Clinical and Microbiological Effects of Localized Ligature-Induced Periodontitis on Non-Ligated Sites in a Monkey Model System

Robert A. Kiel
CLINICAL AND MICROBIOLOGICAL EFFECTS OF LOCALIZED LIGATURE-INDUCED PERIODONTITIS ON NON-LIGATED SITES IN A MONKEY MODEL SYSTEM

Robert A. Kiel, D.M.D.
B.S., Tufts University, 1975
D.M.D., University of Connecticut, 1979

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CLINICAL AND MICROBIOLOGICAL EFFECTS OF LOCALIZED LIGATURE-INDUCED PERIODONTITIS ON NON-LIGATED SITES IN A MONKEY MODEL SYSTEM

Presented by
Robert A. Kiel, D.M.D.

Kenneth S. Kornman, D.D.S., Ph.D.
Paul B. Robertson, D.D.S., M.S.
Alan L. Coykendall, D.M.D., M.S.

The University of Connecticut
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To Nancy
LIST OF ABBREVIATIONS

BAB = basal anaerobic broth
BEN = basal esculin nitrate (broth)
BPB = black pigmented Bacteroides
CABIS = computer assisted bacterial identification system
CFU = colony forming units
ETSA = enriched trypticase soy agar
STB = surface translocating bacteria
TCF = total cultivable flora
TMC = total microscopic count
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INTRODUCTION

It has become increasingly clear that the subgingival microflora of chronic periodontitis in humans represents a very complex combination of predominantly Gram-negative anaerobic rods. The interdependence of these organisms for nutritional requirements, the creation of appropriate environmental conditions for growth, and the host's immune response are some of the potential variables that permit the establishment and maintenance of this complex ecosystem. For reasons that are poorly understood, some of these organisms become more or less numerous as periodontitis occurs. The causes of this alteration in the subgingival microflora are largely unknown. It is not clear whether the proportions of certain bacteria change preceding or following disease production, since longitudinal experiments in the development of chronic periodontitis in humans are unethical. It is also unestablished whether bacterial changes that occur in the development of periodontitis in one area of a subject's mouth can affect adjacent or opposite areas either clinically or microbiologically. Recent reports of the natural history of chronic periodontitis in humans suggested that the disease appeared initially in first molar and incisor regions and involved adjacent sites with time. A similar phenomenon has been been for
Streptococcus mutans, one of the major etiologic agents in smooth surface dental caries.\textsuperscript{87}

It was the purpose of this investigation to examine the effect of localized ligature-induced periodontitis on opposite or contralateral non-ligated sites in monkeys. The ability to disrupt the resident microflora of non-ligated sites in these monkeys by exogenous infection with a suspected periodontal pathogen, Bacteroides gingivalis, was also studied.
LITERATURE REVIEW

There is currently much evidence to suggest that periodontal diseases are caused by microorganisms.\textsuperscript{82,83} Dental plaque, a collection of soft bacterial deposits firmly adherent to the teeth, has long been associated with these diseases. More recently, it has become possible to identify many of the various microbial species in subgingival dental plaque. Identification of all of the organisms in plaque samples has been an immense undertaking until newer techniques became available which allow more rapid identification of organisms.\textsuperscript{3,25}

Relatively few subgingival plaque samples comparing healthy and diseased sites from human patients with chronic periodontitis have been exhaustively analyzed using anaerobic techniques and only cross-sectional information is available. Williams reported that subgingival plaque samples from four patients with 6-8 mm periodontal pockets were composed mainly of Gram-positive rods, especially \textit{Actinomyces}, with about one third of each sample being composed of Gram-negative anaerobic bacteria.\textsuperscript{98} These samples were collected with a sterile curette. In this study, the control site consisted of the "most clinically normal" site available in that patient (<3 mm). Five non-diseased patients were also used as controls. Williams reported that the "normal" and disease site flora within each patient were similar, but both were
different from that of non-diseased patients. Diseased patients seemed to have greater numbers of *Actinomyces* than control subjects. This study, therefore, suggested that "normal" sites in periodontitis patients were different from "normal" sites in periodontitis-free patients.

Another investigation of the microflora of chronic periodontitis reported different patterns and distributions of bacteria. Slots reported that the subgingival flora from 8 patients with advanced periodontitis (5-12 mm periodontal pockets) was composed mainly of Gram-negative anaerobic rods. *Bacteroides gingivalis* was the predominant Gram-negative anaerobe. In this study, surgical access was used to obtain the samples from the pocket base. Samples were collected with a sterile curette. There were no control areas sampled in this study.

White and Mayrand examined the distribution of certain groups of *Bacteroides* in 40 patients with various gingival conditions. Healthy or minimally inflamed sites from the same patients were used as controls. To collect the sample, paper points were placed within the pocket for 10 seconds. Sites with severe inflammation had greater proportions of Gram-negative anaerobic bacteria than less inflamed sites. About one-third of these Gram-negative anaerobic bacteria were *B. gingivalis* and this organism was non-detectable in healthy sulci. *B. gingivalis* was present in sulci that were mildly or moderately inflamed as determined by the Gingival Index. B. *melaninogenicus* ss.
intermedius, however was found in all sites, although in higher percentage in areas of 4-6 mm pockets. Other organisms were not characterized in this study.

Tanner et al. studied the subgingival microflora in four subjects with advanced chronic periodontitis. Control sites consisted of the most healthy site available although the authors admitted that none of the control sites could be considered "healthy" and that one patient had no sites that were considered usable as controls. In diseased sites of these patients, the Gram-negative anaerobes were again the predominant group of organisms. Samples were collected using an oxygen-free gas-flushed syringe. In reporting the distribution of control site flora, the authors grouped the control sites from these patients with control sites from patients with two other clinical conditions. This makes interpretation of the data more difficult. The control sites, however, tended to have decreased proportions of Gram-negative organisms and increased proportions of Gram-positive species. B. gingivalis was again detected primarily in diseased sites.

Spiegel et al. studied the distribution of black pigmented Bacteroides (BPB) from patients with gingivitis as well as various degrees of periodontitis. Control sites again consisted of the most clinically normal site available. The incidence of BPB increased with increasing disease severity. B. gingivalis was detected in some of the minimally inflamed sites examined.
In summary, the literature has very few reports of fully characterized subgingival analyses of healthy sites in patients with chronic periodontitis. The five studies mentioned above are the only ones in the literature that have used careful anaerobic techniques. Two of these studies examined only the distribution of *Bacteroides*. A third had no control subjects. The fourth study mixed the control site data with other control sites from other types of patients. The study by Williams was in fact the only investigation to report control site data in a manner appropriate to the current problem and was unable to show significant differences in control versus diseased sites. This observation was reported, using much less refined techniques, as early as 1950.71 Clearly, then, the relationship of chronic periodontitis and "most normal" or "healthy" sites in the same subject has not been well established.

In an attempt to identify prime causative organisms, several studies have shown that certain microorganisms are capable of inducing periodontitis when implanted in germ-free or conventional animals.15,23,37,38,39,41,43,45,82 However, most of these models are poor substitutes for human periodontitis. Unlike humans, rodents possess a keratinized gingival sulcus. The subgingival microflora of dogs appears to be different from human flora.49,93 The histopathology of periodontitis in dogs differs in many respects from that of humans.68 Additionally, dogs tend to develop gingival recession rather than periodontal pockets,93 thus presenting
a potentially very different ecological environment for study. Currently, there is evidence to suggest that certain monkeys may possess a subgingival flora very similar to that of humans and may be capable of developing ligature-induced rapidly progressing periodontitis that is well suited to experimental study.80,47

Several investigators have studied ligature-induced periodontitis, either intentionally or accidently produced6,13,14,17,29,32,54,72 and showed that this technique was very effective in producing periodontal defects in both humans and animals. The lesions produced in Rhesus monkeys were irreversible and stable and the histopathology was similar to that of human disease.14 The microflora of ligature-induced periodontitis in Macaca arctoides80 and Macaca fascicularis47 has been recently reported and generally resembled human flora. In one study several strains not previously identified in human samples were also described.80 To better understand the sequence of events leading to inflammatory periodontal disease and loss of periodontal attachment, it is necessary to conduct longitudinal investigations. Through these types of investigations it may be possible to determine if the identified flora is pathogenic or merely commensal. Furthermore, studies of plaque of periodontally healthy patients78 as well as patients with gingivitis59,90 suggest that the plaque present in diseased patients does not represent a different flora from periodontally healthy patients, but
differs mainly in the distribution of these organisms. This suggests that chronic periodontitis is not due to a de novo or exogenous infection but rather represents a shift in the natural flora. This fundamental point can best be studied in a longitudinal manner in which it is possible to monitor the microflora prior to and throughout disease development within the same animal. Ideally, that animal should develop disease which clinically and microbiologically closely resembles human disease. For these reasons, the Macaca fascicularis model involving ligature-induced periodontitis appears to be appropriate for the current investigations.

A thorough examination of the experimental design of both previously mentioned longitudinal studies in primates wherein the subgingival microflora was monitored reveals that these investigators performed an exhaustive analysis of the affected sites but did not examine the clinically non-affected sites. If shifts in bacterial flora occurred at other sites in the mouth, the results may take on a very different meaning. Since it has not yet been established what influence either ligature-induced or naturally occurring periodontitis has on non-ligated or "healthy" sites in the same subject, it is impossible to associate specific microflora changes with a diseased state.
GENERAL OBJECTIVE

The general objective of this project was to examine the microflora of periodontitis, especially as related to the changes that occurred in sites that were not clinically involved with active disease. Some of the factors that permit these sites to undergo a change from the non-active to the active state were also investigated. A ligature-induced periodontitis model in monkeys was used. It was hypothesized that the development of large numbers of certain Gram-negative organisms in ligated sites could influence non-ligated sites. Furthermore, this influence could increase the susceptibility of non-ligated sites to develop periodontitis when challenged by large numbers of certain bacteria.
SPECIFIC OBJECTIVES

1. To identify and characterize the microflora in the opposite or contralateral side during the induction, development and establishment of ligature-induced periodontitis in the *Macaca fascicularis* monkey model.

2. To better define the appropriate bacteriological conditions necessary for the establishment of active disease in non-active sites and to determine if direct plaque implantation (such as might occur during certain routine dental manipulations) or implantation of *B. gingivalis* was capable of converting non-active sites to active sites. It was hypothesized that if floral changes occur as a prerequisite to disease production, the ability to produce disease by direct plaque implantation would be limited to a specific time period in the development of control site microflora patterns.

