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A COMPARATIVE STUDY OF PAPER POINTS AS A METHOD OF SAMPLING BACTERIA

by

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ABSTRACT

The purpose of the present study was to investigate absorbent paper points as a method of sampling bacteria found in the gingival sulcus and periodontal pocket.

Five brands of absorbent paper points were selected for the study. These were selected according to set criteria, and purchased from the local dental trade houses. The paper points were measured for selected physical properties , providing potential correlates to the individual brand ability to recover selected bacteria.

The following bacteria, being known associates of the gingival sulcus and periodontal pocket, were selected for the study: Actinomyces viscosus T14, Bacteroides intermedius NCTC 9336, Fusobacterium nucleatum ATCC 25586, Streptococcus sanguis G9b. The individual lyophilised cultures were reconstituted and grown under the appropriate aerobic or anaerobic condition, to provide standard growth curves. At the late log phase of growth, standardised sampling procedures were performed and the resulting cfu/ml for individual points and brands subjected to statistical analysis .

The results show that all brands were sterile as claimed, with one brand possessing antibacterial activity. There were no significant correlations observed between the physical parameters measured and the brand ability to recover the selected bacteria. A wide variance existed within brands and between brands in the bacterial recovery rates. It was recommended that those brands showing the least variance should be selected for bacteriological studies, and that further studies be undertaken to correlate the physical properties of paper points to the recovery rates for periodontal bacteria.

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" Dedicated to my wife and children."

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List of Abbreviations

A. viscosus.	Actinomyces viscosus
B. intermedius	Bacteroides intermedius
CFU / ml	Colony Forming Units per millilitre
F. nucleatum	Fusobacterium nucleatum
OD	Optical Density
P.D.	Produit Dentaire Suisse
Roeko (german)	Roeko (ULM)
S. sanguis	Streptococcus sanguis

CHAPTER ONE

INTRODUCTION

The state of health in man is subject to his ability to maintain a series of equilibriums. In this context, the oral cavity and more specifically, the periodontal tissues are in a state of equilibrium between the host's defence capacity and the periodontal microflora's ability to colonise, attach and remain within the region. The inflamed gingival tissues of gingivitis are an obvious sign of a change in this equilibrium (reviewed by Page 1986). The inflammatory response is reversible without the apparent loss of supporting tissues. Destruction of the periodontal attachment and alveolar bone occur in the various forms of periodontitis. The manner in which the changes in the host's and microflora's equilibrium differ between a reversible inflammatory response in gingivitis and an irreversible destruction of the periodontium in periodontitis is unknown (reviewed by Listgarten 1986).

The host's response to the resident flora is thought to be protective in nature (Listgarten 1986). The cellular, humoral and ancillary components of the immune system provide a complex defence mechanism with the role of excluding the periodontal flora and its products from the tissues. A change in either side of the equilibrium may be sufficient to allow active destruction to occur through a weakened defence system or an increased virulence of the microflora.

The periodontal flora has proved to be a difficult area to research. The technology to study it has been a limiting factor in its investigation. Improved methods to sample, disperse, isolate, and cultivate this diverse group of organisms have found some 300-400 species. Many of these have yet to be identified. The enormous cost and labour intensive procedures limit such studies to a few research centres. These studies have been mainly cross-sectional, with few longitudinal reports being available. They have

shown that great variations can exist between health and disease, and from patient to patient. The sites within a mouth are different, as is the individual site over time (Moore 1987). Herein lies the methodological problem faced by researchers.

Recently, Socransky (1984) proposed that periodontal disease was not a slow continuous process as previously thought (Löe, 1978). Instead he proposed that periodontal disease was episodic and site specific. This theory is now known as the "Burst Theory". The duration and time frame in which active episodes are thought to occur is relatively brief. These episodes are interspersed with lengthy inactive periods. The active episodes may last from days to a few weeks. The theory partially explains the variability seen in the microflora, and requires that the activity status of the site be determined at the time of sampling. In view of this, the data from past studies may be unusable.

The question of disease activity and the determination of its occurrence is actively being researched at present. Fine and Mandel (1986) have reviewed the potential areas of interest with the analysis of gingival crevicular fluid components being promising. This is only a part of the problem being encountered by the microbiological researcher. The methodological aspects and techniques available to remove a segment or all of the subgingival plaque from a site, grow the complete flora and identify it are far from adequate. The total microflora is still to be found, with many micro-organisms requiring highly specialised growth conditions. The literature presently indicates that a small group of bacteria may be the potential pathogens in some forms of periodontal disease (reviewed by Slots 1986b). These bacteria are designated 'pathogens' by virtue of their increased numbers when compared to healthy sites. This concept is itself questionable in view of the symbiotic and antibiotic bacterial relationships. Perhaps the bacteria observed growing in large numbers are only able to do so by a symbiotic relationship with the true pathogen. As Tanner and Goodson (1986), and Moore (1987) have discussed, the widely differing techniques and methodologies do not allow many valid comparisons between studies. A standardised series of techniques are needed to allow this, not only from a research viewpoint, but to allow pooling of the expensive and limited resources presently available. In microbiological studies the sampling technique is the starting point, following on to dispersion, plating and

growth, and culminating in the final collection and analysis of data. Presently the various sampling techniques range from paper points removing a small potentially selective segment of the microflora, to curettes, capable of removing most of the flora within a site. In addition to the size and selectivity of a technique, the use of specialised devices to maintain an anaerobic environment have been employed. The effects of each of the different techniques will require assessment, with the potential existing for a number of different techniques to be used. The ability of paper points to remove a small selective segment of the flora may be advantageous in maintenance testing and longitudinal studies where minimal disturbance to the microflora is required, while the larger capacity of the curettes would be better suited to total cultivable microflora studies.

Recently, the endodontic literature has addressed the properties, both physical and biological of absorbent paper points. It has been shown that a degree of variability exists within and between brands (Möller and Hensten-Pettersen 1985). In view of this, the following study was undertaken to observe in a controlled laboratory situation the variation in physical properties between and within selected brands of absorbent paper points, and additionally to observe the potential effects these variations may have on the ability of the different brands to sample individual pure cultures of known bacteria.

CHAPTER 2.

LITERATURE REVIEW

2.1 THE MICROBIAL RELATIONSHIP

The scientific basis of the relationship of bacterial plaque to the periodontal diseases evolved from the epidemiological studies of the past three decades. A correlation was observed between the level of bacterial deposits, the age of the subject and the severity of periodontal destruction (Lövdal et al. 1959, Russell 1963,).

Schei et al. (1959) in a cross-sectional study of over 700 adults found that increased alveolar bone destruction was associated with poor oral hygiene. Further evidence came from clinical trials which showed the cause and effect relationship between plaque and periodontal disease in humans. Healthy plaque free dentitions were found to develop gingivitis following the abstention from all oral hygiene procedures. The inflammation was resolved following the re-introduction of oral hygiene (Löe et al. 1965, Theilade et al. 1966). Studies on the effect of good oral hygiene provide additional evidence for a bacterial aetiology, showing that periodontal diseases can be slowed or arrested (Lövdal et al. 1959, Suomi et al. 1971, Lindhe and Nyman 1975, Rosling et al. 1976).

Experimental studies on animals, have provided supporting evidence for the human studies. The beagle dog was first described by Saxe (1967) as a suitable experimental model. Experimentally induced gingivitis and periodontitis studies in the beagle dog demonstrate that both gingivitis and periodontitis are bacterially induced diseases, (Lindhe et al. 1973, 1975). The use of antibiotic therapies in animal and human studies provide additional support for a bacterial aetiology (Listgarten et al. 1978b, Listgarten et al.1978c Heijl & Lindhe 1979, Cianco et al. 1981, Lindhe et al.1983). The long-term use of systemic antibiotic therapy has been questioned in light of the similar effects in most periodontal dieases of thorough subgingival debridement (Lindhe et al. 1983). If antibiotics are used alone, the subgingival microflora is found to return within weeks showing similarities to the pre-treatment microflora some months after antibiotic therapy is stopped. Thorough subgingival debridement has been shown to provide longer, more significant changes to the subgingival microflora (Listgarten et al. 1978c, Slots et al. 1979). Both modes of therapy provide further evidence for the primary role being played by the periodontal microflora in the disease process.

Historically, Dayton Miller in 1890 suggested that specific microorganisms were responsible for periodontal disease, although it is only recently that techniques and methods have become available to study the subgingival microflora. The research efforts of the 1890- 1930 period were aligned with a specfic microorganism theory. Various contenders were found and specific treatments performed in an attempt to eliminate them. The lack of success of these treatments led to the period from the 1930's up until the early 1970's being devoted to mixed infection research, resulting in the general

impression that the sheer volume of plaque was important in disease initiation and progression (Socransky et al. 1982).

Loesche (1976), has described two theories in the bacterial aetiology. The Specific Plaque Theory and the Non specific Plaque Theory. The Nonspecific theory was derived from the early epidemiological studies showing a correlation between the volume or mass of plaque, clinical parameters and the degree of severity of periodontal destruction. The Specific Theory indicates that periodontal disease is caused by specific micro-organisms. These may be acting singly or in synergistic combinations.

Theilade (1986) recently reviewed the evidence for each theory and her conclusions have blended the two. The potentially specific diseases of Juvenile Periodontitis and Acute Necrotising Ulcerative Gingivitis (ANUG) have received much attention over the past decade. The studies of Slots et al. (1980) and Mandell (1984) suggest that a specific group of pathogens are present. The potential exists for them to reside in diseased and healthy mouths, which would support both theories. The adult form of periodontitis has been studied using dark field microscopy and microbiological techniques. Each method has found a highly variable and complex microflora with significant differences being observed between subjects, between sites within subjects and within the same site over time (Newman and Socransky 1977, Slots 1979, Lindhe et al. 1980, Tanner et al. 1979, Mousqués et al. 1980).

The problem associated with such studies lies in our inability to state whether the site is active or inactive at the time of sampling. Recently, the long held idea that periodontal disease progressed as a slow and continuous loss of attachment has been questioned (Löe et al. 1978,1986). The disease activity has been described as being episodic and site independent (Socransky et al. 1982, Goodson et al. 1984); requiring the status of the individual site to be known at the time of sampling. The following two studies illustrate the effect that individual site data versus means can create. Evian et al. (1982) using individual site data, found no correlation between the darkfield parameters and the clinical indices being used. A different result to that found by Mousqués et al. (1980) who used pooled data (probing depth and cell counts) from sites with the conclusion that probing depth and spirochaete counts showed a significant correlation. Both studies support an episodic nature to the disease and highlight the problems faced when trying to investigate the microflora at disease active sites when that associated with many inactive sites may "swamp" the results. To date the studies have relied on simple methods to determine the extent and activity status of the disease. The periodontal probe is used to measure clinical attachment level changes, with these being statistically correlated to episodes of disease. This is a crude method with a high degree of inaccuracy, combined with this is some uncertainty in relation to the statistical analysis being used to observe these periods of "activity" (Imrey 1986). To date the disease can still only be diagnosed retrospectively (Haffajee et al. 1983). Other methods are being researched to develop accurate disgnostic methods which will identify periods of disease activity. The techniques range from radioisotope and computer assisted digital subtraction radiographic imaging and quantitation

of alveolar bone changes (Gröndahl et al. 1983, Hausmann et al. 1985, Hausmann et al. 1987) to a wide range of biochemical analyses of gingival crevicular fluid (reviewed by Fine and Mandel,1986).

The cultural studies of adult periodontitis have found great variations in the subgingival microflora. The predominant cultivable studies are limited in the number of patients that can be investigated at any one time by the laboratory manpower and resources available (Tanner et al. 1979, Moore 1982 a&b,1983). This limitation may be statistically significant, with the crude methods of assessing disease type and activity producing sufficient variation to obscure the microbiological results being found.

Moore et al. (1984) used three patients assessed as having similar chronic moderate periodontitis. They found that a statistically significant difference existed between the flora examined from each patient. The three patients also exhibited a variation in the microflora existing within their own mouths when sites were compared over a period of time. Patient A showed little difference in the flora from three sites sampled, with the longitudinal differences providing the greatest variability, whilst patient B had the greatest variation between sites and times. In patient C, a significantly different flora from the other patients was observed. However the microflora did not change markedly over time. This small study exemplifies the problems that must be overcome before accurate assessment of the subgingival flora can be compared to disease status.

The diverse nature of the subgingival microflora is continually being explored with the development of more efficient techniques enabling

recovery of those micro-organisms that exist in small numbers or require a very select ecoclimate. An example is the identification at healthy sites of **Actinobacillus actinomycetemcomitans**, a potential pathogen in juvenile periodontitis. Using selective media, this pathogen was recovered from both healthy and diseased sites where previously the low numbers present were being "swamped" on non-selective media (Zambon et al., 1983). As further developments in culturing techniques occur other microorganisms of high pathogenic potential may be found in similar small numbers. Presently, most research studies have concentrated on observing those microorganisms showing large numerical increases when healthy and diseased sites are compared (Moore et al. 1982, 1983.). This is our apparent measure of pathogenicity.

Moore et al. (1982) have isolated some 264 distinct bacterial groups and species from gingivitis and periodontitis patients while Socransky et al. (1982) suggest that uncultivated spirochaetes and other microorganisms may increase this total to 300 - 400 species. The problem of investigating the relationship of the microflora to the disease state is further compounded by the nature of a potential pathogen's increase in numbers. Is it through superior virulence and ability to survive and colonise, or has the true pathogen provided growth conditions suitable for a symbiotic increase to occur?

The potential perio-pathogenic bacteria number some 30-40 species (Slots 1979). Of these, four bacteria have been chosen for this study. They are **Bacteroides intermedius, Fusobacterium nucleatum, Actinomyces viscosus** and **Streptococcus sanguis.**

The black pigmenting **Bacteroides** are found in low numbers in health and rarely in children. Increased numbers are found after puberty and during pregnancy, suggesting a relationship with steroid hormone levels (Kornman & Loesche 1980). The black pigmenting **Bacteroides** require a source of napthaquinone to colonise and grow, however, host derived oestradiol or progesterone can be used as a substitute. This could possibly explain the appearance of the bacteria after puberty and during pregnancy. The normal source of napthaquinone may be derived from a symbiotic relationship with an anaerobic streptococci and gram positive facultative rods, both of which are found in subgingival plaque (Slots and Genco 1984). The black pigmented **Bacteroides** possess a number of features which assist it in colonising the 'Host'. The capsular material is of a low antigenicity and is used to mask the antigenic lipopolysaccharide moities, avoiding detection by the immune system. The capsule also provides resistance to phagocytosis and complement mediated bacteriolysis by polymorphonuclear leucocytes. A number of degradative enzymes are produced, such as catalase and superoxide dismutase. These may neutralise the intracellular release of hydrogen peroxide and superoxide ions by polymorphonuclear leucocytes. Another group of enzymes have been shown to degrade immunoglobulins. **Bacteroides intermedius** is known to produce an IgG protease (Slots & Genco 1984).

In the case of Adult periodontitis the incidence of **Bacteroides intermedius** is known to increase up to 10 times between non-progressing sites and progressing sites. Slots (1986b) found **Bacteroides intermedius** in 58-89% of progressing sites and in 36- 53% of non-progressing sites, with potentially

different strains being involved in gingivitis and periodontitis. Moore et al. (1982) isolated these bacteria in 26% of periodontitis sites but not in healthy or gingivitis sites. While Loesche et al. (1982) have implicated it in Acute Necrotising Ulcerative Gingivitis, in association with small spirochaetes.

Streptococcus sanguis, a common inhabitant of supra and subgingival plaque (Slots et al. 1978, Theilade et al. 1982), has been found to release a high molecular weight peptide with properties antagonistic towards the black pigmented **Bacteroides.** This potential antibiotic effect was observed by Hillman et al. (1985) in a chronic periodontitis study. They discovered that a number of potential periopathogens including **Bacteroides intermedius** correlated with the absence of the **Viridans Streptococci.** In three patients with intractable periodontitis the inhibitory organisms were found in low numbers compared to healthy mouths. Inactive periodontitis sites were found to have numbers intermediate to the two groups. It is hypothesised that the hydrogen peroxide production of the **Streptococci** inhibits the growth of the suspected pathogens. The symbiotic and antibiotic interactions within the plaque mass may provide future methods of biological plaque control.

Studies on developing plaque and experimental gingivitis have found the **Actinomyces** species to be common microorganisms in the successional change of the flora with time (Theilade et al. 1982). The pathogenic potential of the species has been investigated in various animal studies, in which it has shown significant disease capabilities. The study of Keyes and Jordan (1964) is significant in that the transmissibility of the periodontal 'syndrome' and its relation to bacteria were shown for the first time. The

role of the **Actinomyces** species in human periodontal disease though may be more one of an 'interested onlooker' than an active participant. The limited number of studies on this gram positive microorganism may have overlooked a significant feature of the **Actinomyces** species in the role of plaque co-aggregation and successional growth (Cisar 1982). Future studies may find that the early colonisers are the key to controlling the pathogenic successors (Ellen 1982).

Another potential pathogenic species is the **Fusobacteria.** In particular **Fusobacterium nucleatum** is responsible for production of large quantities of butyric acid, giving the classical "bad breath" odour, and it is associated with disease in other sites of the body. Moore et al.(1982) found that the microorganism was associated with experimental gingivitis, and was the most common isolate in a detailed study of severe periodontitis in young adults (Moore et al. 1982b). Tanner et al. (1984) used cluster analysis to study the relationship of groups of microorganisms to alveolar bone loss in progressive moderate-severe periodontitis. They found that of the three dominant clusters formed, **Fusobacterium nucleatum** was present in each. Cluster 1 was associated with recent bone loss, while clusters 2 & 3 were non-active sites. The association with active and non-active disease sites may be a result of a number of subspecies being grouped together, or a relation with gingival inflammation. Vincent et al. (1985) investigated two different colonial morphologies found on crystal violet-erythromycin agar. A range of immunological and biochemical tests were used, with the finding that both isolates were potentially the same. Clinically the type II isolate was recovered in higher numbers from sites showing a gingival index of two. The authors can give no explaination for the different

colonial morphology.

The four microorganisms mentioned previously, will be used in the study of a sampling method because they are readily found in most adult patients with varying degrees of periodontal disease. This will allow comparative studies to be performed. The collection of the subgingival microflora is the first stage in a microbiological study of periodontal health and disease.

In addition to the methodological and technical difficulties previously discussed, a recent study has implicated the bacteriophage in enhancing the pathogenicity of particular strains of **Actinobacillus actinomycetem-comitans,** (Preus et al. 1987). The study concluded that a change from a temperate to a virulent phage state may increase the pathogenic potential of the microorganism. If phage mediation is an important factor in virulence, and therefore pathogenicity, an additional factor will require investigation.

2.2 SAMPLING METHODS.

The importance of the sampling technique was appreciated by early investigators. Although they were unaware of the complexity or the fastidious nature of the subgingival microflora that was being sampled, the potential difference between the supra and sub-gingival regions was appreciated. Waerhaug and Steen (1952) had proposed that the healthy

gingival sulcus was sterile if free from calculus and deposits. Using sterile steel blades to sample the sulcus, after surface disinfection with an iodine solution, they concluded that bacterial colonisation occured after subgingival calculus had formed. The bacteriological studies that followed showed an awareness of the special nature of the oral microflora. Removal of supragingival plaque and isolation of the sampling site are emphasised by Schultz-Haudt, Bruce, and Bibby (1954), as is the selection of sites. This study shows many similarities to more modern bacteriological studies. They use an aspiration sampling technique as described by Beckwith, Simonton and Rose (1927), in addition to an un-named technique using modified dental instruments. Boyd and Rosenthal (1958) describe a custom made dental explorer being used to retract the gingival tissues while Gavin and Collins (1961) employed a sterile 19 gauge hypodermic needle, serving a dual purpose. The sterile needle was manipulated into the gingival sulcus, avoiding contamination from the supragingival region, and allowed a sterile paper point (un-named brand) to be passed through the needle into the subgingival region.

It is interesting to note that the conclusions drawn from Waerhaug and Steen's (1952) study, indicate that the attempts to eliminate the supragingival flora by disinfection with an iodine solution, most probably had eliminated the subgingival flora as well. Wilkinson (1962), while investigating methods of sampling, raised the question concerning the comparability of results when different sampling techniques were used. His technique employed a 27 gauge needle being passed through the pocket wall, avoiding the supragingival plaque. Following penetration, 0.1ml of sterile distilled water was expelled into the pocket then re-aspirated into a

tuberculin syringe. The aspiration technique was used for a sulcus approach, and compared to the penetration method. Wilkinson's results show that an increased proportion of anaerobes were recovered by the penetration method.

Castro and Going (1964) used three methods to sample the subgingival region by paper points. The gingival tissues were retracted by either a dental scaler, glass tube attached to a vacuum pump or penetrated by a sterile hypodermic needle. The significance of these studies lies in the observation of a flora within the healthy gingival sulcus, disproving the early assumption that the gingival sulcus in the healthy state was sterile. A further modification of the sampling procedure was devised by Kelstrup (1966). This method was an adaptation of the earlier studies using an ether based iodine varnish in preference to an ethanol base, as proposed by Bervell (1964) to sterilise the supragingival region. It was suggested that the ether based material was less likely to flow into the subgingival region and alter the resident microbiota. Sampling was accomplished by coating the tooth and gingival margin with the varnish, then passing a sterile paper point through the dried varnish layer, reducing the potential contamination from supragingival plaque.

2.2.1 SURGICAL TECHNIQUES.

Darwish et al. (1968) used a suture, placed through the gingival tissues enabling the gingival pocket wall to be retracted away from the tooth. The method allowed samples of subgingival plaque to be taken at varying depths

within the pocket, however it also introduced air into the pocket region which changed the anaerobic conditions present. The extraction of a tooth and subsequent sampling of the subgingival plaque has been described by Sabiston and Grigsby (1972). This method allows selective sampling of the plaque levels, however contamination by oxygen, potential disruption of the microflora's architecture and the limited ability to investigate longitudinal changes in the flora preclude its use in modern studies.

An alternative approach to the subgingival area was taken by Slots (1976), in which a semilunar coronally raised flap was used to expose the region. The area was sampled with a sterile curette and processed using continuous anaerobic culturing techniques. Post-operative healing was compromised in 2 out of 6 patients, a point that must be taken into account if this method were to be used for future studies. Healing is occuring in an infected region with the potential existing for soft tissue breakdown. Bleeding into the subgingival region and the physical disruption of the plaque may alter the composition of the sample.

2.2.2 GAS FLUSHED DEVICES.

The use of continuous anaerobic culturing techniques have allowed many more species to be isolated (Slots 1976, Tanner 1979, Moore 1982 a&b,1983). The techniques are compromised by oxygen contamination during the transfer of the subgingival plaque sample from the periodontal pocket to the transport medium, unless special precautions are taken. The problem was addressed by the use of a gas flushed sampling device as devised by Newman and Socransky

(1977). The device contains a barbed broach soldered to a stainless steel wire, the broach being wrapped in calcium alginate fibres. The wire and broach are sealed within the outer glass tube to which a continuous supply of oxygen free gas is connected. The base of the tube is covered by a tinfoil cap. The glass tube is moved into the subgingival region allowing the broach to be pushed through the tinfoil seal, sampling the subgingival plaque without contamination from the supragingival plaque. Following sampling, the broach is withdrawn into the tube and flushed continuously with .the oxygen free gas during transfer to the transport medium. The dispersion fluid usually contains 1% sodium metaphosphate to allow dissolution of the calcium alginate fibres, thus freeing any trapped bacteria.

