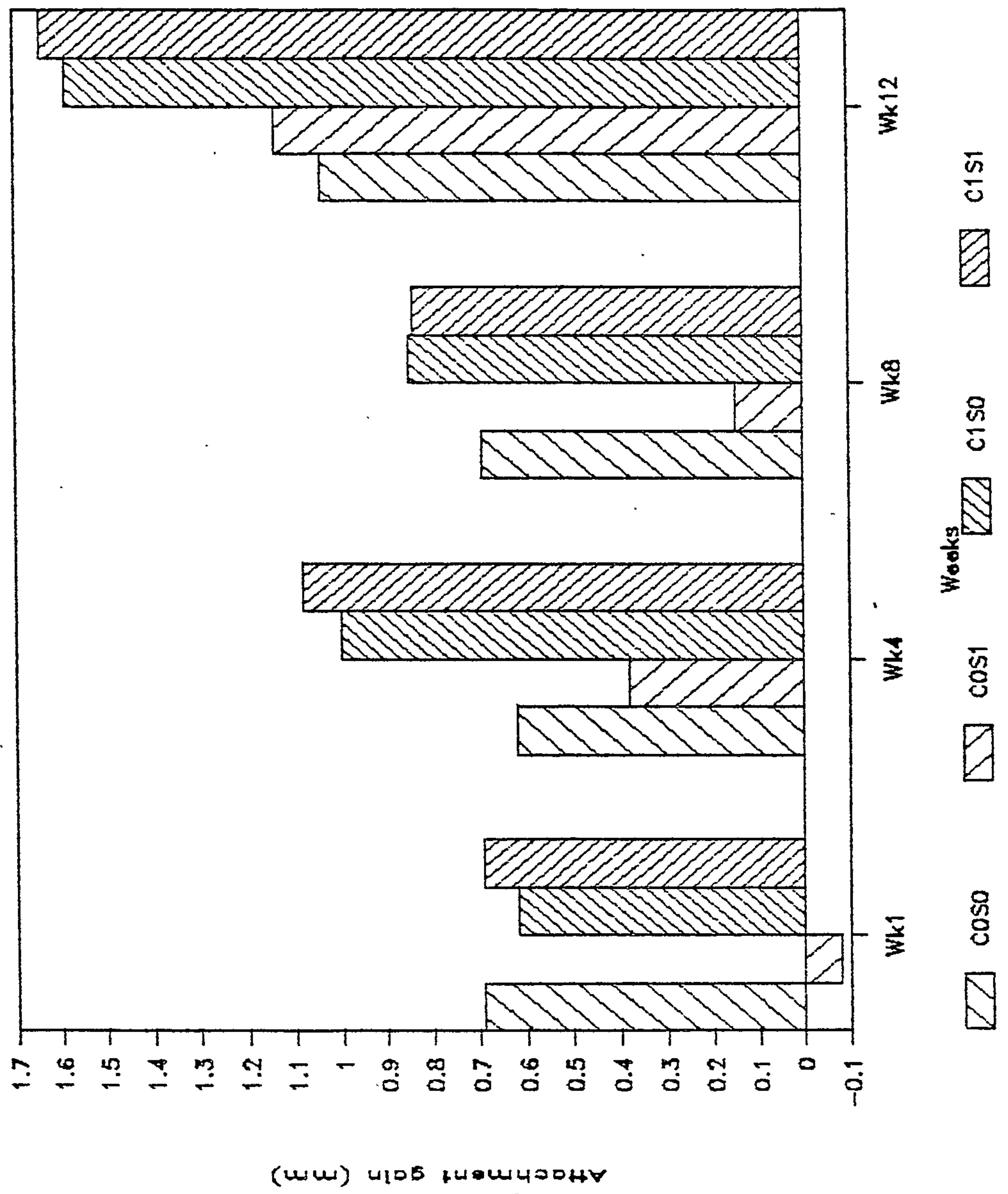


Figure 25

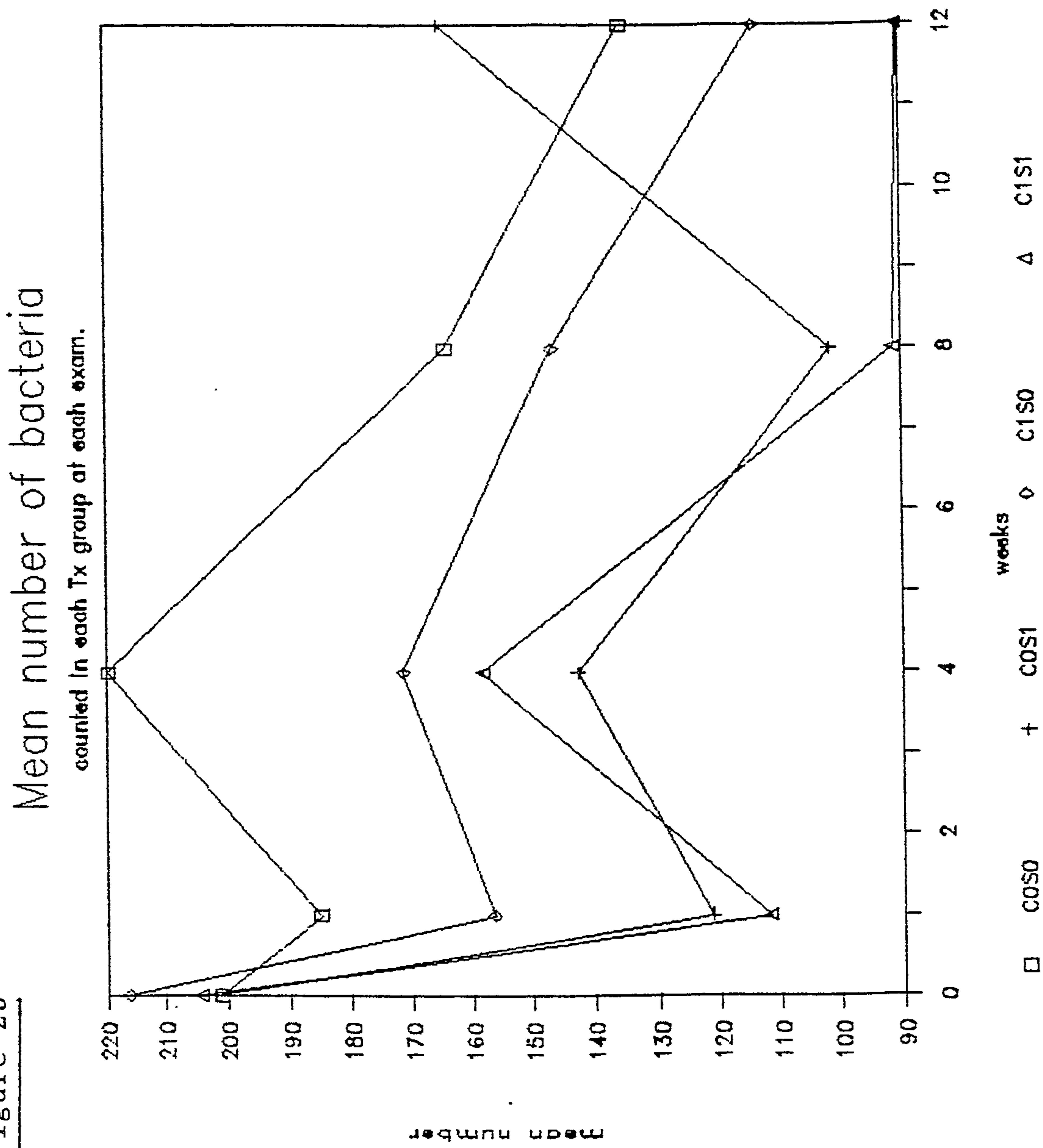


Figure 26

Mean bacterial count per H.P.F.

in each Tx group at each examination.

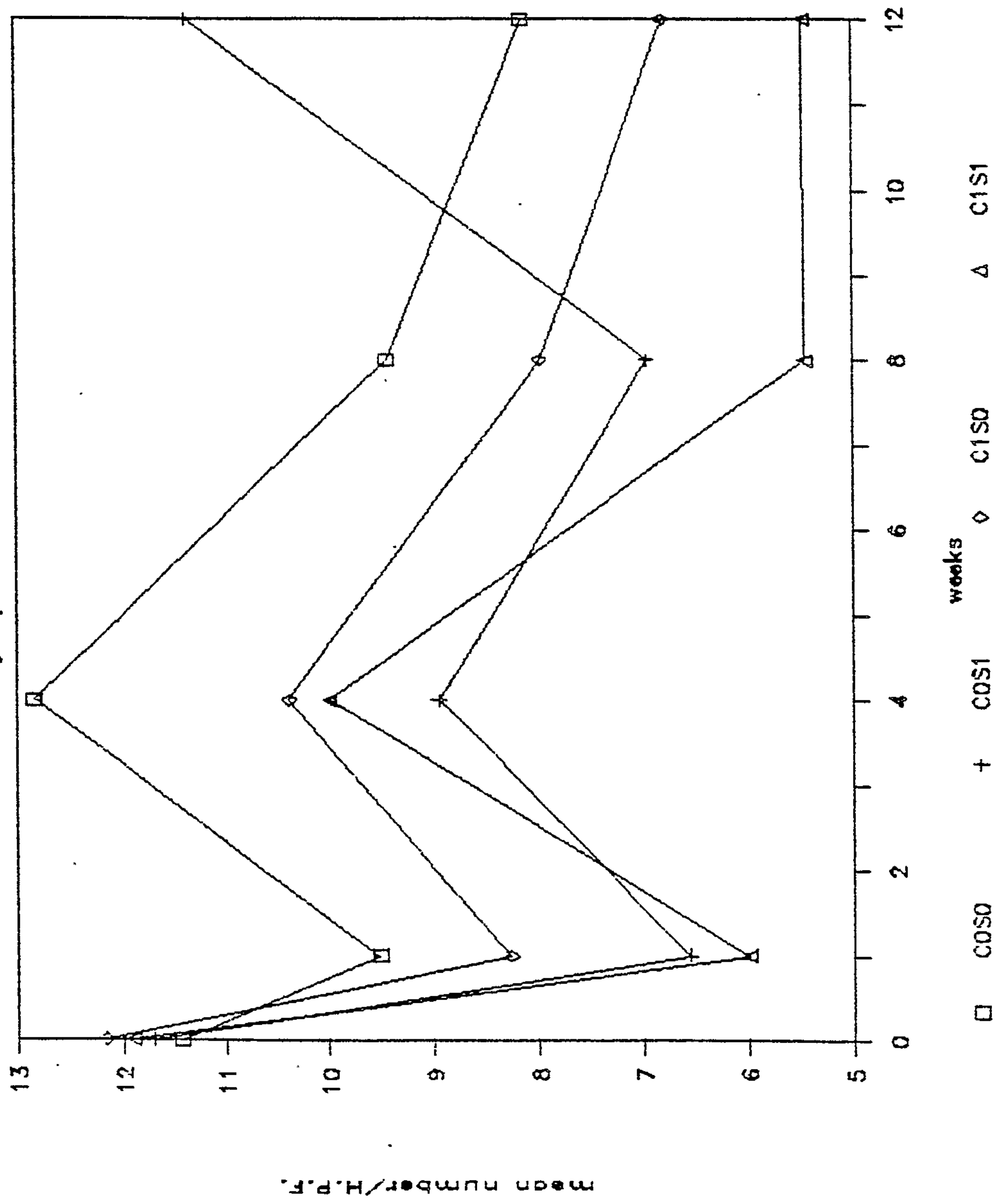


Figure 27.

Mean coccid count per H.P.F.

In each Tx group at each examination.

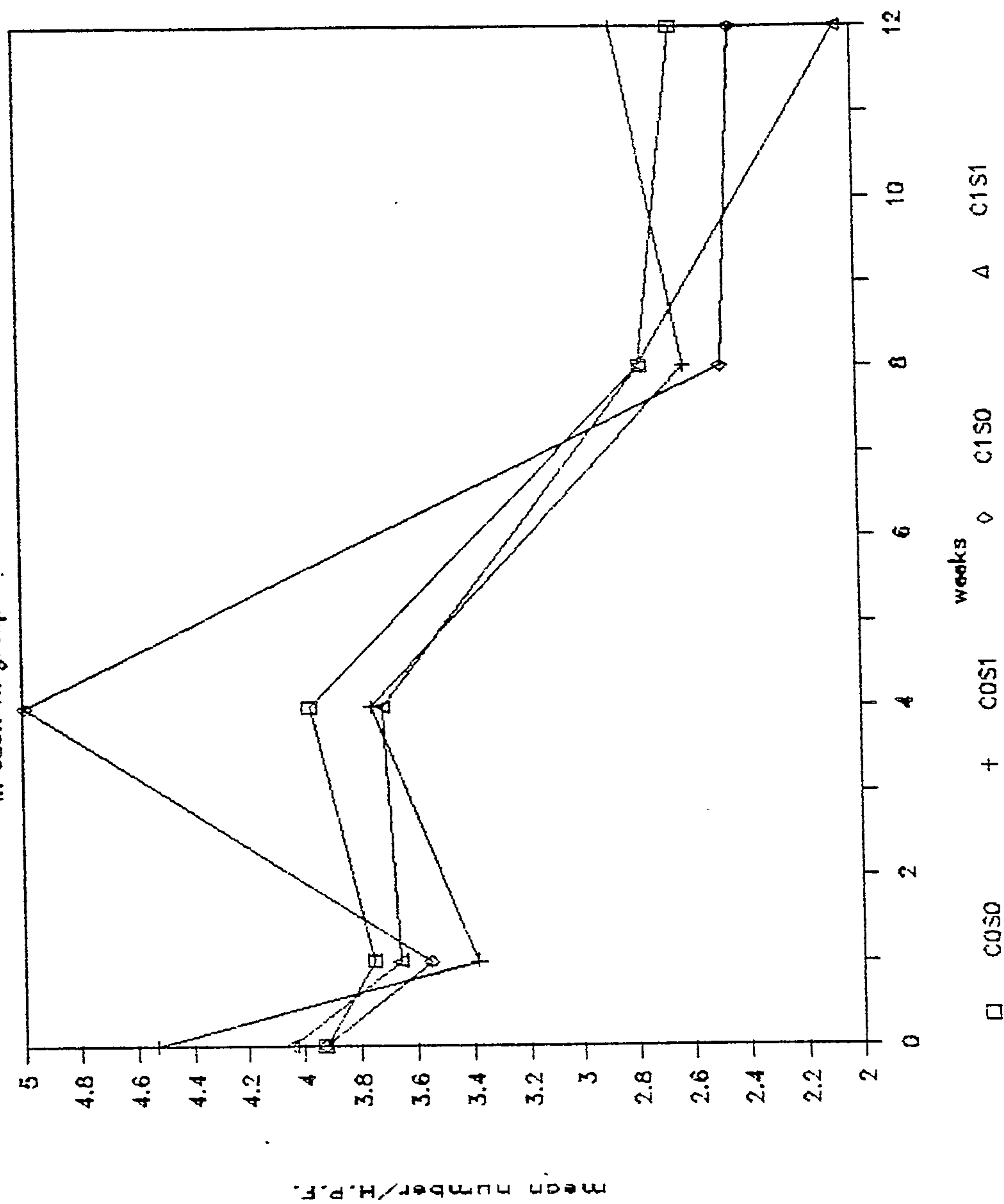


Figure 28

Coccal proportion of count

in each treatment group.

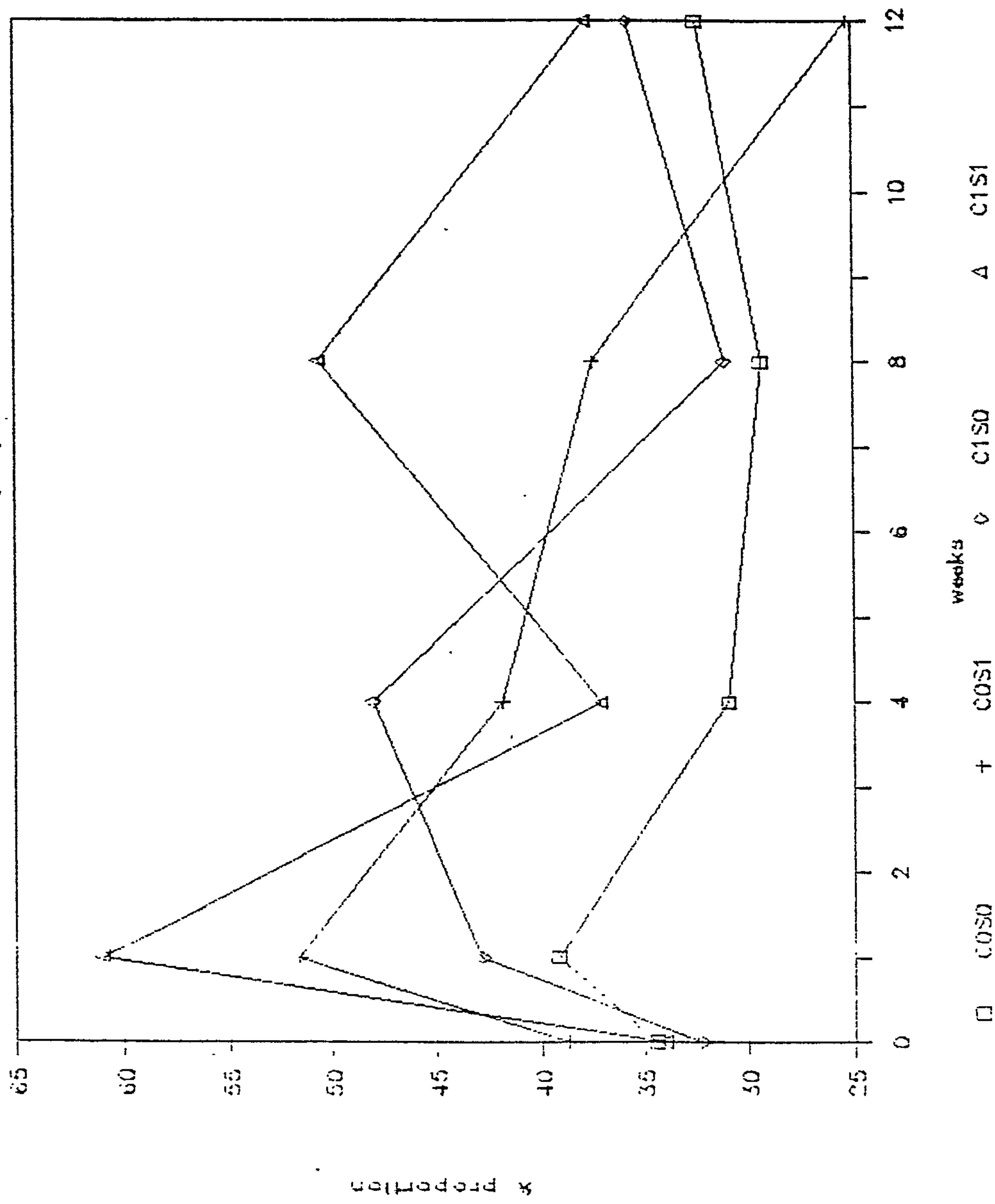


Figure 29

Proportion of coccidial forms

In C1 and C0 treatment groups.