3. To gain evidence as to whether specific microbial patterns appear prior to or as a result of disease.
MATERIALS AND METHODS

Clinical Protocol

The experimental design utilized six Macaca fascicularis monkeys, 4-5 kg. each, with intact dentitions showing minimal occlusal wear (Figure 1). To permit maximum plaque development the animals were placed on a soft diet within a week of arrival consisting of water-soaked standard laboratory monkey chow. After six to eight weeks quarantine, selected teeth received silk ligatures tied just apical to the cementoenamel junction, and the periodontal condition and subgingival plaque were monitored at various time periods by the following methods:

1) Plaque Index
2) Gingival Index
3) Pocket depth and position of the gingival margin relative to the cementoenamel junction
4) Bacterial analysis of subgingival plaque
5) Standardized bitewing radiographs using custom made bite blocks
6) Intraoral photographs

Four monkeys received ligatures on the first and second molars and both premolars of one quadrant. The teeth in the remaining quadrants served as the "non-ligated" sites. The remaining two monkeys served as control for the non-ligated sites and were monitored at identical times as the
experimental group. The animals were anesthetized during recording sessions with I.V. ketamine (15 mg/kg) and atropine (0.04 mg/kg) which was found to provide satisfactory working time for the necessary procedures.

Part A--Site Monitoring

The posterior quadrants were divided into several sites for the purpose of sampling. Two sites per quadrant per animal were monitored one week prior to ligature placement, and then at week 0 (corresponding to the time of ligature placement), weeks 1, 4-6, and 8-11. Other sites were monitored only at weeks 4-6 but not previously. This latter sampling schedule allowed for the evaluation of variations due to the actual procedure of invading a given pocket during sampling.

Part B--Direct Plaque Implantation

To determine the effect of direct plaque implantation in non-ligated sites, a sterile Gracey curette (11/12) was used to collect subgingival plaque from a ligated site or pure cultures of freshly isolated B. gingivalis from a Macaca fascicularis periodontal lesion and were deposited into a non-ligated site at either weeks 0 and 1 or weeks 4 and 5 (Figure 2). These sites were designated as "infected". Contralateral sites were treated identically with a sterile curette and were designated as "sham infected". Both the infected and sham infected sites were monitored four weeks after the first infection (week 4 or 8).
Part C--Control

Two additional animals were monitored on the same schedule as those in Part A (Fig. 1) except that these animals received no ligatures at week 0. These sites were termed "control" sites. These animals also received direct implantation of either B. gingivalis or were "sham infected" with a sterile curette at weeks 0 and 1 or weeks 4 and 5 in a posterior site and were monitored on the same schedule as those in Part B (Fig. 2). These sites were termed "control infected" or "control sham infected" respectively.

Microbial Methods

Sample Collection

Bacterial plaque samples were collected in 4 ml sterile glass vials. Empty sterile vials were taken into a Coy anaerobic chamber, (Coy Manufacturing Co., Ann Arbor, Michigan) and filled with 1.0 ml of prereduced transport fluid without ethylenediamine-tetraacetate (RTF, 57) and capped. Sample vials remained in the anaerobic chamber until immediately prior to use. The anaerobic chamber was maintained as described by Aranki.3

Prior to sampling, supragingival plaque was removed from the sample site with dry sterile gauze. The subgingival plaque was sampled from the base of the pocket by means of three sterile paper points placed into the pocket for 10 seconds. The paper points were then removed and the portion of the point that was subgingival was cut off with
sterile scissors and dropped into the sample vial. The sample vial was then agitated with a vortex mixer for 60 seconds and brought into the anaerobic chamber.

Dispersion, Dilution and Plating

A sterile wire loop was placed into the sample vial and smeared on a glass microscope slide for use in dark-field quantitation of spirochetes. The percent of spirochetes to total cells was determined by counting at least 200 cells per slide. The samples were then dispersed by ultrasonic treatment for 2-3 seconds with a Kontes Cell Disrupter K-881440. The Kontes sonifier was used inside the anaerobic chamber and has been shown to have a wide safety margin in sparing Gram-negative anaerobic bacteria, some of which are highly sensitive to sonication.91

The samples were plated on enriched trypticase soy agar (ETSA). This is a non-selective blood agar enriched to aid growth of anaerobic organisms.92 The media was prereduced in the chamber for at least 24 hours immediately prior to use. The samples were plated on ETSA by means of an automatic diluting and plating device (Spiral Systems, Bethesda, Md.). The Spiral Plater allowed rapid and reproducible plating while giving a two log dilution of the sample on a single plate.28 In addition, well dispersed colonies were produced, allowing greater accuracy in picking single colonies for biochemical tests. The plates were then incubated for 5-7 days at 37°C.
For each sample, plates with well dispersed colonies were selected. A portion of the surface of each plate was scribed by means of a stainless steel template. The inscribed area includes two sectors termed "sector III" and "sector IV". Within sector III, 1.7 μl of the sample dilution was plated in a spiral pattern. Sector IV included 7.3 μl of plated sample. Calculation of the colony forming units (CFU) per ml were performed using these calibrated sector areas. Representative colonies within each sector were subcultured as detailed below. From each plate, colonies in two sectors which included more than 20 colonies per sector were quantitated with the aid of a binocular dissection microscope. The total number of colonies and the number of selected colony types was determined for each sector. After colony morphology type had been determined and recorded, representative colonies of each morphology type were picked and put into separate tubes of basal anaerobic broth with 1% glucose (BAB, 90). After growth in BAB, each tube was checked for purity on ETSA plates. When pure, fresh isolates were picked and placed in BAB and basal esculin nitrate broth (BEN, 57).

Each new BAB culture was handled as follows: Aerobic blood plates were streaked for each BAB sample and incubated in a candle jar. Gram stains were then prepared. A portion of the remaining culture was poured into vials and flash frozen in liquid nitrogen for gas liquid chromatographic analysis of metabolic fatty acid end products.
For each isolate from the BEN medium, biochemical tests for esculin hydrolysis, nitrate reduction, and the presence of catalase were performed as follows:

Two to five drops of each BEN culture were placed in each of three plastic wells. Each well was then tested for one of the following:

**Catalase**: One drop of 30% hydrogen peroxide was added. The appearance of bubbles indicated catalase positive.

**Nitrate reduction**: Two drops of 0.8% sulfanilic acid in 5 N acetic acid (solution A,^33^) and 2 drops of 1.2% dimethyl-alpha-naphthylamine in 5 N acetic acid (solution B,^33^) were added. Red was read as positive for nitrate to nitrite reduction. Crystals of powdered zinc were added to non-red cultures. If the area around the zinc turned pink, it was negative for nitrate reduction. If no color developed, the result was interpreted as "*", indicating the reduction of both nitrate and nitrite.

**Esculin hydrolysis**: Two to three drops ferric ammonium citrate^33^ were added. Black was interpreted as positive, yellow as negative.

The broth remaining in the BEN culture tube was tested for indole production as follows:
One ml xylene was added to each culture. The culture was shaken well and allowed to stand for 2-5 min. Ehrlich Reagent\(^3\) (0.5 ml) was added down the side of each tube. Development of a pink or fuchsia ring within 15 minutes was read as positive.

**Computer Assisted Bacterial Identification System (CABIS)**

The identification of bacteria to the genus or species level is a very labor demanding process. In this study, 111 subgingival plaque samples were analyzed. Each sample contained between 8-15 types of organisms. Identification of each organism required 9-14 separate tests to permit its classification into genus and/or species. Therefore, roughly 15,000-20,000 individual pieces of data were generated. In the past, the processing of this data to permit identification had been done manually in our laboratory. The computer assisted bacterial identification system was developed specifically for this project.

An 8-bit 48-K byte memory table-top computer system that used 5-1/4" magnetic mini-floppy disks was chosen. Programming of the computer was done by the principle investigator in Microsoft Basic\(^R\). This is a powerful high level programming language that provided the features of numeric and alphabet character manipulation required.

All of the available information on each isolate was entered into CABIS. Using criteria previously described\(^4\) which are summarized in Figures 3 through 8, the organisms
were classified to the species level where possible. If not enough data were available for the identification, additional standard biochemical tests (e.g. ammonia, urease, or oxidase production) were suggested by CABIS. The results of these tests were then added and final identifications were made. With CABIS, it was possible to identify most of the bacteria encountered with a high incidence in subgingival plaque to the species or subspecies level.

Statistics

Differences between the mean percentage distributions of bacterial species and differences in pocket depths at different times in the experimental or control animals were compared using the unpaired Student's t-test. The clinical indices were analyzed as dichotomous variables as suggested by Barbano. Differences in the clinical indices were compared using chi-square analysis of the frequency of scores 0-1 or scores 2-3. The data are reported in this thesis as percentage 0-1 or 2-3 for easier comparison.

For all statistical evaluations, p<0.05 was used as the level of confidence.
RESULTS

Ligated Sites

Clinical Results

Four clinical and microbiological stages of disease were evident. Stage I, corresponding to week 0, was characterized by a mean PI = 1.6. The gingival index at this stage was 1.6. The mean pocket depth was $2.2 \pm 0.4$ mm (mean ± standard deviation) (Table 1). There was no clinical recession evident at this stage or throughout any of the recordings during the entire investigation. Stage II, occurring at weeks 1-2, was characterized by a slight increase in the mean PI ($\bar{x} = 2.0$) and a greater increase in the mean GI ($\bar{x} = 2.8$). The mean pocket depth was $3.0 \pm 0.8$ mm which was not statistically different from the pocket depth seen in Stage I. No radiographic bone loss was detected. The tendency to increase mean pocket depth at this stage probably represented the edematous nature of the gingival tissue. Stage III, occurring at weeks 4-6, showed no further significant increase in mean PI ($\bar{x} = 2.0$). The mean GI decreased slightly ($\bar{x} = 2.3$) whereas the mean pocket depth increased significantly to $x = 3.7 \pm 0.8$ mm. Radiographic bone loss was now evident (Fig. 9). The clinical parameters at Stage IV were similar to those at Stage III. The mean PI was 2.0 and the mean GI was 2.3. The mean pocket depth was $3.7 \pm 0.7$ mm. Statistical analysis of the
PI.I. and GI, however, revealed no significant differences throughout the experiment.

Microbiological Results

Stage I

The microbiological composition at ligated sites is summarized in tabular form in Table 2 and graphically in Figure 10. The total cultivable flora (TCF) at Stage I was composed of about one-fourth Gram-positive organisms, primarily cocci. Gram-negative cocci represented only 6.7% of the TCF. The total percentage of Gram-negative cocci did not change significantly throughout the experiment. Gram-negative rods comprised the bulk (approximately two-thirds) of the ligated site TCF throughout the experiment. Black pigmented Bacteroides (BPB) represented 22.0 ± 7.2% of the TCF or about 35% of the total Gram-negative rods. Bacteroides melaninogenicus ss. intermedius comprised the majority of the BPB. Bacteroides gingivalis represented only 2.4 ± 4.5% of the TCF. Surface translocating bacteria (STB, 30) and Gram-negative motile rods represented 4.8 ± 4.2% of the TCF. Fusobacteria represented about 10% of the TCF at Stage I and the anaerobic:facultative ratio was 1.4. Spirochetes represented 14.3 ± 8.3% of the total microscopic flora (TMF) at Stage I.