The above technique is a further step towards the total recovery of all species of microorganisms, however it may possess a number of problems. The chromium in the stainless steel rod and broaches used, is known to undergo catalytic oxidation with moist samples; a reaction which could compromise the effect of the oxygen free gas flushing the tube. The calcium alginate fibres used to wrap the barbed broach are also inhibitory to some anaerobes (Moore, Ranney and Holdeman, 1982).

2.2.3 SCALERS & CURETTES.

The dental scaler and curette are frequently used sampling methods for both cultural and microscopy studies, while **Tables 2.1** and **2.2** indicate other methods in use. The volume of subgingival plaque removed by any particular

sampling method is a controversial point at present. Scalers will remove a larger portion of the microflora than the other techniques currently employed. The potential effect of the different volumes will be discussed in the section on comparative studies.

It would appear that no attempt has been made to standardise the size or shape of the scalers and curettes being used, or the manner in which the instruments are manipulated within the gingival sulcus. The instruments are made from stainless steel or variants of this material, making them prone to the same problems of chromium induced catalytic oxidation with moist samples as is the broach or reamer technique previously discussed. The problem can be avoided by using nickel plated instruments, as advocated by Moore et al. (1982a). The importance of eliminating this effect is particularly obvious in their series of studies (1982a, 1982b, 1983), with the scaler tip remaining in the pre-reduced anaerobically sterilized broth for up to 20 minutes after sampling.

The scaler and curette remove a comparatively large proportion of the flora, a factor that must be considered in the aim of a study. These instruments are used in treatment and produce profound effects on the subgingival microflora. Sampling may therefore constitute a form of treatment in itself. The effect may create long-term disturbances when repeated samplings on the subgingival flora are required in longitudinal studies, producing erroneous observations of the primary effect under study.

Mousquès et al. (1980) investigated the effects of repeated sampling using

Differential Dark Field Microscopy (DDFM). 18 adults with chronic periodontitis were subgingivally sampled with curettes. The results show that a significant change had occurred by day three, with an increased level of coccoid cells that continued through to day forty-two. The spirochaete level decreased at the first week then returned to the baseline region for the remainder of the study. Evian et al. (1982) have observed a different result in the manner in which the microflora underwent changes, however, using DDFM parameters they also found that sampling did effect the subgingival flora. It is apparent that changes do take place following sampling. The variability of the results from the limited studies undertaken suggest that further study is needed. The results of studies involving multiple sampling of individual sites over various time periods, should therefore be viewed with this potential effect in mind .

2.2.4 IRRIGATION TECHNIQUE.

In contrast to the large sample sizes removed by the curette and scaler technique, is the washing method used by Magnusson et al. (1983). This technique was originally devised by Skapski and Lehner (1976) for the sampling of gingival crevicular fluid and its components. Magnusson et al., used a 25µl Hamilton syringe and 25G blunted needle, to sample the base of periodontal pockets by first depositing, then re-aspirating 10µl of a 0.85% NaCl with 1% Gelatin solution. A later study by Magnusson et al. (1985) was undertaken to assess the reproducibility of the washing technique and compare it to the curette technique of sampling. This will be discussed later, under comparative studies. There have been variations of the washing technique, with Goodson et al. (1985) using a 2µl microcapillary tube

Table 2.1

Summary of Different Sampling Techniques for Cultural Studies

SAMPLING TECHNIQUE	DISPERSION METHOD	TRANSPORT/ DISPERSION FLUID	REFERENCE
3 paper points (J&J Fine) 10sec.	vortex 60sec.	VMGA III (Möller 1966)	Bragd et al (1987)
as above	as above	as above	Rosling et al (1983)
as above	as above	as above	Slots et al (1986)
as above	as above	as above	Rosling et al (1986)
as above	as above	Ringers' Soln	Rosling et al (1985)
as above	as above	RTF (Syed & Loesche 1972)	Stabholz et al (1986)
as above	sonication 5sec	RTF	Kornman et al (1981)
1 paper point (un-named fine sterile)30sec	sonication 10sec	RTF	Lang et al (1983)
Scaler (Morse No. 00) (single stroke)	sonication 5sec	¼ strength Ringers'Soln (pre-reduced)	Mandell et al (1986)
Scaler (Morse No.00) Nickel plated (single stroke)	vortex with glass beads (150μm diam) 5-10sec	Pre-reduced anaerobically sterilised broth	Moore et al (1982)
Curette (un-named small)	vortex 20 strokes maximum setting	sterile skim milk	Asikainen et al(1987)
Curette	shaking with glass beads 30sec in Whirlimixer.	RTF soln	Westling (1984)
Gas Flushed anaerobic sampling device.	sonication 30sec	PRAS (pre-reduced anaerobically sterilised)soln.	Newman & Socransky (1977)
Gas flushed anaerobic sampling device	sonication (time unknown)	pre-reduced fluid	Gajewska et al(1983)
as above	sonication 10sec	¼ strength Ringers' soln	Savitt (1984)
as above	microcentrifuge tube; sonicated 10 sec in ultra-sonic water bath	Ringers'soln Pre-reduced anaerobically sterilised	Newman et al (1984)
Wire loop (diam 1.5mm)	sonication 20sec plus vortex 15sec	RTF soln	Svanberg et al (1982)
Coronally raised flap: sampling with curette	sonication 20sec	RTF soln	Slots (1976)

Table 2.2.

Summary of Different Sampling Techniques for DDFM Studies.

SAMPLING TECHNIQUE	DISPERSION	TRANSPORT FLUID	REFERENCES
3 paper points (Maillefer ISO No.25)10sec.	Vortex 20sec.	0.90% NaCl + 1% Gelatin	Beltrami et al (1986)
curette(un-named)	re-aspiration with tuberculin syringe (23G needle) 3x	0.85% NaCl + 1% Gelatin	Listgarten & Hellden (1978a)
curette(un-named)	re-aspiration with sterile pipette 3x	RTF soln.	Westling (1984)
curette (Gracey No.7/8)	shaking	0.85% NaCl +1% Gelatin	Stabholz et al (1986)
(Gracey No. 11/12 or 13/14 or Neos 00 curette)	re-aspiration (25G needle) 3-6,10,15 x	as above	Omar et al (1986)
curette(un-named)	un-named	sterile water	Savitt (1984)
Periodontal probe	re-aspiration with tuberculin syringe 5x	0.85% NaCl + 1% Gelatin	Abbas et al (1986)
Periodontal probe (flattened tip)	re-aspiration with tuberculin syringe (23G needle) 10x	as above	Zappa et al (1986)
Periodontal probe	re-aspiration with tuberculin syringe plus vortex 10sec	BM medium (Shah,1976)	Van der Velden et al (1986)
Hamilton micro-syringe 10μl	not stated	not stated	Magnusson et al(1985)

attached to a 10µl Hamilton microsyringe. For sampling, 2µl of the rinsing solution (10µl of water with 10 units/ml heparin) were used, with 1µl being expelled into the base of the periodontal pocket and re-aspirated by gently pumping the syringe. Following the re-aspiration, the 2µl volume was expelled into the pocket and re-aspirated in the same manner. An alternate method was employed by Lander et al. (1986), using a 1ml insulin syringe and attached 23G needle. The sample was aspirated into 0.5ml of saline, while maintaining the needle tip approximately 1mm from the base of the pocket.

2.2.5 PAPER POINTS

Paper points have been used to perform a number of functions in endodontics, including sampling the root canal for bacteriological purposes (Garber 1963, Olgart 1969,1970). The paper point has also been used for sampling the sub-gingival microflora in earlier bacteriological studies (Bervell 1964, Castro and Going 1964, Gavin and Collins 1961).In more recent studies the paper point technique has been employed in the following areas; a) Studies of the predominant cultivable flora (Slots et al. 1986c). b) The effectiveness of chemotherapeutic techniques and treatment on the subgingival flora (Mashimo et al. 1981, Slots et al 1983, Rosling et al. 1986, Kinder et al. 1986, Christersson et al. 1985, Slots et al. 1985, Beltrami et al.1987.). Stabholz et al. 1986), c) Selective and non-selective culturing of potential periopathogens (Bandt et al. 1982, Zambon et al. 1983, Slots et al.1985, Slots et al.1986). d) Diagnostic studies such as the use of indirect immunofluorescence (Slots 1986.)

The paper point technique has found favour in recent years as a means of sampling the subgingival flora, however, little work has been done on the paper points themselves. A number of studies have appeared in the endodontic literature recently that shed some light on the physical and biological properties of absorbent paper points. The biological effects of selected brands of paper points have been tested for the leachability of a toxic material by Möller & Hensten-Pettersen (1985). Various brands were tested for haemolytic effects using human erythrocytes in a spectrophotometric system to assess the amount of haemolysis. In addition, a tissue culture agarose overlay test was conducted to assess cytotoxic effects on a cultured human epithelial cell line, while chemical tests were performed on eluted materials. The results show that one brand **(PD,Suisse)** gave a moderate response to the cytotoxic tests and a very high result for the haemolytic effects. Further testing using fluorimetric methods showed the brand to contain formaldehyde. The other tested brands contained no formaldehyde and did not give a response to the haemolysis or cytotoxic tests.

The sterility, antibacterial activity and bacterial recovery properties of 4 brands were evaluated by Ørstavik and Möller (1985). The study concluded that all brands were sterile as stated by the manufacturer. The antibacterial activity of each brand was assessed by a gel-diffusion test using blood agar plates seeded with whole saliva. Only the **PD Suisse** brand showed a reproducible zone of inhibition. The bactericidal effect was not observed for a test system using the gram positive coccoid organism **Streptococcus Mutans (OMZ 176)**. The recovery of bacteria from each of the tested brands was greatly reduced for the **PD Suisse** brand. The authors

suggest that the result may be attributed to a lower number of viable bacteria resulting from the effect noted in the gel-diffusion test, or a higher retention rate of microorganisms by this brand.

In a separate study on antibiotic secretion in saliva and gingival crevicular fluid, Rawle et al. (1985) found similar antibacterial effects in a different brand of paper points (**Endic** brand). The effect was noted in a test system using the bacteria, **Sarcina Lutea**, with reproducible zones of inhibition being found around the **Endic** brand. Although it has not been noted in the study, the **PD Suisse** brand also appears to have a faintly defined zone around it.

The physical dimensions and properties of the absorbent paper point have been investigated by the International standards Organisation (ISO). A United States Federal specification, drawn up in 1973, was noted to be so restrictive that only one product is described and the standard does not cover the ISO size or numbering system. The Australian Dental Standards Laboratory produced a draft standard following the acceptance in October 1979 of the requirement for such standardisation. The draft standard AS 2494 (1981), describes the material design and construction of the absorbent paper point, the surface defect allowance, diameter, size and dimension. In addition, the absorption and fibre shedding properties are stated. It is unfortunate that the majority of studies using paper point sampling techniques do not use ISO standardised points. The studies in **Table 2.1** have mainly used the Johnson and Johnson brand, which does not use the ISO system in manufacture. To enable a successful study of a technique, it must be amenable to standardisation for effective comparisons

to be made. The requirements of this study therefore precluded the use of non ISO paper points.

2.3 COMPARATIVE STUDIES

The subject literature contains very few studies on sampling, with few of these being of a comparative nature. The inherent problems of such studies lie in the great variability of the subgingival microflora. As described in section 1.1, the site and time specific nature of the subgingival microflora, the potential selectivity of a particular technique towards different microorganisms, and the effect of different clinicians and laboratory techniques in the manner in which the sample is investigated, provide many inherent variables. For this reason a comparative study should have only the technique as the variable (Tanner & Goodson 1986). The different techniques and variations on them are summarised in **Tables 2.1,& 2.2,.**

In an early study, Wilkinson (1962) draws a comparison between a trans-epithelial approach to the pocket and a gingival sulcus approach for a washing technique. The results of the cultural study performed, would indicate that the transepithelial approach recovered higher numbers of gram negative coccoid organisms (32.9%: 12.1%) from the cultivable flora.

A number of studies have recently been undertaken to compare the size of samples in terms of the recovery of colony forming units (cfu). Kiel and Lang (1983) compared the curette and paper points in 25 patients with

healthy periodontiums and 25 patients with periodontitis. In addition, the study assessed sampling with 1 or 3 paper points and at the 10 and 30 second sampling times. A second sampling was used to estimate the total flora of the pocket. The curette was found to remove between 61- 91% of the flora compared to the paper point samples which removed 7-48%. When more paperpoints were used the recovery rate was higher, however, the increased sampling time from 10 to 30 seconds only showed a higher number of cfu in the healthy sites. The curette was found to recover between 10- 10^3 more cfu/ml than the paperpoints.

The above study also provides information on the selective effect a technique may have for the recovery of microorganisms. This study noted that the paper points recovered greater numbers of black-pigmented Bacteroides as well as a surface translocating bacteria. In view of the interest in the black pigmented Bacteroides as a potential periopathogen (reviewed by Slots 1986b), the selective action of paper points must be considered when interpreting studies that use them as a sampling method.

Moore et al. (1985), using paper points and a scaler found that the scaler only recovered twice as many colony forming units as did the paperpoints. The effect of a small plaque mass available for sampling is a possible cause of this low figure. The study investigated juvenile periodontitis patients, a group in whom the subgingival plaque volume is reported to be significantly smaller than adult periodontitis patients (Waerhaug 1977, Westergaard et al. 1978, Fine & Greene 1984).

Christersson et al. (1985) studied the transmissibility and colonisation of

A. actinomycetemcomitans in localised juvenile periodontitis patients using periodontal probes and paper points for the transmission and sampling methods respectively. In a reproducibility study, the paper point sampling method was repeated 30 minutes after the initial sample. Using average differences between consecutive samples, no statistically significant difference was observe at the p = 0.2 level of significance. Whether this level of significance is acceptable to use is not clear. The probe was found to have recovered between 0 and 21% (mean = 5.7%) of the microorganisms recovered by paper points .

A comparative study was undertaken by Magnusson et al. (1985) investigating the curette and an irrigation device. The irrigation device consisted of a Hamilton microsyringe, and was found to recover higher numbers of coccoid cells, while the other morphotypes studied did not show significant differences. The authors comment on the good reproducibility when the same investigator performed all phases of the study. The effect of operator variable is another area that should be investigated further. In contrast to Mousquès et al.(1980), Magnusson found that 9 repeated samplings over 32 days produced site variations of a similar magnitude to those found when resampling was performed within 30 minutes of the initial sample being taken. They conclude from this evidence that the sampling technique is the major source of variability, with the single sample studies possessing too many variables for predictable analysis. A longitudinal study with repeated samples being taken, is more likely to accurately reflect the change in composition of the microflora.

The previous techniques have no control over the sample size being removed

from the pocket. Gajewska et al. (1983) addressed this problem in a comparative study of the two gas flushed sampling devices. Newman and Socransky's (1977) device which used calcium alginate wrapped barbed broaches, was compared to a standardised reamer device. The reamer device was found to remove approximately ten times more plaque. The device consisted of a size 60 endodontic reamer within a stainless steel tube casing. The reamer had been coated with epoxy resin to within 2mm from the apex. In addition, the stainless steel tubing was graduated to enable accurate placement of the reamer tip within the pocket. A side-arm of the tube allowed oxygen free gas to continuously flush the device. Sampling was accomplished by inserting the tube into the subgingival region, then extending the reamer into the pocket and rotating it 10 times clockwise. The reamer was withdrawn into the tube prior to removal from the subgingival region, thus maintaining a continuous anaerobic environment around the sample. The two devices provide a means of transferring a subgingival sample from periodontal pocket to a transport fluid without contamination from oxygen. The broach technique of Newman and Socransky (1977) does not remove a consistent volume of plaque, while the reamer technique is claimed to provide this. A consistent volume of plaque would allow changes in the density of the subgingival flora to be observed, however the broach technique removes variable volumes and will only allow the flora to be quantified as a mean percent of the total cultivable flora.

In the study, the mean percentage differences were calculated for duplicate samples taken from untreated 6-7mm pockets in chronic periodontitis patients. The mean percentage difference of the pairs sampled for the reamer technique was 76.4%, while the broach technique was 147.3%. The variability of the results is interesting , with the broach technique

ranging between 85.5% to 196.4% and the reamer technique between 0.8% and 159.6%. The greater variability of data for the latter technique, may be explained by the observation that the reamer was removing a greater volume at the first sampling leaving a reduced volume available. The second figures were adjusted by application of a correction factor, resulting in a re-calculated mean percentage difference of 31.7%. This compared favourably to a value of 13.7% resulting from repeated sampling of a known pure culture.

In an attempt to obtain standardised volumes of subgingival plaque Svanberg et al. (1982) used a wire loop. It was felt that the subgingival plaque would be retained within the inner region of the loop and produce a uniform sample size, however no comparative data is presented to substantiate this. The study also employs a "plaque spoon" for supragingival plaque sampling.

As cited in Tanner and Goodson (1986), Socransky (unpublished data) found that the scaler consistently removed between 50-90% of the resident subgingival flora in a resampling study. The sites were consecutively sampled with three different techniques, the order in which the techniques were used did not significantly change the sample size of the scaler. The scaler returned the highest number of cfu, between 10^3-10^5 bacteria. Additional unpublished data by Socransky showed that the paper points were sampling less than 7% of the resident flora, recovering between 10^2- 10^4 bacteria. Also cited in Tanner and Goodson (1986) is a study by Heijl et al. (unpublished), who compared scalers and an irrigation device. The scaler was found to remove approximately three times more bacteria than the irrigation technique.

This observation would be expected in view of the findings from morphological studies, showing that the pocket flora contained an adherent mass of bacteria formed onto the tooth surface with a more fluid mass of microorganisms existing on the gingival pocket side (Listgarten 1976). A comparatively gentle technique such as a paper point would remove mainly the more fluid plaque mass while a scaler or curette would not be selective in this respect. It is apparent from these studies that the scaler or curette, in removing a larger volume, may be more successful in sampling a wider cross-section of the total flora present. It would thus increase the probability of observing the true proportions of the whole plaque mass and reduce the chance of missing a microorganism that exists in small proportions. The potential problem associated with this technique resides in the ability of the sampled flora to reconstitute itself back to the original state. The dark field study of Mousquès et al (1980) investigated the effect of repeated sampling over a period of time. They conclude that changes do occur in the proportion of bacterial morphotypes and that the flora had regenerated back to similar proportions after 42 days. The dark field technique only observes bacterial morphotypes. No comparison can be drawn on cultural studies as a population shift may occur within a morphotype without detection being possible. The dark field technique has been reviewed by Omar and Newman (1986), who note a number of technical difficulties. The sampling methods do not allow the degree of reproducibility to be assessed, as found in the cultural studies. It is suggested this is the major reason that problems exist in quantifying the bacterial morphotypes. In addition, problems in dispersion and production of a monolayer of microrganisms compound the errors and potential for false readings. If a small segment of the microflora is to be observed

longitudinally, then a technique such as the paper point or irrigation device may be more suitable. This approach would be particularly useful when the true periopathogens have been identified. The technique would be expected to allow long term monitoring of the flora for diagnostic and therapeutic means, without creating permanent disturbances.

In view of the differing physical effects of the sampling techniques discussed, each might be expected to possess a degree of selectivity towards a segment of the microflora. The study of Kiel and Lang (1983) indicated that paper points were selective towards the black pigmented **Bacteroides** and a surface translocating microorganism. While Socransky (unpublished data cited in Tanner and Goodson 1986) found that the paper point technique recovered a mean **Bacteroides** count of 14.6% compared to 4.5% for the scaler. Moore et al. (1985) found no apparent difference between a scaler technique and paper points when used to recover **Haemophilus actinomycetemcomitans**. Heijl et al. 1985 (unpublished data) as cited in Tanner and Goodson (1986) found similar recovery rates with darkfield microscopy and selective media culturing when a scaler and an irrigation technique were used consecutively. The study used 118 sites, being sampled firstly by curette then with the irrigation device. The results showed similar recovery rates between 34% of sites for black pigmented **Bacteroides** , while 69% of sites showed a 10% or less variation within the same group. Although there are few studies of a comparative nature, it does suggest that the paperpoint technique is selective for the black pigmented **Bacteroides**.. The investigations of selected bacteria as potential periopathogens (Slots 1986b, Slots and Dahlén 1985, Bragd et al. 1987) would be most susceptible, and caution should be use in the

interpretation of results until the selective effect of the sampling techniques have been further investigated.

2.4 DISPERSION

To allow single cells to be freed from the bacterial mass of the plaque for microscopic or cultural study, various techniques have been employed. However the requirement of a technique is one that produces maximum disruption to the bacterial plaque mass while maintaining maximum viability of all dispersed cells. In view of the numerous techniques used, the ideal method has not yet been found.

The dispersion techniques have been reviewed by Manganiello et al. (1977). The methods include vortex mixing, tissue grinders, hypodermic syringes, blenders, disintegrators, pipetting, shaking with glass beads, and sonication. Chemical and enzymatic methods have also been employed to aid dispersion of bacterial clumps. It was found that anaerobic sonication provided an effective method for dispersion, however the gram negative bacteria, **Veillonella** and **Fusobacteria** were more sensitive than the gram positive bacteria, **Streptococci** and **Actinomyces** . Moore et al. (1982) found the gram negative bacteria to be more sensitive to sonication with the slender rods and filamentous microorganisms being more susceptible to damage and death. The effect became more apparent as the sonication time was increased from 10-30 seconds (Leadbetter & Holt 1974). To overcome the selective reduction of a segment of the microflora, Moore et al. (1982) used vortex mixing after the inclusion of sterile glass beads (110-150μm

diameter) with the sample. The glass beads are thought to be non-selective in the event of microorganisms being trapped between colliding spheres. The technique has allowed an even or greater recovery of microorganisms when compared to sonication. **Tables 2.1,& 2.2,** summarise various methods used. Orams and Newman (1986) recommend that the dispersion technique should be standardised prior to any further studies of the sampling techniques.

2.5 TRANSPORT/DISPERSION SOLUTIONS

A transport or dispersion solution should maintain the microorganisms in a viable state without providing growth factors that allow multiplication to occur (Syed and Loesche 1972). Slots (1986a) describes two types of transport solution. The first is a mineral salt solution and does not encourage microbial growth, while the second solution contains nutritive factors and growth inhibitors.

The various dispersion fluids in use are shown in **Table 2.1, & 2.2.** Manganiello et al. (1977) compared Mycoplasma broth with ¼ strength Ringers' Solution, and found that the Ringer's solution significantly increased the recovery rate. Slots (1986b) compared various transport solutions and found that the survival rate in Reduced Transport Fluid (RTF, Syed and Loesche 1972) was high over short periods, while longer periods of maintenance were more successful in VMG (Möller 1966). Slots (1986b). The better survival rate in VMG may have arisen from the intrinsic properties of the media or the microorganisms may have multiplied.