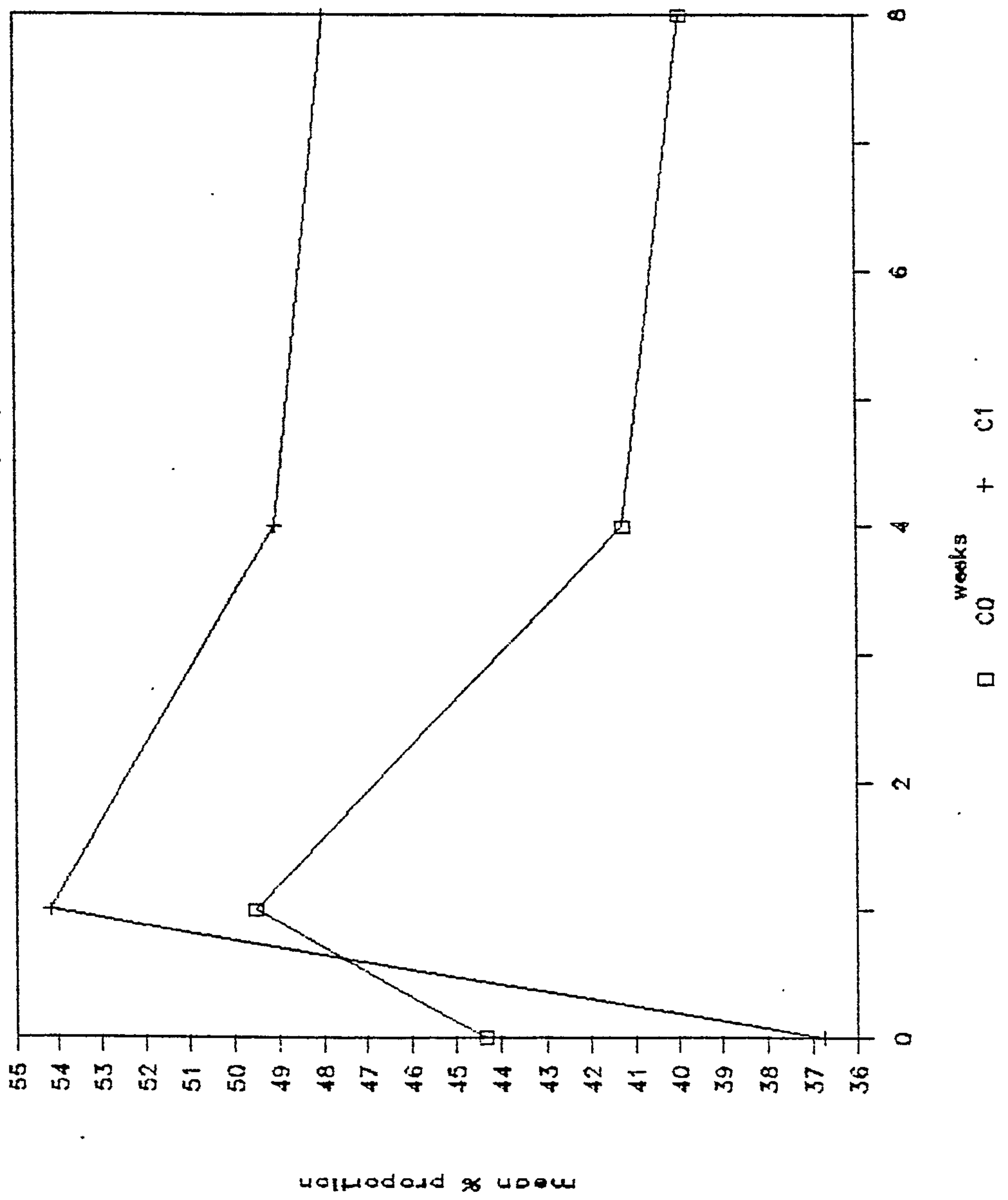


Figure 30

Mean motile count per H.P.F.

In each Tx group at each examination.

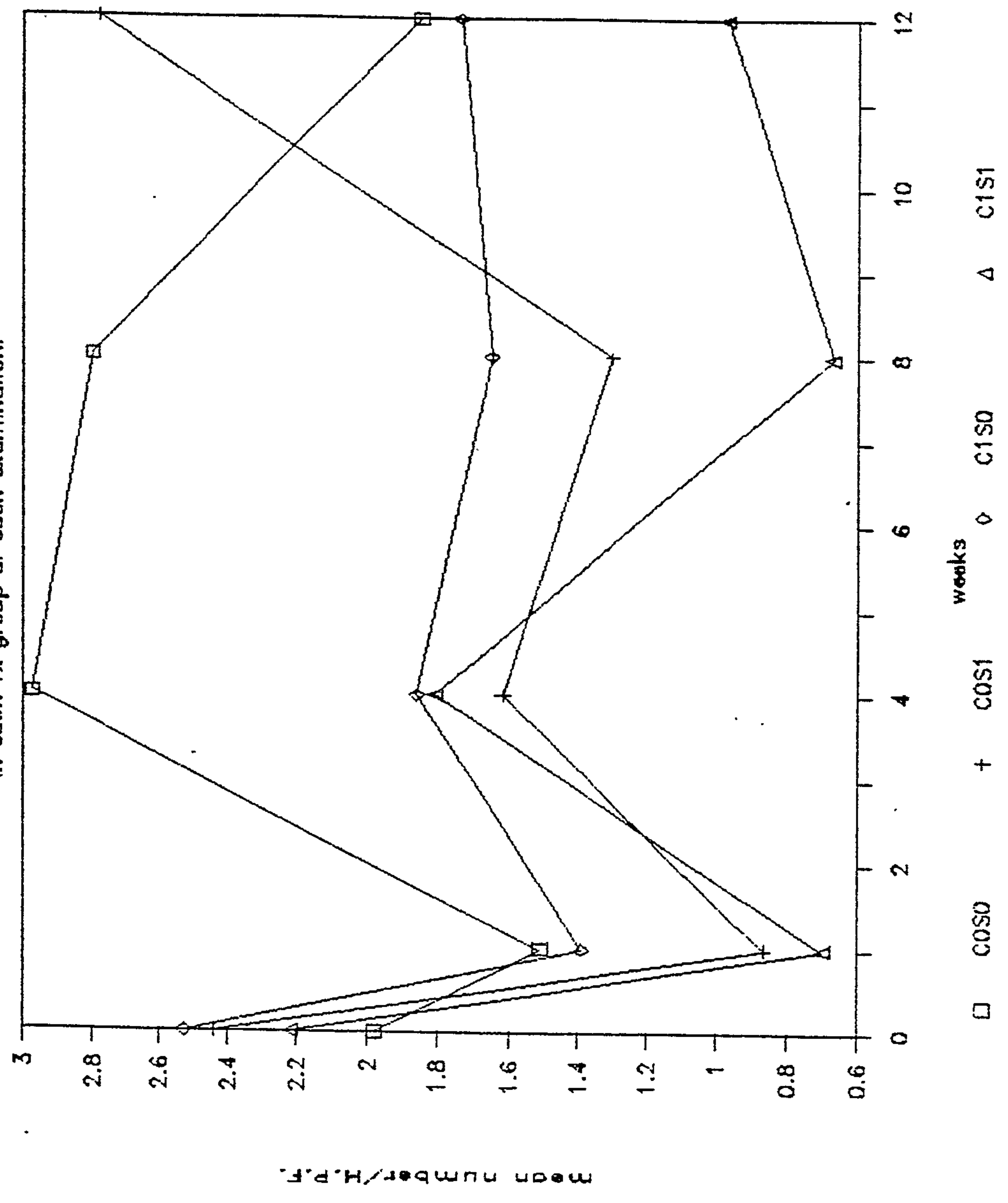


Figure 31

Motile proportion of count
in each treatment group.

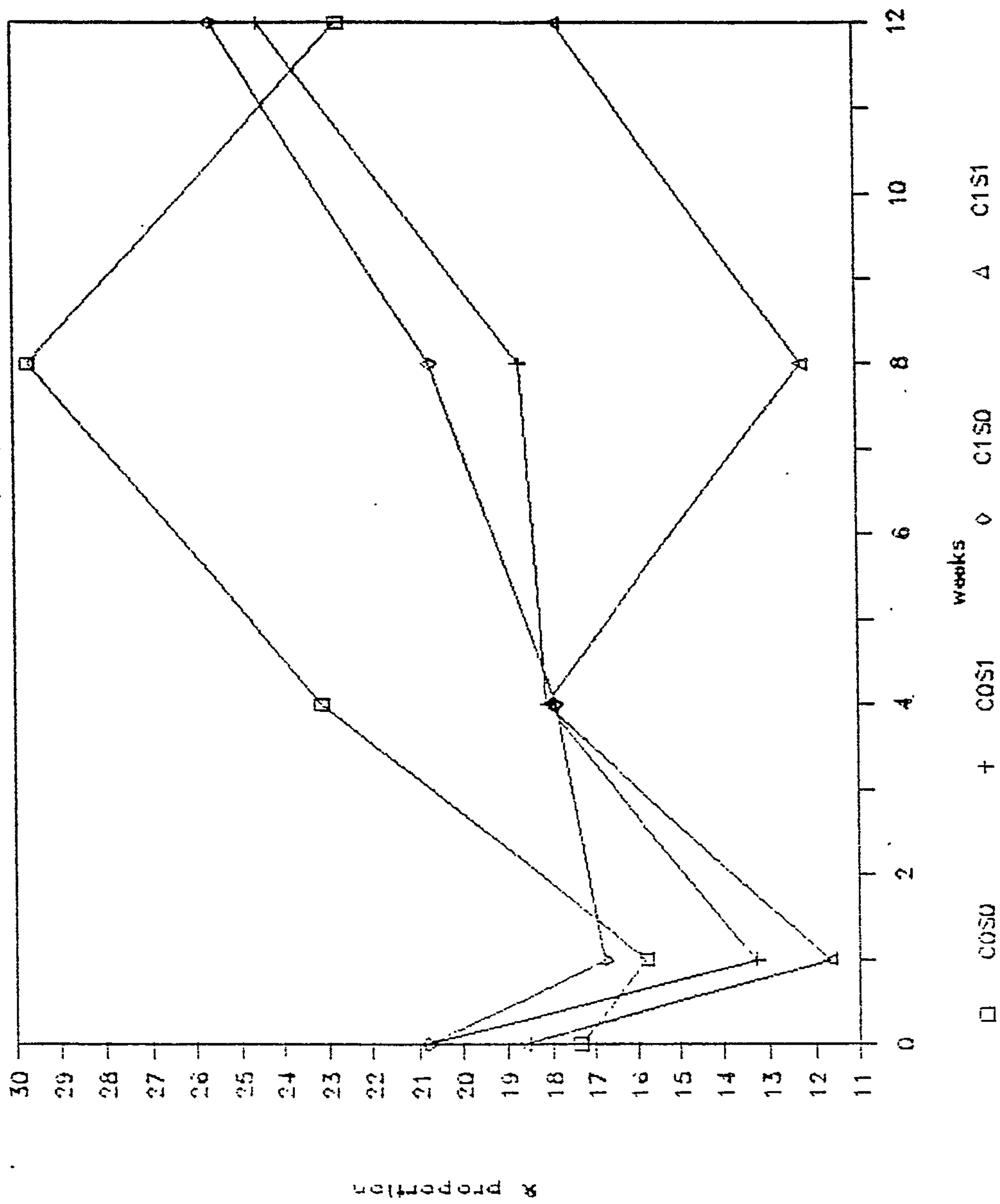


Figure 32

Mean spirochaete count per H.P.F.

in each Tx group at each examination.

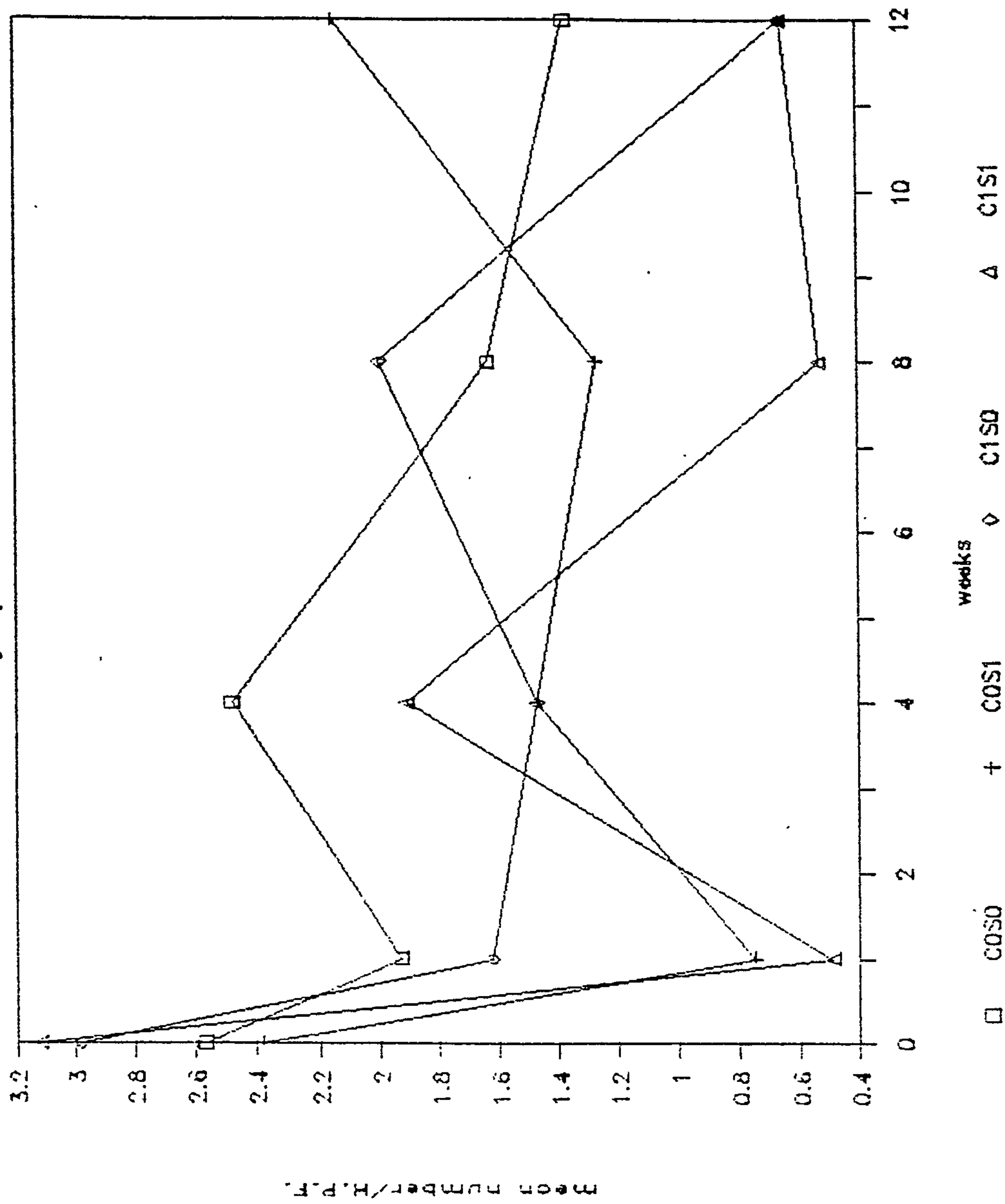


Figure 33

Spirochaete proportion of count

In each Tx group.

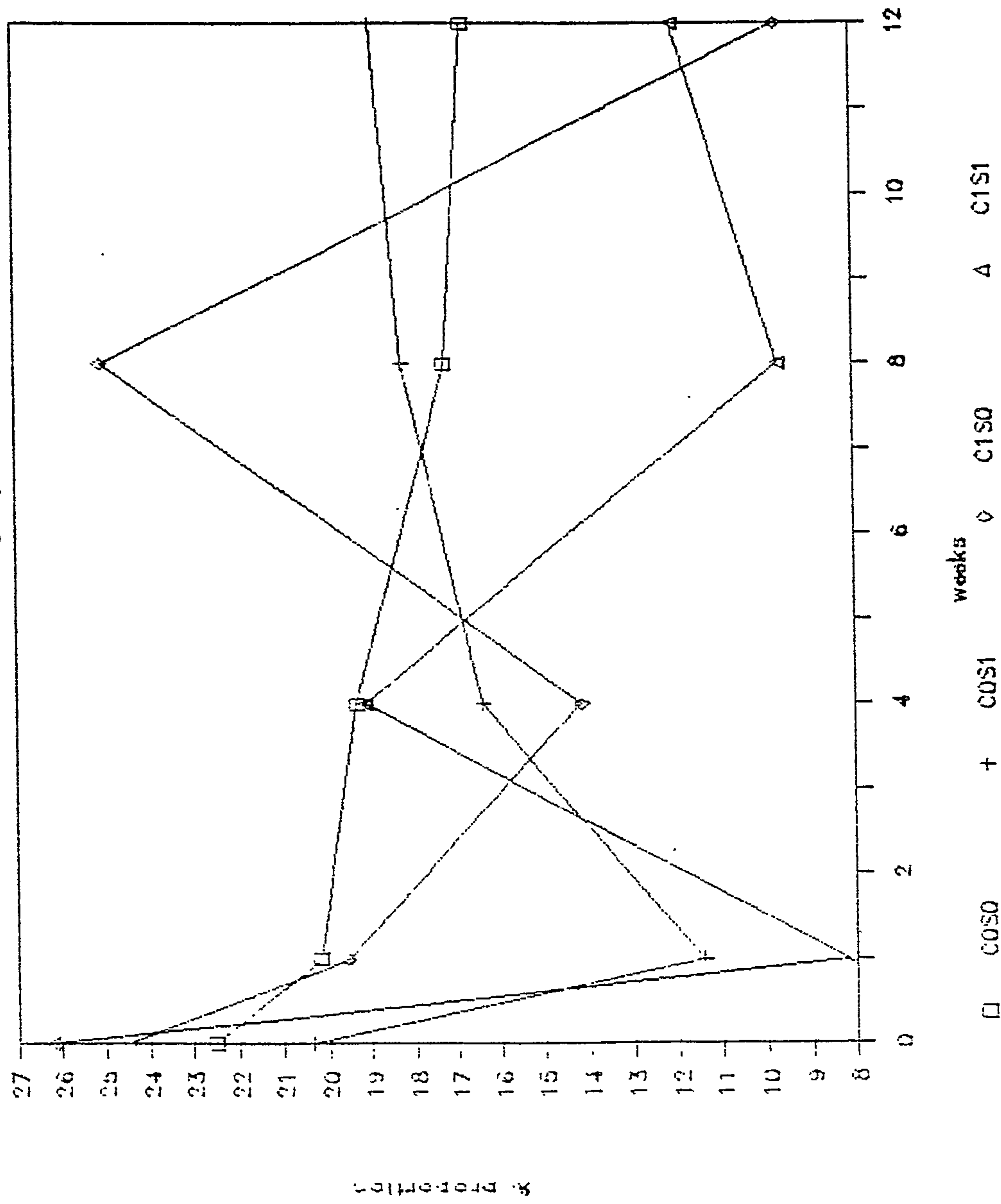


Figure 34

Mean count of others per H.P.F.