Stage II

Stage II was primarily characterized by a four-fold increase in Gram-negative surface translocating bacteria
(STB) and motile rods (Table 2, Fig. 10). Included in this group were Capnocytophaga, Campylobacter, Vibrio, and Selenomonas. This increase appeared to be primarily at the expense of the Gram-positive cocci. Gram-negative cocci and Gram-negative anaerobic rods also decreased slightly. The percentage of the TMF represented by spirochetes also increased significantly approximately two-fold.

**Stage III**

Stage III, which corresponded to clinically detectable bone loss, demonstrated a marked increase in _B. gingivalis_ which now represented nearly one-fourth (23.4 ± 11.0%) of the TCF (Table 2, Fig. 10). The percentage of total Gram-negative rods increased, reflecting the rise in _B. gingivalis_. Gram-positive organisms now represented a total of only 8.2% of the TCF. STB and Gram-negative motile rods decreased slightly. Spirochetes did not change significantly at this stage or for the remainder of the experimental period. The overall anaerobic:facultative ratio increased to 2.4.

**Stage IV**

Stage IV was characterized by minor decreases in the total percentages of Gram-negative rods including BPB, STB, and motile rods (Table 2, Fig. 10). The percentage of Gram-positive organisms increased but remained below Stage I levels. The distribution of bacteria resembled the flora at Stage II except that the levels of _B. gingivalis_ were
still elevated and *B. melaninogenicus* ss. *intermedius* were reduced.

Non-Ligated Sites

Clinical Results

The non-ligated sites did not change clinically during the development of ligature-induced periodontitis in opposite or contralateral quadrants in the same animal (Table 3). There were no significant changes noted in the Pl.I. GI, pocket depth, position of the gingival margin, or radiographic appearance of the interproximal alveolar crests (Fig. 11).

Microbiological Results

As expected, the percentage distribution of the TCF at Stage I for the non-ligated sites was similar to that of the ligated sites since Stage I represented the time of initial ligation (Table 4 and Fig. 12). Stage II, 1-2 weeks post-ligation, did not demonstrate any significant microbiological changes at non-ligated sites from that of Stage I (Table 4).

The non-ligated sites at Stage III showed changes in the percentage of TCF similar to the pattern seen at Stage II in ligated sites (Table 4, Fig. 13). The STB and Gram-negative motile rods increased about three-fold. There was, however, no significant change in the percentage of TMC represented by spirochetes.

Stage IV in non-ligated sites was similar to Stage III in non-ligated sites except that the STB and Gram-negative
motile rods continued to increase and now represented about one-fifth of the TCF (Table 4, Fig. 14). An increase in the percentage of TMC represented by spirochetes relative to Stage I was seen. There were no other significant changes observed.

**Control Sites**

At week 0, the control animals (i.e. those with no ligatures present) resembled the experimental animals at week 0 both clinically (Table 5) and microbiologically (Table 6). There were no changes detected in either clinical or microbiological parameters in these sites throughout the experimental period.

**Infected Sites**

When non-ligated sites in the experimental (ligated) animals were infected at week 0 and again at week 1 with either pure cultures of *B. gingivalis* or plaque from ligated sites and then monitored four weeks later, there were no detectable clinical (Table 7, week 4) or microbiological (Table 8 and Fig. 15) changes from the week 0 (Stage I) conditions. However, when non-ligated sites in experimental animals were infected with either *B. gingivalis* or plaque from ligated sites at week 4 and again at week 5 (Stage III) and then monitored four weeks later, a statistically significant increase in *B. gingivalis* was observed (Table 8 and Fig. 16). No clinical changes were seen, however, four weeks after infection.
Non-ligated sites in experimental animals were sham-infected with sterile curettes at weeks 0 and 1 or weeks 4 and 5 were not significantly different clinically (Table 9) or microbiologically (Table 10) from the non-infected non-ligated sites in these animals. The results from these sites were therefore included with those for the non-ligated sites (Tables 3 and 4).

Sites in control animals that were infected with either pure cultures of *B. gingivalis* (Tables 11 and 12) or with a sterile curette (Table 13 and 14) at weeks 0 and 1 or weeks 4 and 5 were not significantly different clinically or microbiologically from the non-infected sites in the same animals when examined four weeks later. The sham infected site control animal data was combined with the non-infected control animal data in the same manner as the data were handled for the sham infected sites in the experimental animals.

**Effect of Sampling**

In one animal, in which selected sites were sampled either at weeks 0, 1, 2, 4 and 6 or only at weeks 4 or 6 but not previously, it was seen that sites which were samples only once were not significantly different either clinically (Table 15) or microbiologically (Table 16) from those sampled either 3, 4, or 5 times previously during the preceding six weeks. This suggested that an insignificant amount of the total subgingival plaque mass was disturbed or removed during the sampling of a given site with the sampling procedure used in this investigation.
DISCUSSION

In the present investigation non-ligated sites in monkeys underwent changes in their subgingival microflora when ligature-induced periodontitis was produced in contralateral or opposite areas. These changes consisted primarily of increases in the proportion of surface translocating bacteria (STB) and Gram-negative motile rods (Fig. 17). There are no reported longitudinal studies in humans of the subgingival microflora of developing periodontal disease for obvious ethical reasons. Cross-sectional studies in humans of the subgingival microflora in various forms of periodontitis have been carried out. In one study, no controls were used. Two other studies did not report the distribution of Gram-negative organisms other than Bacteroides. A fourth study combined the control site data from patients with several different clinical presentations of periodontitis. However, in all of these studies, the reported distribution of Bacteroides species from chronic periodontitis in humans was similar to the distribution seen in the current model of human disease using the Cynomologus monkey. In both humans and monkeys, saccharolytic BPB (e.g. Bacteroides melaninogenicus ss. intermedius) were associated with gingivitis whereas non-saccharolytic BPB (i.e. B. gingivalis) were associated with periodontitis. Williams reported higher numbers of
Actinomyces species in patients with periodontitis than in patients in periodontal health. Healthy sites from patients without periodontitis were different from healthy sites in patients with periodontitis. The distribution pattern of pigmented Bacteroides species reported in the previous four studies as well as the current study, was not seen in the study of Williams. However, differences in the subgingival microflora of healthy sites in subjects with periodontitis compared to the microflora of healthy sites in subjects with no periodontitis seen by Williams was also seen in the current investigation. Williams reported this difference to be primarily in the distribution of the Gram-positive rods of the Actinomyces genus whereas the current investigation found greater difference in Gram-negative surface translocating bacteria (STB) and Gram-negative motile rods. These differences may be the result of both sampling technique and the technique used to process the samples. In spite of these differences, the currently employed model seems to be a reasonable method for the study of periodontitis.

The subgingival microflora of ligature-induced periodontitis in monkeys in the present investigation was very similar to those previously reported by Slots and Kornman. Neither study, however, reported the subgingival microflora of non-ligated sites. In the present study, the microflora of non-ligated sites in monkeys with ligatures in other sites was different from sites in non-ligated monkeys ("control" sites). Non-ligated sites
developed an increased percentage of Gram-negative STB and motile rods whereas control sites (i.e. from monkeys with no ligatures) did not change during the experimental period.

The distribution pattern of subgingival bacteria in non-ligated sites was similar to that seen just prior to the development of high numbers of *B. gingivalis*, increased pocket depth, and radiographic bone loss observed in ligated sites in the same animal. The ability to infect a non-ligated site with *B. gingivalis* appeared to be augmented by the presence of STB and Gram-negative motile rods since it was not possible to recover high numbers of *B. gingivalis* four weeks after implantation unless implantation occurred at a time when STB and Gram-negative motile rods were increased (Figs. 15 and 16). Gibbons\(^{22}\) reported that it was not possible to establish certain anaerobic organisms in the oral cavity of gnotobiotic animals unless these animals were simultaneously or previously infected with facultative organisms. Gibbons observation and the results of the current study suggest that some nutritional requirement for *B. gingivalis* may be missing in gnotobiotics or periodontally healthy sites and can be supplied by facultative STB or motile rods.

Growth requirements for *Bacteroides melaninogenicus* have been reported to be complex. Growth of pure cultures in *vitro* of *B. melaninogenicus* appears to require two unusual supplements, vitamin K and hemin.\(^{24}\) The action of vitamin K in *B. melaninogenicus* was reported to be
stimulation of the biosynthesis of phosphophingolipids. Vitamin K also appears to serve as the precursor to menaquinone synthesis in anaerobes. Menaquinones serve as electron carriers in the metabolism of certain anaerobes. Succinate can partially replace the dependency for vitamin K in *B. melaninogenicus* as measured by growth curve yields. Radioactivity was detected in the lipid and phospholipid fractions of cells incubated with radiolabelled succinate suggesting this fatty acid is utilized by *B. melaninogenicus*. A related organism and pathogen *Bacteroides fragilis*, has been shown to produce α-ketoglutarate from succinate via α-ketoglutarate synthetase. This pathway may also be an important source of energy in *Bacteroides*. Direct in vivo evidence for the role of succinate in the metabolism of *Bacteroides* was reported by Mayrand and McBride who showed that pure cultures of *B. gingivalis* required *Klebsiella pneumoniae* to produce subcutaneous abscesses in guinea pigs. The dependency on *K. pneumoniae* was related to a substance present in sterilized culture supernatants of *K. pneumoniae* and was identified as succinate. Other organisms including sacchrolytic *B. melaninogenicus*, *E. coli* and *A. viscosus* could also provide supplemental succinate and permit abscess formation in this system, whereas bacterial species that did not produce succinate (i.e. *S. sanguis*, *F. nucleatum*, or *S. albus*) did not permit abscess formation when mixed with *B. gingivalis*. Finally, agar immobilized succinate alone could provide the
necessary supplementation to B. gingivalis for abscess formation in this system.