Möller (1966) developed a number of transport solutions and media for use in endodontic studies. The VMG series were found to provide some growth nutrients, as shown by Möller maintaining a line of **Streptococci** in the solution for approximately 20 years. The VMGA 111 solution contains a reducing agent, allowing the sample to be transferred into the solution in atmospheric air, following which the reducing agents eliminate the free oxygen returning the tube to anaerobic conditions.

RTF solution was chosen for this study, as no growth was required while in the transport solution and all anaerobic procedures were to be performed in an anaerobic atmosphere.

2.6 MEDIA

A single nonselective media for the cultivation of the total subgingival microflora is not available at the present time. Olsen and Socransky (1981) using Brewers'Jars , an anaerobic chamber and Hungate roll tubes found that Trypticase Soy Blood Agar is an efficient nonselective solid media for many of the cultivable subgingival microflora. It was selected for this study on that basis. Slots (1986b) and Moore (1986) have reviewed and discussed the selective and non-selective media in use today. The vast array of media is an indication of the complexity of the ecosystem in the subgingival environment. For further discussion the reader is referred to these articles.

2.7 STUDY AIM

The study was undertaken to investigate one method of sampling that is currently being employed in periodontal research. The study design was employed to limit the number of variables to those associated with the absorbent paper points themselves. The aim was to observe the differences and variability in a qualitative and quantitative manner, and assess the potential effects of these results on the recovery of microorganisms.

CHAPTER 3.

MATERIALS and METHODS

3.1 MATERIALS.

3.1.1 PAPER POINTS.

Five brands of absorbent paper points, also known as endodontic paper points, were selected for the study.

A.**Independent Manufacturer.**

After discussions with the dental trade houses – it became apparent that many brands were proprietary and not generic. To ensure that the studied paper points were manufactured by separate companies, it was decided to use the country of origin as an additional safeguard in their selection.

B.**Metric size.**

The selected manufacturers used the ISO (International Standards Organisation) standards system for the production of the paper points. This system indicates that a size 50 paper point should have a tip diameter of 0.05mm, and size 60 should be 0.06mm, allowing standardisation with endodontic instruments (reamers,files,pluggers). It was decided to use standard size 60 in this study.

C.Sterility.

All brands of the paper points that were selected for this study were prepackaged in sterile containers. The containers varied from sealed paper blister packs and glass vials to sealed plastic trays. The packaging surrounding the paper points contained no information to indicate the method of sterilisation that was used.

Using these selection criteria, the following manufacturers paper points, size 60 were selected for use in this study. The selected paper points were purchased from local dental trade houses, with sufficient being obtained to allow the same lot numbers of each brand to be used for the duration of the study .

PROGRESS. Korean manufacture,glass vials of 50.

PRODUIT DENTAIRE. Suisse (PD),Swiss manufacture,glass vials of 50.

ROEKO (ulm). German manufacture,plastic tray of 100.

ROEKO (usa). USA manufacture by Endodent Inc,USA.Paper Blister pack of 5 per blister.

SURE-ENDO. Japanese manufacture,glass vial of 50.

3.1.2 MICROORGANISMS.

The selected test microorganisms were typed culture strains, kindly supplied from the collection of the Institute of Dental Research, Sydney.

A.**Actinomyces viscosus** T14.

b.**Bacteroides intermedius** NCTC 9336.

C.**Fusobacterium nucleatum** ATCC 25586.

D.**Streptococcus sanguis** G9b.

3.1.3. Recovery.

The test microorganisms were supplied as freeze dried cultures, and were reconstituted under aseptic conditions within a laminar flow cabinet. Following etching with a file the glass vials were fractured by the application of a cherry red hot glass rod to the etched line.

A. viscosus T14 and **S. sanguis** G9b were reconstituted in Tryptone Soy Broth, TSB (Oxoid). While **F. nucleatum** ATCC 25586 and **B. intermedius** NCTC 9336 were reconstituted with pre-reduced Basal Medium, BM (Shah,1976). A measured 0.2ml of the respective solution was introduced to the newly opened vials and gently re-aspirated until all of the contents were

observed to have solubilised. Partial contents (0.1ml) of the individual test microorganisms were then inoculated into separate 16 x 150mm glass tubes (Pyrex No.9825) containing 10 mls of the respective broths. A. **viscosus**, T14 was innoculated to both TSB and pre-reduced TSB (24hours). The remainder of the contents were streaked by sterile platinum loop onto Trypticase Soy Agar (TSA) supplemented with 30 mls of defibrinated sheep blood per litre (TSBA). **F. nucleatum** ATCC 25586 and **B. intermedius** NCTC 9336 were streaked onto pre-reduced Trypticase Soy Blood Agar, TSBA (24hrs). The individual broths and solid media containing **A.viscosus** T14 and **S. sanguis** G9b were grown at 35°C in an incubator under normal atmospheric conditions. The individual broths and prereduced solid media containing **F. nucleatum** ATCC 25586, **B. intermedius** NCTC 9336 and **A. viscosus** T14 were grown at 35 degrees celsius within a Coy® anaerobic glove box (Laboratory Products Inc), containing an atmosphere of 85% nitrogen,10% carbon dioxide and 5% hydrogen.

3.1.4 Media.

Commercially available broth and media were used in addition to specialised broth and media, with the same batch numbers being employed for the duration of the study. Tryptone Soy Broth, TSB (Oxoid,England) was prepared in one litre quantities, being sterilised by autoclaving at 121°C at 15psi for 15 minutes. On cooling, the broth was transferred to sterile sealable Pyrex No. 9825 glass tubes by a sterile pump method, and refrigerated. TSB for anaerobic growth was immediately transferred to the anaerobic glove box

to allow pre-reduction to occur. The broth was discarded if more than four days old.

The Basal Medium (BM), was prepared as per the recommendations of Shah (1976). The broth was sterilised and handled as per the TSB without refrigeration. Following transfer to the sterile glass tubes, it was placed within the anaerobic glove box, stoppers slightly ajar to allow pre-reduction to occur. Unused broth was discarded after four days.

The solid media Trypticase Soy Agar (TSA. Oxoid, England) was prepared and autoclaved in one litre quantities, as per the TSB. Following autoclaving the media was bench cooled to a point slightly above gelation, 30 mls of defibrinated sheep blood (Media Makers.Padstow,NSW), were added per litre, producing Trypticase Soy Blood Agar (TSBA). Plastic petri dishes were filled with 20 mls of the TSBA pumped under aseptic conditions. The TSBA for aerobic growth was refrigerated at 4°C and unused media discarded after four days. The TSBA for anaerobic growth was immediately transferred to the anaerobic glove box for pre-reduction and unused media discarded after four days.

The selective solid media, Crystal Violet-Erythromycin agar (CVE, Walker et al.1979) and Enriched Trypticase Soy Agar media (ETSA, Syed et al. 1980) were prepared and dispensed as per the TSBA. Both selective media were pre-reduced in the anaerobic glove box and unused media discarded after four days.

3.1.5.Serial Dilution.

For all experiments requiring serial dilution and dispersion of the microorganisms onto solid media, a pre-reduced mineral salt solution was used in this study. Dilution blanks were made with Reduced Transport Fluid (RTF, Syed & Loesche 1972) in disposable 10 ml plastic tubes (I.V. Products,Adelaide S.A.). RTF that was not used within 24 hours was discarded.

3.2 METHODS.

3.2.1 Paper Points.

The investigations into the physical dimensions of the absorbent paper points were to study possible size discrepancies from the ISO dimensions. These were assessed within the same batches of the selected brand, and between the brands. The diameters were measured at 1mm and 12mm from the apex, D_1 and D_{12} respectively. The large variation in the tip diameters as found by Edwards and Bandyopadhyay (1981), was avoided by taking the D_1 reading at 1mm from the apex .

The taper was computed in the manner of Edwards and Bandyopadhyay (1981).

$$\text{Taper(t)} = \frac{D_{12} - D_1}{11}$$

The readings were obtained by using a Wild, Heerbrugg, stereomicroscope model N3, with graticule insert, standardised for reading in the 0.01mm range, following calibration with a machined die. A perspex block of the same height was machined to provide a customised holder with milled 1mm and 12mm lines of thickness 0.001mm at right angles to the paper points. The customised holder contained ten slots of depth, 0.1mm. Illumination was by a standard 60w tungsten filament.

One hundred randomly selected paper points of each brand in the ISO sizes 50 and 60 were measured at points D_1 and D_{12}. The presence of foreign objects and loose surface fibres was noted.

3.2.2 ABSORBENCE

A.Rate.

Fifteen paper points of each brand with the taper equal to the mean ± 1 standard deviation (s.d.) of that brand were selected. The absorbence of the paper points were measured as a function of distance and time. The paper points were placed into a standardised holder then immersed 6 ± 0.5mm from the apex into a 2.5% aqueous solution of Brilliant Green.

Sequential photographs were taken at 10 second intervals until 60 seconds had elapsed. The method used to photograph the paper points is as shown in

plate 3.1, while **plate 3.2** shows the steel millimetre ruler attached at the left side of the paper points. It was visible in all photographs. As will be noticed in **plate 3.2**, the steel ruler contained 2.5mm of blank space prior to the gradations commencing. All readings were corrected for this factor as was the depth of the dye solution. Following calibration for co-incidental reading, the steel ruler provided a means of accurately recording the height of the dye solution in all points at each time interval.

The photographic records were taken on 100 ASA Kodak® ektachrome film, a 90mm medical macro lens and ring flash, at a shutter speed of 1/ 125th of a second.

The photographic slides were then projected onto a white screen with the height of the dye solution being read to the nearest ± 0.5mm. It was noted that this level of accuracy was suitable as the dye solution did not penetrate in an even horizontal manner.

PLATE 3.1

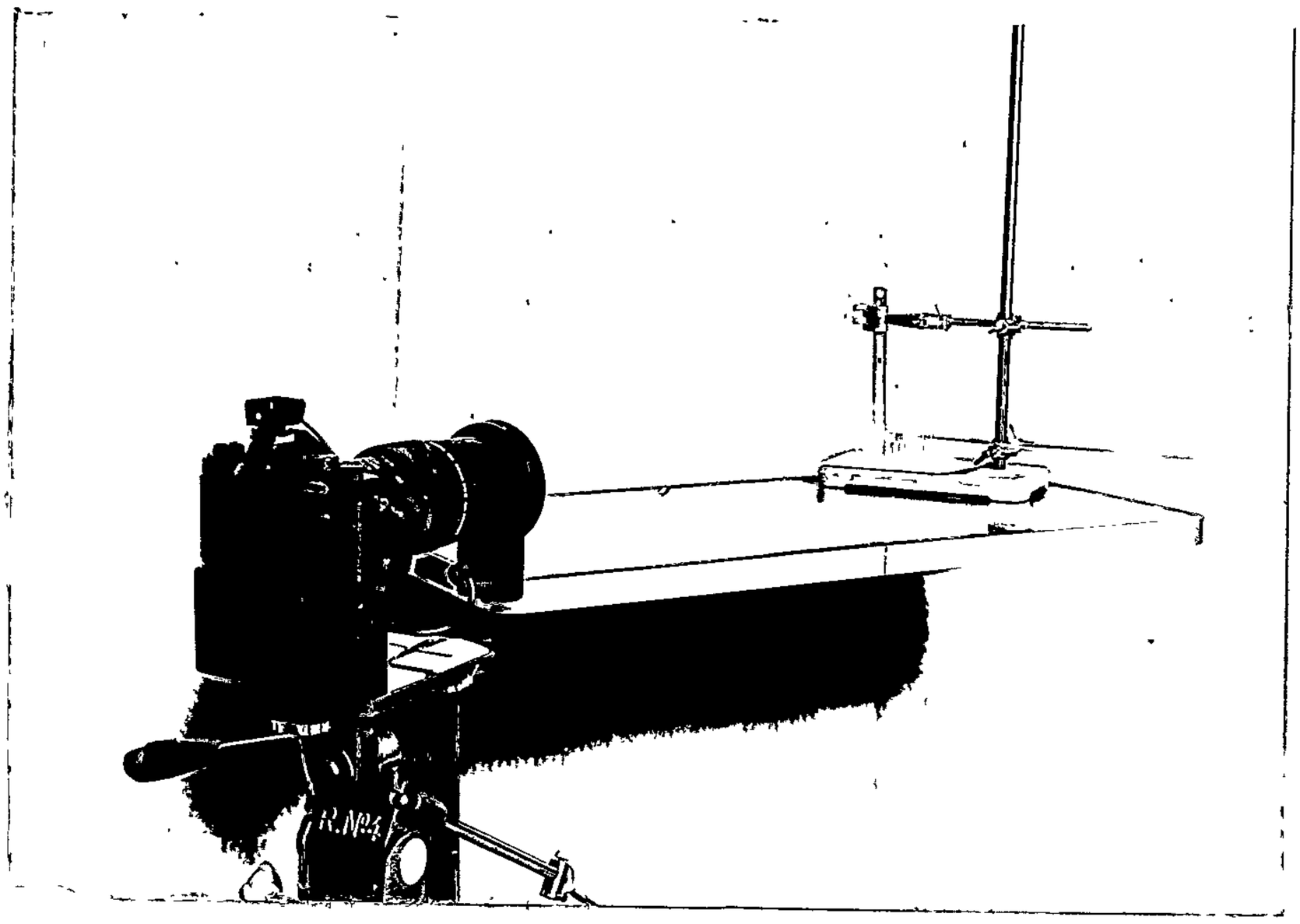


PLATE 3.2

B. Volume.

The approximate volumes absorbed by the paper points were studied by placing increasing volumes from 1µl to 31µl in 2µl steps within microdilution wells (Cook Microtiter System,UK.) and observing the uptake over ten seconds of the 2.5% Brilliant Green dye solution. Sixteen paper points of each brand were selected and tested. The results from the volume absorbed test indicated minimum and maximum values which were found to be approximately 5µl and 20µl respectively.

Prior trial tests had been performed to standardise dilution and plating techniques. Using a 0.1ml sample the serial dilutions were found for each microorganism allowing counts up to 300 colony forming units. The maximum absorbed value of 20µl was used to compute the required dilutions to allow for up to 300 colony forming units per plate and found to be satisfactory.

These were as follows:

a) A. viscosus T14

..... $10^3 - 10^4$

b) F. nucleatum ATCC 25586

..... $10^2 - 10^3$

c) S. Sanguis G9b

..... $10^6 - 10^7$

d) **B. intermedius** NCTC 9336

..... $10^3 - 10^4$

If unequal test and control volumes were used the differential losses through adherence to glassware, pipettes, wire loops and spreaders would be observed. For this reason the 20µl sample was selected for the controls.

3.3. CULTURAL STUDIES.

3.3.1 Growth Conditions.

Aerobic growth, which included both broth and solid media, was carried out at 35°C in an incubator at normal atmospheric conditions.

A. viscosus T14 and **S. sanguis** G9b were always grown in TSB being subcultured and tested when needed onto TSBA. After initial trials, it was observed that **A. viscosus** T14 grew more consistently and faster in the anaerobic glove box. All further study with this microorganism occured within this environment.

Anaerobic growth conditions, which included both broth and solid media, were carried out at at 35°C in a COY® Anaerobic glove box (Laboratory Products Inc.) containing 85% nitrogen, 10% carbon dioxide and 5% hydrogen. The anaerobic environment within the glove box was maintained in accordance with the guidelines for anaerobic culturing supplied by the American

Society for Microbiology, 1980. The platinum-palladium catalyst, a reducing agent, and silica gel trays, for water absorption, were closely monitored and changed as required.

The oxidation-reduction potential (E_h)sensitive indicators, resazurin, and methylene blue, were closely monitored. The glove box conditions were maintained with resazurin and methylene blue being colourless. Resazurin changes to colourless at E_h-42 mv, and methylene blue at - 11 mv.

F. nucleatum ATCC 25586 and **B. intermedius** NCTC 9336 were grown in pre-reduced BM and subcultured onto pre-reduced TSBA for experimental purposes. **A. viscosus** T14 was grown in pre-reduced TSB and subcultured onto pre-reduced TSBA.

F. nucleatum ATCC 25586 and **B. intermedius** NCTC 9336 were incubated for 7-10 days, while **A. viscosus** T14 and **S. sanguis** G9b were incubated for 48 hours, allowing maximum colony formation. The resulting colonies were counted under an illuminated magnifying lens up to a maximum of 300 colonies per plate, after which the plate was recorded as 300^{+}.

3.3.2 Colony Purity.

The Gram positive facultative coccus selected for this study, **S. sanguis** G9b, was frequently checked for purity by Gram staining and the use of the Rapid "STR" system (IDR.Atlanta, Georgia.) for selected biochemical reactions.

The Gram positive facultative branching rod, A. viscosus T14, a recognised dental plaque microorganism, was frequently checked for purity by Gram stain, the catalase test and colony morphology.

The Gram negative staining fusiform, F. nucleatum ATCC 25586 was checked by colony morphology on TSBA, Gram stain and on a selective media containing Crystal Violet and Erythromycin (Walker et al,1979). In addition, F. nucleatum ATCC 25586 was positive for the indole production test.

The Gram negative cocco-bacillus, B. intermedius NCTC 9336 was frequently checked for purity by the Gram stain reaction and the distinctive round glossy black colonies that develop on TSBA and ETSA (at 4 days).

3.3.3 Growth Curves.

The growth curves for each microorganism were plotted as a function of absorbence and time . Colonies of the microorganisms were freshly grown in the respective media and conditions, to provide fresh innoculums (0.1mls) into 10mls of sterile broth in glass tubes (Pyrex No.9825). The resulting change in absorbence with growth, was measured spectrophotometrically at a wavelength of 600nm, using a dual beam spectrophotometer (Spectronic 20, Bausch and Lombe, New York). Control tubes of un-innoculated media allowed calibration of the instrument and comparison of the change in optical density (OD).

The glass tubes were all calibrated for co-incident baseline readings prior

to innoculation. Relocation markings were placed on all tubes. The uneven texture of tubes was observed to create large discrepancies in the baseline readings, requiring this initial calibration.

At selected time intervals the tubes were removed from the respective aerobic or anaerobic environments, vortexed for 30 seconds, then let stand for an additional 15 seconds to allow air bubbles and turbulence to subside. The tubes were placed within the spectrophotometer, and absorbence read as the peak value within 15 seconds.

At selected times, 1ml samples of the broth were removed for serial dilution (in 1/10th steps) in RTF. The serial dilutions were sampled using a sterile micropipette, with 0.1ml being spread onto duplicate TSBA plates via glass rod spreaders. The procedure was performed at the laboratory bench for **S. sanguis** G9b, and within the anaerobic glove box for **A. viscosus** T14, **F. nucleatum** ATCC 25586 and **B. intermedius** NCTC 9336.

The resulting colony forming units per ml(cfu/ml) were counted and transformed to the logarithmic values (log_{10}). These values were plotted against the absorbence to indicate the various log growth phases for each test microorganism. The growth curves for each of the test microorganisms are shown in **figures 3.1, 3.2, 3.3** and **3.4** .

3.3.4. Paper points Sampling Studies.

Five paper points of each brand with taper dimensions within the brand mean ± 1s.d. and ISO size 60 , were selected under aseptic conditions in a

laminar flow cabinet for the aerobic culturing study. The same selection criteria was used for the anaerobic studies, with the containers being left slightly opened for 30 minutes within the anaerobic glove-box to allow for pre-reduction of the paper points prior to commencement. To maintain sterility it was not possible to accurately measure the paper points using the die. The points were observed within the containers to identify paper points of the required dimensions. For this reason, the absolute measurements of the paper points could not be read. The re-sterilisation procedure would require a technique that could be expected not to alter the physical properties of the paper points. The method suited to this study would be gamma radiation. It was felt that this would be impractical in view of the small number of items and the observations of Edwards and Bandyopdhyay (1981) showing no correlation between physical dimension and absorbence

FIGURE 3.1

LOG GROWTH CURVE: A. viscosus T14.

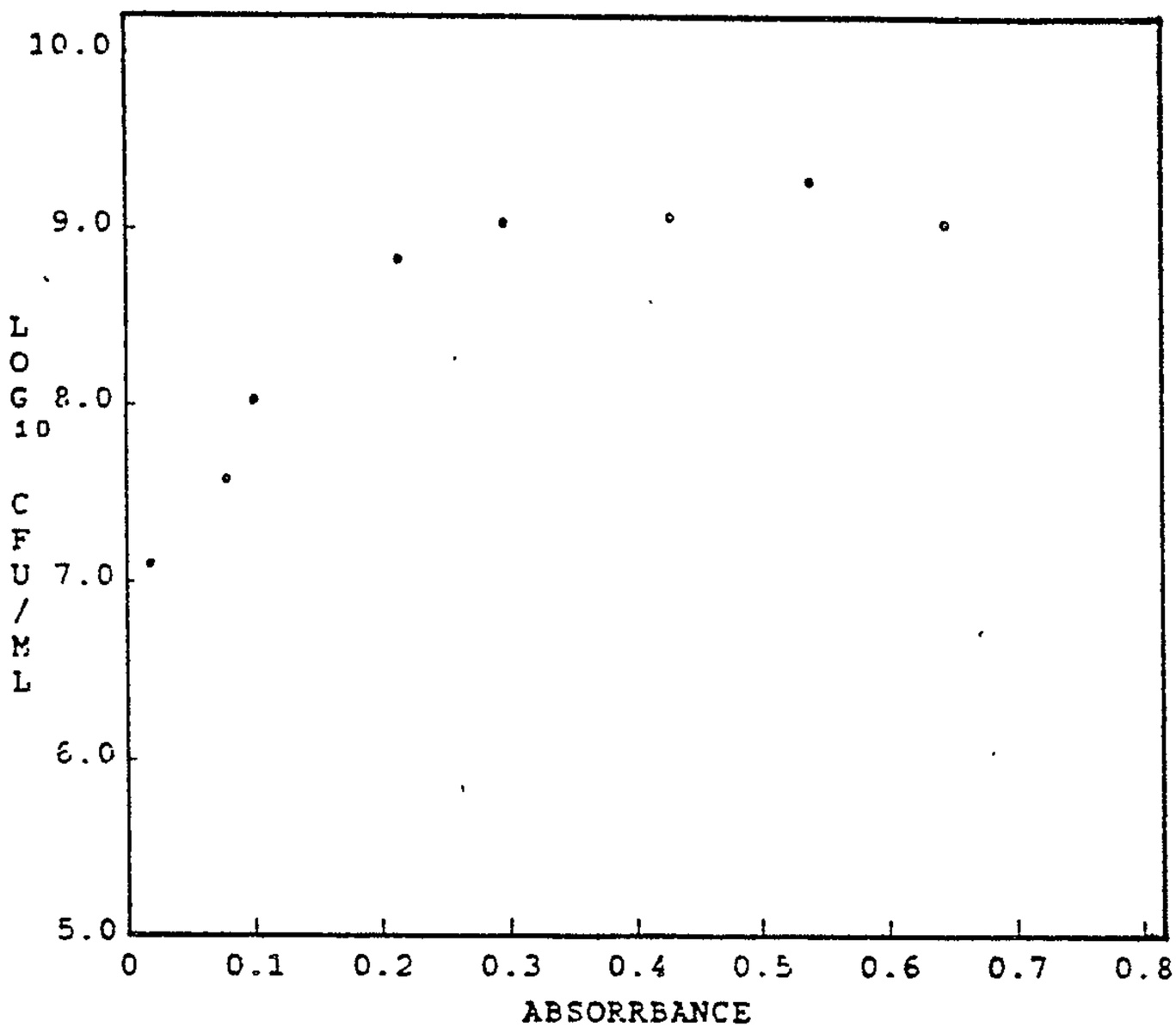


FIGURE 3.2

LOG GROWTH CURVE: S. Sanguis G9b.