In each Tx group at each examination.

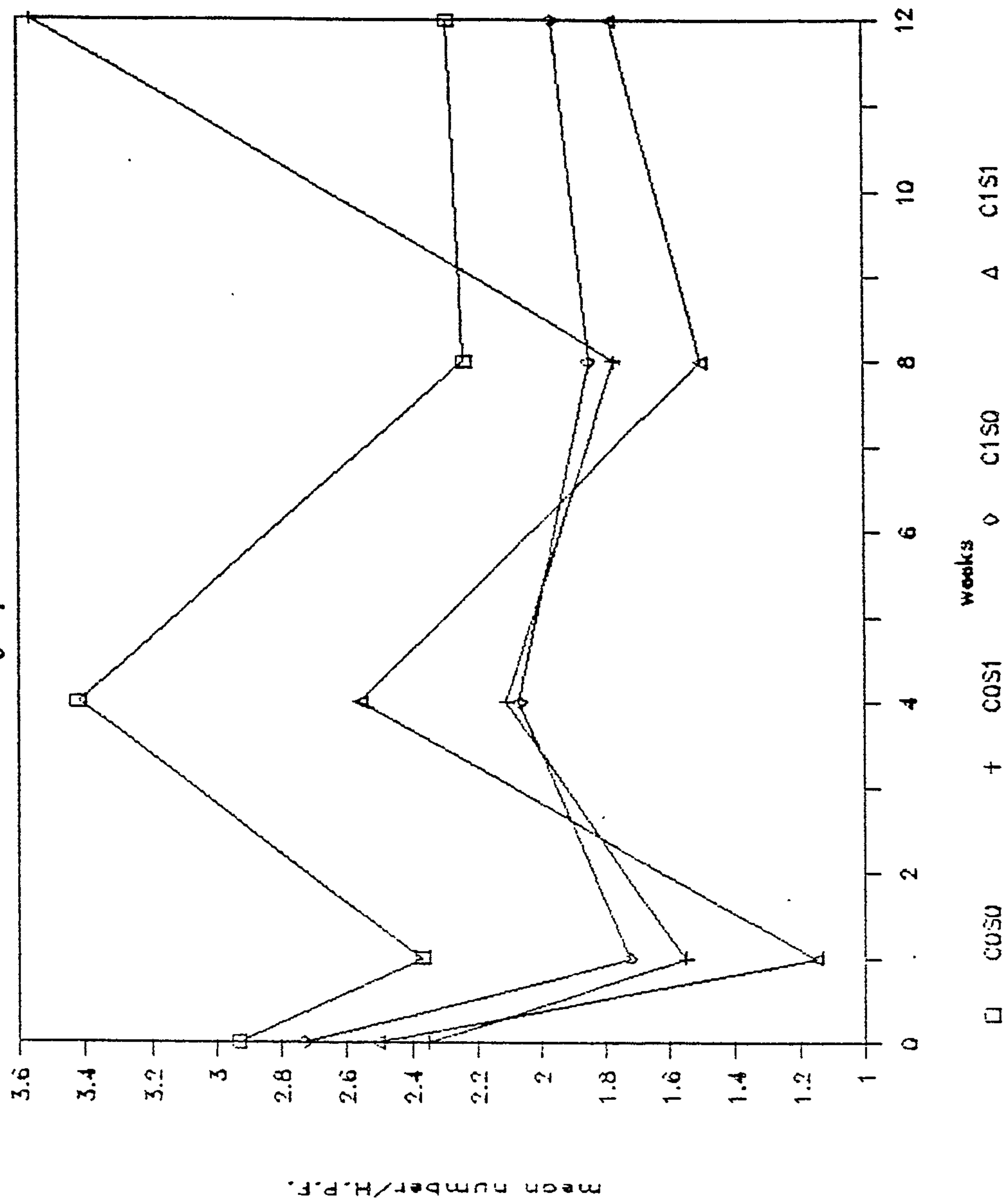


Figure 35
Proportion of 'others' in count
In each treatment group.

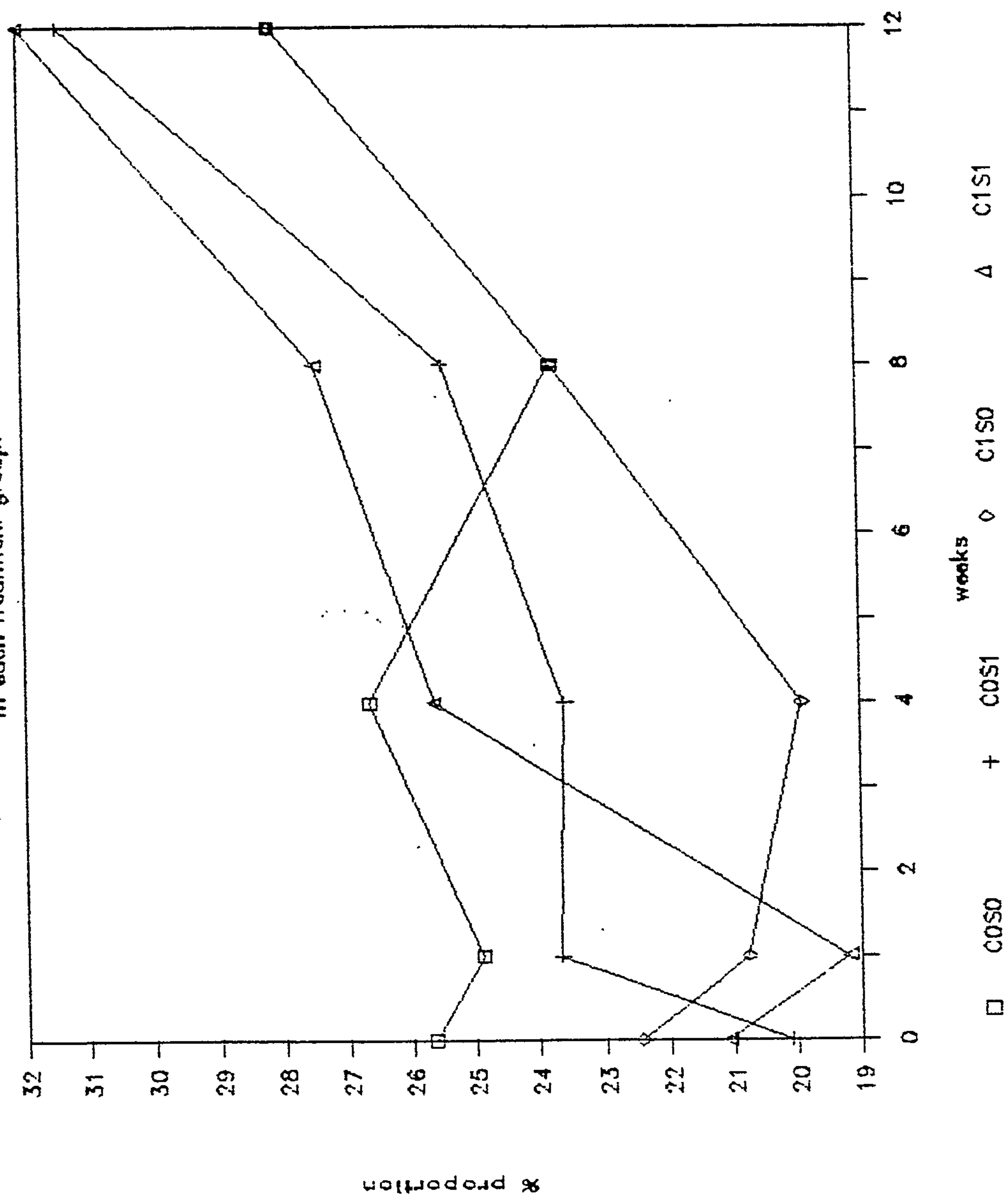


Figure 36

COSO Treatment Group
proportions of bacterial types.

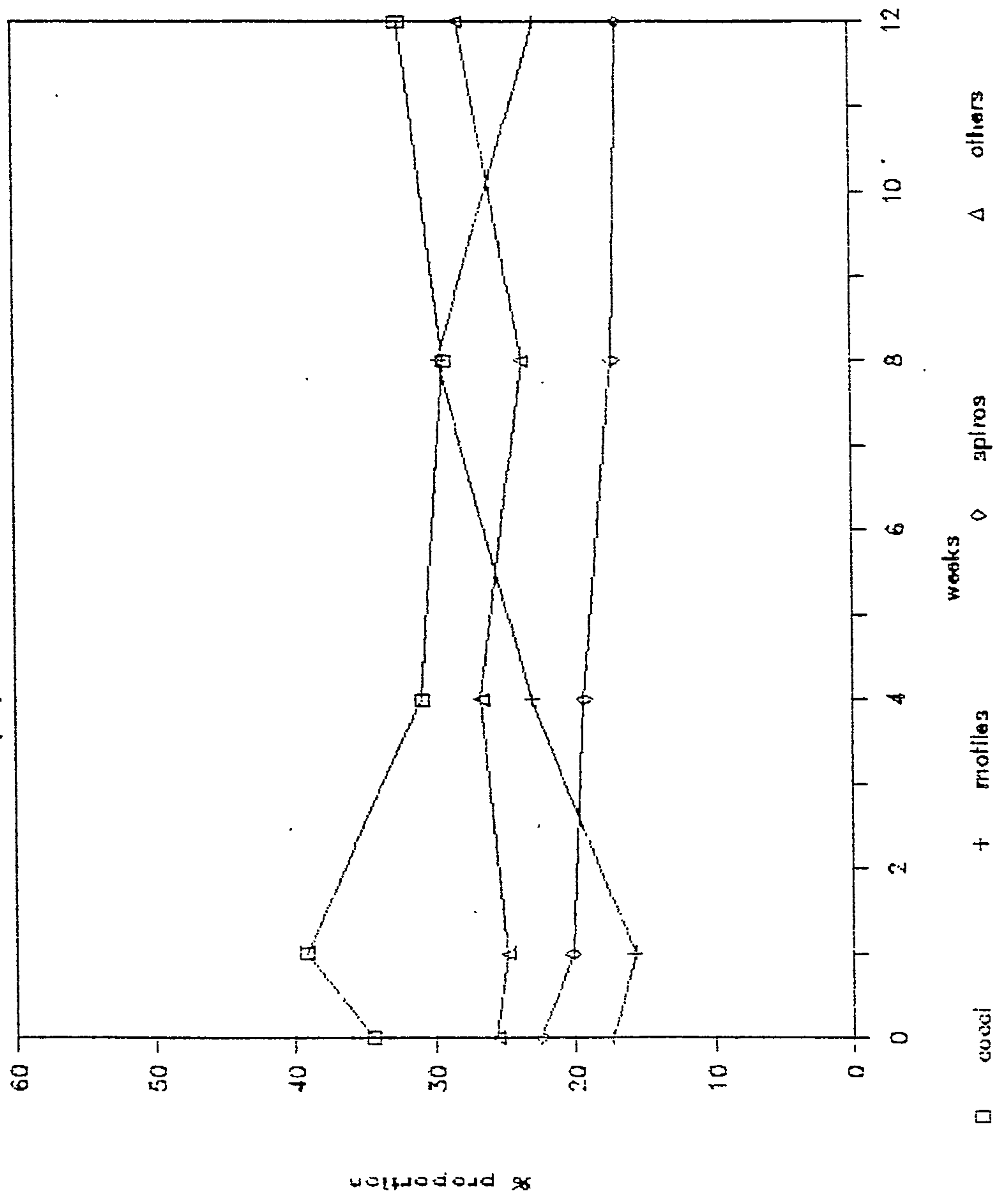


Figure 37

COS1 Treatment Group

proportions of bacterial types.

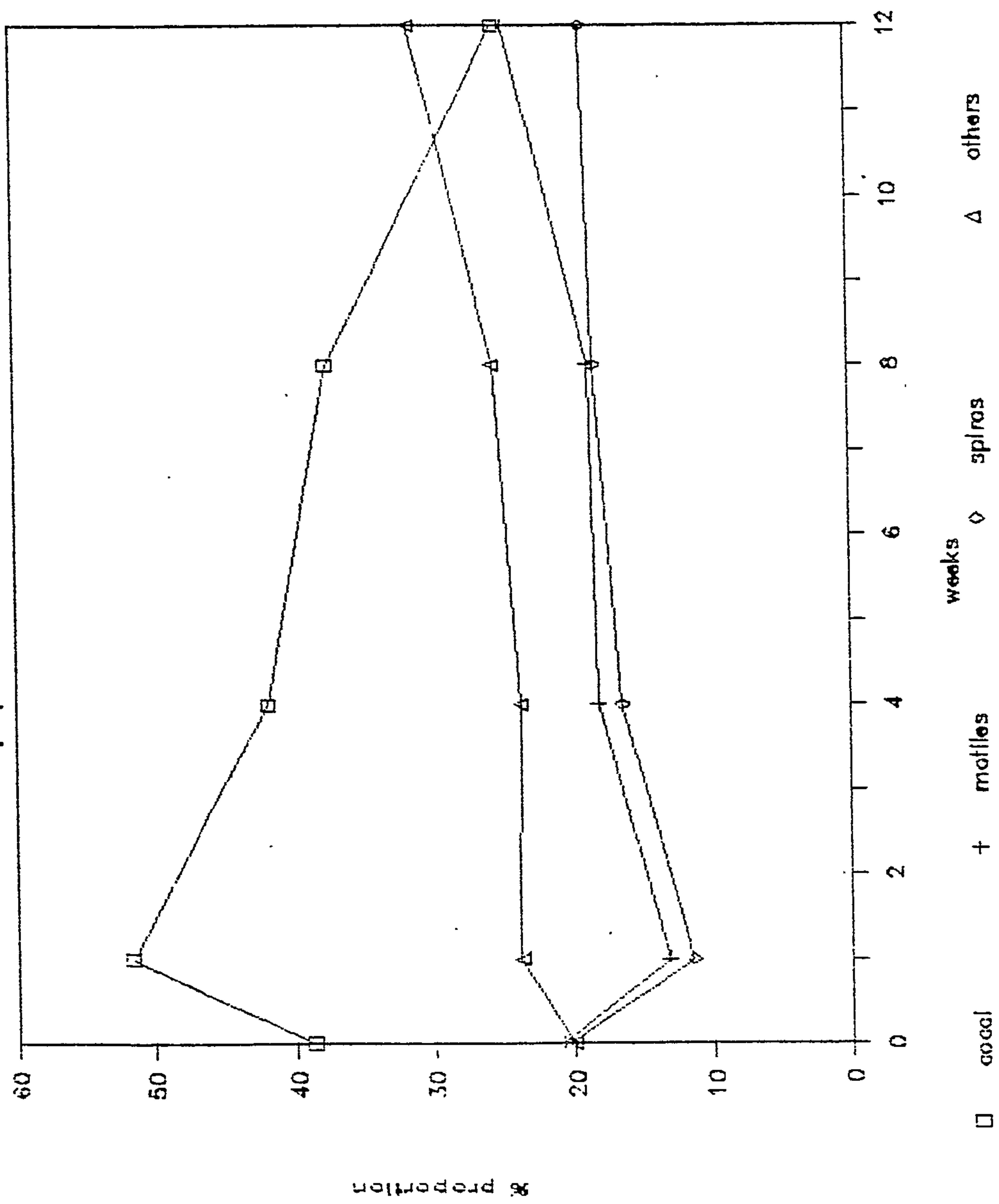


Figure 38

C1S0 Treatment Group proportions of bacterial types.