Sources of succinate in the gingival sulcus are not difficult to find. Succinate is an intermediate metabolite in the Kreb's cycle of many facultative organisms. Additionally, succinate may be produced in large quantities by many of the STB and motile rods characteristic of the Stage II ligated flora or Stage III and IV non-ligated flora in the current study. 33  *Selenomonas* and anaerobic *Vibrio* species can produce succinate via fumarate reduction that utilizes a cytochrome b electron transport system. 19  *Vibrio succinogenes* can produce fumarate from formate via formate dehydrogenase 50 and succinate from fumarate via cytochrome-dependent fumarate reduction. 40  *Bacteroides corrodens* has been shown to utilize formate or hydrogen as electron donors in the formation of fumarate from malate and succinate from fumarate. 81  *Actinomyces* species produce formate and anaerobic cocci produce hydrogen which was shown to be utilized by *Vibrio succinogenes in vitro*. 35  These sources of electron donors are examples of potential metabolic interactions capable of supplying succinate in vivo to *Bacteroides* in the gingival sulcus. Finally, *Campylobacter sputorum* has also been shown to produce large amounts of succinate from fumarate. 18  It is evident then, that many of the STB and motile rods, particularly *Campylobacter, Vibrio, Anaerovibrio, Capnocytophaga* and *Selenomons*, are capable of supplying nutritional supplementation of
succinate to \textit{Bacteroides}. Whether this phenomenon actually occurs \textit{in vivo} is not clear. The current observation that increased proportions of this group of organisms preceded the establishment of high proportions of \textit{B. gingivalis} in ligated sites or in exogenously infected non-ligated sites supports the role of \textit{in vivo} interaction. However, it was also seen that in non-ligated sites, even in Stages III and IV in which higher proportions of these organisms were present, the interaction of succinate producers and \textit{B. gingivalis}, which was usually detectable in very low proportions in non-ligated sites, was not sufficient to permit the development of increased numbers of \textit{B. gingivalis} and subsequent periodontitis. This could be due to the fact that the observation period may have been inadequate to detect such changes since the nonligated sites were not monitored for longer than 8-11 weeks. In the absence of the changes induced by the ligature, \textit{B. gingivalis} may require a much longer time to become prominent in the subgingival ecosystem. Infected non-ligated sites, however, could permit high numbers of \textit{B. gingivalis} to become established due to some minimum required infectious dose that is exceeded by the infectious challenge. Similarly, studies of the establishment of the human cariogen \textit{S. mutans} in artificial fissures have demonstrated that a minimum of $10^4$ CFU/ml of saliva was required for early colonization of the fissures.\textsuperscript{88} Lower salivary levels of \textit{S. mutans} were capable of establishing in the artificial fissure if given a much longer
time. This may occur as a result of changes within the fissure brought about by other organisms which favors later colonization by \textit{S. mutans}.\cite{88}

The observation of decreasing levels of STB and motile rods during the development and establishment of high proportions of BPB or \textit{B. gingivalis} suggests an antagonistic interaction between these groups of organisms (Figs. 16 and 17). \textit{B. gingivalis} produces large amounts of butyrate as a metabolic end product\cite{33} and this product may be in part responsible for this interaction. A similar relationship of two groups of bacteria was seen by Lee and Gemmill\cite{151} who reported that in the developing intestinal flora of weanling mice, the predominantly facultative coliform flora seen at 8-12 days after birth decreases 10,000 fold with the establishment of an anaerobic fusiform flora that develops when the mouse begins to eat solid food. Fatty acid analyses of the mouse cecal content and the bacterial metabolites of the intestinal flora revealed the presence of large amounts of butyrate. Feeding penicillin to these mice caused a decrease in the levels of anaerobic fusiforms and the levels of butyrate in cecal contents along with an increase in coliforms. Other volatile fatty acids analyzed in this study did not correlate well with levels of either group of organisms. Similarly, Bonhoff et al\cite{10} attempted to produce experimental \textit{Salmonella} enteritis by oral inoculation of \textit{S. enteriditis} in adult mice. The results of this study suggested that an \textit{in vivo} inhibition of \textit{Salmonella
multiplication occurred since supernatants of heat killed extracts of colon contents were inhibitory to *S. enteriditis* growth *in vitro*, as were anaerobic cultures of colon contents. Butyrate recovered from colon contents or butyrate produced from *Bacteroides* isolated from the colon was inhibitory to growth of *S. enteriditis*. Pretreatment of these mice with streptomycin decreased the oral I.D.50 by $10^5$ and decreased the levels of butyrate in the colon.\textsuperscript{11} In this latter experiment, succinate was capable of overcoming the inhibitory effect of butyrate *in vivo*. This may be due to the ability of *S. enteriditis* to utilize succinate as a sole carbon source.\textsuperscript{11} Butyric acid has been shown to decrease the growth of *E. coli* *in vitro*.\textsuperscript{8} Dietary changes in humans capable of altering butyrate concentrations in the intestine were also shown to be associated with changes in intestinal flora.\textsuperscript{9} Butyrate has been shown to increase gingival inflammation in dogs when applied topically twice per day\textsuperscript{75} and the incidence of butyric acid producing bacteria has been correlated with the severity of periodontitis in humans.\textsuperscript{65}

The interrelationship of *B. gingivalis*, STB, and Gram-negative motile rods may be affected by factors other than those described above. Oxidation-reduction potential in the gingival sulcus may be important.\textsuperscript{44} Facultative organisms are capable of tolerating less negative redox potentials than strict anaerobes and may be capable of lowering the Eh in the sulcus. This could then permit establishment of *B*.
gingivalis. A similar phenomenon was reported by Socransky et al.\textsuperscript{85} who showed growth dependency on controlled (i.e. lowered) oxidation-reduction potential for Treponema microdentium, an oral spirochete.

Certain strains of non-oral Vibrio have been shown to produce an extracellular hemolysin.\textsuperscript{34,64} If oral Vibrio produce similar exotoxins, a readily available potential nutrient source for heme could be present in vivo. Certainly, the inflammation associated with gingivitis and periodontitis could supply blood cells and other potential nutrients to the subgingival flora. Bacteroides fragilis has been shown to produce succinate from glucose supplemented growth media only when hemin was present in the culture.\textsuperscript{61}

Bacteriocins, compounds produced by one bacterial species that are capable of inhibiting other species, represent another source of bacterial interaction. Oral strains of Streptococci have been shown to produce bacteriocins that were active against various Gram-positive and Gram-negative organisms.\textsuperscript{7,16,31} S. mutans produces a bacteriocin which is capable of inhibiting colonization of gnotobiotic rats by A. viscosus.\textsuperscript{70} Bacteriocin producing strains of S. mutans were shown to establish in specific pathogen free Osborne Mendel rats previously infected with A. viscosus or S. mutans if introduced one day after infection with these two organisms.\textsuperscript{95} These authors suggested that bacteriocin inhibition may only be effective if the "microbial equilibrium"
or "climax community" has not previously established. *B. gingivalis* and *B. melaninogenicus* ss. *melaninogenicus* produce low molecular weight substances capable of inhibiting the growth of *Neisseria gonorrhoea* in vitro. Actinomyces odontolyticus produces a bacteriocin active against *Lactobacillus*. Non-oral strains of *Vibrio* and *Bacteroides fragilis* also produce bacteriocins.

The role of bacteriocins in vivo, however, is not clear. Bacterial species sensitive to bacteriocins produced by oral streptococci have been shown to persist in large numbers by Kelstrup, who suggested that this may be due to bacteriocin inactivation by proteolytic salivary enzymes. The presence of salivary proteolytic enzymes in subgingival areas is not likely since gingival crevicular fluid flow tends to be unfavorable to ingress of saliva. Proteolytic enzymes released by inflammatory cells, however, certainly could be an important factor in the activity of bacteriocins. Bacteriocin sensitive species persist in large numbers in the intestinal flora even though bacteriocin producing species of *Bacteroides* can be isolated from these sites. These bacteriocins were only produced during the log phase of growing cultures. This suggests that if bacteriocins are effective in vivo, they may be present during the establishment of high numbers of an organism in a given site but not subsequently.

Other factors that may effect the observed interaction of organisms in vivo are suggested by studies of the
inhibition of polymorphonuclear leukocyte (PMN) phagocytosis 
*in vitro* by obligate anaerobes.\(^{36}\) It was shown that *B.
melaninogenicus* was capable of inhibiting PMN phagocytosis 
of certain aerobes. Similarly, oral *Capnocytophaga* species 
may be associated with defects in the morphology and function 
of polymorphonuclear leukocytes.\(^{73}\) In an experimentally induced 
mouse groin abscess model, it was shown that 
\(\beta\)-lactamase production by *Bacteroides fragilis* was capable 
of protecting penicillin sensitive strains of *Fusobacteria* 
from the antibiotic's activity.\(^{97}\) The range of bacterial 
and host interactions in the gingival sulcus are obviously 
very complex and it is likely that some or all of these factors could account for the observed results of the current investigation.

The observed result of changes in otherwise healthy sites during the development of periodontitis in distant sites suggests that diseased and healthy sites do not occur independently. Split mouth studies of periodontitis in which one side serves as the experimental area and one side as control may therefore be improper since sites in the same mouth may not be truly independent of each other. For example, longitudinal studies of various treatment modalities for periodontitis\(^{46}\) using a split mouth design have shown that there were only very minor differences in the 8 year follow-up measurements of clinical parameters between the treatment modalities used. Although the author ascribed these results to the effect of the therapy used, it is
possible, in light of the results of the current study, that
the elimination of pathology by one particularly effective
treatment method could effect the results of the remaining
areas since these areas may not be independent. Conversely,
the lack of elimination of disease associated bacterial
species by one treatment method could permit bacterial re-
population of the contralateral side and subsequent masking
of the effectiveness of the other method. Clinical studies
of the effectiveness of treating gingival recession with
free gingival grafts in a split mouth designed study showed
that no difference existed between experimental and control
areas within the same mouth.\textsuperscript{20} Thus, lack of independence
observed in the current study between sites in the same
mouth makes the results of split mouth studies difficult to
interpret.

The ability of motile bacteria to influence adjacent or
contralateral areas is not surprising. Many of the species
in the STB and motile rod group have clearly demonstrable
organs of motility that could account for their movement to
distant sites. Although organs of motility cannot be demon-
strated for certain STB,\textsuperscript{30} time-lapse photography studies
have revealed that many of these organisms are capable of
gliding over agar surfaces.\textsuperscript{1} One species of STB, \textit{Capnocyto-
phaga}, has been shown to be present primarily in supraging-
ival or slightly subgingival plaque from patients with
severe periodontitis.\textsuperscript{69} The transmissibility of this
organism may thus be facilitated by its location in plaque.
Monkeys in animal care facilities have been observed by caretakers to pick at oral appliances or ligatures on their teeth. Mastication of food combined with the lack of plaque control in these animals may also be capable of transferring organisms to distant sites. Transmissibility of other STB (Campylobacter species) between humans\textsuperscript{76} as well as animal to human\textsuperscript{89} has been reported.