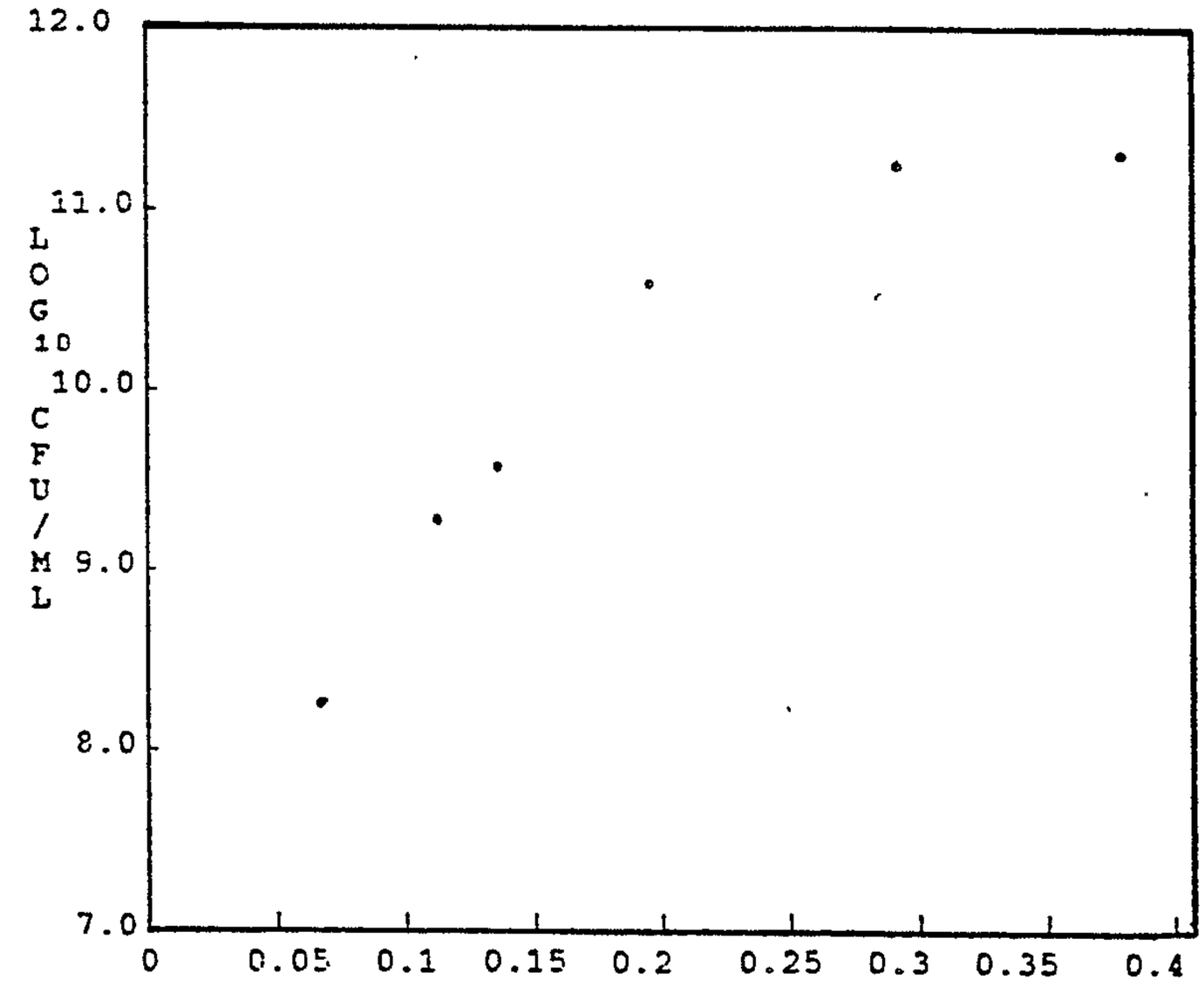


FIGURE 3.3

LOG GROWTH CURVE: B. intermedius NCTC 9336.

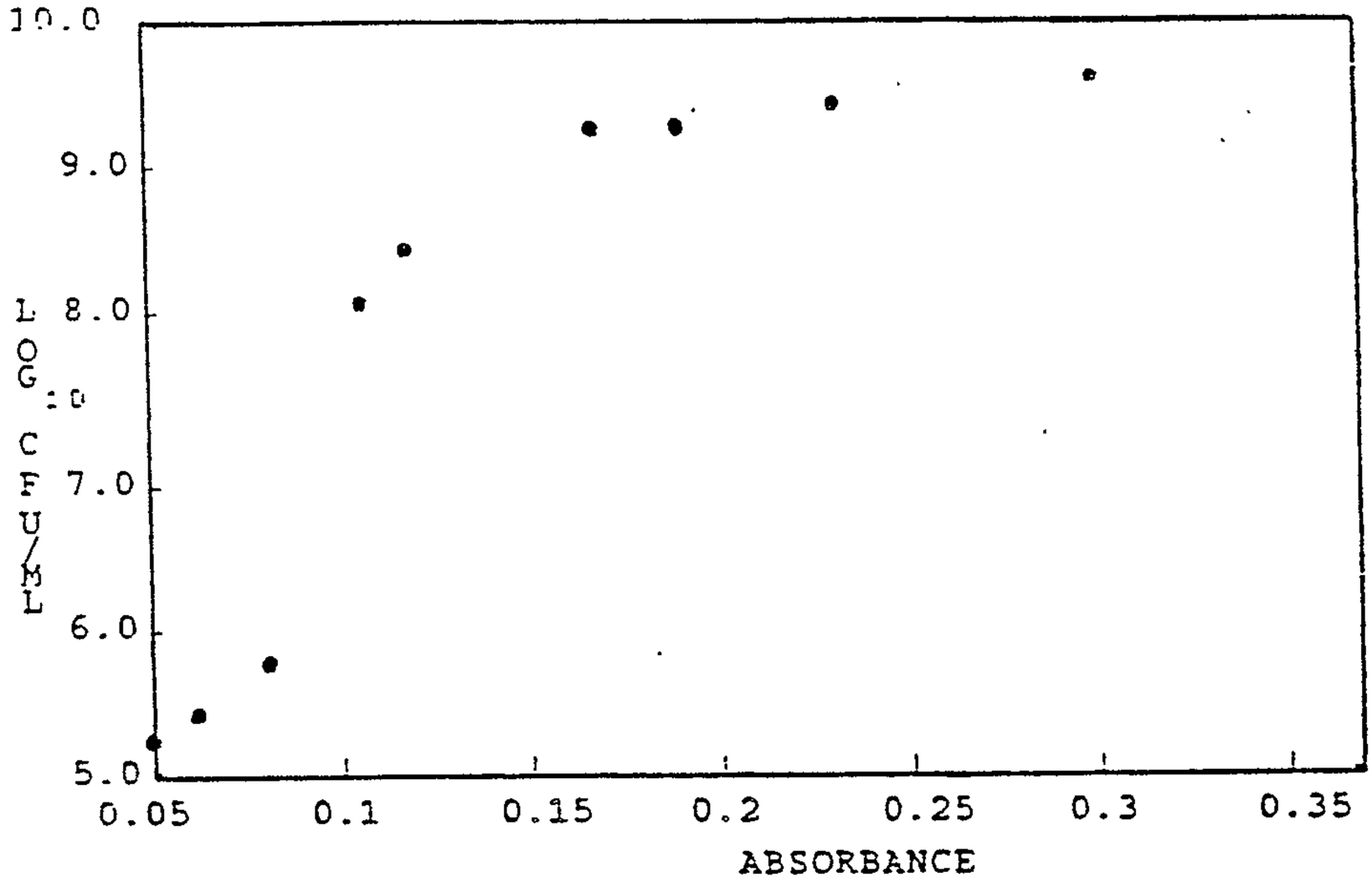
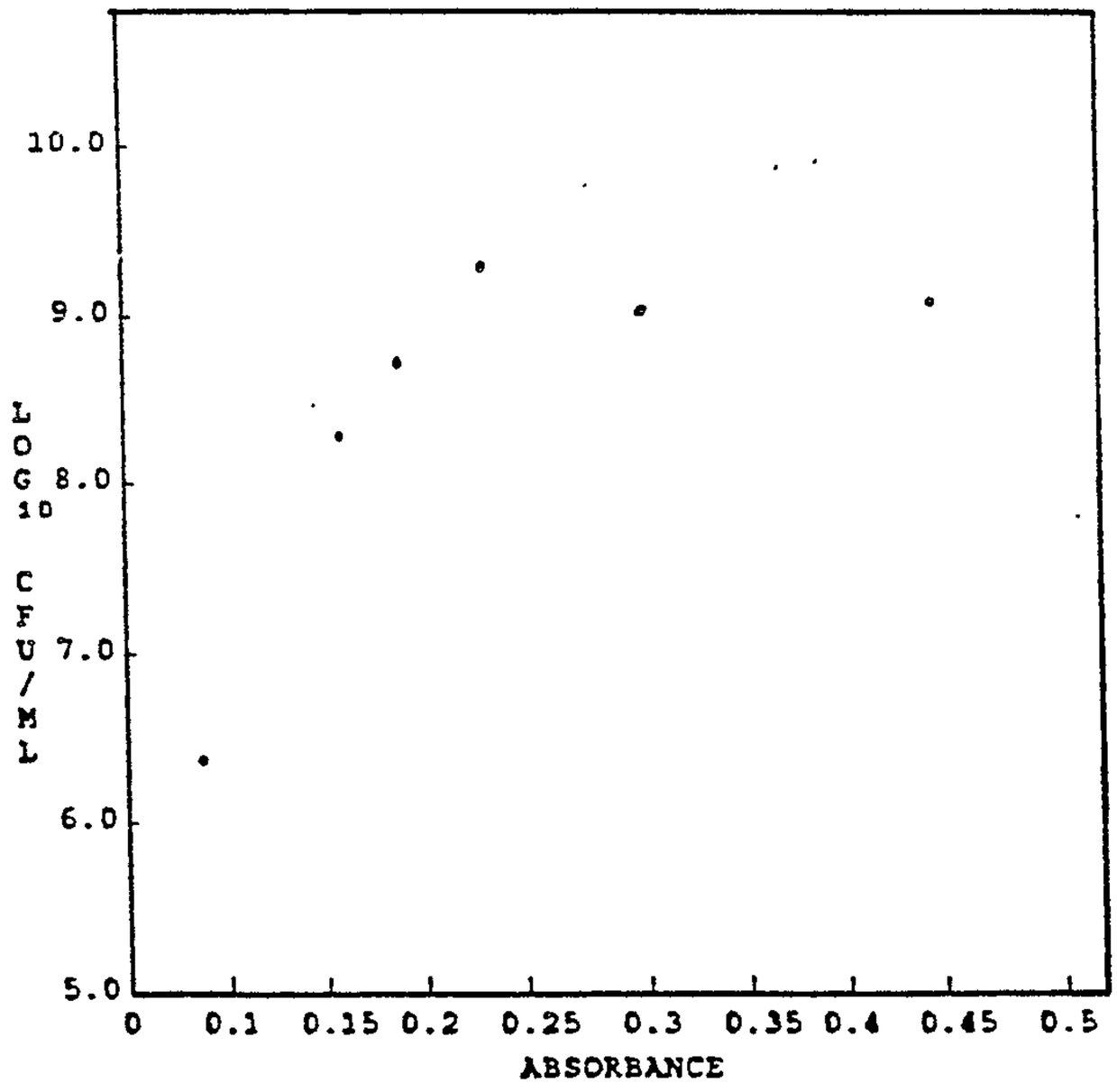


FIGURE 3.4

LOG GROWTH CURVE: F. Nucleatum ATCC 25586.



The test bacteria were grown to the required optical density corresponding to the late log growth phase and known concentrations of microorganisms. The 25 paper points were then placed individually into separate wells of a microdilution blank to which 200µl of the culture media had been added. It had been determined that 200µl would fill a well to 6 ± 0.5mm depth. The paper points were immersed to the depth of 6 ± 0.5mm, for a timed 10 second interval. Each paper point was placed into 10ml of RTF following sampling. The control well contained 200µl of the culture media, after the sampling time of 10 seconds, 20µl were micropipetted into 10ml of RTF. All resulting 27 tubes were vortexed for 60 seconds.

The sampling procedure and vortexing, were performed in the anaerobic glove box for the anaerobic bacteria and at the laboratory bench for the aerobic bacteria.

The tubes were given a further 10 second vortexing period prior to serial dilution in 1/10th steps for plating. The plating order is as shown in **Table 3.1.** This method was considered to avoid bias in the time each brand of paper point spent in the RTF, producing a uniform death rate across the brands. The tubes of RTF were maintained in a water bath at 35°celsius.

Dispersion was performed by glass rod spreading of 0.1ml of the RTF solution onto the media plates. Each dilution was duplicated, as were the control plates. The control plates were taken at the start of dispersion and at the completion, allowing the death rate to be observed. The total time from sampling to the end of plating was 2 hours 15 minutes ±10 minutes.

TABLE 3.1

Table 3.1 Sampling and Plating order.

Brand	Order*				
Control(start)	1				
PD	2	7	12	17	22
PROGRESS	3	8	13	18	23
ROEKO(usa)	4	9	14	19	24
ROEKO(ulm)	5	10	15	20	25
SURE-ENDO	6	11	16	21	26
Control(end)					27

The sampling and plating order commenced at No.1.

Counting of the colony forming units was manually performed using an illuminated magnifying lens. The first 300 cfu were counted, additional uncounted units were indicated by a superscript 300^{+}.

3.3.5 Controls.

The control samples for the cultural studies were taken to represent the bacterial recovery rate of the maximum volume of the most absorbent point. To allow for a relative bacterial loss rate between the controls and paper point samples, the 20 µl volume was selected in preference to the 1ml sample. The loss rate via adherence to plastic and glassware while within the RTF, would exhibit a closer relation than if relatively unequal sample sizes were to be used. This effect was studied by Manganiello et al. (1977), and shown to account for approximately a 5 percent loss of viable microorganisms. The relative magnitude of this loss rate increased as the culture solution underwent higher dilutions.

The volume of fluid absorbed was only measured in a crude fashion. It was accepted that the cfu obtained from the paper point sampling could be higher than the control. This eventuality was not considered important as the controls were designed to monitor bacterial death rate and relative loss rate. The control samples were taken at the beginning and end of sampling, allowing the death rate while being maintained in the RTF to be observed.

3.4 Biological Activity.

Ørstavik and Möller(1985), Möller & Hensten-Pettersen (1985) and Rawle, Adams & Witherley(1985), have reported some features which test the biological activity of endodontic (absorbent) paper points. These studies included tests for haemolytic and antibacterial activity, as well as the determination of the sterility of prepackaged paper points. These properties were tested for the 5 brands.

3.4.1 Haemolytic Activity.

Five paper points of each brand were placed onto the surface of each of three TSBA media plates. The plates were incubated at 35°C for a 24 hour period and the resulting zones of haemolysis observed and recorded photographically.

3.4.2. Antibacterial Activity.

The antibacterial activity of each brand of paper point was tested by laying 5 points onto the surface of each of three TSBA plates. The procedure was repeated for each brand. Each plate had been seeded with a lawn of an indicator strain of Staphylococcus aureus , kindly supplied by the Institute of Dental Research, Sydney, and was incubated aerobically at 35°C for twelve hours. The resulting zones of inhibition were observed and photographed.

3.4.3. Sterility.

The sterility of sixteen, randomly selected paper points of each brand was tested by placing individual points into glass tubes containing 9ml of TSB medium and then incubating eight tubes aerobically at 35°C for seven days, and eight tubes anaerobically at 35°C for seven days. The optical density (OD), was measured against control tubes of the medium without paper points, at daily intervals. Any indication of growth under either condition was to be plated out onto TSBA plates for culturing aerobically and anaerobically.

3.5. Microscopy.

This aspect of the study included an examination by light microscopy of the paper points following sampling of each bacterial culture. It was considered to be of importance to determine the position of the bacteria in relation to the layers of the cellulose sheets comprising the paper point. Two methods were used to prepare the paper points following a ten second sampling period in 200µl of the cultured media, grown to late log growth phase and contained in a microdilution blank well.

3.5.1.

The first method involved direct freeze drying for 12 hours, followed by routine histological preparation in paraffin wax, sectioning and then staining with haematoxylin and eosin.

3.5.2.

The second method used fixation in paraformaldehyde/lysine periodate, with washing in 0.1M sodium cacodylate prior to dehydration in acetone and embedding in glycol methacrylate. The sections were stained with haematoxylin and eosin.

The test microorganisms were not observed within the body of the paper point or upon the surface, in either method.

3.6. Statistical Analysis.

The statistical analysis involved both parametric and non-parametric analysis of the data generated by the study. The comparison of absorbence and paper point brand was computed at the time intervals 10, 30 and 60 seconds. The analysis observed the variance between brands and between time frames for the optimum time point at which least variance would exist. Bartlett's Test for common variance was performed at each time interval.

The colony forming unit counts were compared between brands and between the different test microorganisms, using the one way analysis of variance (ANOVA) and the Kruskal-Wallis test. The comparison within a brand, between the different microorganisms was performed using the Friedman two way analysis of variance. In addition to these analyses, a parallel regression model was developed. The model was designed to take into consideration the

effect of the death rate of each microorganism on the relative time a particular paper point spent in the RTF.

The analyses were performed by the University Computing Service, University of Sydney, NSW, using the programmes of the Statistical Package for the Social Sciences (SPSS) on a Vax mainframe computer.

CHAPTER 4.

RESULTS.

4.1 PAPER POINTS

The results of the investigation on the paper points found that none of the brands were consistently within the physical dimensions suggested by the International Standards Organisation (ISO). The contents of **Table 4.1**, showing taper, mean measurements of D_1 and D_{12}, the range for all brands and the computed ideal ISO measurements, indicate significant variation from the standard. The taper values, when compared to the **ISO** standard, show the **PROGRESS** brand to have a significant variation. The remaining 4 brands tested were within the 50% tolerance limit. The computed **ISO** diameters for D_1 and D_{12}, when compared to the 5 tested brands, indicate that only the two **ROEKO** brands ($\mu \pm 1sd$) did not lie within the value, as shown by **figure 4.1.** These two brands were below the computed ISO values. The range for all tested brands at the D_1 and D_{12} measurements were wide, the D_{12} range of the **ROEKO**(ulm) brand being the only range not to include the ISO value within it.

Table 4.1 Physical Dimensions.

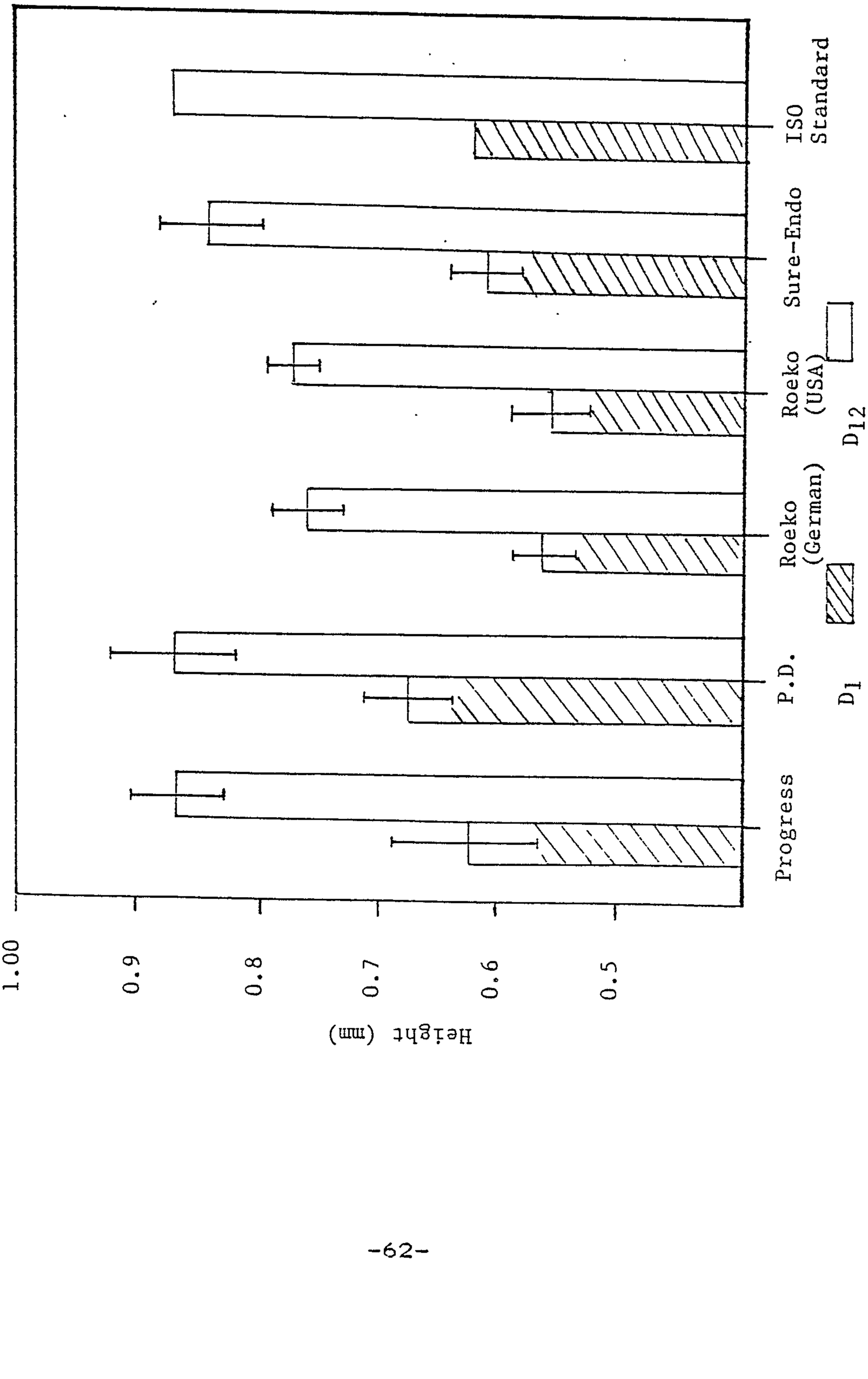
Brand*	taper $_{\mp 25\,pct.}$	Diameter (mm) $D_{1\,\mu\mp 1\,sd.}$	$D_{12\,\mu\mp 1\,sd.}$	Range (mm) D_1	D_{12}
ISO(Ideal)	0.0200 ± 0.005	0.62	0.84		
PD	0.0198 ± 0.009	0.62 ± 0.06	0.86 ± 0.04	0.55 0.84	0.77 0.97
PROGRESS	0.0167 ± 0.006	0.67 ± 0.04	0.86 ± 0.05	0.55 0.73	0.75 0.99
ROEKO(usa)	0.0189 ± 0.006	0.56 ± 0.03	0.75 ± 0.03	0.51 0.66	0.68 0.88
ROEKO(ulm)	0.0187 ± 0.006	0.55 ± 0.03	0.76 ± 0.02	0.48 0.70	0.70 0.81
SURE-ENDO	0.0203 ± 0.004	0.61 ± 0.03	0.84 ± 0.04	0.55 0.73	0.77 0.97

* ISO size 60 paper points.