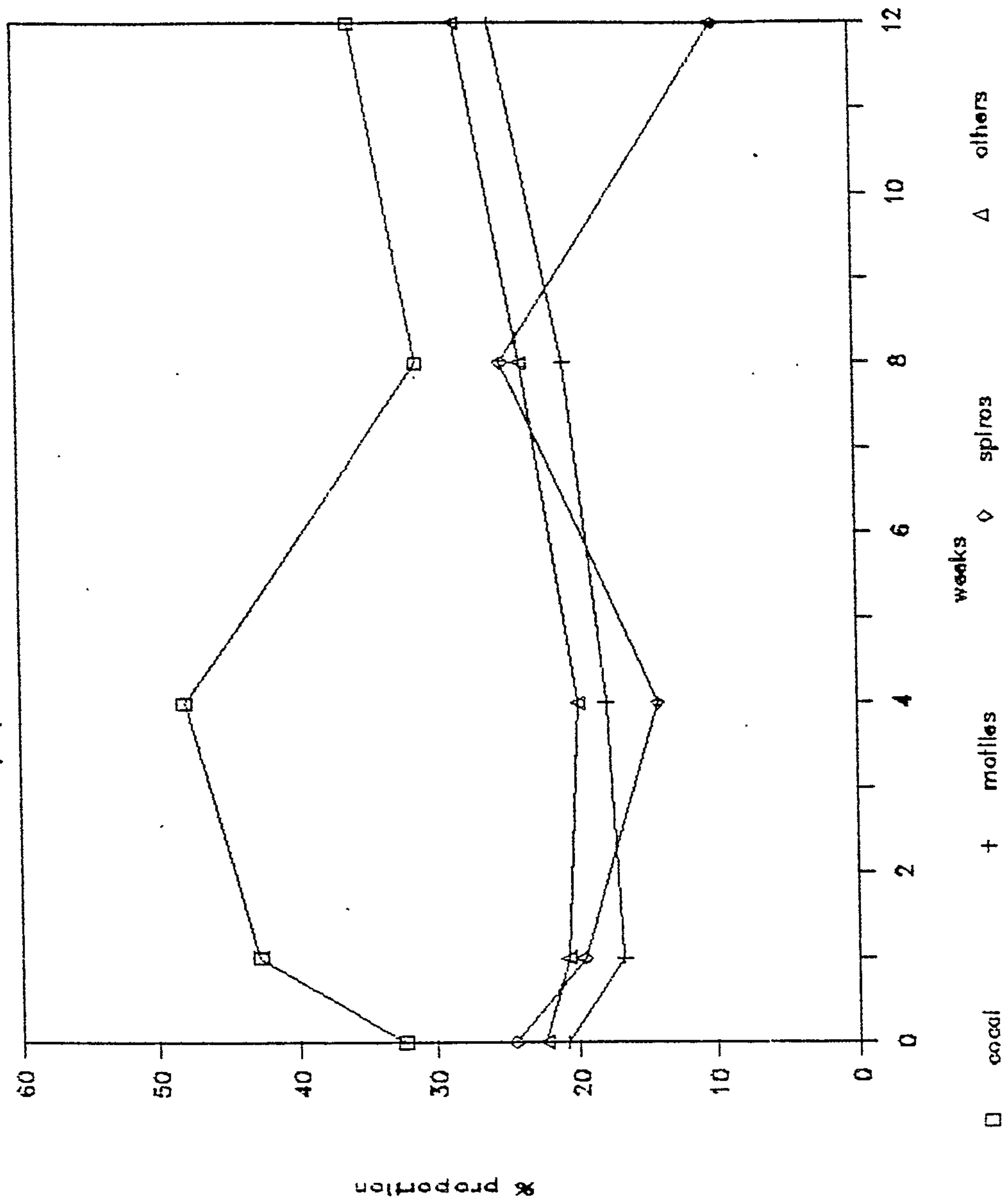
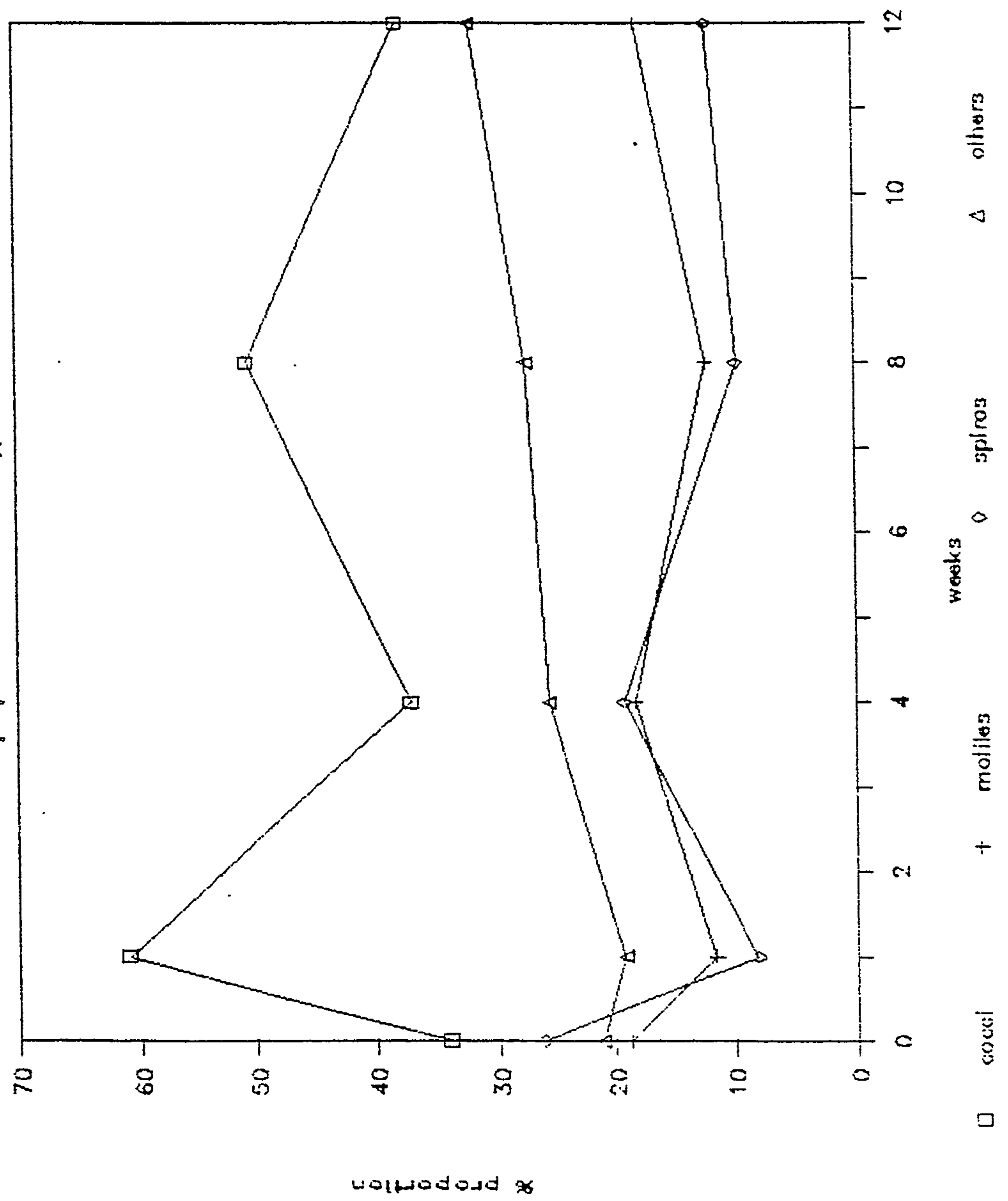


Figure 39

C1S1 Treatment Group proportions of bacterial types.



CHAPTER FIVE

DISCUSSION

5.1 Clinical Findings

Nature abounds with shifting patterns of response. In an experiment one attempts to create a microcosm of the real world, manipulate variables, observe outcomes and add to knowledge. A critical issue is the extent to which the study mirrors reality. Thus, in a clinical trial such as this, the changes that are seen may be a product of the experimental method, a response to treatment or a combination of the two.

Changes in the parameters measured were indicative of a general improvement in periodontal health over the study period in all treatment groups, even in the control group.

There are three possible reasons for this. The first of these is the Hawthorne Effect which occurs when subjects' knowledge of participation in a clinical trial causes them to alter their behaviour, thus producing changes in the dependent variables and threatening the internal and external validity of the study (*Darby and Bowen, 1980*).

One could argue that the fact that the subjects agreed to participate in a clinical trial for three months for no tangible reward in itself sets them apart, as being compliant and motivated and, perhaps, more inclined to change their behaviour.

Oral hygiene was the single factor that was mainly under the control of the subjects in this trial. All subjects received oral hygiene instruction at the start of the study and there was a statistically

significant improvement in the proportion of sites scoring PlI(0) in the COS1 and C1S1 groups over the first eight weeks. However, this improvement was mainly at the expense of sites that had scored PlI(1) originally. Also, the C1S1 group had greater potential for improvement than the COS1 group because the C1S1 group had a lower proportion of sites scoring PlI(0) at baseline. In addition, the frequency of sites scoring PlI(2,3) remained fairly constant in all the groups which would suggest that a single episode of oral hygiene instruction, unreinforced, was not particularly beneficial. In view of the small changes that occurred, the Hawthorne Effect appears to have made little impact. The Hawthorne Effect, if it appeared, should have been spread evenly across the groups.

The second possibility is that all the treatments, including that received by the control, were effective in improving periodontal health to some extent. The control group received subgingival irrigation with saline which may have disrupted or washed away the loosely adherent subgingival microflora. Although it has been shown that pulsated jet irrigation will remove materia alba but not adherent supragingival plaque, the subgingival microflora is quite different in structure and composition to supragingival plaque (*Listgarten et al., 1976; Addy et al., 1983*). If the physical effect of subgingival irrigation disrupted the subgingival microflora sufficiently then this would result in some improvement in the periodontal condition.

The third factor to consider is that the measurement of biological parameters is, in itself, invasive (*Listgarten, 1986*). Each time a site was sampled for dark field microscopy, the supragingival plaque was removed and the subgingival microflora was sampled. This would have caused disruption to the microflora and the simple physical

removal of bacteria was likely to improve the periodontal condition somewhat. Whilst other methods of sampling would seem less invasive, some bacteria would still have been removed by methods, such as using paper points, which have their own limitations (for review see *Tanner and Goodson, 1986*).

The findings in this clinical trial concur with existing knowledge. That is, they indicate that both mechanical and chemical plaque control effect an improvement in periodontal health (*Listgarten et al., 1978; Axelsson and Lindhe, 1978, 1981, Hellden et al., 1979*). However, the key issue here was that of the relative benefit, a ranking, of treatments. The evidence gained from this study suggests that subgingival irrigation with chlorhexidine does not confer additional benefit of significance over that of scaling and root planing alone in subjects with adult periodontitis. Although the microbial parameters examined were suggestive of some additional benefits, this was not statistically significant.

Some adjunctive effect did appear to occur when the effect on gingival inflammation was examined. The statistically significant results in the COS1 and C1S1 groups, where the proportion of sites scoring GI(0) increased over weeks 0 to 8, suggest that scaling and subgingival irrigation compared with chlorhexidine reduced gingival inflammation. Significance was retained for the full twelve weeks in the C1S1 group alone and this is suggestive of additional therapeutic gain being derived from the joint therapies. However, the C1S1 group had greater potential for improvement than the COS1 group as the C1S1 group had a lower proportion of sites scoring GI(0) at baseline.

To show a true treatment effect one should ideally have no significant

difference between the parameters at baseline and have a significant difference after treatment has been carried out. The small sample group and the wide spread of scores for the Plaque and Gingival Indices resulted in small differences between groups being statistically significant. Thus, the treatment groups appeared to be quite dissimilar when the Plaque and Gingival Indices were considered at baseline.

Concurrent with the general improvement in the Plaque Index and the Gingival Index was a general reduction in the mean proportion of sites exhibiting bleeding on probing to the base of the pocket. Indicative of a reduction in inflammation, bleeding on probing provides a more adequate assessment of the histological and physiological state of the base of a pocket than the Gingival Index. Unfortunately, its high specificity in combination with its low sensitivity limits its use as a predictor and indicator of disease activity.

Likewise, there was a generalized reduction in mean probing depths, which was statistically significant, over the twelve weeks. This was probably related to resolution of inflammation.

Two of the cardinal signs of inflammation are swelling and redness - 'tumor' and 'calor' - products of increased vascularity and blood vessel permeability. Removal of the factors that induced the inflammatory response, bacteria, resulted in decreased oedema and improved tissue tone. As the tissues shrank and the likelihood decreased of the probe penetrating the attachment, the probing depths reduced. The mean probing depths reduced by 1.15-1.53 mm and this is similar to the reduction reported in other studies (*Morrison et al., 1980; Badersten et al., 1981*).

Doubtless it is possible (but not probable) that examiner error led to the results that were observed as intra-examiner error of $\pm 1\text{mm}$ in probing measurements is not uncommon. This can be influenced by factors such as probe size, angulation, pressure and calibration. However, one would expect that such error would be random. Additionally, in this study the same probe was used for a subject at each examination. There would have had to be a consistent underestimation of pocket depth in the measurement after the baseline examination in order to imply a generalized reduction in mean probing depths. *Haffajee and co-workers (1983)* recognized the possibility of intra-examiner or inter-examiner error introducing bias and suggested that there should be variation greater than three standard deviations from the original mean before claiming true changes in probing depths or attachment levels.

Socransky and co-workers (1984) have also considered the episodic nature of periodontitis and have proposed the burst theories as an appropriate disease model. What was seen at the time, to be an innovative approach is not new to medicine as this type of model has long been accepted in chronic inflammatory diseases such as rheumatoid arthritis.

If we accept the model then it is likely that probing depths will increase or decrease at a site over a period of time, and that the use of mean values would tend to obscure this variation. In addition, sites with greater initial probing depths have greater potential to reduce than relatively shallower sites. Their contribution could disproportionately influence mean values. Thus, the pooling of data for statistical analysis may explain some of the findings in this trial.

Scaling and subgingival irrigation with chlorhexidine are essentially non-selective methods of controlling the microflora and, over the twelve weeks, the COS1 group had a greater reduction in mean probing depth than the C1S0 group probably because scaling removes the surface deposits and roughness that provides sites for bacterial retention.

Although the C1S1 group had a greater decrease in mean probing depth than the other treatment groups, the lack of statistical significance in this is not surprising. The mean decrease in the C1S1 group was 1.53 mm at week 12 in comparison to 1.20 mm in the COS1 group. Certainly there was a clinical improvement but it was only marginally more than the mean change that one might ordinarily expect as a result of scaling and root planing and, as previously mentioned, the pooling of data places limitations on the extent to which inferences may be drawn from the results.

It was interesting to note that, at week 1, the COS1 group, which received conventional conservative therapy, lost attachment. This may have occurred as a result of tissue disruption during instrumentation close to the base of the pocket although the same effect was not noted in the C1S1 group.

Many of the points raised in relation to mean probing depth apply equally when considering attachment levels. There was a statistically significant mean gain in attachment in all treatment groups over the period of study but no significant differences between groups. In fact, the mean gain in attachment seemed quite variable until week 12 when all treatment groups improved. This was possibly due to the exiting from the study of subjects 5 and 8 after week 8. Both subjects had a mean loss of attachment of 9 mm at the outset which

could have biased mean values. In addition, subject 8's upper right first molar spontaneously exfoliated in week 11. Both it and the contralateral first molar had been losing attachment and his removal from the study could have strongly influenced mean values in such a small sample group.

When this study was designed it represented an approach to real and anticipated difficulties such as finding large enough treatment groups and cross-matching subjects. Thus, each subject in this trial had four treatments and four experimental sites sharing the oral hygiene practices and immune response of the host. To say that the experimental sites were completely independent of each other is to deny the plainly apparent. There is a high intraclass correlation between sites in an individual, as has been pointed out by *Imrey* and acknowledged by *Haffajee and co-workers (Imrey, 1986; Haffajee et al., 1985)*.

However, the use of different subjects for the four treatment groups would have introduced its own difficulties when an indication of the relative benefit or ranking of the treatments could be obtained using the current study design.

5.2 Microbiological Findings

The microbiological findings in this study were that there were both quantitative and qualitative changes in the subgingival microflora as a result of treatment.

Scaling and root planing remove bacteria and accretions that enhance the potential for microbial repopulation whereas chlorhexidine acts as an antiseptic and kills bacteria by adversely affecting cell membrane permeability and disrupting pathways of cellular metabolism.

The physical effect of subgingival irrigation was common to all groups and could have contributed to the generalized improvement that was seen although doubts about this possibility have been expressed in the literature (*MacAlpine et al., 1985*).

Each experimental site was sampled at each examination and this too would have altered the ecology, thus leading to a decline in mean bacterial numbers counted.

There was a noticeable reduction in the mean bacterial number counted at week 1 following treatment and this was in line with the observations made in previous studies (*Listgarten et al., 1978; Schiött et al., 1970*). However, at week 4 there was an increase in the mean bacterial number counted in all treatment groups, followed by a decrease at week 8. Two subjects were omitted from microbiological assessment at week 8, with four more omitted at week 12 for various reasons.

Examination of the raw scores indicates that the subjects who did not complete the trial were those who tended to have a larger number of bacteria present in their samples. Thus, at week 8 and at week 12 the mean numbers counted decreased again in all but the COS1 group as the remaining subjects were those who tended to have a lower number of bacteria in their samples.

The increase in the mean number of bacteria counted in the COS1 group at week 12 is difficult to explain. It could be due to chance variation or a real return to baseline values if, for example, operator variables resulted in scaling only reducing the mean bacterial count for 8 weeks. It is important to note, though, that

this apparent aberration occurs within what is still a relatively narrow range i.e. $5.47 \pm 1.38 - 11.35 \pm 8.96$ bacteria per H.P.F.

Since the mean number of bacteria decreased markedly between weeks 0 and 1, the proportions of bacterial morphotypes present were considered to be more relevant in comparing treatments.

A higher proportion of coccal forms is associated with a younger plaque and periodontal health in contrast to the lower proportion of coccal forms that could be expected to be present in the subgingival microflora of adult periodontitis (*Addy et al., 1983; Slots, 1977a; Slots, 1977b*). The mean proportion of coccal forms increased after treatment, indicating that a less pathogenic flora was present. The highly statistically significant results between weeks 0 and 8 in the groups that were subgingivally irrigated with chlorhexidine (C1S0, C1S1) suggests benefit from treatment. However, the lack of significance between weeks 0 and 12 implies that the effect might have dissipated by week 12. As scaling alone can disrupt the microflora for up to four months, it might be expected that chlorhexidine would not confer additional benefit beyond scaling. This was confirmed for this trial when a comparison was made between the COS1 and C1S1 groups. The effect of subgingival irrigation with chlorhexidine was found to be less for teeth that were scaled than for those that were not scaled, with respect to the mean proportion of coccal forms.

The decrease in mean proportions of motile forms following treatment is in accordance with previously published work (*Khoo and Newman, 1983; Rosling et al., 1982*). The effect of scaling was weakly significant, the weakness possibly being a function of the small number of motiles counted after treatment in a small sample group.

The proportion of spirochaetes also dropped after treatment and this correlates with a decrease in the proportion of sites bleeding on probing to the base of the pocket (*Listgarten and Levin, 1981*). The weakly significant interaction of subgingival irrigation with chlorhexidine and scaling should be interpreted with caution. It suggests that the combination of treatments induced change in the subgingival environment so that natural antagonists of spirochaetes were encouraged or the conditions made unfavourable for their proliferation. Although the small sample and weak significance limits inference, further investigation is warranted.

Others were bacteria that did not belong to any of the other three morphological groupings. These have not been implicated, as a group, in either health or disease in the literature. Since the group could have contained species as diverse as *Actinomyces viscosus* and *Bacteroides gingivalis* - both rods - the lack of statistically significant change in proportions is not unexpected.

The microbiological assessment was more susceptible to methodological errors than the clinical assessment as sampling for dark field microscopy in itself disrupts the development of the microflora (*Listgarten, 1986*). Recently reviewed, the limitations of sampling for dark field microscopy may be unavoidable but it does, nonetheless, provide a simple method of assessing the type of microflora present (*Tanner and Goodson, 1986*).

It has been suggested that the survival time for bacteria in saline is limited (*Lander et al., 1986*). For this reason, bacteria were counted within an hour of sampling. Time also governed the number of fields that could be counted.

Since there are millions of bacteria in any one site, ten or twenty fields might not be truly representative of the microflora in that pocket. Many of the subgingival species are obligate anaerobes and it is likely that they would die soon after sampling even if the curette had collected sufficient numbers of bacteria typical of the site. As a result the proportion of, for example, motiles, would be underestimated. This type of error would be common to all four treatment groups and could be reduced by increasing the size of the sample.

Despite these factors it is possible to establish some ranking of the four treatments. The COSO group showed little variation from baseline to week 12. Therapy induced similar changes in the COS1 and C1SO groups but the effect on the mean proportions of bacterial types appeared to be prolonged to week 4 in the C1SO group. Both showed a return to baseline values by week 12. The C1S1 group showed a much more dramatic change in mean bacterial proportions between week 0 and week 1 but these returned to baseline values by week 4. Thus, the use of subgingival irrigation with chlorhexidine did not seem to result in benefit additional to that derived from scaling and root planing alone, at least in terms of producing a microflora dominated by coccal forms for a prolonged period.