The changes that occurred in non-ligated sites in this study were not due to the soft diet alone as reported by Slots\textsuperscript{80}. In that study, the animals were placed on soft diets only one week prior to ligation. In the present study, the soft diet was started 5-8 weeks prior to ligation. More importantly, the changes observed in non-ligated sites were significantly different from the control animals in which no ligatures were placed but were fed a soft diet for an identical time period as the experimental animals.

Several studies that investigated radionuclide imaging of healing dental extraction sites have shown a biphasic pattern of increased isotope uptake in the healing socket\textsuperscript{26,60} indicating increased bone metabolism in these areas. However, an identical increase in radionuclide uptake was also observed in contralateral areas. These changes were less pronounced than those seen in the extraction site. These observations suggest that the production of inflammation as occurred during healing (which may not be different from the production of inflammation associated with ligature-induced periodontitis) may be capable of
causing an increase in inflammation or metabolic activity in distant sites. The increase in inflammation in distant sites may provide altered local environmental factors or nutrients that permit changes in the resident microflora of gingival sulci.

The increased susceptibility to exogenous infection observed in the present study suggests that it may be possible to infect certain sites in human patients if large masses of plaque rich in \textit{B. gingivalis} are transferred into otherwise healthy sites when active periodontitis exists in other areas of the mouth. This could occur during certain routine dental manipulations including periodontal probing or subgingival scaling. \textit{In vitro} and \textit{in vivo} studies of the ability of \textit{B. gingivalis} to attach to various oral surfaces have suggested that these organisms attached well to Gram-positive organisms or preformed plaque and that this attachment was not affected by serum or saliva.\textsuperscript{79} Serum and saliva markedly reduced the attachment of this organism to hydroxyapatite or epithelial cells. In the present investigation, the ability to establish \textit{B. gingivalis} by direct subgingival implantation also appeared to be dependent upon certain changes in the pre-existing plaque. These changes may permit the establishment of \textit{B. gingivalis} by increasing its ability to attach to pre-existing plaque. The intraoral transmission of the caries-associated pathogen \textit{Streptococcus mutans} by a dental explorer has been demonstrated.\textsuperscript{58} The ability to transmit periodontitis-associated organisms by
periodontal probing was recently suggested by Barnett who demonstrated by electron microscopy that probes placed into periodontal pockets greater than six millimeters and immediately fixed upon removal from the pocket had many bacterial types adherent to the probe. Many of these specimens contained predominantly Gram-negative rods and flagellated filaments. In view of the current findings, inoculation and subsequent development of periodontitis in apparently healthy sites in patients with active periodontitis seems possible.
CONCLUSIONS

1. Placement of silk ligatures in monkeys resulted in certain specific shifts in the subgingival microflora. In ligated sites, an immediate (1-2 week post-ligation) four-fold increase in surface translocating bacteria (STB) and Gram-negative motile rods occurred. This was followed by an increase in *B. gingivalis* and a simultaneous decrease in STB and Gram-negative motile rods at 4-6 weeks post-ligation. Clinically demonstrable bone loss was evident at this stage.

2. The development of localized ligature-induced periodontitis in monkeys resulted in changes in the subgingival microflora at non-ligated "healthy" sites. These changes consisted of an increase in STB and Gram-negative motile rods after 4-6 weeks. No clinical changes were noted in nonligated sites.

3. The ability to establish high proportions of *B. gingivalis* in non-ligated sites by direct implantation was possible in the presence of high proportions of STB and Gram-negative motile rods. Levels of these STB and Gram-negative motile rods decreased as *B. gingivalis* increased.
FUTURE INVESTIGATIONS

The appearance of STB and Gram-negative motile rods in non-ligated sites seen in this investigation could be due to several mechanisms. Future studies could be directed towards investigating these mechanisms. The ability of these organisms to translocate or to be motile may permit them to eventually establish in a distant or adjacent site. In monkeys, the act of mastication or manual fidgeting also may be capable of transmitting organisms to other sites if the load of cells in ligated sites is increased sufficiently (i.e. Stage II). These points could be investigated by more specific adjacent versus distant site monitoring when ligatures are placed, or by tube feeding or restraint collar experiments. For example, areas near ligated sites may develop increased numbers of STB and motile rods sooner than sites more distantly located from the ligature if transmission occurs by bacterial motility alone.

Certain oral microorganisms may be capable of causing suppression of host responses.\textsuperscript{36,73} This suppression, acting either locally or systemically, could permit increases in the percentage of these organisms in nearby or distant sites. The bacterial pattern seen in the ligated Stage II sites could predispose the subject to the non-ligated Stage III flora in distant sites by the suppression of host responses. Monitoring of certain host responses to
these STB and Gram-negative motile rods during disease development could clarify this possibility.

The ability to contaminate an otherwise "healthy" site in a periodontitis patient will be difficult to clarify in humans and may be impossible for ethical reasons. However, certain operators routinely disinfect their dental instruments between sites within a patient's mouth using solutions such as povidone-iodine. Careful comparisons of these dentists' patients with other patients not receiving this type of attention would be useful.

To investigate the importance of the metabolic by-products succinate and butyrate, it may be possible to alter the subgingival microflora by local application of these acids into the gingival sulcus. This could be performed by either subgingival irrigation or by acid impregnated ligatures which release their products over an extended time period. In this manner, the ability to establish *B. gingivalis* infection in these sites with exogenously supplemented succinate could be studied. The ability of butyrate to inhibit STB and Gram-negative motile rods could also be studied in this system.

The results of this study suggest that this animal model system may be useful as an *in vivo* method for testing pathogenic mechanisms in *B. gingivalis* and other organisms. Cells with specific defects in certain functions (such as collagenase defective mutants) could be implanted in non-ligated sites. The relative importance of bacterial
collagenase might then be assessed. Other members of the subgingival flora from ligated sites might also be examined for their ability to induce periodontitis. This system represents a model for the study of periodontitis much more like that of humans than do gnotobiotic rodent or Beagle dog experimental systems. An organism must compete for survival in the sulcus of the monkey in much the same environment as in humans.

Host factors, such as immune response or local or systemic steroid levels, could be manipulated in these monkeys and the ability to infect sites with *B. gingivalis* or other organisms could be further studied. Studies such as these could help to clarify the relative importance of specific bacteria, certain bacterial characteristics of a given organism, and host factors in the etiology of chronic periodontitis.

The sampling technique used in this study employed sterile absorbant paper points to collect subgingival plaque. This technique may not be capable of detecting increases in the number of organisms present in a site since the absorbancy of the paper point may reach its saturation level. Thus, decreases in percentages of species could occur while the actual number of organisms may increase. In similar studies, the saturation limit of paper point sampling appeared to be approximately $5 \times 10^7$ CFU/ml which is above the levels found in samples from the current study. This suggests that the sampling technique used in the
current study was capable of detecting changes in CFU/ml if they had occurred. However, there are no published studies that have examined the ability of any of the sampling methods currently in use to detect changes in the number of organisms in a subgingival site. Further studies of the sensitivity and limitations of these sampling techniques are needed.
APPENDIX

The following is a brief description of the major features of the computer assisted bacterial identification system.

The program is divided into eight routines that can be accessed in any sequence.

The "A" routine allows the user to enter the initial data generated when a plaque sample has been subcultured to begin the separation and purification of the isolates in that sample. The information stored for each sample includes its identification number (an integer), the date it was processed, the dilution used to plate the sample, and a quantitative and qualitative description of the isolates on the plate. This information is stored on a data disk to be accessed in the future as more data on the samples becomes available.

The "D" routine permits the user to add data to the existing files for all isolates previously entered with the "A" routine. These data include items such as biochemical test results and Gram-stain. The data can be entered either by adding all of the data for one isolate at once, or by adding the results of a single biochemical test for all isolates. Isolates are identified by adding a decimal portion to the integer representing the sample. (For example, the isolates from sample 2000 would be numbered 2000.01,
The data files are accessed in such a way that existing data is located almost instantaneously and does not require time consuming sequential searching of all the existing data.

The "I" routine is used to identify the isolates using all of the available data. This routine uses a series of branched logic statements as shown in Figures 3 through 8. When insufficient data exists to permit identification to the species level, the user is notified which additional tests could be performed that will permit further identification. In addition, an "N" is placed in the row corresponding to the unidentified isolate and column of the needed test. These isolates can later be sorted for using the "S" routine (see below). In these cases, an identification such as "Gram-negative anaerobic rod" is made until the additional test data is available. Likewise, if the computer cannot make a specific identification based on the schemes used, a more general identification is given to that isolate.

The "S" routine permits the user to sort through the existing data files to produce a composite listing of all of the existing data plus identification of the organisms if known (Fig. 18). It can also produce a cross-reference list of all organisms with a specific characteristic. For example, a list of all isolates that were Gram-positive anaerobic rods, nitrate positive and indole negative could be generated. The computer is capable of rapidly searching through thousands of isolate data files to sort out the
desired organisms. It will list their numbers and biochemical profiles on one page. This particular feature is especially useful in helping to recognize patterns of organisms that appear throughout a series of samples.

The "G" routine is a bookkeeping type of program that stores the location of individual vials in a 7 x 7 holed carton. Each vial contains 2 ml aliquot of an isolate in culture medium that is frozen for additional tests needed to identify a given organism. If the "I" (Identification) routine indicates that additional tests are needed, the user then uses the "G" routine to locate the frozen sample which can be thawed and purified (if needed).

The "Z" routine produces a summary of the data for a given sample. The calculations for Colony Forming Units per ml of sample are calculated from the information entered in the "A" routine. The percent distribution of Gram-positive and negative cocci and rods are determined, along with the percent distribution for each species in the sample (Fig. 19). Multiple samples can be summarized and the mean and standard deviations are calculated for the group of samples.

The last two routines are disk or program manipulation routines. The "R" routine permits the user to exchange data disks to process additional data. (Each 5-1/4" disk holds all of the information for approximately 1000 isolates.) The "E" routine exits from the program.