FIGURE 4.1

PAPER POINT MEAN DIAMETERS ± 1 STANDARD DEVIATION

Size 60 n = 100



4.2. ABSORBANCE

The absorbance study indicated that the method of manufacture may create differences in the manner in which the dye solution moved within the tested paper points. The two **ROEKO** and the **PROGRESS** brands, were consistently even in the manner in which the dye solution rose in the paper points. The **PD** and **SURE-ENDO** brands showed large variations, with the dye solution moving in a random manner through the points. It was also observed that embedded impurities provided obstacles to the dye solution as it moved upwardly within the paper points, an example is shown in **Plate 4.1.** The angular manner in which the dye solution is moving required that ± 0.5mm be allowed in the measuring procedure.

4.2.1 Time

The results of the investigation into the absorbance of the tested paper points were analysed at the 10, 30 and 60 second time intervals. The analysis was performed to provide a sampling time, where the least variability between the brands was occuring. This approach was designed to provide a baseline for additional study on the effect of different sampling times on the relative recovery rates of test microorganisms. The mean values for the observed time intervals are shown in **Table 4.2,** while **figures 4.2** shows a difference between PD and **SURE-ENDO** brands, with the

PLATE 4.1

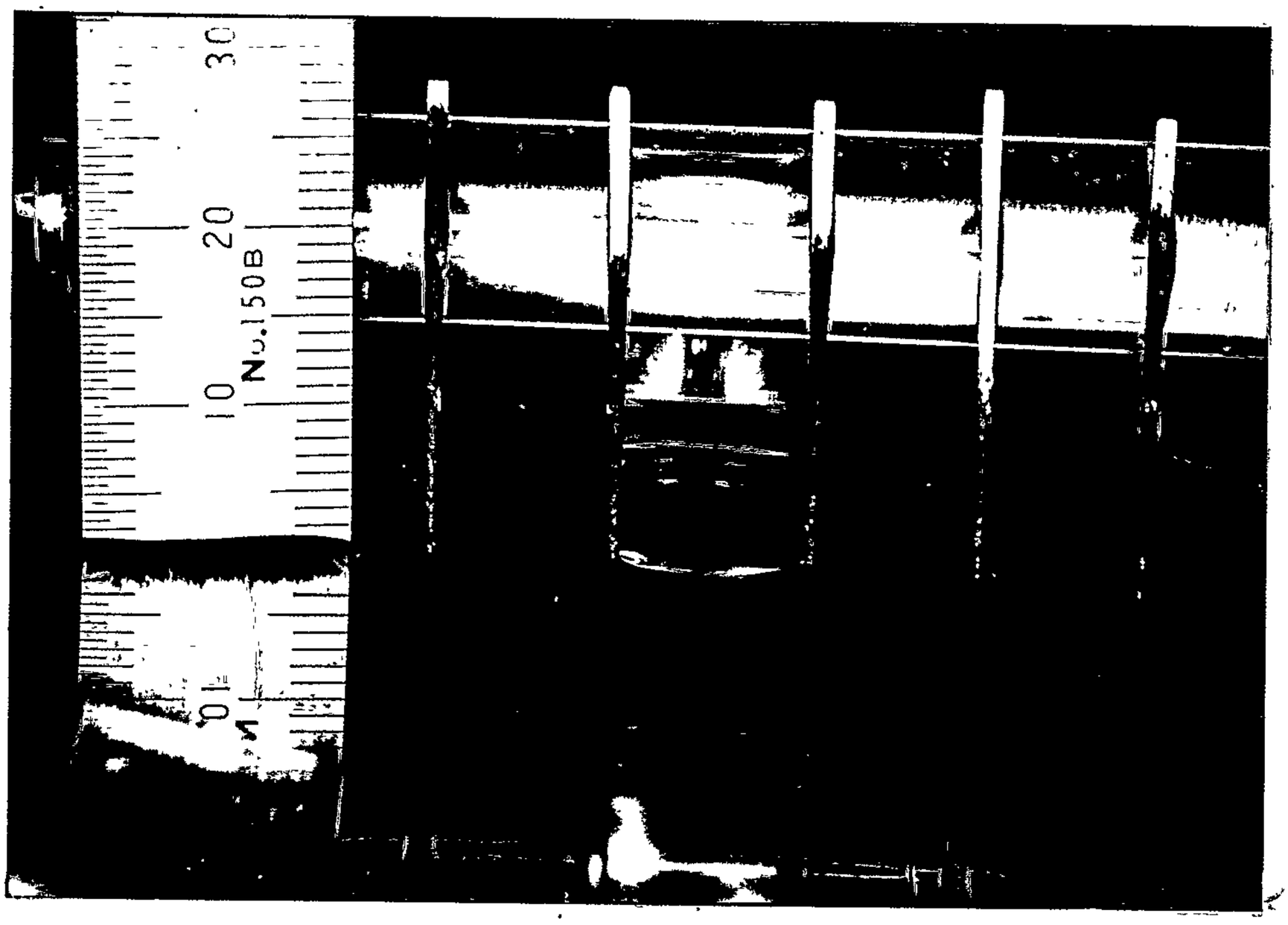


TABLE 4.2

Table 4.2 Mean Absorbance (mm).

Brand	Means 10 (sec)	30 (sec)	60 (sec)	Variance 10 (sec)	30 (sec)	60 (sec)
PD	7.433	9.667	13.933	1.96	4.738	4.674
PROGRESS	8.233	14.067	20.2	5.745	10.46	16.064
ROEKO(usa)	9.1	12.933	16.533	0.507	1.531	2.41
ROEKO(ulm)	8.767	12.833	16.133	0.352	0.774	1.267
SURE-ENDO	11.9	17.867	23.4	5.007	8.552	17.221

mean values alone showing the similarity between the two ROEKO brands and the apparent early (up to 30 seconds) relation of the PROGRESS brand. **Figure 4.2** demonstrates the degree of overlap that occurred between the brands and has been separated into the individual brands; in **figures 4.3, 4.4, 4.5, 4.6 and 4.7.** The uniform nature of the two ROEKO brands is evident in **figures 4.5, 4.6.** The variability of individual points within the other three brands can be observed in **figures 4.3, 4.4 and 4.7.**

The Bartlett's Chi-square test for common variance was performed at each time interval.The test assumes that each brand has the same variance. .

using the formula: $$V = \frac{[70Ln(MSE)-14\Sigma Lns^2}{1+{}^1/_{12}({}^1/_{70}+{}^4/_{14})}$$

where the MSE is the mean squared error, and s^2 is the variance.