CHAPTER SIX

CONCLUSIONS

Based on the findings in this clinical trial, the following may be concluded regarding the effect of a single subgingival irrigation with 0.2% chlorhexidine with and without scaling and root planing, in moderate to severe adult periodontitis:

- 1) scaling and root planing was effective in producing resolution of inflammation and what is regarded as a less pathogenic flora;
- 2) a single subgingival irrigation with 0.2% chlorhexidine was not as effective as scaling and root planing in producing resolution of inflammation and a less pathogenic flora;
- 3) there was no additive effect of significance on clinical and microbiological parameters when scaling and root planing was combined with a single subgingival irrigation with 0.2% chlorhexidine although trends were suggestive of some additional benefit;
- 4) further investigation is warranted in sites such as furcations where mechanical debridement is difficult but where subgingival irrigation with 0.2% chlorhexidine could be employed in chemical control of periodontal pathogens.

CHAPTER SEVEN

REFERENCES:

Addy, M., & Roberts, W.R. (1981): The use of polymethylmethacrylate to compare the adsorption and staining reactions of some cationic antiseptics.

Journal of Periodontology 52: 380-385.

Addy, M. & Moran, J. (1983): Comparison of plaque accumulation after topical application and mouth rinsing with chlorhexidine gluconate.

Journal of Clinical Periodontology 10: 69-71.

Addy, M., Newman, H., Langeroudi, M. & Gho, J.G.L., (1983): Dark-field microscopy of the microflora of plaque.

British Dental Journal 155: 269-273.

Addy, M. & Langeroudi, M., (1984): Comparison of the immediate effects on the sub-gingival microflora of acrylic strips containing 40% chlorhexidine, metronidazole or tetracycline.

Journal of Clinical Periodontology 11: 379-386.

Addy, M., Hassan, H., Moran, J., & Wade, W. (1985): A comparison of the effects of metronidazole (M), tetracycline (T) and chlorhexidine (C) containing acrylic strips and root planing (RP) on chronic periodontal pockets.

Journal of Dental Research 64: abstract 175.

Addy, M. & Hunter, L., (1987): The effects of a 0.2% chlorhexidine gluconate mouthrinse on plaque, toothstaining and candida in aphthous ulcer patients. A double-blind placebo-controlled cross-over study.

Journal of Clinical Periodontology 14: 267-273.

Addy, M. (1986): Chlorhexidine compared with other locally delivered antimicrobials.

Journal of Clinical Periodontology 13: 957-964.

Addy, M., Jenkins, S., & Newcombe, R. (1989): Studies on the effect of toothpaste rinses on plaque regrowth (I). Influence of surfactants on chlorhexidine efficacy.

Journal of Clinical Periodontology 16: 380-384.

Aleo, J.J., De Renzis, F.A., Farber, P.A. & Varboncoeur, A.P. (1974): The presence and biologic activity of cementum-bound endotoxin.

Journal of Periodontology 45: 672-675.

Allenspach-Petrzilka, G.E. & Guggenheim, G. (1983): Bacterial invasion of the periodontium; an important factor in the pathogenesis of periodontal disease?

Journal of Clinical Periodontology 10: 609-617.

Armitage, G.C. & Christie, T.M. (1973)a: Structural changes in exposed human cementum. I. Light microscopic observations.

Journal of Periodontal Research 8: 343-355.

Armitage, G.C. & Christie, T.M. (1973)b: Structural changes in exposed human cementum. II. Electron microscopic observations.

Journal of Periodontal Research 8: 356-365.

Ashley, F.P., Skinner, A., Jackson, P.Y. & Wilson, R.F. (1984)a:
Effect of a 0.1% cetylpyridinium chloride mouthrinse on
the accumulation and biomechanical composition of dental
plaque in young adults.

Caries Research 18: 465-471.

Ashley, F.P., Skinner, A., Jackson, P.Y. & Wilson, R.F. (1984)b:
the effect of a 0.1% cetylpyridinium chloride mouthrinse
on plaque and gingivitis in adult subjects.

British Dental Journal 157: 191-196.

Axelsson, P. & Lindhe, J. (1974): The effect of a preventive
programme on dental plaque, gingivitis and caries in
schoolchildren. Results after one and two years.

Journal of Clinical Periodontology 1: 126-138.

Axelsson, P. & Lindhe, J. (1978): Effect of controlled oral
hygiene procedures on caries and periodontal disease in
adults.

Journal of Clinical Periodontology 5: 133-151.

Axelsson, P. & Lindhe, J. (1981): Effect of controlled oral hygiene
procedures on caries and periodontal disease in adults.
Results after 6 years.

Journal of Clinical Periodontology 8: 239-248

Aziz-Gandour, I.A. & Newman, H.N. (1986): The effects of a
simplified oral hygiene regime plus supragingival
irrigation with chlorhexidine or metronidazole on chronic
inflammatory periodontal disease.

Journal of Clinical Periodontology 1: 228-236.

Badersten, A., Nilveus, R. & Egelberg, J. (1981): Effect of non-surgical periodontal therapy. I. Moderately advanced periodontitis.

Journal of Clinical Periodontology 8: 57-72.

Bailey, L. (1989): Direct plaque removal by a pre-brushing dental rinse.

Clinical Preventive Dentistry 11: 21-27.

Bakaeen, G.S. & Strahan, J.D. (1980): Effects of a 1% chlorhexidine gel during the healing phase after inverse bevel mucogingival flap surgery.

Journal of Clinical Periodontology 7: 20-25.

Bass, C. (1951): A previously undescribed demonstrable pathologic condition in exposed cementum and the underlying dentine.

Oral Surgery 4: 641-652.

Bergenholtz, A., Gustafsson, L.B., Segerlund, N., Hagberg, C. & Nygaard Ostby, P. (1984): Role of brushing technique and toothbrush design in plaque removal.

Scandinavian Journal of Dental Research 92: 344-351.

Bonesvoll, P., Lokken, P., Rolla, G. & Paus, P.N. (1974): Retention of chlorhexidine in the human oral cavity after mouthrinses.

Archives of Oral Biology 19: 209-212.

Bower, R.C. (1979): Furcation morphology relative to periodontal treatment. Furcation root surface anatomy.

Journal of Periodontology 50: 366-374.

- Braatz, L., Garrett, S., Claffey, N. & Egelberg, J. (1985):
Antimicrobial irrigation of deep pockets to supplement
non-surgical periodontal therapy. II. Daily irrigation.
Journal of Clinical Periodontology 12: 630-638.
- Cercek, J.F., Kiger, R.D., Garrett, S. & Egelberg J. (1983):
Relative effects of plaque control and instrumentation on
the clinical parameters of human periodontal disease.
Journal of Clinical Periodontology 10: 46-56.
- Ciancio, S.G. (1986): Chemotherapeutic agents and periodontal
therapy. Their impact on clinical practice.
Journal of Periodontology 57: 108-111..
- Ciancio, S.G. Mather, M.L. Zambon, J.J. & Reynolds, H.S. (1989):
Effect of a chemotherapeutic agent delivered by an oral
irrigation device on plaque, gingivitis, and subgingival
microflora.
Journal of Periodontology 60: 310-315.
- Ciancio, S.G. & Nisengard, R.: Control and prevention of perio-
dental disease. (In Newman, M.G. & Nisengard, R.,
ed. Oral Microbiology and Immunology: Philadelphia,
W.B. Saunders Company, 1988: p.439-446).
- Cohen-Cole, S.A. Cogen, R.B., Stevens, A.W., Kirk, K., Gaitan,
E., Bird, J., Cooksey, R. & Freeman, A. (1983):
Psychiatric, psychosocial, and endocrine correlates of
acute necrotizing ulcerative gingivitis (trench mouth):
a preliminary report.
Psychiatric Medicine 1: 215-225.

Costerton, J.W., Irvin, R.T. & Cheng, K.J. (1981): The bacterial glycocalyx in nature and disease.

Annual Review of Microbiology 35: 299-324.

Cotton, W.R. (1988): Guest editorial: How far can a frog jump?

A current assessment of pulp biology research.

Journal of Dental Research 67: 1251.

Coventry, J. & Newman, H.N. (1982): Experimental use of a slow release device employing chlorhexidine gluconate in areas of acute periodontal inflammation.

Journal of Clinical Periodontology 9: 129-133.

Daly, C., Seymour, G. & Kieser, J. (1980): Bacterial endotoxin - a role in chronic inflammatory periodontal disease?

Journal of Oral Pathology 9: 1-15.

Daly, C.G., Seymour, G.J., Kieser, J.B. & Corbet, E.F. (1982):

Histological assessment of periodontally involved cementum.

Journal of Clinical Periodontology 9: 266-274.

Darby, M.L. & Bowen, D.M.:

Research Methods for Oral Health Professionals:

St. Louis, C.V. Mosby (1980), pp 55-63.

Davies, G.E., Francis, J., Martin, A.R., Rose, F.L. & Swain, G.

(1954) 1:6-Di-4'-Chlorophenyldiguanidohexane

("Hibitane"): Laboratory investigation of a new anti-bacterial agent of high potency.

British Journal of Pharmacology 9: 192-198.

- Dzink, J.L. & Socransky, S.S. (1985): Comparative *in vitro* activity of sanguinarine against oral microbial isolates. *Antimicrobial Agents and Chemotherapy* 27: 663-665.
- Egelberg, J. (1965): Local effect of diet on plaque formation and development of gingivitis in dogs. I. Effect of hard and soft diets. *Odontologisk Revy* 16: 31-41.
- Elin, R. & Wolff, S., (1973): Non-specificity of the limulus amoebocyte lysate test: positive reactions with polynucleotides and proteins. *Journal of Infectious Diseases* 128: 349-352.
- Etemadzadeh, H. & Ainamo, J. (1987): Lacking anti-plaque efficacy of 2 sanguinarine mouthrinses. *Journal of Clinical Periodontology* 14: 176-180.
- Fardal, G. & Turnbull, R.S. (1986): A review of the literature on use of chlorhexidine in dentistry. *Journal of the American Dental Association* 112: 863-869.
- Flotra, L., Gjermo, P., Rolla, G. & Waerhaug, J. (1971): Side effects of chlorhexidine mouth washes. *Scandinavian Journal of Dental Research* 79: 119-125.
- Flotra, L., Gjermo, P., Rolla, G. & Waerhaug, J. (1972): A 4-month study on the effect of chlorhexidine mouth washes on 50 soldiers. *Journal of Dental Research* 80: 10-17.

Foulkes, D.M. (1973): Some toxicological observations on chlorhexidine.

Journal of Periodontal Research 8: Supplement 12: 55-57.

Gabler, W.L. Bullock, W.W., Creamer, H.R., (1987): The influence of chlorhexidine on superoxide generation by induced human neutrophils.

Journal of Periodontal Research 22: 445-450.

Gad, T., (1968): Periodontal disease in dogs. I. Clinical investigation.

Journal of Periodontal Research 3: 268-272.

Gardner, J.F. : Chlorhexidine (pp 251-270) (In Block, S.S.:

Disinfection, Sterilization and Preservation:

Philadelphia, Lea and Febiger, 1983).

Gibbons, R.J. and van Houte, J., (1973): On the formation of dental plaques.

Journal of Periodontology 44: 6:347-360.

Gillett, R and Johnson, N.W. (1982): Bacterial invasion of the periodontium in a case of juvenile periodontitis.

Journal of Clinical Periodontology 9: 93-100.

Gjerme, P., (1974): Chlorhexidine in dental practice.

Journal of Clinical Periodontology 1: 143-152.

Glickman, I., (1972): Chlorhexidine. (In

Clinical Periodontology, 4th Ed. London:

W.B. Saunders Co.(p. 196).)

Gold., S.I. (1985)a: Periodontics. The past. Part (I) Early Sources.

Journal of Clinical Periodontology 12: 79-87.

Gold., S.I., (1985)b: Periodontics. The past. Part (II). The development of modern periodontics.

Journal of Clinical Periodontology 12: 171-189.

Gold, S.I., (1985)c: Periodontics. The past. Microbiology. Part (III).

Journal of Clinical Periodontology 12: 257-269.

Golub, L.M. Goodson, J.M., Lee, H.M., Vidal, A.M., McNamara, T.F., and Ramamurthy, N.S., (1985): Tetracyclines inhibit tissue collagenases. Effects of ingested low-dose and local delivery systems.

Journal of Periodontal Research 56: Supplement:93-97.

Goodson, J.M., Haffajee, A.D., & Socransky, S.S., (1979): Periodontal therapy by local delivery of tetracycline.

Journal of Clinical Periodontology 6: 83-92.

Greene, J.C., and Vermillion, J.R., (1960): The oral hygiene index: A method for classifying oral hygiene status.

Journal of the American Dental Association 61: 173-179.

Grossman, E., Meckel, A.H., Isaacs, R.L., Ferretti, G.A., Sturzenberger, O.P., Bollmer, B.W., Moore, D.J. Lijana, R.C., & Manhart, M.D., (1989): A clinical comparison of antibacterial mouthrinses: Effects of chlorhexidine, phenolics and sanguinarine on dental plaque and gingivitis.

Journal of Periodontology 60: 435-440.

- Haffajee, A.D., Socransky, S.S. and Goodson, J.M. (1983):
Comparison of different data analyses for detecting
changes in attachment level.
Journal of Clinical Periodontology 10: 298-310.
- Haffajee, A.D., Socransky, S.S., Goodson, J.M., and Lindhe, J.,
(1985): Intraclass correlations of periodontal measure-
ments.
Journal of Clinical Periodontology 12: 216-224.
- Hamp, S.E. Lindhe, J. and Löe, H. (1973): Long-term effect of
chlorhexidine on developing gingivitis in the beagle dog.
Journal of Periodontal Research 8: 63-70
- Hansen, F., Gjermo, P., (1971): The plaque-removing effect of
four toothbrushing methods.
Scandinavian Journal of Dental Research 79: 502-506.
- Harrap, G.J., Saxton, C.A. & Best, J.S. (1982): Inhibition of
plaque growth by zinc salt.
Journal of Periodontal Research 18: 634-642.
- Haskel, E., Esquenasi, J. & Yussim, L., (1986): Effects of
subgingival chlorhexidine irrigation in chronic moderate
periodontitis.
Journal of Periodontology 57: 5:305-310
- Hatfield, C.G. & Baumhammers, A., (1971): Cytotoxic effects of
periodontally involved surfaces of human teeth.
Archives of Oral Biology 16: 465-468.

Hedberg, A.R. (1987): The effects of fluorides on oral micro-organisms. A thesis in the Department of Preventive Dentistry.

University of Sydney.

Hellden, L.B., Listgarten, M.A., & Lindhe, J., (1979):

The effect of tetracycline and/or scaling on human periodontal disease.

Journal of Clinical Periodontology 6: 222-230.

Heyden, G. (1973): Relation between locally high concentration of chlorhexidine and staining as seen in the clinic.

Journal of Periodontal Research 8: Supplement 12, 76-80.

Hill, R.W. Ramfjord, S.P., Morrison, E.C., Appelberry, E.A.,

Caffesse, R.G., Kerry, G.J., & Nissle, R.R. (1981):

Four types of periodontal treatment compared over two years.

Journal of Periodontology 52: 655-662.

Hill, H.C., Levi, P.A. & Glickman, I., (1973): The effects of waxed and unwaxed dental floss on interdental plaque accumulation and interdental gingival health.

Journal of Periodontology 44: 411-413.

Hirschfeld, L. & Wasserman, B., (1978): A long-term survey of tooth loss in 600 treated periodontal patients.

Journal of Periodontology 49: 225-237.

Hirst, R.C., (1972): Chlorhexidine: A review of the literature.

Periodontal Abstracts 20: 2:52-58

- Holt, S.C., Ebersole, J., Felton, J., Brunxvold, M. and Kornman, K.S., (1988): Implantation of bacteroides gingivalis in non-human primates initiates progression of periodontitis.
Science 1: 55-57.
- Hull, P.S., (1980): Chemical inhibition of plaque.
Journal of Clinical Periodontology 7: 431-442.
- Imrey, P.B., (1986): Considerations in the statistical analysis of clinical trials in periodontitis.
Journal of Clinical Periodontology 13: 517-528.
- Jenkins, S., Addy, M., & Newcombe, R., (1989): Studies on the effect of toothpaste rinses on plaque regrowth. (II). Triclosan with and without zinc citrate formulation.
Journal of Clinical Periodontology 16: 385-387.
- Jones, S.J., Lozdan, J., & Boyde, A., (1972): Tooth surfaces treated *in situ* with periodontal instruments. Scanning electron microscopic studies.
British Dental Journal 132: 57-64.
- Jones, W.A. & O'Leary, T.J., (1978): The effectiveness of *in vivo* root planing in removing bacterial endotoxin from the roots of periodontally involved teeth.
Journal of Periodontology 49: 337-342.
- Jordan, H.V. & Keyes, P.H., (1964): Aerobic, gram-positive, filamentous bacteria as etiologic agents of experimental periodontal disease in hamsters.
Archives of Oral Biology 9: 401-414.

- Kennedy, J.E. & Polson, A.M. (1973): Experimental marginal periodontitis in squirrel monekys.
Journal of Periodontology 44: 140-144.
- Kinane, D.F., Cullen, C.F., Johnston, F.A. & Evans, C.F. (1989): Neutrophil chemotactic behaviour in patients with early-onset forms of periodontitis. (I). Leading front analysis in Boyden chambers.
Journal of Clinical Periodontology 16: 242-246.
- Khoo, J.G.L. & Newman, H.N., (1983): Subgingival plaque control by a simplified oral hygiene regime plus local chlorhexidine or metronidazole.
Journal of Periodontal Research 18: 607-619.
- Kornman, K.S.,: Antimicrobial Agents. State-of-the-Science review. (p.121-142). (In Loe, H., & Kleinman, D., ed. *Dental Plaque Control Measures and Oral Hygiene Practices.*, Oxford, IRL Press Limited, 1986).
- Lander, P.E., Newcomb., G.M. Seymour, G.J., & Powell, R.N., (1986): The antimicrobial and clinical effects of a single subgingival irrigation of chlorhexidine in advanced periodontal lesions.
Journal of Clinical Periodontology 13: 74-80.
- Lang, N.P., Catalanotto, F.A., Knopfli, R.U., Antczak, A.A.A., (1988): Quality-specific taste impairment following the application of chlorhexidine digluconate mouthrinses.
Journal of Clinical Periodontology 15: 43-48.

Langa, H.,: *Relative Analgesia in Dental Practice; Inhalation and Sedation with Nitrous Oxide*: Philadelphia, W.B. Saunders Co., 2nd Ed. 1976. (p. 8-11).

Lavine, W.S. Maderaco, E.G., Stolman, J., Ward, P.A. Cogen, R.B., Greenblatt, I. & Robertson, P.B., (1979): Impaired neutrophil chemotaxis in patients with juvenile and rapidly progressing periodontitis.
Journal of Periodontal Research 14: 10-19.

Leon, L.E., and Vogel, R.I., (1981): A comparison of the effectiveness of hand scaling and ultrasonic debridement in furcations as evaluated by differential dark-field microscopy.
Journal of Periodontology 52: 119-123.