The program code is on the following pages.
10 CLEAR 1000
20 TS$=CHR$(27):RU$=TS$="":UR$=TS$="":PRINT TS$;CHR$(69)
30 PRINT TAB(5):RU$;"**** UCHC DEPARTMENT OF PERIODONTICS BACTERIAL"
40 PRINT " CATALOG PROGRAM ****":UR$
50 PRINT:PRINT:ANS=""
60 INPUT "DID YOU "LOAD LP" PRIOR TO ENTERING MBASIC <Y or N> <Y> "":AN$=
70 IF AN$="Y" THEN 80 ELSE 110
80 PRINT CHR$(7):
90 PRINT:PRINT:PRINTRU$;" REMEMBER -- YOU ARE NOT ENTITLED TO HARD COPY!!! ":UR$
100 PRINT UR$;GOTO 120
110 PRINT:PRINT RU$;"GOOD":UR$
120 PRINT:PRINT:PRINT:GOTO 160
130 DT$="CLOSE":PRINT TS$;CHR$(69)
140 PRINT TAB(25):RU$;" *** COMMAND LEVEL ***":UR$
150 PRINT:PRINT
160 RR$="":RS$="":A = ADJUST SAMPLE, ISOLATES AND COLONY COUNTS"
170 BS$="D = ADD DATA TO PREVIOUSLY ENTERED ISOLATES"
180 CS$="E = EXIT FROM PROGRAM"
190 DS$="G = ADD/FIND G.L.C. VIAL STORAGE DATA"
200 ES$="I = IDENTIFY/SPECIATE ISOLATES"
210 RS$="R = MOUNT OR REMOUNT DATA DISK"
220 FS$="S = SORT/FIND/CROSS REFERENCE ISOLATES"
230 GS$="Z = DATA SUMMARY AND STATISTICAL ANALYSES"
240 PRINT AS$;PRINT BS$;PRINT CS$;PRINT DS$;PRINT ES$;PRINT RS$;PRINT FS$;PRINT GS$
250 PRINT:PRINT
260 PRINT "ENTER LETTER INDICATING YOUR CHOICE: ":;RR$=INPUT$(1):PRINTRR$
270 IF RR$="A" GOTO 490
280 IF RR$="D" GOTO 1300
290 IF RR$="E" THEN PRINT:PRINT:PRINT TS$;CHR$(69):CLOSE:END
300 IF RR$="G" THEN RUN "SV1;GLCPROG.BAS"
310 IF RR$="I" THEN RUN "HHA2.BAS"
320 IF RR$="S" GOTO 2650
330 IF RR$="Z" THEN RUN "CRUNCH.BAS"
340 IF RR$="R" THEN 390
350 PRINT CHR$(7):
360 PRINT:PRINT RU$;"NO SUCH RESPONSE. PLEASE TRY AGAIN.":UR$
370 PRINT
380 GOTO 240
390 GOTO 130:
400 REM -- FIND APPROPRIATE FILE NAME FOR ISOLATE
410 IF IN$:<=1000 THEN KEYS=MID$(IN$,2,2)
420 IF IN$:=1000 THEN KEYS=MID$(IN$,2,3)
430 FILNAME$="SV1;BK\+KEYS++.DAT"
440 OPEN "R",2,FILNAME$
450 RETURN
460 "THE A ROUTINE"
470 ""
480 ""
490 PRINT TAB(15):RU$;"*** ADDING NEW 'SAMPLE' INFORMATION ***":UR$
500 PRINT:PRINT:PRINT "YOU ARE ABOUT TO ENTER NEW SAMPLES TO THE FILES."
510 PRINT:PRINT "TYPE '0' TO BAIL OUT"
520 PRINT:INPUT "ENTER SAMPLE NUMBER (WITHOUT DECIMAL)":IN$:
530 IF IN$="0" GOTO 130
540 IF IN$>:1000 GOTO 500
550 IF IN$=1000 GOTO 500
560 PRINT:PRINT CHR$(7):RU$;"SAMPLE NUMBER TOO LOW.":UR$
570 PRINT "TRY AGAIN.":UR$
580 PRINT:GOTO 510
590 PRINT:PRINT "ENTER DATE PLATE WAS PICKED AS FOLLOWS:"
600 PRINT "DD-MM-M-Y" <(i.e. 01-JAN-80) <"DT$" >":INPUT DT$
PRINT "ENTER SOURCE (UP TO 10 CHARACTERS)" ;"$S$"
PRINT "ENTER DILUTION (i.e. 2/2, 1/2, OR STO)" ;"$DL$"
PRINT "ENTER SECTORS (i.e. III, IV, OR T)" ;"$S$"
PRINT "ENTER TOTAL ISOLATES (i.e. X/##)" ;"$T$"
PRINT "ENTER TOTAL PICKED (i.e. X/##)" ;"$T$"
PRINT "DATE PICKED:" ;"$D$"
PRINT "SOURCE:" ;"$S$"
PRINT "DILUTION:" ;"$DL$"
PRINT "SECTORS:" ;"$S$"
PRINT "TOTAL ISOLATES:" ;"$T$"
PRINT "TOTAL PICKED:" ;"$T$"
PRINT "CORRECT <Y or N> <Y>" ;"$A$"
IF $A$="N" GOTO 520
Q=STR$(NM)$ Q$=RIGHT$(Q$, LEN(Q$, 1))
OPEN "R", W 1., "$S$SRMPL" +Q$, "DRT"
FIELD 1., 4 NR$, 28I NR
I NM$=STR$(NM)$
GET #1, 1
NR%=NR%+1
PUT #1, 1 NR$=STR$(NR%)
GET #1, CL%
NR%=NR%+1
PUT #1, 1 NR%=NR%+1
FIELD #1, 4 NR%, 28I NR, 4M% 8S Z%, 5 8S R1%, 9 AS R2%, 10 AS R3%, 3 AS R4%, 7 AS R5%, 5 AS R6%, 3 AS R7%
GET #1, 1
RSET R1$=IN$#
RSET R2$=DT$#LSET R3$=S$#LSET R4$=DL$#LSET R5$=S$#LSET R6$=TI$#LSET R7$=TP$
REM--PUT NEED SAMPLE INFO INTO FILE AT REC # NR%+1
PUT #1, 1
GOSUB 410 :REM--FIND FILENAME (<FILNAM>)
FOR X=0.01 TO .3 STEP .01
INUM$=STR$(IN$#)+MID$(STR$(X),2,3)
IF LEN(INUM$)>LEN(ZSTR$(IN$#)) THEN INUM$=INUM$+"0"
PRINT "ISOLATE:" INUM$
FOR X=0.01 TO .3 STEP .01
IF CO$=".." GOTO 1200
IF CO$=".." GOTO 1010
DE$="" PRINT "ENTER COLONY DESCRIPTION NUMBER (3-DIGIT NUMBER)" ;"$I$"
PRINT "ENTER NUMBER OF COLONIES:" INUM$
PRINT "COLONY DESCRIPTION NUMBER:" ;"$I$"
PRINT "CORRECT <Y or N> <Y>" ;"$A$"
IF $A$="N" GOTO 1010
FIELD #1, 4 AS NR$, 248 AS T$
GET #2, 2
HR%=URAL(NR$)+1
RSET NR$=STR$(HR$)
PUT #2, 2
DUX%=NR%+1
CL%=DUX%+62+1
IF UD%=DUX% THEN UD%=DUX%+62
FIELD #2, 4 AS NR$, 4UD% AS T$, 4 AS ND$#
GET #2, CL#;LSET NO$=RIGHT$(INUM$, 4)
500 PUT #2, CL%
510 RN%=DU$<4+3:DM%=DU$ MOD 4
520 FIELD #2:DM%=$6 AS TS:7 AS RN%,2 AS RC$:3 AS RD$:44 AS SP$
530 GET #2, RN%
540 RSET RN%=RIGHT$(INUM$,7)
550 RSET RC%=C0$
560 LSET RD$=DE$
570 LSET SP$=
580 PUT #2, RN%
590 NEXT X
600 CLOSE
610 PRINT:PRINT
620 AR#$="Y": INPUT "ANY MORE SAMPLES <Y or N> <Y> ":AH#
630 IF AH#$="Y" GOTO 490
640 GOTO 130
650 / "THE D ROUTINE"
660 /
670 PRINT TS$:CHR$(69)
680 PRINT TAB(25):RU#; "*** DATA ADDITION *** ":UR$
690 PRINT:PRINT
700 PRINT "YOU ARE NOW ABOUT TO ADD DATA <SUCH AS GRAM STAIN>"
710 PRINT "BIOCHEMICAL, pH, G.L.C., etc...) TO PREVIOUSLY ENTERED ISOLATES."
720 AN#="Y":Z%=0
730 PRINT:"DO YOU WISH TO ADD ONLY ONE TYPE OF DATA <Y or N> <Y> ":
740 INPUT AN#:PRINT:IF AN#="N" THEN Z%=1
750 IF Z%=1 GOTO 1570
760 PRINT:"PLEASE SELECT ONE OF THE FOLLOWING"
770 PRINT:"1 - GRAM STAIN <COLOR and MORPHOLOGY>"
780 PRINT"2 - pH and AEROBIC GROWTH"
790 PRINT"3 - ESCULIN, NITRATE, CATALASE, and INDOLE"
800 PRINT"4 - G.L.C. only"
810 PRINT "5 - "$RU$: "NO "$UR$: GROWTH ON PLATE"
820 PRINT:"PLEASE ENTER DATA TYPE (i.e. '1', '2', '3', '4', or '5'):
830 Z%=Z%+1
840 IF Z%>1 AND Z%<7 THEN 1570
850 PRINT:GOSUB 4120:IF QF$<Z: "Y" THEN 1430 ELSE 1
860 PRINT:PRINT:"TO BAIL OUT"
870 IN=0:PRINT:"ENTER ISOLATE NUMBER (XXXX...XXX)":IN
880 NN#=STR$(IN..14):IF LEN(NN#)=INSTR(NN#., ",")+1 THEN NN#=NN#+"0"
890 IN=0 GOTO 130
900 IF IN<10000! GOTO 1650
910 PRINT:CHR$(7)
920 PRINT:"ENTER ISOLATE NUMBER. TRY AGAIN."UR$
930 PRINT GOTO 1410
940 PRINT:"FIND APPROPRIATE FILE AND OPEN IT"
950 Q#=#STR$(IN..10)
960 IN=VAL(RIGHT$(STR$(IN..4)):IF IN>10! THEN IN=VAL(RIGHT$(STR$(IN..3))
970 OPEN "R",2,"SV1:EK"+RIGHT$(Q#;LEN(Q#)-1)".DAT"
980 SE#=#RIGHT$(NN#..4)
990 FOR II=1 TO 2
1000 FIELD #2, 4 AS HR$:248 AS T#1GET #2,II
1010 IF T#0 THEN I=I+3:41GOTO 1770
1020 NEXT II
1030 PRINTCHR$(7):RU$: "NO SUCH ISOLATE. TRY AGAIN OR BAIL OUT."UR#:PRINT
1040 GOTO 1410
1050 REM-- FIND APPROPRIATE FILE AND OPEN IT
1060 Q#=#STR$(IN..10)
1070 IN=VAL(RIGHT$(STR$(IN..4)):IF IN>10! THEN IN=VAL(RIGHT$(STR$(IN..3))
1080 OPEN "R",2,"SV1:EK"+RIGHT$(Q#;LEN(Q#)-1)".DAT"
1090 SE#=#RIGHT$(NN#..4)
1100 FOR II=1 TO 2
1110 FIELD #2, 4 AS HR$:248 AS T#1GET #2,II
1120 IF T#0 THEN I=I+3:41GOTO 1770
1130 NEXT II
1140 PRINTCHR$(7):RU$: "NO SUCH ISOLATE. TRY AGAIN OR BAIL OUT."UR#:PRINT
1150 CLOSE
1160 GOTO1410
1170 X=X+3:1+II-1)
1180 T=T/4:D=INT(T):T=T-D:RN%=D+3
1190 IF T=0 THEN DM=0 ELSE DM=1/(.25-T)
1800 FIELD #2,56<DM> AS Z%$,7 AS RN$,2 AS RC$,3 AS RD$,1 AS R1$,1 AS R2$,1 AS R3$,1 AS R4$,4 AS R5$,1 AS R6$,1 AS R7$,1 AS R8$,1 AS R9$,1 AS R10$,1 AS S1$,6 AS S2$,1 AS S3$,1 AS S4$,1 AS S5$,1 AS S6$,10 AS S7$,10 AS S8$