FIGURE 4.2
PAPER POINT ABSORBANCE
Means ± 1 Standard Deviation

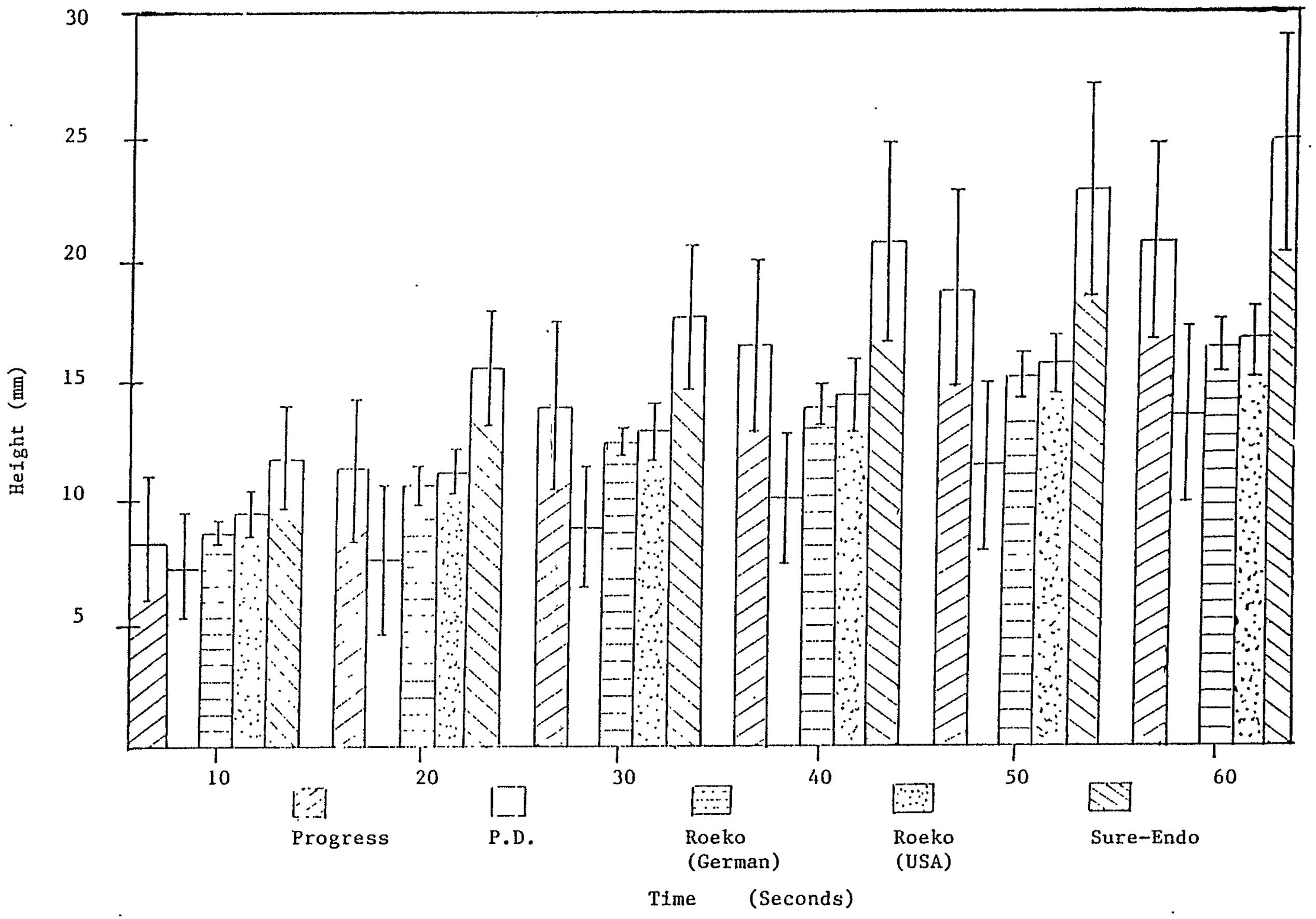


FIGURE 4.3
PROGRESS PAPER POINT ABSORBANCE
Mean ± 1 Standard Deviation

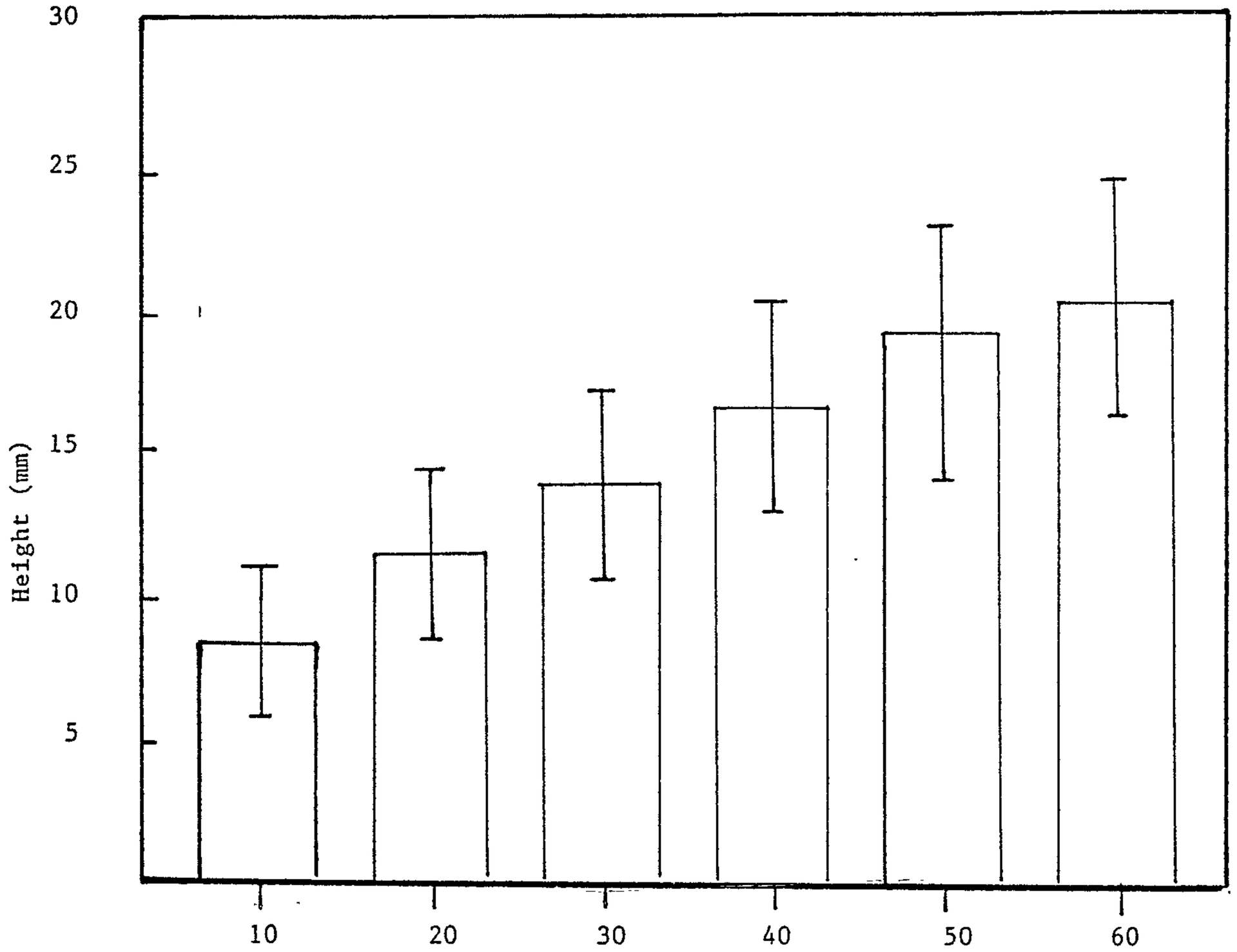


FIGURE 4.4
P.D. PAPER POINT ABSORBANCE
Mean ± 1 Standard Deviation

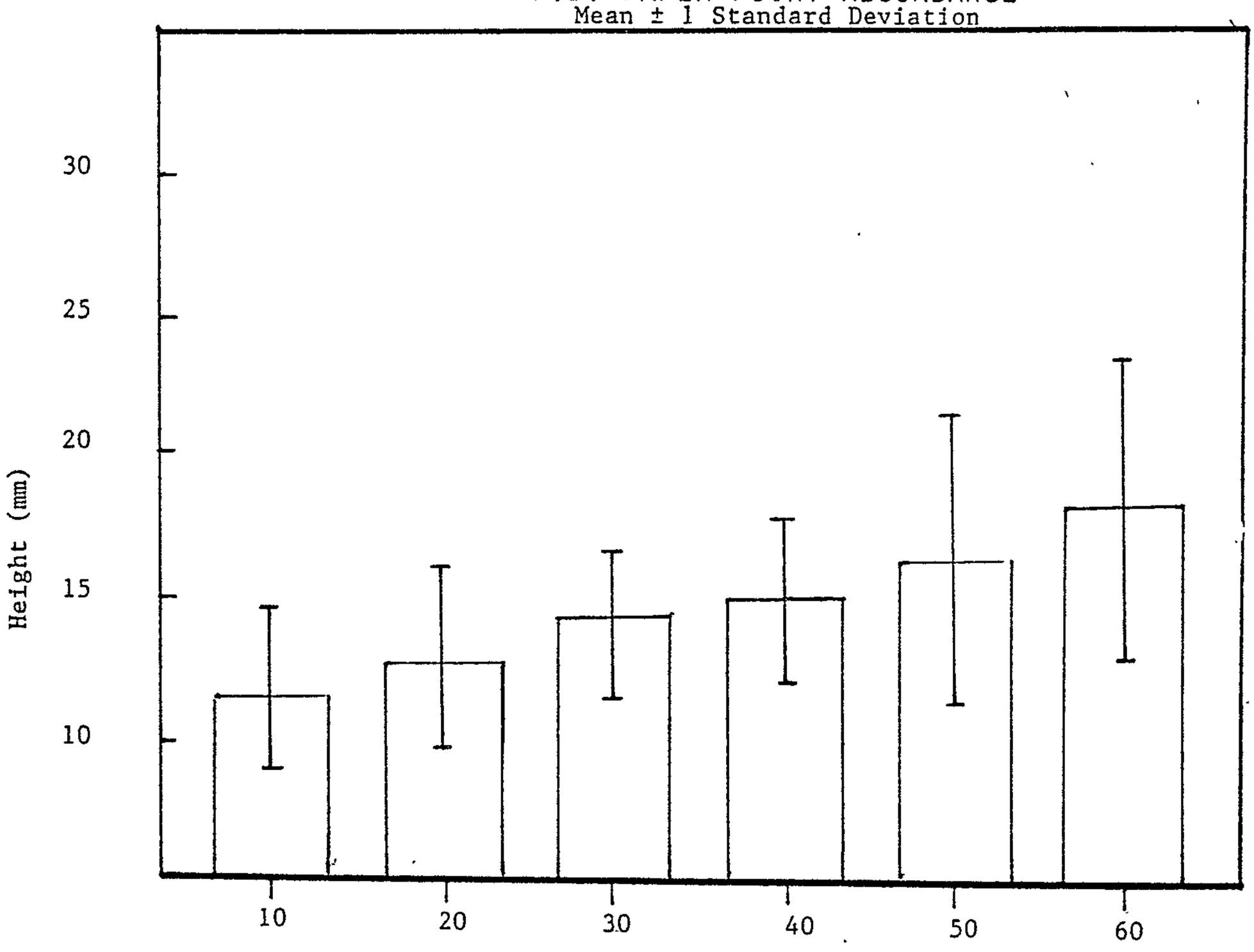


FIGURE 4.5
ROEKO (GERMAN) PAPER POINT ABSORBANCE
Mean ± 1 Standard Deviation

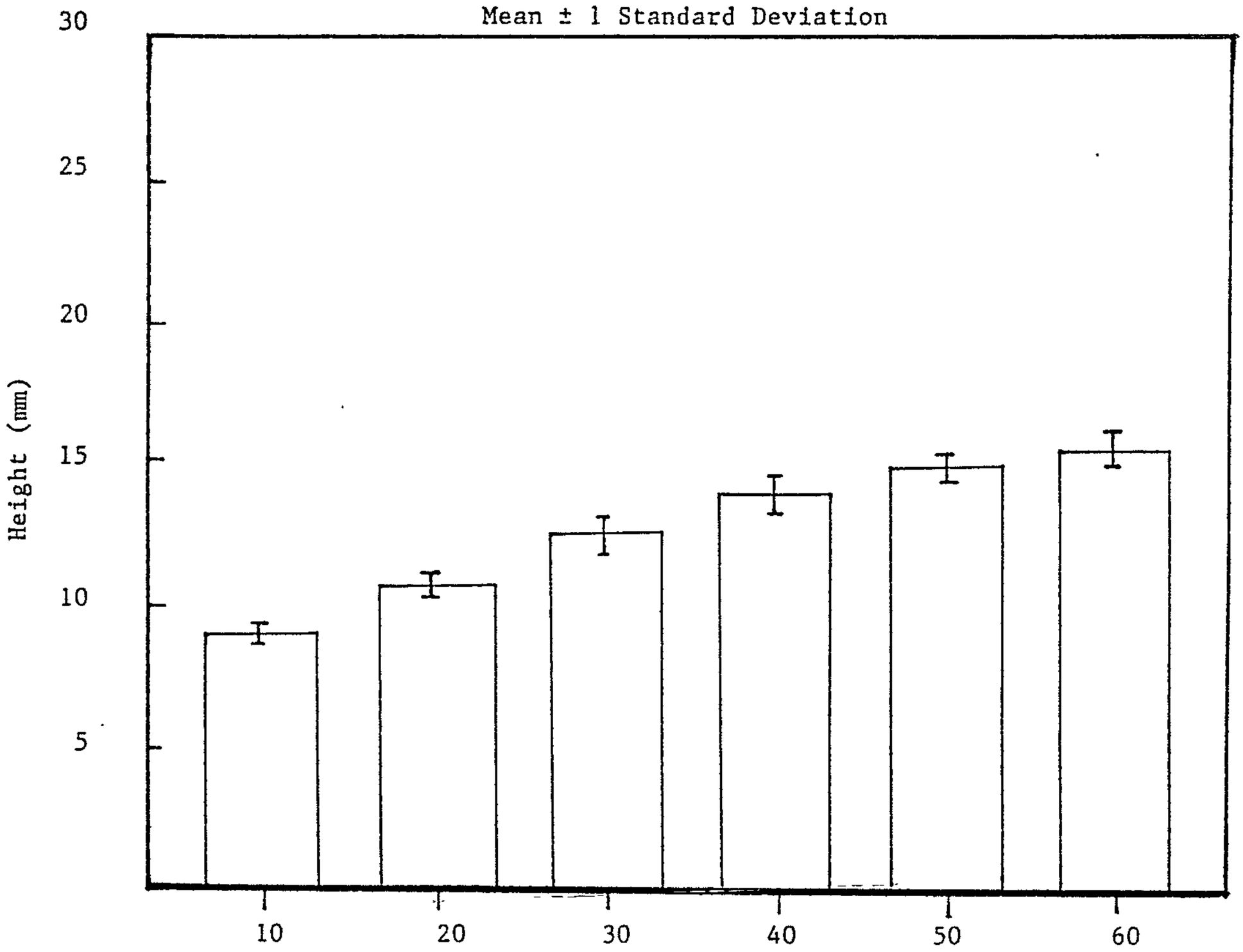


FIGURE 4.6

ROEKO (USA) PAPER POINT ABSORBANCE

Mean ± 1 Standard Deviation

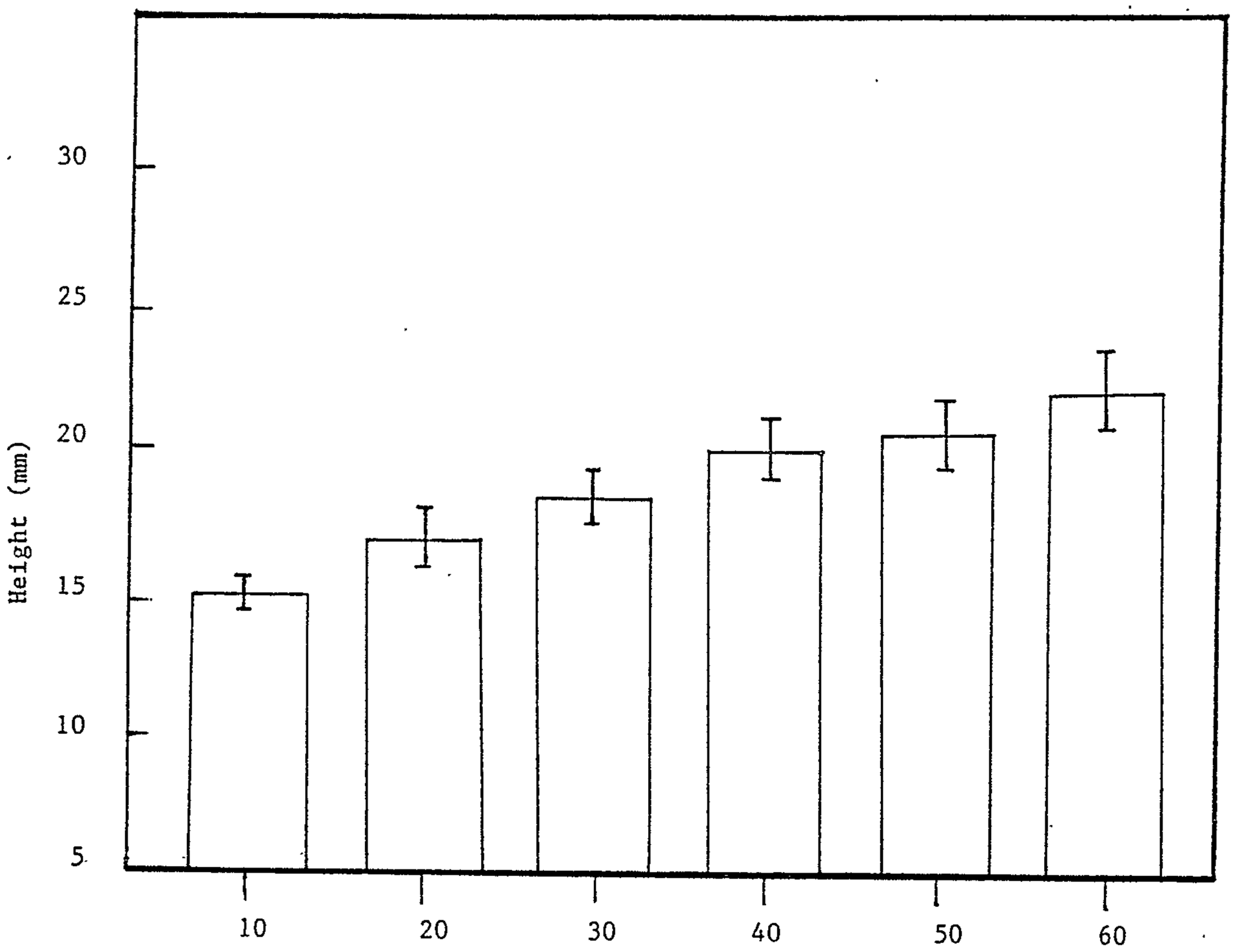
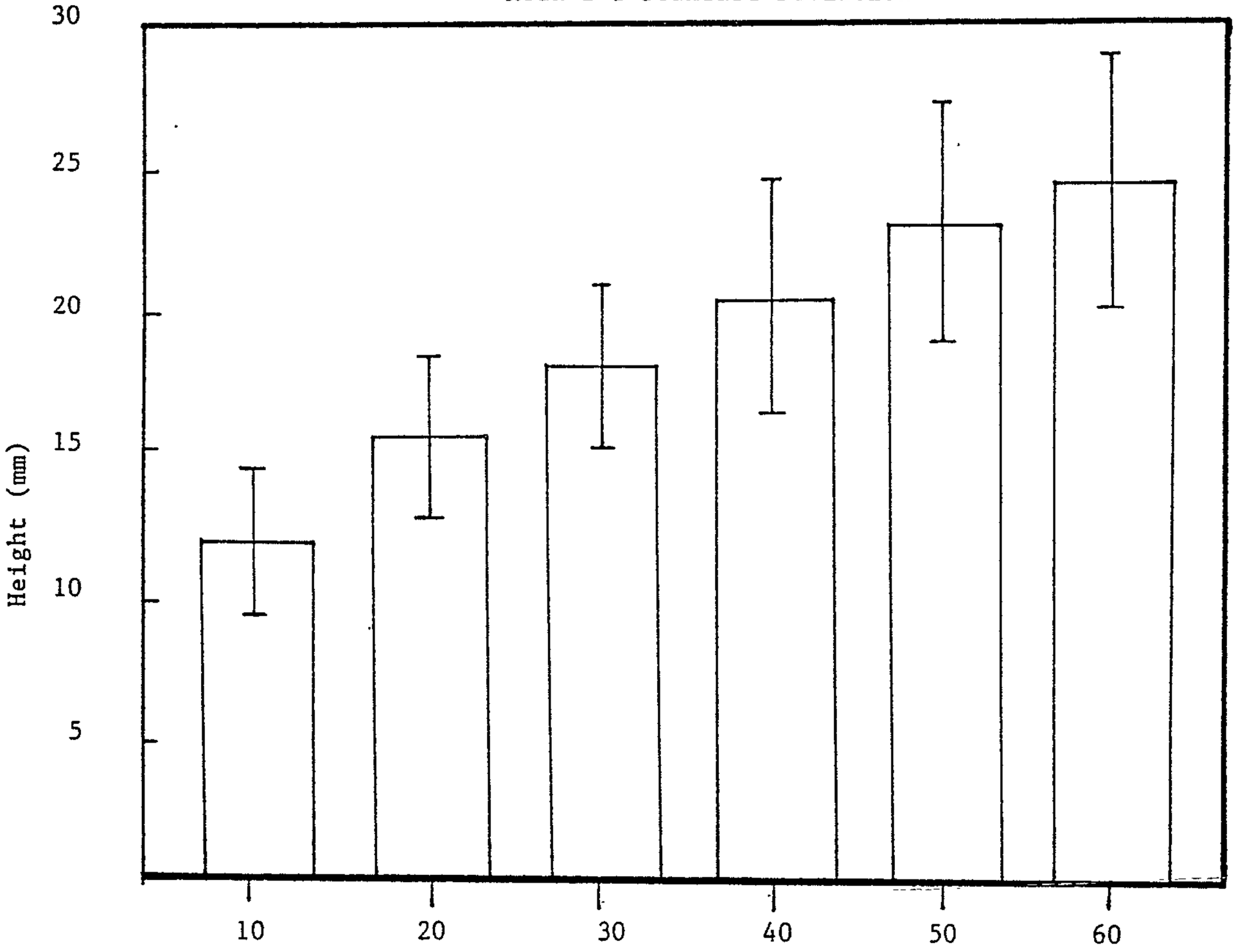


FIGURE 4.7
SURE-ENDO PAPER POINT ABSORBANCE
Mean ± 1 Standard Deviation



4.2.2 10 Seconds

The MSE at 10 seconds = 2.7143.

Therefore, V = 36.658 ,cf χ^2_4

and $\chi^2_4(.9995) = 70.0$.

The significance probability of this test is less than 0.0005. Indicating a highly significant difference exists in the variation of the tested brands. In view of the result, the analysis of variance test (ANOVA), was not justified.

The means and variances of the 5 brands, at 10 seconds are shown in **Table 4.2** . The two **ROEKO** brands show the least variability as there is no evidence to support a claim of any difference between them. This was tested by taking the null hypothesis ;

H_o: $\sigma^2_{R\{USA\}} = \sigma^2_{R\{ULM\}} = \underline{0.0507} = 1.44$ p = 0.352

The p value was greater than 0.25 (from $F_{14,14}$ tables). The null hypothesis was accepted. The **PROGRESS** and **SURE-ENDO** brands have the greatest variation.

The 95% confidence intervals for the mean absorbance at 10 seconds :

$y_i \pm t_{14}(.975)\underline{s}_i$, $t_{14}(.975) = 2.145$

are shown in **Table 4.4.** The mean confidence interval for the **SURE-ENDO** does not overlap the other test brand intervals. This indicates a higher mean absorbance than the other tested brands. The two **ROEKO** brands can be taken as having the same mean absorbance and variability at 10 seconds. The PD brand was lowest overlapping the 3 middle brands.

4.2.3. 30 Seconds

The Bartletts's test for common variance indicates an MSE of 5.211. V = 27.79 , cf χ^2_4 p < 0.0005.

The p value indicates that significant differences in the variability exist at the 30 second time interval.The two **ROEKO** brands were least variable at this time interval and could be taken as having the same variance.

$S^2_{R\{USA\}} / S^2_{R\{ULM\}} = 1.978$, cf $F_{14,14}$

p = 0.11

Table 4.4 Absorbance (95% confidence intervals).

Brand	Absorbance (mm) 10 sec.	30 sec.
PD	7.433 ± 0.775 (6.65,8.21)	9.667 ± 1.206 (8.46,10.87)
PROGRESS	8.233 ± 1.327 (6.91,9.56)	14.067 ± 1.791 (12.28,15.86)
ROEKO(usa)	9.1 ± 0.394 (8.71,9.49)	12.933 ± 0.685 (12.25,13.62)
ROEKO(ulm)	8.767 ± 0.329 (8.44,9.10)	12.833 ± 0.487 (12.35,13.32)
SURE-ENDO	11.9 ± 1.239 (10.66,13.14)	17.867 ± 1.619 (16.25,19.49)

Table 4.4 Continued.

Brand	Absorbance (mm) 60 (sec)
PD	13.933 ± 1.197 (12.74,15.13)
PROGRESS	20.2 ± 2.220 (17.98,22.42)
ROEKO(usa)	16.533 ± 0.860 (15.67,17.39)
ROEKO(ulm)	16.133 ± 0.623 (15.51,16.76)
SURE-ENDO	23.4 ± 2.298 (21.10,25.70)

The 95% confidence intervals for the mean absorbance at 30 seconds are shown in **Table 4.4.** The **PD** brand has shown significantly less mean absorbance than the other tested brands.The **SURE-ENDO** brand continued to show maximum mean absorbance and the two **ROEKO** brands could be taken as having the same mean absorbance and variability. The **PROGRESS** brand was similar to the two **ROEKO** but more variable.

4.2.4 60 Seconds

The data recorded at 60 seconds produced an MSE of 8.327.The variance, V was 31.64 with a very significant p value of p << 0.0005. This indicates that significant differences exist in the variability of the brands. The two **ROEKO** brands were the least variable and as found at the 10 and 30 second intervals they could be taken as having the same variance.

$S^2_{R\{USA\}} / S^2_{R\{ULM\}} = 1.90$, cf $F_{14,14}$

$p = 0.12$

The 95% confidence intervals for mean absorbance at 60 seconds are shown in **Table 4.4.** The **PD** brand shows no overlap with the other brands and has the lowest mean absorbance. The two **ROEKO** brands could be taken as equal and were less than **PROGRESS** and **SURE-ENDO.** The latter two brands now show substantial overlap.

4.3 BACTERIAL COUNTS

The time that any particular paper point spent in the reduced transport fluid (RTF) was considered to be a potential source of bias. The bias would arise from the death rate during the experimental procedure not being applied equally between brands. The sampling and plating order (Table 4.3) used was considered to reduce this potential source of bias.

For the analysis of the bacterial count data generated from the 4 test microorganisms and 5 brands of paper points, a parallel regression model was developed. The model was required to statistically assess any influence that death rate may have had on the structured sampling procedure and the resulting bacterial counts. It was assumed that the number of bacteria at risk (or available in the pool) varies linearly with time. Using this assumption, a parallel regression model was constructed for each microorganism.

The control start and control end bacterial counts provided the endpoint data for the counts to be compared with the slopes of the regression lines.

TABLE 4.3

Sampling and Plating order.

Brand	Order*				
Control(start)	1				
PD	2	7	12	17	22
PROGRESS	3	8	13	18	23
ROEKO(usa)	4	9	14	19	24
ROEKO(ulm)	5	10	15	20	25
SURE-ENDO	6	11	16	21	26
Control(end)					27

The sampling and plating order commenced at No.1.

Therefore at time X_i, brand J yields Y_{ij} bacteria.

assume: $Y_{ij} = \alpha_{0j} + \alpha_{1j}x_i + \Sigma_{ij}$

where $_j = 1,2....5$

$_i = 1,2....5$

$x_i = _j + 5(_i-1)$

$\Sigma_{ij} \cong n(o,\sigma^2)$

$_j = 1 =$ PROGRESS

2 = PD

3 = ROEKO(ULM)

4 = ROEKO(USA

5 = SURE-ENDO

The model was applied to the 4 different test bacteria and expressed as a p value.

4.3.1 Streptococcus. sanguis G9b

The test for parallel regression (ie that the change in number varies over time in the same way for all brands) gave an F value of 0.709 ,cf $F_{14,15}$. The p value is > 0.5 with p = 0.60. There is no significant difference in brand count dependent on time. The test for dependence on time of the mean bacterial counts gave an F value of 1.29,cf $F_{5,15}$. The p value was >0.25, with p = 0.32 concluding that no variation was present in the mean bacterial counts with time.

The results of the averagé bacterial counts for the tested brands are shown

in **Table 4.5** and indicate the following ranking.

PD < ROEKO(ulm) < SURE-ENDO < PROGRESS/ROEKO(usa)

The difference between **PROGRESS** and **ROEKO(usa)** gave an F value of 0.238, cf $F_{1,20}$. The p value being > 0.5, with p = 0.63. It was concluded that the two brands had the same mean bacterial counts. The variance estimate was $(23.53)^2$.

4.3.2 Actinomyces viscosus T14

The test for parallel regressions gave an F value of 3.208, cf $F_{4,15}$. The p value of 0.043 indicated a marginal significance. The result was influenced by the strong dependence of the **ROEKO(usa)** data on time. The test for dependence on time, F = 2.69 cf $F_{5,15}$, gave a p value of 0.0625 demonstrating that overall there was no dependence on time. While the test for overall differences between brands indicated a highly significant difference existed with F = 10.17, cf $F_{4,20}$,and a p value of p < 0.0002.

The average bacterial counts for the 5 tested brands are shown in **Table 4.5**, producing the following rankings;

PD < PROGRESS/SURE-ENDO/ROEKO(ulm) < ROEKO(usa)

TABLE 4.5

BACTERIAL RECOVERY COUNTS.

Mean ± 1 Standard Deviation

Actinomyces Viscosus T14

	10^3	10^3 dup	10^4	10^4 dup
Progress	69 ± 45	72 ± 65	9 ± 11	9 ± 10
P.D.	9 ± 5	10 ± 6	2 ± 1	4 ± 1
Roeko (german)	89 ± 56	79 ± 52	16 ± 13	12 ± 10
Roeko (usa)	188 ± 66	187 ± 59	221 ± 6	9 ± 7
Sure-endo	71 ± 41	49 ± 16	6 ± 5	6 ± 4

Bacteroides Intermedius NCTC 9336

	10^3	10^3 dup	10^4	10^4 dup
Progress	141 ± 45	130 ± 60	38 ± 51	47 ± 44
P.D.	12 ± 6	7 ± 8	3 ± 1	2 ± 1
Roeko (german)	81 ± 12	78 ± 28	9 ± 4	12 ± 6
Roeko (usa)	114 ± 6	118 ± 14	18 ± 6	20 ± 6
Sure-Endo	69 ± 29	71 ± 23	10 ± 4	11 ± 7

Fusobacterium Nucleatum ATCC 25586

	10^2	10^2 dup	10^3	10^3 dup
Progress	261 ± 68	272 ± 61	124 ± 120	119 ± 117
P.D.	85 ± 45	76 ± 33	8 ± 7	10 ± 8
Roeko (german)	222 ± 89	234 ± 61	41 ± 11	45 ± 23
Roeko (usa)	241 ± 81	234 ± 90	102 ± 27	83 ± 23
Sure-Endo	251 ± 32	230 ± 44	60 ± 55	54 ± 51

Streptococcus Sanguis G9b

	10^6	10^6 dup	10^7	10^7 dup
Progress	261 ± 27	233 ± 34	31 ± 17	44 ± 12
P.D.	51 ± 38	54 ± 33	4 ± 2	4 ± 3
Roeko (german)	154 ± 27	149 ± 38	27 ± 14	24 ± 8
Roeko (usa)	238 ± 25	241 ± 22	53 ± 16	49 ± 20
Sure-Endo	173 ± 39	184 ± 25	41 ± 15	42 ± 18

The test for differences between the three brands ranked in the middle order could be taken as having the same mean bacterial counts for this test microorganism. The F value of 0.335, cf $F_{2,20}$ gave a p value > 0.6, with p = 0.72 .

4.3.3 Bacteroides intermedius NCTC 9336

The test for parallel regressions for this test microorgan-ism produced an F value of 0.177, cf $F_{4,15}$ and a p value > 0.9 with p = 0.95. The test for dependence on time was not significant with a p = 0.41.The test for overall differences in the brands gave an F value of 15.58, cf $F_{4,20}$ producing a p < 0.0001.There were significant differences between the brands.

The average bacterial counts (**Table 4.5**) produced the following rankings;

PD < ROEKO(ulm)/SURE-ENDO < ROEKO(usa) < PROGRESS

The difference between the **SURE-ENDO** and **ROEKO(ulm)** was tested producing an F value of 0.324,cf $F_{1,20}$ and a p value > 0.5 with p = 0.58.This indicated that the two brands could be taken as having the same mean bacterial counts.

4.3.4 Fusobacterium nucleatum ATCC 25586

The test for parallel regressions produced an F value of 0.774, cf $F_{4,15}$.

The p value being > 0.5 and equal to 0.56 indicates that there is no dependence on time. The test for overall differences in the brand counts produced an F value = 2.226, cf $F_{4,19}$ with a p value = 0.10. The result demonstrates a significant difference in the brand counts. The test for dependence on time produced a p value = 0.89 and indicated that time was not a significant factor in this study. The variance estimate $(70.885)^2$ for this study was higher than the other test microorganisms. The bacterial counts showed such large variations within the tested brands that no significant differences were evident. The test for dependence on time produced an F value = 0.019, cf $F_{1,23}$ and p = 0.89. There was no dependence on time evident.

In summary, there was no dependence on time indicating that the sampling procedure was suitable for this study. **Table 4.6** outlines the significant differences between brands, based on the mean bacterial counts. The trend present, shows that PD brand gave the lowest mean bacterial counts for all 4 test microorganisms, while no clear trend emerged or could be observed in the other four brands tested. The variance estimates (**Table 4.7**), show the comparatively large variance of the bacterial counts for **F. nucleatum** ATCC 25586 , making analysis of the data statistically unreliable.

TABLE 4.6

Rank Analysis

Microorganism	Brand by rank
A. viscosus T14	PD < SURE-ENDO/PROGRESS/ROEKO(ULM) < ROEKO(USA)
S. sanquis G9b	PD < ROEKO(ULM) < SURE-ENDO < PROGRESS/ROEKO(USA)
B. intermedius NCTC 9336	PD < SURE-ENDO/ROEKO(ULM) < ROEKO(USA) < PROGRESS
F. nucleatum ATCC 25586	Data too variable for ranking.

TABLE 4.7

Variance Estimates.

Microorganism	Variance Estimates (σ^2)
A. viscosus T14	$(44.27)^2$
S. sanquis G9b	$(23.53)^2$
B. intermedius NCTC 9336	$(26.77)^2$
F. nucleatum ATCC 25586	$(70.885)^2$

Figures 4.8, 4.9, 4.10 and 4.11 are the bacterial recovery values for the two dilutions within the counting range. The two values have been obtained from the duplicate platings at each dilution. The similarity from a visual inspection of the graphs, between the two dilutions, indicates that the experimental method used provided a constant and reliable technique for this study. The apparently large variations that can be observed within a particular brand would be attributed to the variability of the paper points ability to recover the test bacteria, and hence the large variance estimates. The **non parametric Kruskal–Wallis test** for one way analysis of variance was performed on the data to test the hypothesis that the bacterial counts of the 4 test microorganisms are distributed in the same way. The analysis was performed on the mid ranks of the averaged scores. The results are summarised in **Table 4.8.**

The K value in each case, is testing the hypothesis that the bacteria counts are distributed in the same way for each brand. K is compared with a χ^2_4 to calculate the p value.