Lie, T., and Meyer, K., (1977): Calculus removal and loss of tooth substance in response to different periodontal instruments. A scanning electron microscope study.
Journal of Clinical Periodontology 4: 250-262.

Lindhe, J. & Nyman, S., (1984): Long-term maintenance of patients treated for advanced periodontal disease.
Journal of Clinical Periodontology 11: 504-514.

Lindhe, J. & Nyman, S., (1975): The effect of plaque control and surgical pocket elimination on the establishment and maintenance of periodontal health. A longitudinal study of periodontal therapy in cases of advanced disease.
Journal of Clinical Periodontology 2: 67-79.

Lindhe, J. & Axelsson, P., (1975): Effect of proper oral hygiene on gingivitis and dental caries in Swedish school-children.
Community Dental Oral Epidemiology 3: 150-155.

Lindhe, J., Westfelt, E., Nyman, S., Socransky, S.S., Heijl, L. & Bratthall, G., (1982): Healing following surgical/non-surgical treatment of periodontal disease. A clinical study.
Journal of Clinical Periodontology 9: 115-128.

Lindhe, J., Westfelt, E., Nyman, S., Socransky, S.S., & Haffajee, A.D., (1984): Long-term effect of surgical/non-surgical treatment of periodontal disease.
Journal of Clinical Periodontology 11: 448-458.

Lindhe, J.: *Textbook of Clinical Periodontology*: Copenhagen, Munksgaard, 2nd Ed., 1989.

Lindhe, J., Liljenberg, G., Adielson, B., Borjesson, I., (1983): Use of metronidazole as a probe in the study of human periodontal disease.
Journal of Clinical Periodontology 10: 100-112.

Lindhe, J., Hamp, S.E., & Löe, H., (1973): Experimental periodontitis in the beagle dog.

Journal of Periodontal Research 8: 1-10.

Listgarten, M.A., Mayo, H. & Tremblay, R., (1975): Development of dental plaque on epoxy resin crowns in man. A light and electron microscopic study.

Journal of Periodontology 46: 10-26.

Listgarten, M.A., Levin, S., Schifter, C.C., Sullivan, P., Evian, C.I. & Rosenberg, E.S., (1984): Comparative differential dark-field microscopy of subgingival bacteria from tooth surfaces with recent evidence of recurring periodontitis and from non-affected surfaces.

Journal of Periodontology 55: 398-401.

Listgarten, M.A., Grossberg, D., Schwimer, C., Vito, A. & Gaffar, A., (1989): Effect of subgingival irrigation with tetrapotassium peroxydiphosphate on scaled and untreated periodontal pockets.

Journal of Periodontology 60: 4-11.

Listgarten, M.A., Lindhe, J. & Hellden, L., (1978): Effect of tetracycline and/or scaling on human periodontal disease. Clinical, microbiological, and histological observations.

Journal of Clinical Periodontology 5: 246-271.

Listgarten, M.A. & Levin, S., (1981): Positive correlation between the proportions of subgingival spirochaetes and motile bacteria and susceptibility of human subjects to periodontal deterioration.

Journal of Clinical Periodontology 8: 122-138.

Listgarten, M.A., (1976): Structure of the microbial flora associated with periodontal health and disease in man.
Journal of Periodontology 47: 1-17.

Listgarten, M.A., (1986): Direct microscopy of periodontal pathogens.
Oral Microbiology and Immunology 1: 31-36.

Löe, H., Schiott, C.R., Glavind, L. and Karring, T., (1976):
Two years' oral use of chlorhexidine in man. I. General design and clinical effects.
Journal of Periodontology 17: 135-144.

Löe, H., (1967): The gingival index, the plaque index and the retention index system.
Journal of Periodontology 38: 610-616.

Löe, H. and Schiott, C.R., (1970): The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man.
Journal of Periodontal Research 5: 79-83.

Löe, H., Theilade, E. & Jensen, S.B., (1965): Experimental gingivitis in man.
Journal of Periodontology 36: 177-187.

- Löe, H., Anerud, A., Boysen, H., and Smith, M., (1978)a: The natural history of periodontal disease in man. Study design and baseline data.
Journal of Periodontal Research 13: 550-562.
- Löe, H. Anerud, A., Boysen, H., and Smith, M., (1978)b: The natural history of periodontal disease in man. Tooth mortality rates before 40 years of age.
Journal of Periodontal Research 13: 563-572.
- Löe., H. Anerud, A., Boysen, H. and Smith, M., (1978)c: The natural history of periodontal disease in man. The rate of periodontal destruction before 40 years of age.
Journal of Periodontology 49: 607-620.
- Loesche, W.J., Syed, S.A., Schmidt, E. & Morrison, E.C. (1985):
Bacterial profiles of subgingival plaques in periodontics.
Journal of Periodontology 56: 447-456.
- Lovdal, A., Arno, A., Schei, O. & Waerhaug, J., (1961):
Combined effect of subgingival scaling and controlled oral hygiene on the incidence of gingivitis.
Acta Odontologica Scandinavica 19: 537-555.
- MacAlpine, R., Magnusson, I., Kiger, R., Crigger, M., Garrett, S., & Egelberg, J., ((1985): Antimicrobial irrigation of deep pockets to supplement oral hygiene instruction and root debridement.
Journal of Clinical Periodontology 12: 568-577.

Magnusson I., Lindhe, J., Yoneyama, T., & Liljenberg, B., (1984):
Recolonization of a subgingival microbiota following
scaling in deep pockets.

Journal of Clinical Periodontology 11: 193-207.

Mandel, I.D., (1988): Chemotherapeutic agents for controlling
plaque and gingivitis.

Journal of Clinical Periodontology 15: 488-498.

Marshall-Day, C.D., & Shourie, K.L., (1949): A roentgenographic
survey of periodontal disease in India.

Journal of the American Dental Association 39: 572-588.

Marshall-Day, C.D., Stephens, R.G. and Quigley, F., (1955):
Periodontal disease: prevalence and incidence.

Journal of Periodontology 26: 185-203.

Moore, J., Wilson, M. and Kieser, J.B., (1986): The distribution of
bacterial lipopolysaccharide (endotoxin) in relation to perio-
dontally involved root surfaces.

Journal of Clinical Periodontology 13: 748-751.

Moore, W.E.C., (1987): Microbiology of periodontal disease.

Journal of Periodontal Research 22: 335-341.

Moore, W.E.C., Holdeman, L.V., Cato, E.P., Good, I.J., Smith,
E.P., Ranney, R.R. & Falcanis, K.G., (1984): Variation
in periodontal floras.

Infection and Immunity: 720-726.

Morrison, E.C. Ramfjord, W.P. & Hill, R.S. (1980): Short-term effects of initial, non-surgical periodontal treatment (hygienic phase).

Journal of Clinical Periodontology 7: 199-211.

Mousques, T., Listgarten, M.A. & Phillips, R.W., (1980): Effect of scaling and root planing on the composition of the human subgingival microbial flora.

Journal of Periodontology 15: 144-151.

Muller, H.P. & Flores-De-Jacoby, L., (1985): The composition of the subgingival microflora of young adults suffering from juvenile periodontitis.

Journal of Clinical Periodontology 12: 113-123.

Newman, M.G. & Socransky, S.S., (1977): Predominant cultivable microbiota in periodontosis.

Journal of Periodontal Research 12: 120-128.

Newman, M.G. & Nisengard, R.: (ed). *Oral Microbiology and Immunology*. Philadelphia, W.B. Saunders Co., 1988: (pp 411-445).

Nishimine, D., O'Leary, T.J., (1979): Hand instrumentation versus ultrasonics in the removal of endotoxins from root surfaces.

Journal of Periodontology 50: 345-349.

Nyman, S., Lindhe, J. & Rosling, B., (1977): Periodontal Surgery in plaque-infected dentitions.

Journal of Clinical Periodontology 4: 240-249.

- Nyman, S., Westfelt, E., Sarhed, G., & Karring, T., (1988): Role of "diseased" root cementum in healing following treatment of periodontal disease. A clinical study.
Journal of Clinical Periodontology 15: 464-468.
- Nyman, S., Lindhe, J., Janson, J.C., (1972): The effect of a bacterial dextranase on human dental plaque formation and gingivitis development.
Odontologisk Revy 23: 243-252.
- Pader, M.: *Oral Hygiene Products and Practice*: New York, Marcel Dekker Inc., 1st Ed., 1987: (pp. 313-381).
- Page, R.C. & Schroeder, H.E.: *Periodontitis in man and other animals; A comparative Review*: Basel, New York, Karger, 1982.
- Page, R.C., Sims, T.J., Geissler, F., Altman, L.C. & Baab, D.A., (1984): Abnormal leukocyte motility in patients with early-onset periodontitis.
Journal of Periodontal Research 19: 591-594.
- Palomo, F., Wantland, L., Sanchez, A., De Vizio, W., Carter, W. & Baines, E. (1989): The effect of a dentifrice containing triclosan and a copolymer on plaque and gingivitis formation.
American Journal of Dentistry 2: Special Issue: 231-237.
- Pitcher, G.R., Newman, H.N. & Strahan, J.D., (1980): Access to subgingival plaque by a disclosing agent using mouthrinsing and direct irrigation.
Journal of Clinical Periodontology 7: 300-308.

Preus, H.R., Olsen, I. & Gjermo, P., (1987): Bacteriophage infection
- A possible mechanism for increased virulence of bacteria
associated with rapidly destructive periodontitis.

Acta Odontologica Scandinavica 45: 49-54.

Ramfjord, S.P., Morrison, E.C., Burgett, F.G., Nissle, R.R., Schick, R.A.,
Zann, G.J. and Knowles, J.W., (1982): Oral hygiene and maintenance
of periodontal support.

Journal of Periodontology 53: 26-30.

Rees, T.D. & Orth., C.F., (1986): Oral ulcerations with use of
hydrogen peroxide.

Journal of Periodontology 57: 689-692.

Riffle A., (1956): Radical subgingival curettage.

Journal of Periodontology 27: 102-118.

Roberts, W.R. & Addy, M., (1987) or (1981): Comparison of the
in vivo and *in vitro* antibacterial properties of anti-
septic mouthrinses containing chlorhexidine, alexidine,
cetylpyridinium chloride and hexetidine. Relevance to
mode of action.

Journal of Clinical Periodontology 8: 295-310.

Rogers, A.H., Zilm, P.S., Gully, N.J. & Pfennig, A.L., (1987):
Chlorhexidine affects arginine metabolism as well as
glycolysis in a strain of *Streptococcus Sanguis*.

Oral Microbiology and Immunology 2: 178-182.

Rölla, G., Löe, H. and Schiott, C.R., (1970): The affinity of
chlorhexidine for hydroxyapatite and salivary mucins.

Journal of Periodontal Research 5: 90-95.

Rölla, G., Löe, H., and Schiott, C., (1976): Retention of chlorhexidine in the human oral cavity.

Archives of Oral Biology 16:1109-1116.

Rosling, B.G., Slots, J., Christersson, L.A., Gröndahl, H.G., & Genco, R.J., (1986): Topical and antimicrobial therapy and diagnosis of subgingival bacteria in the management of inflammatory periodontal disease.

Journal of Clinical Periodontology 13: 975-981.

Rovin, S., Costich, E.R. & Gordon, H.A., (1966): The influence of bacteria and irritation in the initiation of periodontal disease in germfree and conventional rats.

Journal of Periodontal Research 1: 193-203.

Russell, A.L., (1956): A System of classification and scoring for prevalence surveys of periodontal disease.

Journal of Dental Research 35: 350-359.

Russell, A.L., (1957)a: A social factor associated with the severity of periodontal disease.

Journal of Dental Research 36: 922-926.

Russell, A.L., (1957)b: Some epidemiological characteristics of periodontal disease in a series of urban populations.

Journal of Periodontology 28: 286-293.

Russell, A.L., (1963): International nutrition surveys: A summary of preliminary dental findings.

Journal of Dental Research 42: 232-244.

Saglie, R., Newman, M.G., Carranza, Jr., F.A. & Pattison, G.L., (1982):

Bacterial invasion of gingiva in advanced periodontitis in humans.

Journal of Periodontology 53: 217-222.

Saxe, S.R., Greene, J.C., Bohannon, H.M., Vermillion, J.R., (1967):

Oral debris, calculus, and periodontal disease in the beagle dog.

Journal of Periodontology 5: 5:217-225

Saxton, C.A. Svatun, B. & Lloyd, A.M., (1988): Antiplaque effects and mode of action of a combination of zinc citrate and a nonionic antimicrobial agent.

Scandinavian Journal of Dental Research 96: 212-217.

Schiott, C.R., Löe, H., Jensen, M., Kilian, M., Davies, R.M. &

Glavind, K., (1970): The effect of chlorhexidine mouth-rinses on the human oral flora.

Journal of Periodontal Research 5: 84-89.

Schiott, C.R. (1973): Effect of chlorhexidine on the microflora of the oral cavity.

Journal of Periodontal Research 8: Suppl. 12: 7-10.

Selvig, K.A., (1966): Ultrastructural changes in cementum and adjacent connective tissue in periodontal disease.

Acta Odontologica Scandinavica 24: 459-500.

Selvig, K.A., & Hals, E., (1977): Periodontally diseased cementum studies by correlated microradiography, electron probe analysis and electron microscopy.

Journal of Periodontal Research 12: 419-429.

Silness, J. & Loe, H., (1964): Periodontal disease in pregnancy.

II. Correlation between oral hygiene and periodontal condition.

Acta Odontologica Scandinavica 24: 747-759.

Slots, J. (1977)a: Microflora in the healthy gingival sulcus in man.

Scandinavian Journal of Dental Research 85: 247-254.

Slots, J., (1979): Subgingival microflora and periodontal disease.

Journal of Clinical Periodontology 6: 351-382.

Slots, J., (1977)b: The predominant cultivable microflora of advanced periodontitis.

Scandinavian Journal of Dental Research 85: 114-121.

Slots, J., Rams, T.E. and Listgarten, M.A., (1988): Yeasts, enteric rods and pseudomonads in the subgingival flora of severe adult periodontitis.

Oral Microbiology and Immunology 3: 47-52.

Socransky, S.E., Manganiello, A.D., Propas, D., Oram, V. and van Houte, J., (1977): Bacteriological studies of developing supragingival dental plaque.

Journal of Periodontal Research 12: 90-106.

Socransky, S.S., Tanner, A.C.R., Haffajee, A.D., Hillman, J.D. & Goodson, J.M. : Present status of studies on the microbial etiology of periodontal diseases. (In *Host-parasite interactions in periodontal diseases*. Genco, R.J. & Mergenhagen, S.E. ed. American Society for Microbiology, Washington, DC., 1982. pp. 1-12).

Socransky, S.S., (1977): Microbiology of periodontal disease - present status and future considerations.

Journal of Periodontology 48: 497-502.

Socransky, S.S., Haffajee, A.D., Goodson, J.M. & Lindhe, J., (1984): New concepts of destructive periodontal disease.

Journal of Clinical Periodontology 11: 21-32.

Soh, L.L., Newman, H.N. & Strahan, J.D., (1982): Effects of subgingival chlorhexidine irrigation on periodontal inflammation.

Journal of Clinical Periodontology 9: 66-74.

Southard, S.R., Drisko, C.L., Killoy, W.J., Cobb, C.M. & Tira, D.E., (1989): The effect of 2% chlorhexidine digluconate irrigation on clinical parameters and the level of bacteroides gingivalis in periodontal pockets.

Journal of Periodontology 60: 302-309.

Stanier, R.Y., Adelberg, E.A., Ingraham, J.L. *General Microbiology*, 4th Ed., The MacMillan Press Ltd., London. 1977. pp.2-4.

Suomi, J.D., Leatherwood, E.C. and Chang, J.J., (1973): A follow-up study of former participants in a controlled oral hygiene study.

Journal of Periodontology 44: 662-666.

Suomi, J.D., Greene, J.C. Vermillion, J.R., Chang, J.J. and Leatherwood, E.C. (1969): The effect of controlled oral hygiene procedures on the progression of periodontal disease in adults.

Journal of Periodontology 40: 416-420.

- Suomi, J.D., Greene, J.C. Vermillion, J.R., Doyle, J., Chang, J.J. & Leatherwood, E.C., (1971): The effect of controlled oral hygiene procedures on the progression of periodontal disease in adults: Results after third and final year.
Journal of Periodontology 42: 152-160.
- Sweeney, E.A., Alcoforado, G.A.P., Nyman, S. & Slots, J., (1987): Prevalence and microbiology of localized prepubertal periodontitis.
Oral Microbiology and Immunology 2: 65-70.
- Tanner, A.C.R., & Goodson, J.M. (1986): Sampling of microorganisms associated with periodontal disease.
Oral Microbiology and Immunology 1: 15-20.
- Theilade, E., (1986): The non-specific theory in microbial etiology of inflammatory periodontal disease.
Journal of Clinical Periodontology 13: 905-911.
- Theilade, E., Wright, W.H., Jensen, S.B. & Löe, H., (1966): Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation.
Journal of Periodontal Research 1. 1-13.
- Tonelli, P.M., Hume, W.R., Kenney, E.B., (1983): Chlorhexidine: A review of the literature.
Periodontal Abstracts 31: 5-10.
- Van Dyke, T.E. Levine, M.J. and Genco., R.J., (1985): Neutrophil function and oral disease.
Journal of Oral Pathology 14: 95-120.

Van Palenstein Helderman, W.H., (1981): Microbial etiology of periodontal disease.

Journal of Clinical Periodontology 8: 261-280.

Vogel, R.J., Sullivan, A.J., Pascuzzi, J.N. & Deasy, M.J., (1975): Evaluation of cleansing devices in the maintenance of interproximal gingival health.

Journal of Periodontology 46: 745-747.

Waerhaug, J., (1952): The gingival pocket. Anatomy, deepening and elimination.

Odontologisk Tidskrift 60: Supplement 1.

Waerhaug, M., Gjermo, P., Rolla, G. & Johansen, J.R., (1984): Comparison of the effect of chlorhexidine and CUS04 on plaque formation and development of gingivitis.

Journal of Clinical Periodontology 11: 176-180.

Watts, E.A. & Newman, H.N. (1986): Clinical effects on chronic periodontitis of a simplified system of oral hygiene including subgingival pulsated jet irrigation with chlorhexidine.

Journal of Clinical Periodontology 13: 666-670.

Wennstrom, J. & Lindhe, J., (1985): The effect of mouthrinses on parameters characterizing human periodontal disease.

Journal of Clinical Periodontology 13: 86-93.

Westfelt, E., Nyman, S., Lindhe, J. & Socransky, S.S., (1983): Use of chlorhexidine as a plaque control measure following surgical treatment of periodontal disease.

Journal of Clinical Periodontology 10: 22-36.

Wolffe, G.N., (1976): An evaluation of proximal surface cleansing agents.