1810 GET #2,RH
1820 IF Z%=6 THEN NER$=""GOTO 2060
1830 PRINT: PRINT "HERE IS THE EXISTING DATA FOR ISOLATE: ";RU$;R2$;S4$;
1840 PRINT "IF CURRENT DATA IS O.K., OR IF NO NEW DATA, JUST HIT <CR>.
1850 PRINT: PRINT "ISOLATE: ";RU$;R3$;S1$;
1860 ON Z% GOTO 1870,1890,2100,2170,2340
1870 PRINT "NUMBER OF COLONIES: ";RU$;RC$;S4$;
1880 ON Z% GOTO 1890,1910,2100,2170,2340
1890 LSET RC$=NER$;NER$=""GOTO 1870
1900 PRINT "COLONY DESCRIPTION NUMBER: ";RU$;RD$;S4$;
1910 INPUT NER$;IF NER$="" THEN 1930
1920 LSET RD$=NER$;NER$=""GOTO 1900
1930 PRINT "GRAM STAIN <+ OR ->: ";RU$;R1$;S1$;
1940 INPUT NER$;IF NER$="" THEN 1960
1950 IF NER$="" GOTO 1970
1960 LSET R1$=NER$;NER$=""GOTO 1930
1970 PRINT "GRAM STAIN MORPHOLOGY (FROM 1 TO 8): ";RU$;R2$;S1$;
1980 INPUT NER$;IF NER$="" THEN 1910
1990 IF NER$="" GOTO 2030
2000 IF NER$="" GOTO 2060
2010 IF VAL(NER$)<1 OR VAL(NER$)>8 GOTO 1970
2020 LSET R2$=NER$;NER$=""GOTO 1970
2030 IF Z%=2 GOTO 2560
2040 PRINT "GROWTH ON PLATE <+ OR ->: ";RU$;R3$;S1$;
2050 ON NER$;IF NER$="" THEN 2070 ELSE 2040
2060 PRINT "GROWTH IN BRAIN <+ OR ->: ";RU$;R4$;S1$;
2070 INPUT NER$;IF NER$="" THEN 1970
2080 LSET R4$=NER$;NER$=""GOTO 2070
2090 LSET R3$=NER$;NER$=""GOTO 2070
2100 INPUT NER$;IF NER$="" THEN 2010
2110 IF Z%=2 GOTO 2560
2120 LSET R3$=NER$;NER$=""GOTO 2070
2130 IF Z%=3 GOTO 2560
2140 PRINT "GROWTH IN BEN <+ OR ->: ";RU$;R5$;S1$;
2150 INPUT NER$;IF NER$="" THEN 2010
2160 LSET R5$=NER$;NER$=""GOTO 2140
2170 PRINT "ESCELIN <+ OR ->: ";RU$;R7$;S1$;
2180 INPUT NER$;IF NER$="" THEN 2020
2190 LSET R7$=NER$;NER$=""GOTO 2170
2200 PRINT "NITRATE <+ OR ->: ";RU$;R8$;S1$;
2210 INPUT NER$;IF NER$="" THEN 2020
2220 LSET R8$=NER$;NER$=""GOTO 2200
2230 PRINT "CATALASE <+ OR ->: ";RU$;R9$;S1$;
2240 INPUT NER$;IF NER$="" THEN 2020
2250 LSET R9$=NER$;NER$=""GOTO 2230
2260 PRINT "INDOLE <+ OR ->: ";RU$;R1$;S1$;
2270 INPUT NER$;IF NER$="" THEN 2020
2280 LSET R1$=NER$;NER$=""GOTO 2260
2290 IF Z%=4 GOTO 2560
2300 PRINT "AEROBIC GROWTH <+ OR ->: ";RU$;S1$;S1$;
2310 LSET S1$=NER$;NER$=""GOTO 2290
2320 IF Z%=5 GOTO 2560
2330 LSET S2$=NER$;NER$=""GOTO 2320
2340 PRINT "G.L.C. <UP TO 6 LETTERS>: ";RU$;S2$;S1$;
2350 INPUT NER$;IF NER$="" THEN 2020
2360 LSET S2$=NER$;NER$=""GOTO 2350
2370 IF Z%=5 THEN 2560
2380 PRINT "AMMONIA PRODUCTION <+ OR ->"; "\$RU\$S3\$\$URS; 2390 INPUT NER$;IF NER$="" GOTO 2410 2400 LSET S3$=NER$;NER$=""";GOTO 2380 2410 PRINT "OXIDASE <+ OR ->"; "\$RU\$S4\$\$URS; 2420 INPUT NER$;IF NER$="" GOTO 2440 2430 LSET S4$=NER$;NER$=""";GOTO 2410 2440 PRINT "UREASE <+ OR ->"; "\$RU\$S5\$\$URS; 2450 INPUT NER$;IF NER$="" GOTO 2470 2460 LSET S5$=NER$;NER$=""";GOTO 2420 2470 PRINT "MOTILITY <+ OR ->"; "\$RU\$S6\$\$URS; 2480 INPUT NER$;IF NER$="" GOTO 2500 2490 LSET S6$=NER$;NER$=""";GOTO 2470 2500 PRINT RU$;"GENUS: ";S7$;UR$; 2510 INPUT HERS; IF HERS="" GOTO 2530 2520 LSET S7$=HER$;HER$=""";GOTO 2510 2530 PRINT RU$;"SPECIES: ";S8$;UR$; 2540 INPUT NER$;IF NER$="" GOTO 2560 2550 LSET S8$=NER$;NER$=""";GOTO 2540 2560 PRINT RU$;"ANY MORE DATA OR CORRECTIONS FOR ISOLATE: ";\$RU\$S7\$\$URS; 2570 ANS=""";INPUT "<V or N> <N> "\$HAN$ 2580 IF ANS=""";GOTO 1830 2590 PUT #2,2R 2600 CLOSE #2 2610 GOTO 1570 2620 \" THE 'S' ROUTINE \" 2630 \" 2640 \" 2650 PRINT TS$;"E";CLEAR 1000;TS$=CHR$(27);RU$=TS$;"P";UR$=TS$;"4" 2660 PRINT TAB(17);RU$;*** SORT / CROSS REFERENCING ROUTINE *** " 2670 PRINTRU$;"PRINTPRINT 2680 PRINT"THIS ROUTINE WILL SORT THROUGH THE EXISTING DATA FILES" 2690 PRINT"AND CREATE A CROSS-REFERENCED LIST BASED UPON A SET OF CHUSED "; \" CRITERIA. \" 2700 PRINT"A SPECIFIC ISOLATE RANGE MUST BE INDICATED FOR THE SEARCH.";\" :PRINT 2710 ANS=""";INPUT"DO YOU WISH TO PROCEED <V or N> <V> "\$HAN$ 2720 IF ANS=""";GOTO 130 2730 PRINT TS$;"E";"THIS ROUTINE WORKS AS FOLLOWS:" 2740 PRINT"PRINT"YOU WILL BE PRESENTED WITH EACH OF THE CRITERIA 2750 PRINT"USED FOR ISOLATE IDENTIFICATION, FOLLOWED BY A 'Y' " 2760 PRINT"AT THAT POINT, PLEASE INDICATE THE CONDITION OF THAT "; \" :PRINT"CRITERION YOU WISH TO SORT BY." 2770 PRINT"IF YOU DO NOT CARE ABOUT THE CONDITION OF A 2780 PRINT"PARTICULAR CRITERION, JUST HIT <CR>.";PRINT 2790 GOSUB 4220 ;REM HARDCOPY 2800 PRINT;PRINT"WOULD YOU RATHER HAVE A COMPLETE, NON-SPECIFIC" 2810 PRINT"LISTING OF ALL CURRENT DATA ON A GIVEN ISOLATE RANGE <Y or N> <Y> "; 2820 INPUT AJ#;""";INPUT AJ# 2830 IF AJ#<>"N" GOTO 3070 2840 PRINT;PRINT"COLONY DESCRIPTION NUMBER (3-DIGIT NUMBER, USE * FOR WILD") ; ";PRINT 2850 AR$=""";PRINT"GRAM STAIN ";JAR$;"INPUT F1$ 2860 INPUT "GRAM STAIN MORPHOLOGY <FROM 1 TO 7> ";JF2$ 2870 PRINT "GROWTH ON PLATE ";JAR$;"INPUT F3$ 2880 PRINT "GROWTH IN 'BAB' ";JAR$;"INPUT F4$ 2890 INPUT "MINIMUM pH ( < >0 ) ";JF5$ 2890 INPUT "MAXIMUM pH ( < =14.0 ) ";JF6$ 2910 PRINT"GROWTH IN 'BEN' ";JAR$;"INPUT F6$ 2920 PRINT"ESCULIN ";JAR$;"INPUT F7$ 2930 PRINT"NITRATE ";JAR$;"OR + ";JAR$;"INPUT F8$
PRINT"INDOLE";JAR$;:INPUT F9$
PRINT"CATALASE";JAR$;:INPUT F0$
PRINT"AEROBIC GROWTH";JAR$;:INPUT G1$
PRINT"G.L.C. (CALL ISOLATES WITH 'AT LEAST' THE ACIDS REQUESTED WILL BE"
"PRINTED.)"
INPUT "ENTER UP TO 6 (SIX) LETTERS: ";G2$
PRINT"AMMONIA";JAR$;:INPUT G3$
PRINT"UREASE";JAR$;:INPUT G4$
PRINT"OXIDASE";JAR$;:INPUT G5$
PRINT"MOTILITY";JAR$;:INPUT G6$
PRINT "GENUS (PLEASE USE ONLY CORRECT GENUS/SPECIES FORMATTING )":JUR$
PRINT "SPECIES (PLEASE USE ONLY CORRECT FORMAT) ":INPUT G8$
PRINT"PRINT ARE YOU SATISFIED WITH THESE ":
"CRITERIA <Y or N> ";UPS; B'Y'$="": INPUT B'Y$
IF B'Y$="N" GOTO 2658
GOSUB 4388 REM--REQUEST RFINGE
GOSUB 4518: REM--PRIHT RFINGE
FF=8
GOSUB 4568
IF FIJ$="N" THEN GOSUB 3148
GOTO 3298
IF HC=1" THEN GOTO 3218
PRINT ............
PRINT" ISOLFITE HUM COL &:1 G:MI G:BE:1 C: FI H
PRINT" ILIM COL r:,,ES ST I:0 F': Fi PH E S hl Fi E GLC: H :.-:', F.: Of GEML!,,,
" SPECIES
PRINT ............
PRINT#3,"I SOLFITE IHUM COL GI'II GMI G:BE:1 C: FI H
PRINT#3,NUMBER \:COL\:DES\:ST\:IM\:OP\:PA\:] h\:EiS\:I11\:IME\:E* GLC \:HI\:XI\:R0\:I":
" GENUS \:SPECIES ";
PRINT#3,\:E\:C\:IT\:I\:T\:I\:R\:I\:I\: ;
PRINT#3,"I SOLFITE IHUM COL GI'II GMI G:BE:1 C: FI H
PRINT#3,NUMBER \:COL\:DES\:ST\:IM\:OP\:PA\:] h\:EiS\:I11\:IME\:E* GLC \:HI\:XI\:R0\:I":
" GENUS \:SPECIES ";
PRINT#3,\:E\:C\:IT\:I\:T\:I\:R\:I\:I\: ;
PRINT#3,FIELD#2,4 AS FR$,248 AS T$;GET #2,I:F$1=T$;NEXT I
FOR I=1TO2:FIELD#2,I=FR$,I+1:FIELD#2,=T$;NEXT I
FOR I=1TO2:FIELD#2,=T$;NEXT I
IF B=0 THEN 4040
DA=VAL(<KEY$)>10+B
DA=VAL(<KEY$)>10+B
DA=VAL(<KEY$)>10+B
IF DA<LO OR DA>HNO GOTO 4030
X=1+62*(J-1)-1:T=X/4:D=1 INT(T):T=T-D:RN=3+D
IF T=0 THEN DM=1 ELSE DM=1/\ (.25/T)
FIELD #2,56*(DM) AS 2X,.7 AS RN,.2 AS RC,.3 AS RD,.1 AS R1,.1 AS R2,.1 AS R3,.1 AS R4,.4 AS R5,.1 AS R6,.1 AS R7,.1 AS R8,.1 AS R9,.1 AS RO,.1 AS 816.6 AS S2,.1 AS S3,.1 AS S4,.1 AS S5,.1 AS S6,.1 AS S7,.10 AS S8,.1
GET #2,RN
IF AJ$<>"N" GOTO 3968
IF E1$="" GOTO 3460
IF LEFT$ (E1$.,1)="*" GOTO 3420
IF LEFT$ (E1$.,1)<>LEFT$ (RD$,1) THEN 4030
3420 IF MID$(EI$, 2, 1) = "*" THEN 3440
3430 IF MID$(EI$, 2, 1) = MID$(RD$, 2, 1) THEN 4030
3440 IF RIGHT$(EI$, 1) = "*" THEN 3460
3450 IF RIGHT$(RD$, 1) = "*" THEN 4030
3460 IF F1$ = "" THEN 3480
3470 IF F1$ <> R1$ THEN 4030
3480 IF F2$ = "" THEN 3500
3490 IF F2$ <> R2$ THEN 4030
3500 IF F3$ = "" THEN 3520
3510 IF F3$ <> R3$ THEN 4030
3520 IF F4$ = "" THEN 3540
3530 IF F4$ <> R4$ THEN 4030
3540 IF F5$ = "" THEN 3560
3550 IF VAL(RS$) <> VAL(FS$) THEN 4030
3560 IF FF$ = "" THEN 3580
3570 IF VAL(RS$) <> VAL(FS$) THEN 4030
3580 IF F6$ = "" THEN 3600
3590 IF F6$ <> R6$ THEN 4030
3600 IF F7$ = "" THEN 3620
3610 IF F7$ <> R7$ THEN 4030
3620 IF F8$ = "" THEN 3640
3630 IF F8$ <> R8$ THEN 4030
3640 IF F9$ = "" THEN 3660
3650 IF F9$ <> R9$ THEN 4030
3660 IF F0$ = "" THEN 3680
3670 IF F0$ <> R0$ THEN 4030
3680 IF GI$ = "" THEN 3700
3690 IF GI$ <> SI$ THEN 4030
3700 IF G2$ = "" THEN 3720
3710 DIM GG$(5), SS$(5)
3720 FOR GI% = 0 TO 5
3730 GG$(GI%) = MID$(G2$, GI% + 1, 1)
3740 SS$(GI%) = MID$(S2$, GI% + 1, 1)
3750 NEXT GI%
3760 FOR GI% = 0 TO 5
3770 FOR GJ% = 0 TO 5
3780 IF GG$(GI%) = "" THEN 3830
3790 IF GG$(GI%) <> SS$(GJ%) THEN 3820
3800 NEXT GJ%
3810 ERASE GG$, SS$; GOTO 4030
3820 NEXT GI%
3830 ERASE GG$, SS$
3840 IF G3$ = "" THEN 3860
3850 IF G3$ <> S3$ THEN 4030
3860 IF G4$ = "" THEN 3880
3870 IF G4$ <> S4$ THEN 4030
3880 IF G5$ = "" THEN 3900
3890 IF G5$ <> S5$ THEN 4030
3900 IF G6$ = "" THEN 3920
3910 IF G6$ <> S6$ THEN 4030
3920 IF G7$ = "" THEN 3940
3930 IF G7$ <> S7$ THEN 4030
3940 IF G8$ = "" THEN 3960
3950 IF G8$ <> S8$ THEN 4030
3960 IF R7$ = "*" AND INT(VAL(RN$)) > INT(LA$) THEN GOSUB 3140
3970 LA$ = INT(VAL(RN$))
3980 IF HC = 1 THEN 4010
3990 PRINT " /*
" /* !" " " " " " " " " " !" " /*
4000 PRINT RN$; " " RC$; " " RD$; " " RI$; " " R2$; " " R3$; " " R4$; " /*
55