The ranking of the tested brands, according to the average ranks for each bacteria type is shown in **Table 4.9.** The Friedman statistic was calculated to test the hypothesis that the rankings are randomly assigned in each of the test microorganism studies. The hypothesis was accepted that no

FIGURE 4.8
BACTERIAL RECOVERY: ACTINOMYCES VISCOSUS T14
Mean ± 1 Standard Deviation n = 5

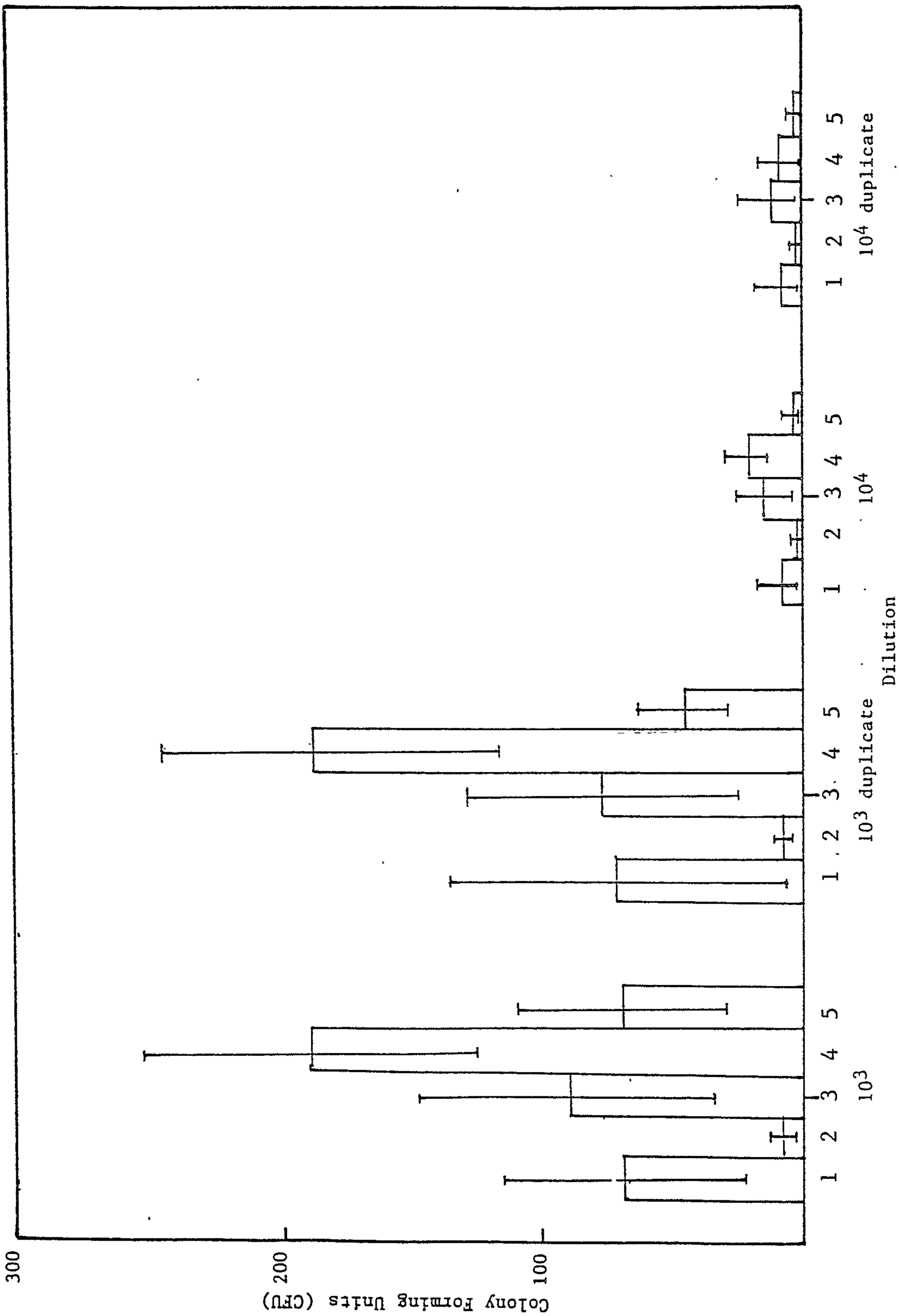


FIGURE 4.9
BACTERIAL RECOVERY: BACTEROIDES INTERMEDIUS NCTC 9336
Mean ± 1 Standard Deviation n = 5

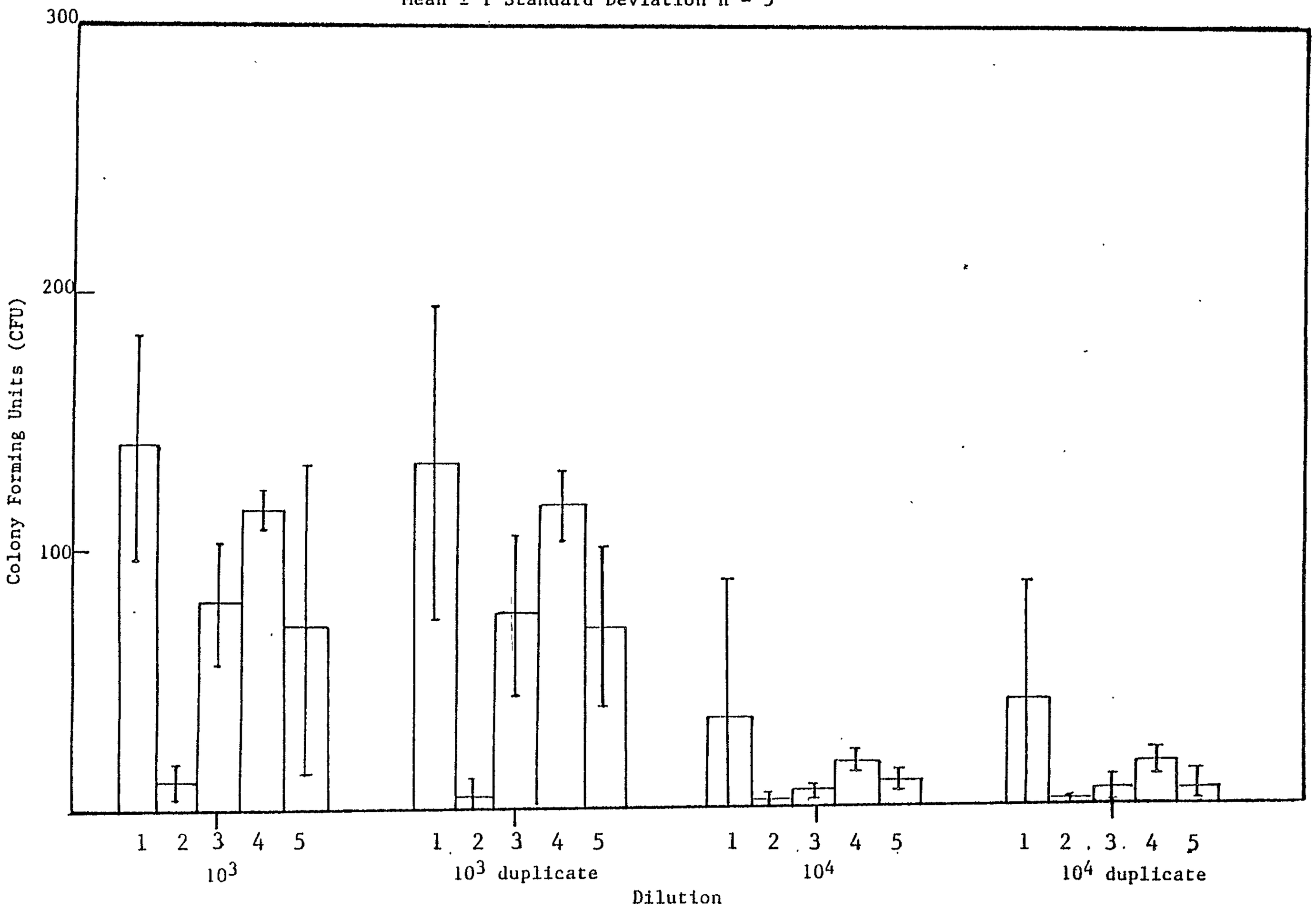


FIGURE 10.
BACTERIAL RECOVERY: FUSOBACTERIUM NUCLEATUM ATCC 25586
Mean ± 1 Standard Deviation n = 5

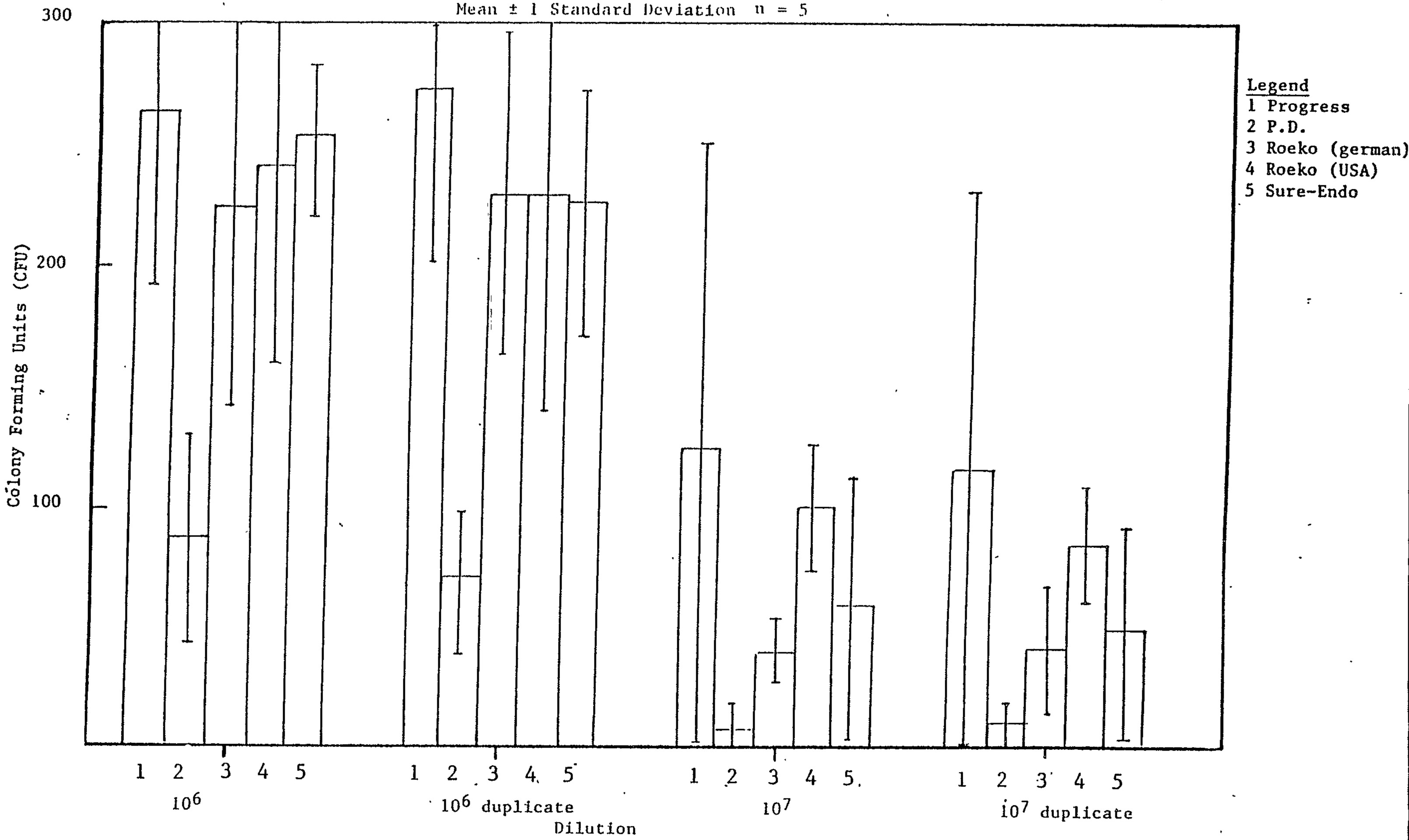


FIGURE 4.11
BACTERIAL RECOVERY: STREPTOCOCCUS SANGUIS G9B
Mean ± 1 Standard Deviation n = 5

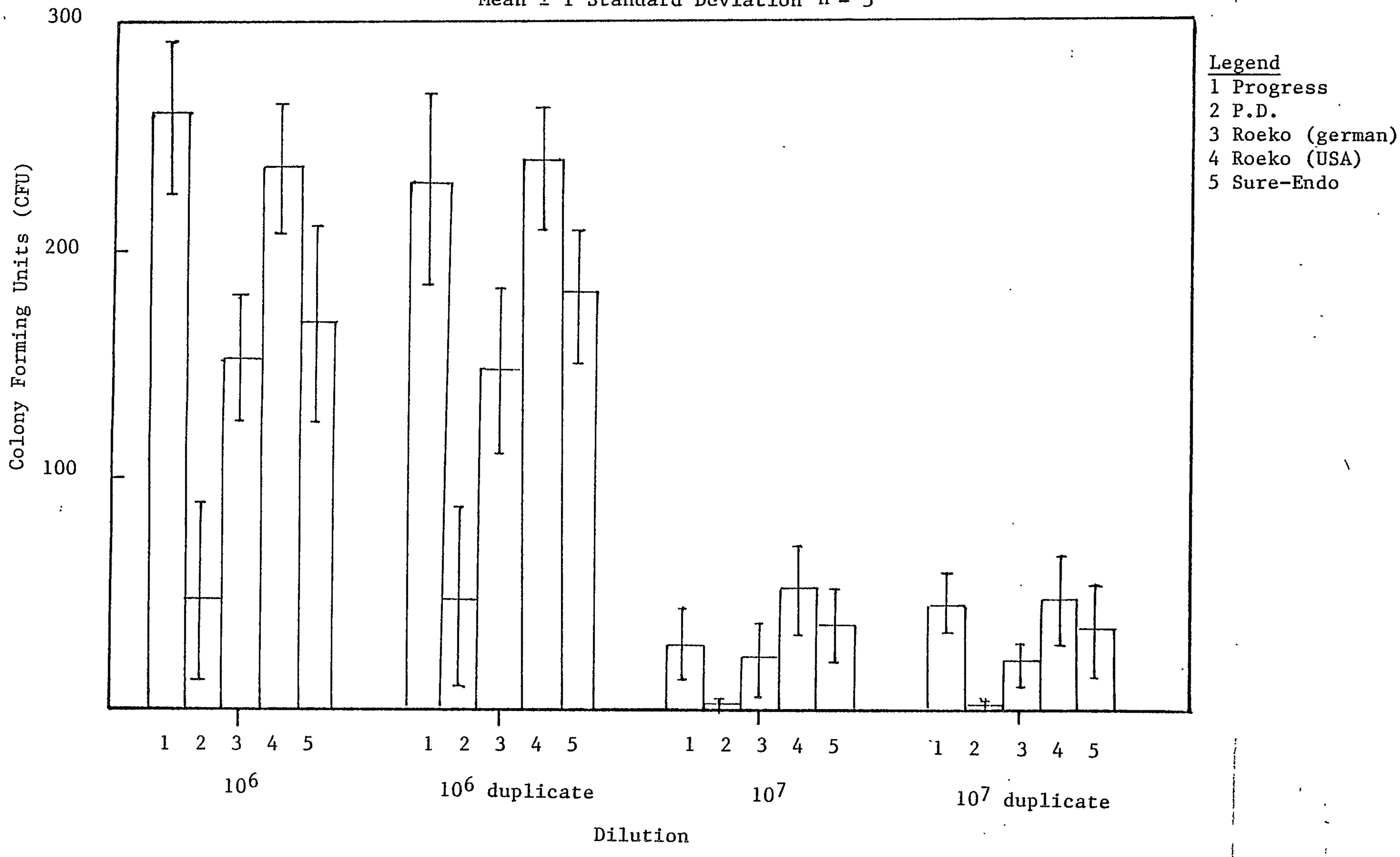


TABLE 4.8

Kruskal-Wallis Ranking Scores.

Microorganism	Brand						
	PD	PROGRESS	ROEKO (usa)	ROEKO (ulm)	SURE-ENDO	K	p
A. viscosus T14	15	62	111	75	62	17.479	0.0015
S. sanquis G9b	15	105	100	43	62	21.48	0.0008
B. intermedius NCTC 9336	15	101	99.5	43	62	19.29	0.0003
F. nucleatum ATCC 25586	16	83	86	71	69	11.88	0.018

TABLE 4.9

Kruskal-Wallis Ranks.

Microorganism	Brand				
	PD	PROGRESS	ROEKO (usa)	ROEKO (ulm)	SURE-ENDO
A. viscosus T14	1	2.5	5	4	2.5
S. sanquis G9b	1	5	4	2	3
B. intermedius NCTC 9336	1	5	4	3	2
F. nucleatum ATCC 25586	1	4	5	3	2
TOTALS	4	16.5	18	12	9.5

consistent ranking pattern exists. The only brand showing a consistent ranking being PD brand, with the lowest ranking.

Overall the PD brand gave the lowest average bacteria counts for each of the 4 tested microorganisms, it also had the lowest mean absorbance at the 10, 30 and 60 second time intervals. Although the **SURE-ENDO** brand had the highest mean absorbance (significantly higher at the 10 second interval). it did not give the highest mean absorbance count for any of the tested microorganisms.

4.4 BIOLOGICAL ACTIVITY

The biological properties of each brand of paper point were tested for sterility, antibacterial and haemolytic activity.

4.4.1 Sterility

The sterility of 16 randomly sampled paper points per brand, were tested for aerobic and anaerobic growth. There were no positive cultures obtained after 7 days, for any of the 5 tested brands.

4.4.2 Antibacterial Activity

The antibacterial activity was tested for by inhibition of growth around an indicator strain of **Staphylococcus aureus**. The PD brand paper point consistently produced a clear zone of inhibition on the lawn, as shown in **plate 4.2.** The size of the zone increased up to day 3, then remained static to day 7. It was also observed that some paper points of the **PROGRESS** brand appeared to have promoted additional growth around them when compared with the other brands, as shown in **plate 4.3.** This observation was not investigated further.

4.4.3 Haemolytic Activity

The PD brand paper points consistently produced a zone of haemolysis on blood agar plates, and can be observed in **plates 4.4** and **4.5** . The other tested brands did not produce this effect.