Journal of Clinical Periodontology 3: 148-156.

World Health Organization: *Epidemiology, etiology and prevention of periodontal diseases.*

Technical Report Series: No. 621. (1978).

Appendix I. Probing Pocket Depths

Probing pocket depth in COS0 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	5	5	5	4	4
2	6	5	6	5	5
3	8	6	6	7	6
4	6	5	5	5	5
5	6	6	5	5	
6	6	6	8	7	7
7	6	6	6	6	6
8	8	6	9	7	
9	6	5	4	4	4
10	7	5	5	5	5
11	6	7	7	6	5
12	7	6	5	6	6
13	6	5	4	4	4
Total	83	73	75	71	57
Mean	6.38	5.62	5.77	5.46	5.18
S.D.	0.84	0.62	1.42	1.08	0.94

Probing pocket depths (mm) in COS1 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	6	6	6	6	7
2	7	7	7	7	7
3	6	5	5	5	4
4	7	6	5	6	5
5	7	6	6	5	
6	7	5	6	7	5
7	7	8	7	8	6
8	8	9	10	8	
9	5	4	3	4	3
10	6	5	5	5	5
11	6	5	5	5	5
12	6	5	6	5	5
13	5	6	5	5	5
Total	83	77	76	76	57
Mean	6.38	5.92	5.85	5.85	5.18
S.D.	0.84	1.33	1.56	1.23	1.11

Appendix I. Probing Pocket Depths

Probing pocket depths (mm) in C1S0 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	6	6	5	4	5
2	6	7	6	6	4
3	6	5	5	5	5
4	6	4	4	4	4
5	7	7	5	6	
6	7	6	7	7	6
7	6	6	6	5	6
8	7	6	5	6	
9	5	5	4	4	4
10	6	5	5	5	5
11	6	6	8	8	7
12	6	5	5	6	5
13	6	5	4	4	4
Total	80	73	69	70	55
Mean	6.15	5.62	5.31	5.38	5.00
S.D.	0.53	0.84	1.14	1.21	0.95

Probing pocket depths (mm) in C1S1 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	6	5	4	4	3
2	6	5	6	6	5
3	6	5	4	6	4
4	6	4	4	5	4
5	6	7	6	8	
6	7	6	6	6	7
7	7	6	6	5	5
8	9	9	10	8	
9	5	4	3	4	3
10	6	5	5	5	5
11	7	6	6	6	6
12	8	7	7	7	7
13	6	6	6	5	6
Total	85	75	73	75	55
Mean	6.54	5.77	5.62	5.77	5.00
S.D.	1.01	1.31	1.69	1.25	1.35

Appendix II. Attachment Levels

Attachment levels: attachment levels (in mm) in COS0 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	9	7	7	8	7
2	6	6	6	6	6
3	7	6	6	7	6
4	6	5	5	5	5
5	8	8	8	8	
6	6	7	8	7	7
7	6	6	6	6	6
8	9	7	10	8	
9	7	6	5	5	5
10	7	6	5	5	6
11	8	7	7	6	6
12	8	8	7	8	8
13	8	7	7	7	7
Total	95	86	87	86	69
Mean	7.31	6.62	6.69	6.62	6.27
S.D.	1.07	0.84	1.38	1.15	0.86

Attachment levels: attachment levels (in mm) in COS1 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	8	8	8	10	9
2	7	7	7	8	7
3	6	6	5	5	4
4	7	6	5	6	5
5	10	10	11	10	
6	7	9	6	7	6
7	7	8	7	8	6
8	10	10	11	8	
9	5	5	3	4	4
10	7	7	7	7	7
11	6	5	5	5	5
12	7	7	7	7	7
13	7	7	7	7	7
Total	94	95	89	92	67
Mean	7.23	7.31	6.85	7.08	6.09
S.D.	1.37	1.59	2.18	1.73	1.44

Appendix II. Attachment Levels

Attachment levels: attachment levels (in mm) in C1S0 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	6	6	6	5	6
2	6	7	6	6	4
3	6	6	6	5	5
4	6	6	5	4	4
5	10	8	7	9	
6	10	6	7	7	7
7	6	6	6	6	6
8	8	7	6	7	
9	6	6	5	5	4
10	6	6	5	6	6
11	8	8	8	8	7
12	8	7	7	8	7
13	8	7	7	7	6
Total	94	86	81	83	62
Mean	7.23	6.61	6.23	6.38	5.64
S.D.	1.48	0.74	0.89	1.39	1.15

Attachment levels: attachment levels (in mm) in C1S1 treatment group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	8	7	7	7	6
2	6	5	6	6	5
3	6	6	5	6	5
4	6	4	4	5	4
5	8	8	8	10	
6	10	9	7	7	7
7	7	6	6	6	5
8	9	10	10	8	
9	6	5	3	4	4
10	6	6	6	6	6
11	8	6	6	6	6
12	10	9	8	9	9
13	7	7	7	6	7
Total	97	88	83	86	64
Mean	7.46	6.77	6.38	6.62	5.82
S.D.	1.45	1.72	1.73	1.55	1.40

Appendix III. Total Bacterial Numbers

Total number of bacteria counted in COS0 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	150	72	262	56	72
2	152	123	107	102	122
3	718	419	431	280	164
4	112	125	168	59	146
5	44	118	135	47	
6	227	151	194		
7	188	158	368	106	
8	152	505	232	140	
9	244	120	168	195	
10	162	114	86	167	122
11	116	91	406	264	240
12	60	140	126	390	81
13	288	267	171		
Total	2613	2403	2854	1806	947
n	13	13	13	11	7
Mean	201.00	184.85	219.54	164.18	135.29
S.D.	163.18	127.28	110.31	109.69	52.39

Total number of bacteria counted in COS1 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	132	127	104	203	119
2	146	105	95	140	239
3	757	209	262	108	54
4	127	124	127	69	115
5	59	63	242	75	
6	300	125	193		
7	112	83	60	80	
8	261	153	105	116	
9	119	67	144	75	
10	222	223	127	134	315
11	117	78	58	61	226
12	81	64	82	57	87
13	193	154	256		
Total	2626	1575	1855	1118	1155
n	13	13	13	11	7
Mean	202.00	121.15	142.69	101.64	165.00
S.D.	173.77	50.69	69.53	42.25	88.41

Appendix III. Total Bacterial Numbers

Total number of bacteria counted in C1S0 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	177	119	227	33	44
2	151	188	96	87	132
3	686	299	111	485	205
4	130	100	94	86	73
5	73	96	91	30	
6	247	107	380		
7	201	80	150	66	
8	149	167	102	131	
9	190	108	157	152	
10	103	113	152	79	110
11	258	298	282	406	140
12	136	98	224	59	93
13	310	261	164		
Total	2811	2034	2230	1614	797
n	13	13	13	11	7
Mean	216.23	156.46	171.54	146.73	113.86
S.D.	154.46	76.75	82.88	146.02	48.28

Total number of bacteria counted in C1S1 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	119	104	326	49	55
2	261	83	229	78	81
3	563	100	107	117	75
4	114	78	178	94	92
5	110	81	66	65	
6	287	54	173		
7	112	100	167	84	
8	224	289	98	122	
9	200	75	144	154	
10	205	115	147	83	77
11	102	69	120	74	53
12	99	68	142	85	201
13	265	237	161		
Total	2661	1453	2058	1005	634
n	13	13	13	11	7
Mean	204.69	111.77	158.31	91.36	90.57
S.D.	123.16	67.25	62.43	28.06	46.89

Appendix III. Total Bacterial Numbers

Mean number of bacteria counted per H.P.F. in COS0 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	15.00	7.20	26.20	5.60	7.20
2	7.60	6.15	5.35	5.10	6.10
3	35.90	20.95	21.55	14.00	8.20
4	5.60	6.25	8.40	2.95	7.30
5	4.40	5.90	13.50	4.70	
6	11.35	7.55	9.70		
7	9.40	7.90	18.40	5.30	
8	7.60	25.25	11.60	7.00	
9	12.20	6.00	8.40	9.75	
10	16.20	5.70	8.60	16.70	12.20
11	5.80	4.55	20.30	13.20	12.00
12	3.00	7.00	6.30	19.50	4.05
13	14.40	13.35	8.55		
Total	148.45	123.75	166.85	103.80	57.05
n	13	13	13	11	7
Mean	11.42	9.52	12.83	9.44	8.15
S.D.	8.15	6.19	6.37	5.31	2.77

Mean number of bacteria counted per H.P.F. in COS1 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	13.20	12.70	10.40	20.30	11.90
2	7.30	5.25	4.75	7.00	11.95
3	37.85	10.45	13.10	5.40	2.70
4	6.35	6.20	6.35	3.45	5.75
5	5.90	3.15	24.20	7.50	
6	15.00	6.25	9.65		
7	5.60	4.15	3.00	4.00	
8	13.05	7.65	5.25	5.80	
9	5.95	3.35	7.20	3.75	
10	22.20	11.15	12.70	13.40	31.50
11	5.85	3.90	2.90	3.05	11.30
12	4.05	3.20	4.10	2.85	4.35
13	9.65	7.70	12.80		
Total	151.95	85.10	116.40	76.50	79.45
n	13	13	13	11	7
Mean	11.69	6.55	8.95	6.95	11.35
S.D.	9.02	3.09	5.67	5.11	8.96

Appendix III. Total Bacterial Numbers

Mean number of bacteria counted per H.P.F. in C1S0 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	17.70	11.90	22.70	3.30	4.40
2	7.55	9.40	4.80	4.35	6.60
3	34.30	14.95	5.55	24.25	10.25
4	6.50	5.00	4.70	4.30	3.65
5	7.30	4.80	9.10	3.00	
6	12.35	5.35	19.00		
7	10.05	4.00	7.50	3.30	
8	7.45	8.35	5.10	6.55	
9	9.50	5.40	7.85	7.60	
10	10.30	5.65	15.20	7.90	11.00
11	12.90	14.90	14.10	20.30	7.00
12	6.80	4.90	11.20	2.95	4.65
13	15.50	13.05	8.20		
Total	158.20	107.65	135.00	87.80	47.55
n	13	13	13	11	7
Mean	12.17	8.28	10.38	7.98	6.79
S.D.	7.21	3.94	5.54	7.00	2.67

Total number of bacteria counted per H.P.F. in C1S1 treatment group at weeks 0,1,4,8 and 12.

Subject	Week 0	Week 1	Week 4	Week 8	Week 12
1	11.90	10.40	32.60	4.90	5.50
2	13.05	4.15	11.45	3.90	4.05
3	28.15	5.00	5.35	5.85	3.75
4	5.70	3.90	8.90	4.70	4.60
5	11.00	4.05	6.60	6.50	
6	14.35	2.70	8.65		
7	5.60	5.00	8.35	4.20	
8	11.20	14.45	4.90	6.10	
9	10.00	3.75	7.20	7.70	
10	20.50	5.75	14.70	8.30	7.70
11	5.10	3.45	6.00	3.70	2.65
12	4.95	3.40	7.10	4.25	10.05
13	13.25	11.85	8.05		
Total	154.75	77.85	129.85	60.10	38.30
n	13	13	13	11	7
Mean	11.90	5.99	9.99	5.46	5.47
S.D.	6.35	3.59	6.99	1.48	2.38

Appendix IV. Coccid Forms

Coccid forms: number in samples from COS0 Tx group
at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	54	45	50	29	24
2	47	23	42	31	34
3	135	83	67	54	76
4	42	36	51	13	21
5	27	60	41	15	
6	101	98	88		
7	83	42	136	41	
8	58	217	117	70	
9	104	102	109	89	
10	44	57	17	33	47
11	66	55	66	62	58
12	38	46	37	96	38
13	98	66	102		
Total	897	930	923	533	298
n	13	13	13	11	7
Mean	69.00	71.54	71.00	48.45	42.57
S.D.	31.08	47.57	34.79	26.86	18.05

Coccid forms: number in samples from COS1 Tx group
at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	58	42	31	40	22
2	43	31	39	43	69
3	135	82	80	36	29
4	34	60	33	30	26
5	36	52	56	16	
6	107	95	77		
7	39	45	40	33	
8	185	104	101	50	
9	95	52	105	66	
10	65	63	33	55	63
11	90	65	45	53	65
12	39	53	54	41	42
13	92	92	163		
Total	1018	836	857	463	316
n	13	13	13	11	7
Mean	78.31	64.31	65.92	42.09	45.14
S.D.	43.59	21.53	37.17	13.05	18.73

Appendix IV. Coccid Forms

Coccid forms: number in samples from C1S0 Tx group
at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	70	35	165	27	37
2	37	41	49	17	35
3	164	119	36	89	74
4	30	44	46	37	38
5	43	57	48	20	
6	109	93	126		
7	65	40	111	41	
8	53	136	98	63	
9	84	81	112	64	
10	30	56	38	30	25
11	83	75	60	46	33
12	33	33	52	34	36
13	77	78	105		
Total	878	888	1046	468	278
n	13	13	13	11	7
Mean	67.54	68.31	80.46	42.55	39.71
S.D.	36.58	31.42	39.51	20.70	14.56

Coccid forms: mean number in samples from C1S1 Tx group
at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	60	94	39	35	28
2	43	28	32	27	33
3	97	72	54	55	48
4	35	33	45	48	33
5	41	64	31	18	
6	119	42	121		
7	39	41	89	35	
8	136	207	94	70	
9	108	53	105	118	
10	45	44	38	24	27
11	66	54	91	58	38
12	52	59	55	44	26
13	67	64	63		
Total	908	855	857	532	233
n	13	13	13	11	7
Mean	69.85	65.77	65.92	48.36	33.29
S.D.	32.53	44.11	29.21	26.63	7.17

Appendix IV. Coccid Forms

Coccid forms: mean number per H.P.F. in COS0 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	5.40	4.50	5.00	2.90	2.40
2	2.35	1.15	2.10	1.55	1.70
3	6.75	4.15	3.35	2.70	3.80
4	2.10	1.80	2.55	0.65	1.05
5	2.70	3.00	4.10	1.50	
6	5.05	4.90	4.40		
7	4.15	2.10	6.80	2.05	
8	2.90	10.85	5.85	3.50	
9	5.20	5.10	5.45	4.45	
10	4.40	2.85	1.70	3.30	4.70
11	3.30	2.75	3.30	3.10	2.90
12	1.90	2.30	1.85	4.80	1.90
13	4.90	3.30	5.10		
Total	51.10	48.75	51.55	30.50	18.45
n	13	13	13	11	7
Mean	3.93	3.75	3.97	2.77	2.64
S.D.	1.44	2.36	1.58	1.21	1.18

Coccid forms: mean number per H.P.F. in COS1 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	5.80	4.20	3.10	4.00	2.20
2	2.15	1.55	1.85	2.15	3.45
3	6.75	4.10	4.00	1.80	1.45
4	1.70	3.00	1.65	1.50	1.30
5	3.60	2.60	5.60	1.60	
6	5.35	4.75	3.85		
7	1.95	2.25	2.00	1.65	
8	9.25	5.20	5.05	2.50	
9	4.75	2.60	5.25	3.30	
10	6.50	3.15	3.30	5.50	6.30
11	4.50	3.25	2.25	2.65	3.25
12	1.95	2.65	2.70	2.05	2.10
13	4.60	4.60	8.15		
Total	58.85	43.90	48.75	28.70	20.05
n	13	13	13	11	7
Mean	4.53	3.38	3.75	2.61	2.86
S.D.	2.17	1.05	1.79	1.17	1.59

Appendix IV. Coccid Forms

Coccid forms: mean number per H.P.F. in C1S0 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	7.00	3.50	16.50	2.70	3.70
2	1.85	2.05	2.45	0.85	1.75
3	8.20	5.95	1.80	4.45	3.70
4	1.50	2.20	2.30	1.85	1.90
5	4.30	2.85	4.80	2.00	
6	5.45	4.65	6.30		
7	3.25	2.00	5.55	2.05	
8	2.65	6.80	4.90	3.15	
9	4.20	4.05	5.60	3.20	
10	3.00	2.80	3.80	3.00	2.50
11	4.15	3.75	3.00	2.30	1.65
12	1.65	1.65	2.60	1.70	1.80
13	3.85	3.90	5.25		
Total	51.05	46.15	64.85	27.25	17.00
n	13	13	13	11	7
Mean	3.93	3.55	4.99	2.48	2.43
S.D.	1.93	1.50	3.62	0.92	0.84

Coccid forms: mean number per H.P.F. in C1S1 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	6.00	9.40	3.90	3.50	2.80
2	2.15	1.40	1.60	1.35	1.65
3	4.85	3.60	2.70	2.75	2.40
4	1.75	1.65	2.25	2.40	1.65
5	4.10	3.20	3.10	1.80	
6	5.95	2.20	6.05		
7	1.95	2.05	4.45	1.75	
8	6.80	10.35	4.70	3.50	
9	5.40	2.65	5.25	5.90	
10	4.50	2.20	3.80	2.40	2.70
11	3.30	2.70	4.55	2.90	1.90
12	2.60	2.95	2.75	2.20	1.30
13	3.35	3.20	3.15		
Total	52.70	47.55	48.25	30.45	14.40
n	13	13	13	11	7
Mean	4.05	3.66	3.71	2.77	2.06
S.D.	1.62	2.73	1.22	1.19	0.54

Appendix V. Motile Forms

Motiles: numbers of motiles in COS0 treatment group samples at weeks 0,1,4,8 & 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	31	7	80	14	15
2	28	33	22	34	20
3	149	122	85	90	44
4	13	18	21	11	44
5	1	26	32	13	
6	17	25	16		
7	30	34	111	21	
8	30	54	21	7	
9	47	0	6	24	
10	53	15	35	70	28
11	15	9	149	100	56
12	4	29	30	135	9
13	12	13	18		
Total	430	385	626	519	216
n	13	13	13	11	7
Mean	33.08	29.62	48.15	47.18	30.86
S.D.	36.57	29.91	42.18	42.05	16.18

Motiles: numbers of motiles in COS1 treatment group samples at weeks 0,1,4,8 & 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	22	33	28	57	30
2	36	17	2	34	44
3	228	64	37	20	9
4	25	5	22	6	38
5	5	7	66	23	
6	50	7	27		
7	25	7	5	9	
8	6	5	2	0	
9	4	0	10	0	
10	74	43	56	28	87
11	5	0	4	1	57
12	21	1	13	1	6
13	32	3	0		
Total	533	192	272	179	271
n	13	13	13	11	7
Mean	41.00	14.77	20.92	16.27	38.71
S.D.	57.31	19.00	20.54	17.38	25.98

Appendix V. Motile Forms

Motiles: numbers of motiles in C1S0 treatment group samples at weeks 0,1,4,8 & 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	46	41	22	1	0
2	38	54	17	28	37
3	179	44	23	138	60
4	30	7	17	22	9
5	7	10	15	5	
6	17	7	39		
7	53	11	15	5	
8	31	4	0	4	
9	22	5	15	11	
10	28	25	58	27	39
11	55	80	88	82	44
12	41	10	78	6	15
13	31	22	2		
Total	578	320	389	329	204
n	13	13	13	11	7
Mean	44.46	24.62	29.92	29.91	29.14
S.D.	42.66	22.57	26.81	40.62	19.95