PLATE 4.2

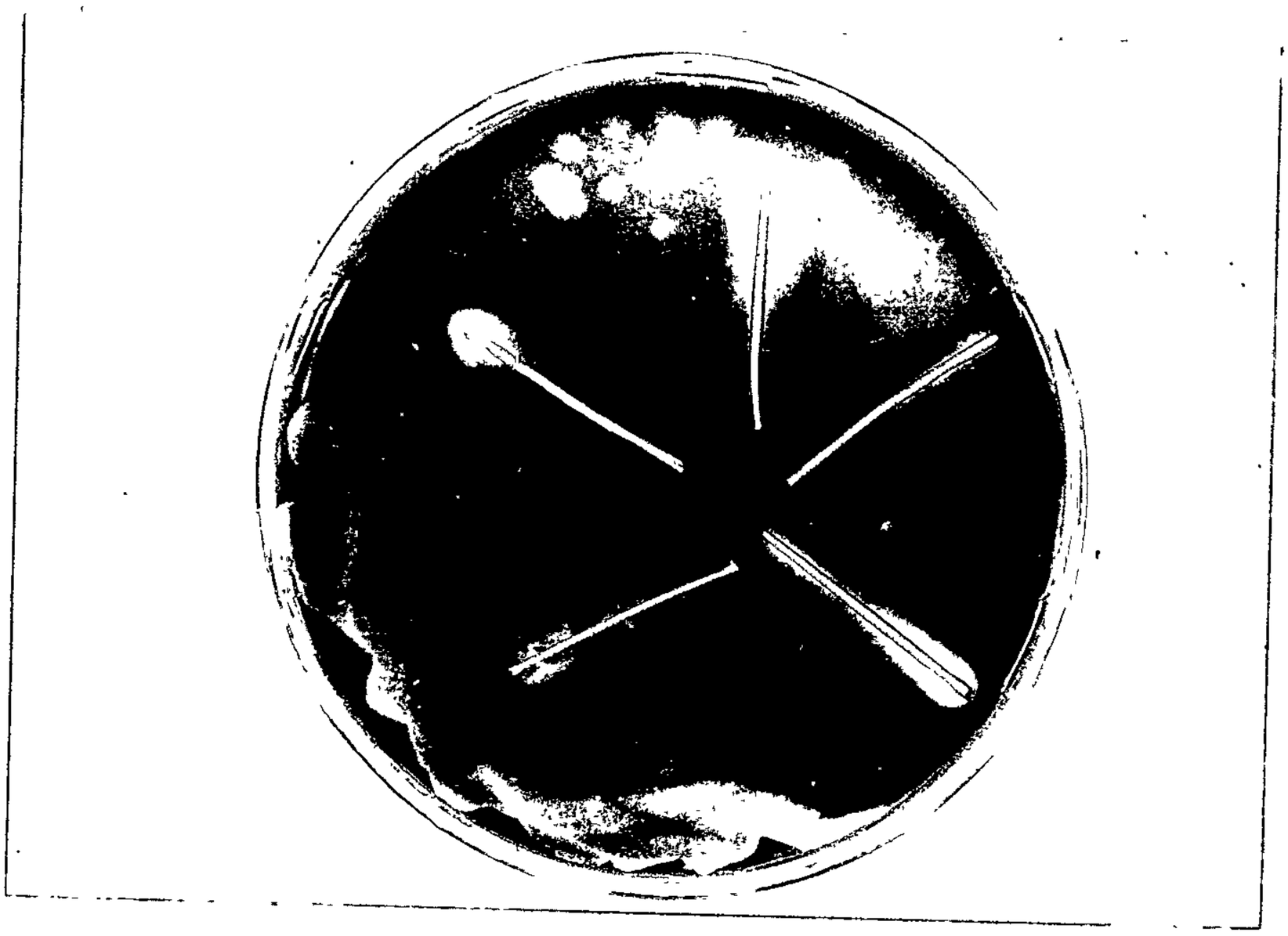


PLATE 4.3

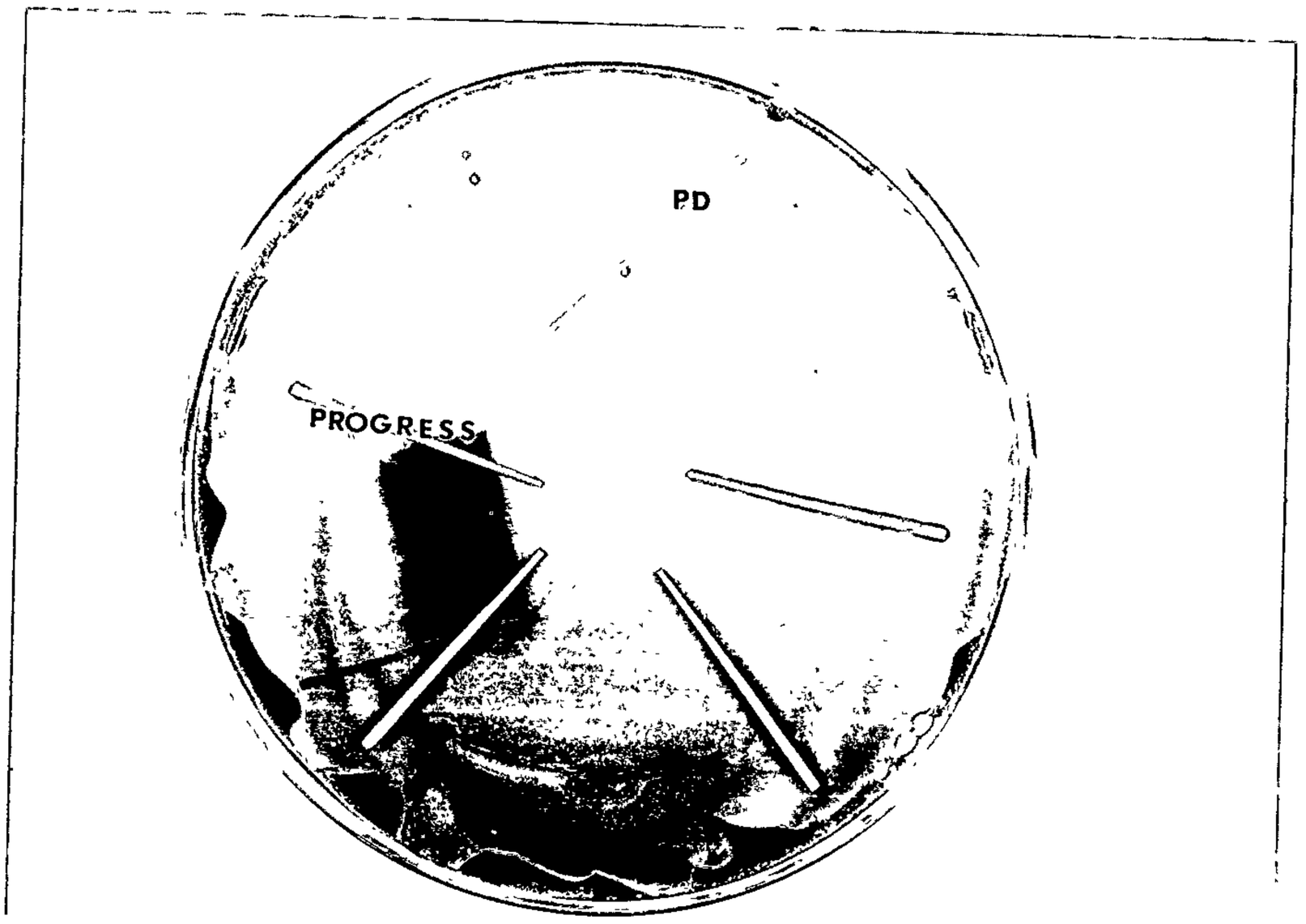


PLATE 4.4

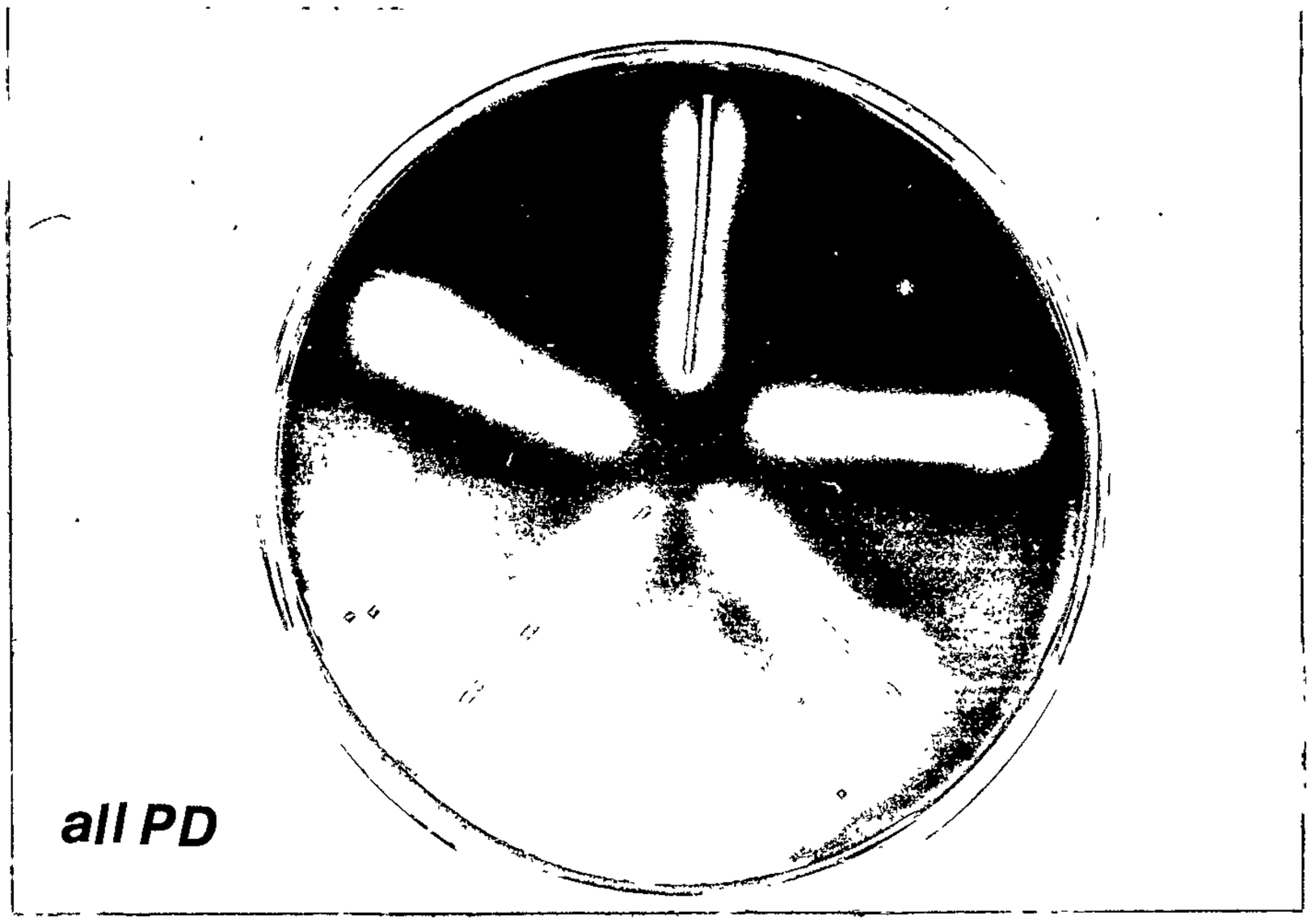
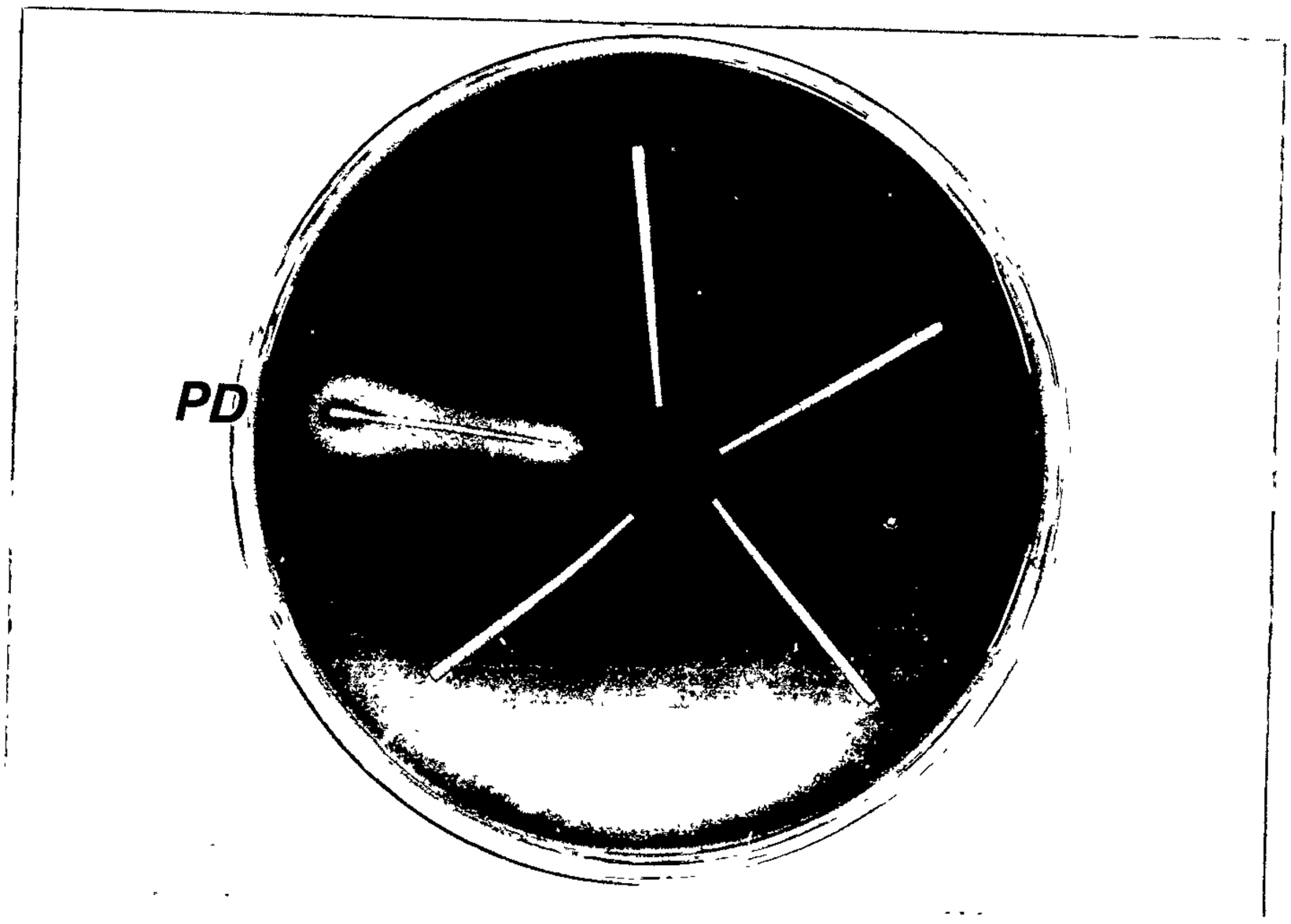


PLATE 4.5



CHAPTER 5.

DISCUSSION

The results from this study have shown that the physical and biological properties of the tested brands of paper points are not uniform. The study of Edwards and Bandyopadhyay (1981) indicates the manner in which suitable test methods were devised to evaluate the absorbent paper point. They found that there was a large variation in the physical properties. The results of taper, and the diameters at D_1 and D_{12} from this study have shown a closer correlation to the ISO standards. The large variation observed for individual brands, in addition to the large ranges found, (ie the D_1 and D_{12} of the PD brand overlapping) would indicate that the manufacturers of these brands are not maintaining adequate quality control.

The aim of the study on absorbence was to identify the brands with the lowest and the highest mean absorbence, at the three different sampling times. The lowest and highest mean absorbent brands were to form the basis of the next stage of the study. To sample known pure bacterial cultures, observing the relative recovery rates and testing the hypothesis that bacterial recovery rates are a function of absorbence and sampling time. The results from the absorbence study identified the P.D. brand as the paper point brand with the lowest rate of absorbence. The SURE-ENDO brand was identified as having the highest rate of absorbence. This correlation was apparent at the three time intervals

investigated, with the significance of the difference becoming more pronounced as time passed. The degree of overlap that existed at the 10 second interval decreased at the 30 second interval, while the 60 second interval showed a clear and significant difference between the lowest and the highest absorbence values.

The correlation of the absorbence data to the physical dimensions of the paper points indicated that no correlation existed. This was confirmed by the study of Edwards and Bandyopadhyay (1981). The physical dimensions measured in this and the latter study, may not relate to those properties involved in the physical retention of the microorganisms. The potential factors are discussed further on with suggested areas to be the subject of future investigations.

The study has shown a relation of the absorbence to the bacterial recovery rates observed for the 4 test microorganisms. The comparison of the bacterial counts recovered from the 10 second sampling time indicates that the PD brand gave the lowest bacterial recovery rate and the lowest absorbence. The **SURE-ENDO** brand did not give the highest recovery rate for any of the 4 test microorganisms. The Kruskal-Wallis Test rankings within each test microorganism show significant differences between some brands, while the Friedman Test indicated that significant differences exist between the mean bacterial counts for each brand over the 4 test microorganisms. In the interpretation of these results the small number of paper points used in each brand must be observed. The large variance estimates for each test microorganism indicate that although the mean values show significant differences, a wide range of values within a

particular paper point brand can be expected. The clinical significance of this would suggest that among the selection criteria for a particular brand of paper point for clinical studies, the brand showing the least variance would be most suited. The acceptance of such a brand would reduce one potential source of variation between clinical studies of the subgingival microflora. The potential selectivity of that brand would need to be taken into consideration.

If the low absorbence rate and bacterial recovery rate of the PD brand are compared to the SURE-ENDO brand with the highest absorbence rate and middle ranking bacterial recovery rate, it can be inferred , that absorbence does not play an important role in the relative bacterial recovery rates. The comparative analyses performed in this study do not show significant correlations between the absorbence data and the bacterial recovery rates. If the absorbence rate, as it has been measured in this study is an accurate measure of volume, then additional studies at the 30 and 60 second sampling time intervals, where the mean absorbence values are more widely spread, may predict a closer relation of the two parameters being investigated here. The ranking of the 5 tested brands may not show the same degree of overlapping as has occured at the 10 second interval.

The present study has investigated one property of the paper points, absorbence. An additional property that has not been investigated is adherence. Although some preliminary work on the position of the test microorganisms in relation to the paper point has been attempted, it will require additional study to clarify this point. The question of the relative importance of adherence and absorbence may be answered by the

demonstration of the microorganisms on the surface only or uniformly distributed within the body of the paper point. In addition to this, investigation into the residual numbers, positions and types of microorganisms following the different methods of dispersion may indicate further sources of variation. The latter point may be as important as the original comment on adherence, the degree of retention of microorganisms after dispersion may indicate that a specific property of the cellulose fibres and /or the method of manufacture, such as density, fibre orientation or the angle at which the paper point is wound, will determine the sampling properties of that particular brand. This also will include the variabilility within the brand, a factor that this study has shown to be evident.

If it is felt that adherence of the microorganisms to the cellulose fibres of the paper point is of more importance than the volume absorbed, then microscopy studies using techniques causing minimal disturbance to this relationship must be used. The microscopy techniques used in this study have resulted in clear fields without bacteria being observed. Even though a freeze drying technique was employed, the final staining and embedding techniques used in this study have disturbed the relationship of the test microorganisms to the paper points. The scanning electron microscope may provide more information on the relationship.

The apparent relative selectivity of a brand for a particular test microorganism has been shown in this study. If the data for PD brand is excluded, then a general trend would indicate that the **SURE-ENDO** brand recovers the least number of bacteria, the **ROEKO(ulm)** and **PROGRESS** brands

would occupy the middle rank and the **ROEKO(usa)** brand is the highest. The test for significant differences between brands within each test microorganism indicates that some brands may be more selective for particular bacteria. This should be viewed with caution, the small number of paper points used in this study does not exclude the potential variation that would be found in a larger sample study.

The biological properties of the paper points in this study were tested for sterility, antibacterial and haemolytic activity. The manufacturers claim of sterility for all of the 5 tested brands was confirmed in the study. The method of sterilisation is not displayed on the packaging, a point that would be of assistance for selection of suitable brands in bacteriological studies. The use of gamma sterilisation would be the least invasive method available, reducing the risk of contamination with residual chemicals.

The antibacterial activity of a particular brand of paper point was observed in the study of Rawle, Adams and Witherley (1985). They describe a zone of inhibition observed around Endic brand paper points used in the study. Included in the study are the **PD** brand paper points with a zone of inhibition , not as large as the Endic paper point but still visible. The study of Möller and Hensten-Pettersen (1985), identified the **PD** brand as containing a leachable cytotoxic material. The material was identified as formaldehyde, using fluorimetric methods. Ørstavik and Möller (1985) investigated this effect in a study on the biological activity of endodontic (absorbent) paper points. They have concluded that **PD** brand paper points have an antibacterial effect, in addition to a very low bacterial recovery rate. These additional properties of PD brand have been

confirmed in this study. The antibacterial effect, even though it was with a test strain of microorganism and not one of the 4 test microorganisms and the haemolytic effect, were both observed.

The biological properties of the tested paper points indicate that the use of the PD brand is contra-indicated for bacteriological studies. The confirmation in this study of the haemolytic effect and the significantly reduced mean bacterial recovery rates as have been found in another study may significantly effect the results of qualitative and quantitative microbiological studies on plaque bacteria.

Edwards and Bandyopadhyay (1981), investigated the fibre shedding properties of selected paper points and commented on the possible consequences of leaving fibres within the periapical tissues. The consequences of wood fibres from disposable surgical drapes entering surgical wounds, leading to intense inflammation were discussed by Dragan (1979). The effect of fibres being shed within the periodontal pocket or tissues during a sampling procedure may also lead to an intense inflammatory response. The fibre shedding property of the tested brands of paper points was not investigated in this study, however it should be considered in future in vivo studies.

This study was designed to provide the baseline data for additional studies on the effect of paper points as a sampling method. In view of the variable sampling times used in microbiological studies of periodontal pocket bacteria, further studies with sampling times of 30 and 60 seconds will provide information on the effect of time on bacterial recovery rates.

It was originally envisaged that an in vivo study of the most and least absorbant paper point brands be investigated as a method of sampling subgingival bacteria for Differential Dark Field Microscopy (DDFM). The study would use the recommended sampling time as indicated by the mean bacterial recovery count data from the three sampling times, to sample periodontal pockets of greater than or equal to 6mm. A paper point of the least and most absorbent brands would be inserted simultaneously into the same area of the pocket for the recommended time. Using sterile conditions, both points would be divided into three equal segments and prepared for DDFM, the results being compared for significant differences between brands, and between segments within and between brands. The objective being to test the resulting differences against the absorbence. In addition a method of selectively sampling different levels of a periodontal pocket, would be tested. The confirmation of PD brand as being unsuitable for bacteriological studies, combined with the results of the absorbence and bacterial recovery studies, suggested that this study should be performed after additional investigation of the physical properties of paper points has been done.

The variability of the mean bacterial counts noted within some brands and the inability to correlate a physical property to the data would suggest that further property/s are involved in the paper point sampling method. The **ROEKO(usa)** brand would appear to be more consistent over the range of test bacteria, with the **ROEKO(ulm)** and **PROGRESS** brands being similar. While the **SURE-ENDO** brand was consistently lower than the other three.

CHAPTER 6

CONCLUSION

An attempt has been made to correlate the physical properties of 5 brands of absorbent paper points, to mean absorbence values and mean bacterial recovery counts as found in this study. The results show no significant correlation between the physical dimensions measured to the absorbence or the recovery rates .

The trends observed indicate that one brand of paper point is not recommended for bacteriological studies. The remaining 4 brands tested produce variable results with the variances of the absorbence study and the bacterial recovery study being large enough to show significant overlap of the mean data. There may be a degree of selectivity occurring between brand type and test microorganism though, this will require a larger number of paper points to be used for a significant effect to be observed.

The overall trend observed would indicate that the brand/s showing the better consistency (least variability) should be used for bacteriological studies. In this study the **ROEKO (usa)** brand, ISO No.60 would be the most suitable paper point, followed equally by the **ROEKO (ulm)** and **PROGRESS** brands, then the **SURE-ENDO** brand. The **PD** brand of paper point is not recommended for use in bacteriological studies.

CHAPTER 7.

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APPENDIX 1

Actinomyces viscocus T14

	Colony forming units (cfu).	
PAPER POINTS	10^3	10^4
PROGRESS		
1	57/ 31	3/ 1
2	36/ 36	2/ 5
3	52/ 49	3/ 7
4	53/ 59	8/ 9
5	149/ 189	29/ 27
P.D.		
1	5/ 15	1/ 2
2	7/ 8	1/ 1
3	12/ 19	2/ 1
4	18/ 9	2/ 4
5	5/ 2	3/ 2
ROEKO ULM		
1	73/ 47	27/ 8
2	181/169	11/ 9
3	94/ 67	33/30
4	28/ 39	3/ 2
5	69/ 74	8/11
ROEKO USA		
1	213/230	29/29
2	281/262	24/19
3	178/173	26/16
4	151/166	13/12
5	118/107	17/19
SURE-ENDO		
1	45/ 49	4/ 7
2	71/ 59	7/ 2
3	143/ 70	3/11
4	47/ 29	3/ 4
5	52/ 40	14/ 4

Control 20µl Start $289/268 \times 10^3$,
$\mu = 2.7 \times 10^5$
$= 1.3 \times 10^7$ /ml

Control 20µl End $238/229 \times 10^3$
$\mu = 2.3 \times 10^5$
$= 1.1 \times 10^7$ /ml

Optical Density = 0.08. Known cfu = 2.5×10^8 /ml

Bacteroides intermedius NCTC 9336

	Colony forming units (cfu).	
PAPER POINTS	10^3	10^4
PROGRESS		
1	138/123	21/46
2	116/121	18/23
3	120/ 91	9/26
4	219/234	131/125
5	112/ 83	14/ 17
P.D.		
1	10/ 9	2/ 2
2	23/ 16	1/ 3
3	13/ 11	4/ 1
4	10/ 7	1/ 1
5	6/ 1	1/ 1
ROEKO ULM		
1	79/ 62	8/ 9
2	83/ 51	14/ 6
3	64/ 75	5/11
4	87/ 93	11/13
5	96/111	7/21
ROEKO USA		
1	117/110	14/15
2	112/119	16/22
3	104/126	13/19
4	119/102	24/16
5	118/137	24/29
SURE-ENDO		
1	41/ 60	7/ 9
2	53/ 62	13/ 4
3	116/111	16/20
4	73/ 68	11/15
5	64/ 55	7/ 8
Control 20 µl Start		286/291 x 10^4
		µ = 2.8 x 10^6
		= 1.4 x 10^8 /ml
Control 20µl End		187/176 x 10^4 ,
		µ = 1.8 x 10^6
		= 9.0 x 10^7 /ml
Optical Density =0.19.	Known cfu = 2.1 x 10^9 /ml	

Fusobacterium nucleatum ATCC 25586

PAPER POINTS	Colony forming units (cfu). 10^2	10^3	10^4
PROGRESS			
1	143/164	19/ 11	
2	300+/300+	102/ 87	
3	" "	300+/300+	(46/26)
4	" "	184/166	
5	262/300+	15/ 34	
P.D.			
1	94/ 93	5/ 8	
2	59/ 47	6/ 5	
3	109/ 92	11/16	
4	139/114	19/12	
5	25/ 37	1/ 1	
ROEKO ULM			
1	300+/300+	50/62	
2	" "	44/67	
3	91/167	24/14	
4	249/261	50/57	
5	174/196	41/29	
ROEKO USA			
1	300+/300+	113/102	
2	" "	178/144	
3	" "	142/111	
4	146/127	56/ 49	
5	162/147	23/ 9	
SURE-ENDO			
1	269/251	38/ 43	
2	221/204	46/ 11	
3	241/196	44/ 53	
4	228/203	16/ 23	
5	300+/300+	157/142	

Control 20µl Start 227/243 x 10^3 ,
µ = 2.3 x 10^5
= 1.1 x 10^7 /ml

Control 20µl End 215/236 x 10^3 ,
µ = 2.2 x 10^5
= 1.1 x 10^7 /ml

Optical Density = 0.16 Known cfu = 2.0 x 10^8 /ml

Streptococcus sanguis.G9b

	Colony forming units (cfu)	
PAPER POINTS	10^6	10^7
PROGRESS		
1	287/249	36/43
2	219/256	19/25
3	248/197	31/46
4	281/267	59/47
5	271/198	13/59
PD.		
1	45/ 57	4/ 9
2	49/ 46	7/ 6
3	117/109	4/ 3
4	26/ 39	2/ 1
5	21/ 19	2/ 2
ROEKO ULM		
1	167/186	14/26
2	192/149	43/37
3	144/186	17/28
4	119/125	42/19
5	148/ 99	21/14
ROEKO USA		
1	257/241	62/46
2	219/276	44/26
3	238/227	52/45
4	271/243	76/82
5	209/218	35/49
SURE-ENDO		
1	187/176	19/21
2	215/149	31/43
3	109/219	52/38
4	175/186	46/39
5	182/194	57/71

Control 20µl Start

286/291 x 10^6
$\mu = 2.9 \times 10^8$
$= 1.45 \times 10^{10}$/ml

Control 20µl End

249/223 x 10^6
$\mu = 2.3 \times 10^8$
$= 1.15 \times 10^{10}$/ml

Optical Density = 0.20. Known cfu = 5×10^{10}/ml