Motiles: numbers of motiles in C1S1 treatment group samples at weeks 0,1,4,8 & 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	9	8	76	8	13
2	76	21	65	19	10
3	149	5	13	7	2
4	23	6	22	3	7
5	33	7	10	18	
6	13	0	9		
7	25	25	24	15	
8	19	14	0	0	
9	25	4	10	0	
10	63	30	56	21	12
11	3	7	3	0	3
12	17	1	33	9	64
13	16	45	7		
Total	471	173	328	100	111
n	13	13	13	11	7
Mean	36.23	13.31	25.23	9.09	15.86
S.D.	38.14	12.78	24.05	7.66	20.04

Appendix V. Motile Forms

Motiles: mean numbers of motiles per H.P.F. in COS0 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	3.10	0.70	8.00	1.40	1.50
2	1.40	1.65	1.10	1.70	1.00
3	7.45	6.10	4.25	4.50	2.20
4	0.65	0.90	1.05	0.55	2.20
5	0.10	1.30	3.20	1.30	
6	0.85	1.25	0.80		
7	1.50	1.70	5.55	1.05	
8	1.50	2.70	1.05	0.35	
9	2.35	0.00	0.30	1.20	
10	5.30	0.75	3.50	7.00	2.80
11	0.75	0.45	7.45	5.00	2.80
12	0.20	1.45	1.50	6.75	0.45
13	0.60	0.65	0.90		
Total	25.75	19.60	38.65	30.80	12.95
n	13	13	13	11	7
Mean	1.98	1.51	2.97	2.80	1.85
S.D.	2.08	1.48	2.53	2.39	0.83

Motiles: mean numbers of motiles per H.P.F. in COS1 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	2.20	3.30	2.80	5.70	3.00
2	1.80	0.85	0.10	1.70	2.20
3	11.40	3.20	1.85	1.00	0.45
4	1.25	0.25	1.10	0.30	1.95
5	0.50	0.35	6.60	2.30	
6	2.50	0.35	1.35		
7	1.25	0.35	0.25	0.45	
8	0.30	0.25	0.10	0.00	
9	0.20	0.00	0.50	0.00	
10	7.40	2.15	5.60	2.80	8.70
11	0.25	0.00	0.20	0.05	2.85
12	1.05	0.05	0.65	0.05	0.30
13	1.60	0.15	0.00		
Total	31.70	11.25	21.10	14.35	19.45
n	13	13	13	11	7
Mean	2.44	0.87	1.62	1.30	2.78
S.D.	3.15	1.15	2.07	2.07	2.61

Appendix V. Motile Forms

Motiles: mean numbers of motiles per H.P.F. in C1S0 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	4.60	4.10	2.20	0.10	0.00
2	1.90	2.70	0.85	1.40	1.85
3	8.95	2.20	1.15	6.90	3.00
4	1.50	0.35	0.85	1.10	0.45
5	0.70	0.50	1.50	0.50	
6	0.85	0.35	1.95		
7	2.65	0.55	0.75	0.25	
8	1.55	0.20	0.00	0.20	
9	1.10	0.25	0.75	0.55	
10	2.80	1.25	5.80	2.70	3.90
11	2.75	4.00	4.40	4.10	2.20
12	2.05	0.50	3.90	0.30	0.75
13	1.55	1.10	0.10		
Total	32.95	18.05	24.20	18.10	12.15
n	13	13	13	11	7
Mean	2.53	1.39	1.86	1.65	1.74
S.D.	2.10	1.35	1.71	2.04	1.32

Motiles: mean numbers of motiles per H.P.F. in C1S1 Tx group at weeks 0,1,4,8 and 12.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	0.90	0.80	7.60	0.80	1.30
2	3.80	1.05	3.25	0.95	0.50
3	7.45	0.25	0.65	0.35	0.10
4	1.15	0.30	1.10	0.15	0.35
5	3.30	0.35	1.00	1.80	
6	0.65	0.00	0.45		
7	1.25	1.25	1.20	0.75	
8	0.95	0.70	0.00	0.00	
9	1.25	0.20	0.50	0.00	
10	6.30	1.50	5.60	2.10	1.20
11	0.15	0.35	0.15	0.00	0.15
12	0.85	0.05	1.65	0.45	3.20
13	0.80	2.25	0.35		
Total	28.80	9.05	23.50	7.35	6.80
n	13	13	13	11	7
Mean	2.22	0.70	1.81	0.67	0.97
S.D.	2.23	0.64	2.23	0.69	1.01

Appendix VI. Spirochaetes

Spirochaetes: numbers of spirochaetes in COS0 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	24	4	54	1	10
2	22	33	8	2	42
3	237	131	182	75	7
4	11	16	9	9	29
5	2	2	7	7	
6	80	4	45		
7	7	13	4	5	
8	38	122	51	39	
9	54	9	23	49	
10	23	20	15	22	15
11	10	11	118	55	64
12	6	29	22	64	0
13	106	103	31		
Total	620	497	569	328	167
n	13	13	13	11	7
Mean	47.69	38.23	43.77	29.82	23.86
S.D.	62.32	45.27	49.69	26.17	21.01

Spirochaetes: numbers of spirochaetes in COS1 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	18	35	12	77	50
2	21	5	8	11	21
3	254	9	65	13	3
4	28	8	28	7	14
5	10	2	66	16	
6	114	7	38		
7	8	10	0	5	
8	34	12	2	39	
9	5	5	13	0	
10	32	51	14	9	69
11	3	1	1	1	11
12	4	1	4	0	14
13	27	15	38		
Total	558	161	289	178	182
n	13	13	13	11	7
Mean	42.92	12.38	22.23	16.18	26.00
S.D.	66.93	14.01	22.23	21.89	22.34

Appendix VI. Spirochaetes

Spirochaetes: numbers of spirochaetes in C1S0 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	29	13	18	0	4
2	26	40	4	5	7
3	199	92	12	159	16
4	18	0	3	0	0
5	5	11	3	1	
6	104	3	126		
7	12	5	4	3	
8	34	5	0	26	
9	56	14	6	34	
10	15	10	18	2	10
11	58	90	81	199	20
12	36	28	49	10	21
13	133	98	19		
Total	725	409	343	439	78
n	13	13	13	11	7
Mean	55.77	31.46	26.38	39.91	11.14
S.D.	54.67	35.44	36.19	66.97	7.49

Spirochaetes: numbers of spirochaetes in C1S1 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	30	0	123	1	3
2	78	12	45	6	7
3	217	6	22	25	4
4	19	0	21	5	5
5	19	1	2	5	
6	133	0	8		
7	8	2	5	3	
8	36	11	0	35	
9	36	3	13	3	
10	40	5	20	8	7
11	10	0	9	2	1
12	13	2	28	10	56
13	84	86	56		
Total	723	128	352	103	83
n	13	13	13	11	7
Mean	55.62	9.85	27.08	9.36	11.86
S.D.	58.08	22.33	31.88	10.26	18.13

Appendix VI. Spirochaetes

Spirochaetes: mean numbers of spirochaetes per H.P.F. in C0S0 Tx group at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	2.40	0.40	5.40	0.10	1.00
2	1.10	1.65	0.40	0.10	2.10
3	11.85	6.55	9.10	3.75	0.35
4	0.55	0.80	0.45	0.45	1.45
5	0.20	0.10	0.70	0.70	
6	4.00	0.20	2.25		
7	0.35	0.65	0.20	0.25	
8	1.90	6.10	2.55	1.95	
9	2.70	0.45	1.15	2.45	
10	2.30	1.00	1.50	2.20	1.50
11	0.50	0.55	5.90	2.75	3.20
12	0.30	1.45	1.10	3.20	0.00
13	5.30	5.15	1.55		
Total	33.45	25.05	32.25	17.90	9.60
n	13	13	13	11	7
Mean	2.57	1.93	2.48	1.63	1.37
S.D.	3.07	2.25	2.58	1.31	1.00

Spirochaetes: mean number of spirochaetes per H.P.F. in C0S1 Tx group at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	1.80	3.50	1.20	7.70	5.00
2	1.05	0.25	0.40	0.55	1.05
3	12.70	0.45	3.25	0.65	0.15
4	1.40	0.40	1.40	0.35	0.70
5	1.00	0.10	6.60	1.60	
6	5.70	0.35	1.90		
7	0.40	0.50	0.00	0.25	
8	1.70	0.60	0.10	1.95	
9	0.25	0.25	0.65	0.00	
10	3.20	2.55	1.40	0.90	6.90
11	0.15	0.05	0.05	0.05	0.55
12	0.20	0.05	0.20	0.00	0.70
13	1.35	0.75	1.90		
Total	30.90	9.80	19.05	14.00	15.05
n	13	13	13	11	7
Mean	2.38	0.75	1.47	1.27	2.15
S.D.	3.32	1.01	1.74	2.12	2.47

Appendix VI. Spirochaetes

Spirochaetes: mean number of spirochaetes per H.P.F. in C1S0 Tx group at each examinationn.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	2.90	1.30	1.80	0.00	0.40
2	1.30	2.00	0.20	0.25	0.35
3	9.95	4.60	0.60	7.80	0.80
4	0.90	0.00	0.15	0.00	0.00
5	0.50	0.55	0.30	0.10	
6	5.20	0.15	6.30		
7	0.60	0.25	0.20	0.15	
8	1.70	0.25	0.00	1.30	
9	2.80	0.70	0.30	1.70	
10	1.50	0.50	1.80	0.20	1.00
11	2.90	4.50	4.05	9.95	1.00
12	1.80	1.40	2.45	0.50	1.05
13	6.65	4.90	0.95		
Total	38.70	21.10	19.10	21.95	4.60
n	13	13	13	11	7
Mean	2.98	1.62	1.47	2.00	0.66
S.D.	2.65	1.75	1.80	3.32	0.38

Spirochaetes: mean number of spirochaetes per H.P.F. in C1S1 Tx group at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	3.00	0.00	12.30	0.10	0.30
2	3.90	0.60	2.25	0.30	0.35
3	10.85	0.30	1.10	1.25	0.20
4	0.95	0.00	1.05	0.25	0.25
5	1.90	0.05	0.20	0.50	
6	6.65	0.00	0.40		
7	0.40	0.10	0.25	0.15	
8	1.80	0.55	0.00	1.75	
9	1.80	0.15	0.65	0.15	
10	4.00	0.25	2.00	0.80	0.70
11	0.50	0.00	0.45	0.10	0.05
12	0.65	0.10	1.40	0.50	2.80
13	4.20	4.30	2.80		
Total	40.60	6.40	24.85	5.85	4.65
n	13	13	13	11	7
Mean	3.12	0.49	1.91	0.53	0.66
S.D.	2.84	1.12	3.11	0.51	0.89

Appendix VII. Others

Others: numbers of bacteria that were not cocci, motiles or spirochaetes in COS0 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	41	16	78	12	23
2	55	34	35	35	26
3	197	83	97	61	37
4	46	55	87	26	52
5	14	30	55	12	
6	29	24	45		
7	68	69	117	39	
8	26	112	43	24	
9	39	9	30	33	
10	42	22	19	42	32
11	25	26	73	47	62
12	12	36	37	95	34
13	72	85	20		
Total	666	601	736	426	266
n	13	13	13	11	7
Mean	51.23	46.23	56.62	35.50	38.00
S.D.	45.65	30.49	29.83	24.10	13.06

Others: numbers of bacteria that were not cocci, motiles or spirochaetes in COS1 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	34	17	33	29	17
2	46	52	46	52	105
3	140	54	80	39	13
4	40	51	44	26	37
5	8	2	54	20	
6	29	16	51		
7	40	21	15	33	
8	36	32	0	27	
9	15	10	16	9	
10	51	66	24	42	96
11	19	12	8	6	93
12	17	9	11	15	25
13	42	44	55		
Total	517	386	437	298	386
n	13	13	13	11	7
Mean	39.77	29.69	33.62	27.09	55.14
S.D.	31.56	20.36	22.59	13.46	37.90

Appendix VII. Others

Others: numbers of bacteria that were not cocci, motiles or spirochaetes in C1S0 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	32	30	22	5	3
2	50	53	26	37	53
3	144	44	40	99	55
4	52	49	28	27	26
5	18	18	25	4	
6	17	4	89		
7	71	24	20	17	
8	31	22	4	38	
9	28	8	24	43	
10	30	22	38	20	36
11	62	53	53	79	43
12	26	27	45	9	21
13	69	63	38		
Total	630	417	452	378	237
n	13	13	13	11	7
Mean	48.46	32.08	34.77	34.36	33.86
S.D.	32.83	17.81	19.78	29.01	17.24

Others: numbers of bacteria that were not cocci, motiles or spirochaetes in C1S1 Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	20	2	88	5	11
2	64	22	87	26	31
3	100	17	18	30	21
4	37	39	90	38	47
5	17	9	23	24	
6	22	12	35		
7	40	32	49	31	
8	33	57	4	17	
9	31	15	16	33	
10	57	36	33	30	31
11	23	8	17	14	11
12	17	6	26	22	55
13	98	42	35		
Total	559	297	521	270	207
n	13	13	13	11	7
Mean	43.00	22.85	40.08	24.55	29.57
S.D.	27.63	16.16	28.50	9.11	15.66

Appendix VII. Others

Others: mean numbers of others per H.P.F. in COS0
Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	4.10	1.60	7.80	1.20	2.30
2	2.75	1.70	1.75	1.75	1.30
3	9.85	4.15	4.85	3.05	1.85
4	2.30	2.75	4.35	1.30	2.60
5	1.40	1.50	5.50	1.20	
6	1.45	1.20	2.25		
7	3.40	3.45	5.85	1.95	
8	1.30	5.60	2.15	1.20	
9	1.95	0.45	1.50	1.65	
10	4.20	1.10	1.90	4.20	3.20
11	1.25	1.30	3.65	2.35	3.10
12	0.60	1.80	1.85	4.75	1.70
13	3.60	4.25	1.00		
Total	38.15	30.85	44.40	24.60	16.05
n	13	13	13	11	7
Mean	2.93	2.37	3.42	2.24	2.29
S.D.	2.29	1.48	2.01	1.30	0.67

Others: mean numbers of others per H.P.F. in COS1
Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	3.40	1.70	3.30	2.90	1.70
2	2.30	2.60	2.30	2.60	5.25
3	7.00	2.70	4.00	1.95	0.65
4	2.00	2.55	2.20	1.30	1.85
5	0.80	0.10	5.40	2.00	
6	1.45	0.80	2.55		
7	2.00	1.05	0.75	1.65	
8	1.80	1.60	0.00	1.35	
9	0.75	0.50	0.80	0.45	
10	5.10	3.30	2.40	4.20	9.60
11	0.95	0.60	0.40	0.30	4.65
12	0.85	0.45	0.55	0.75	1.25
13	2.10	2.20	2.75		
Total	30.50	20.15	27.40	19.45	24.95
n	13	13	13	11	7
Mean	2.35	1.55	2.11	1.77	3.56
S.D.	1.77	1.00	1.52	1.10	2.95

Appendix VII. Others

Others: mean numbers of others per H.P.F. in C1S0
Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	3.20	3.00	2.20	0.50	0.30
2	2.50	2.65	1.30	1.85	2.65
3	7.20	2.20	2.00	4.95	2.75
4	2.60	2.45	1.40	1.35	1.30
5	1.80	0.90	2.50	0.40	
6	0.85	0.20	4.45		
7	3.55	1.20	1.00	0.85	
8	1.55	1.10	0.20	1.90	
9	1.40	0.40	1.20	2.15	
10	3.00	1.10	3.80	2.00	3.60
11	3.10	2.65	2.65	3.95	2.15
12	1.30	1.35	2.25	0.45	1.05
13	3.45	3.15	1.90		
Total	35.50	22.35	26.85	20.35	13.80
n	13	13	13	11	7
Mean	2.73	1.72	2.07	1.85	1.97
S.D.	1.55	0.96	1.10	1.39	1.06

Others: mean numbers of others per H.P.F. in C1S1
Tx group samples at each examination.

Subject	Wk0	Wk1	Wk4	Wk8	Wk12
1	2.00	0.20	8.80	0.50	1.10
2	3.20	1.10	4.35	1.30	1.55
3	5.00	0.85	0.90	1.50	1.05
4	1.85	1.95	4.50	1.90	2.35
5	1.70	0.45	2.30	2.40	
6	1.10	0.60	1.75		
7	2.00	1.60	2.45	1.55	
8	1.65	2.85	0.20	0.85	
9	1.55	0.75	0.80	1.65	
10	5.70	1.80	3.30	3.00	3.10
11	1.15	0.40	0.85	0.70	0.55
12	0.85	0.30	1.30	1.10	2.75
13	4.90	2.10	1.75		
Total	32.65	14.95	33.25	16.45	12.45
n	13	13	13	11	7
Mean	2.51	1.15	2.56	1.50	1.78
S.D.	1.58	0.80	2.21	0.71	0.89

Appendix VIII. Statistical Summary

Cochran's Q-Test: Plaque, Gingival and Bleeding Indices.

Variable	Effect	Weeks	TxGroup	df	p-value
PLI(0)	increase	0,1,4,8	C0S1,C1S1	3	0.05
	increase	0,1,4,8,12	C1S1	4	0.05
GI(0)	increase	0,1,4,8	C0S1,C1S1	3	0.05
	increase	0,1,4,8,12	C1S1	4	0.05
BI(1)	decrease	0,1,4,8,12	C0S0	4	0.025

Appendix VIII. Statistical Summary

Analysis of variance- probing pocket depth (PPD) and attachment level (AL).

Variable	Effect	Weeks	TxGroup	df	f	p	G*	H#
PPD	decrease	0,1,4,8	all	3	9.28	0.0001	0.0005	0.0002
	decrease	0,1,4,8,12	all	4	14.2	0.0000	0.0000	0.0000
AL	gain	0,1,4,8	all	3	9.55	0.0001	0.0004	0.0001
	gain	0,1,4,8,12	all	4	16.3	0.0000	0.0000	0.0000

Analysis of variance-decrease in proportions of bacterial types.

Type	Weeks	TxGroup	df	f	p	G*	H#
Cocci	0,1,4,8	C1S0,C1S1	3	8.37	0.0003	0.0008	0.0003
	0,1,4,8	C1S0,C1S1	3	6.27	0.0020	0.0047	0.0020
Motile forms	0,1,4,8,12	C1S1,C0S1	4	3.15	0.0324	0.0785	0.0490
Spirochaetes	0,1,4,8,12	C1S1	4	3.53	0.0210	0.0570	0.0289

G* = Greenhouse-Geisser probability

H# = Huynh-Feldt probability

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