GOTO 4030

4010 PRINT "#", """"""""""""""""""""""""""""""""""
""
""
""
""

""

""

""

4020 PRINT "#", """"""""""""""""""""""""""""""""""
""
""
""
""

""

""

""

4030 NEXT I:NEXT J

4040 GOSUB 4620

4050 HIKEY$=LEFT$(HN$,2)

4060 IF VAL(HNN$)<1000 THEN HIKEY$=LEFT$(HN$,3)

4070 IF VAL(LOKEY$)<VAL(HIKEY$) GOTO 4090

4080 GOSUB 4560:GOTO 3290

4090 CLOSE:PRINT:PRINT "SEARCH FINISHED";

4100 PRINT:PRINT "MORE SORTING "V or- "N: ....

4110 IF LN$=" " THEN 2650 ELSE 130

4120 OF$="N":AX$="N"

4130 PRINT

4140 PRINT CHR$(7):RU$; " """""""""""""""""""""""""

4150 PRINT "T"... AGAIN. "

4160 PRINT:PRINT "ARE YOU TRYING TO QUIT "V or "N" "V"

4170 AX$=""""INPUT AX$"

4180 IF AX$=""""V" GOTO 4200

4190 OF$="V"

4200 RETURN

4210 REM--REQUEST HARD COPY FLAG SUBROUTINE

4220 AX$=""""INPUT"DO YOU WANT HARD COPY OF LIST "V or "N" "N"

4230 IF AX$="V" GOTO 4260

4240 HC=0:

4250 PRINT:RETURN

4260 HC=1:

4270 PRINT:RA$="""

4280 INPUT "DID YOU "LOAD" LP" PRIOR TO RUNNING THIS PROGRAM "V or "N" "N"

4290 IF RA$="V" GOTO 4330

4300 PRINT:CHR$(7):RU$; " "SORRY. LINE PRINTER IS NOT AVAILABLE"

4310 PRINT " FOR YOU. "RU$"

4320 PRINT:GOTO 4240

4330 PRINT:PRINT:CHR$(7):RU$; " "PLEASE BE SURE THAT THE POWER ON OFF".

4340 PRINT:"LINE SWITCHES",

4350 PRINT"ARE PROPERLY SET. "PRINT "THANKS. "RU$"

4360 CLOSE #3:OPEN "0":#3:"LP:LP.DUC"

4370 PRINT:RETURN

4380 PRINT:"ENTER THE "RU$" LOWEST "RU$"; ""SAMPLE NUMBER OF THE RANGE TO BE SEARCHED"

4390 INPUT "(NO DECIMAL PLEASE)";LN$:LO=VAL(LN$)

4400 PRINT:"ENTER THE "RU$" HIGHEST "RU$"; ""SAMPLE NUMBER OF THE RANGE TO BE SEARCHED"

4410 INPUT "(NO DECIMAL PLEASE)";HN$:

4420 IF VAL(LNN$)<=VAL(HN$) GOTO 4490

4430 GOSUB 4120:IF OFS$="V" THEN 130 ELSE 4380

4440 IF VAL(LNN$)<100 OR VAL(LNN$)>10000 THEN GOSUB 4120 ELSE 4460

4450 IF OFS$="V" THEN 130 ELSE 4380

4460 LOKEY$=LEFT$(LNN$,3)

4470 IF VAL(LNN$)<1000 THEN LOKEY$=LEFT$(LNN$,2)

4480 LNO=VAL(LNN$)

4490 HNO=VAL(HN$)+1

4500 RETURN

4510 IF HC=1 GOTO 4540

4520 PRINT TSS$;CHR$(69)

4530 PRINT"THE SELECTED RANGE IS FROM "LN$:" TO "HN$:"PRINT:GOTO 4550
4540 PRINT #3, PRINT #3, "THE SELECTED RANGE IS FROM " ; LN$ ; " TO " ; HN$ ; 
4550 PRINT #3, 
4560 FILNAM$ = "SY1: BK" + LOKEY$$ " .DAT "
4570 OPEN "R" ; 2 , FILNAM$
4580 FIELD #2, 4 AS NR$
4590 GET #2, 1
4600 NR% = VAL ( NR$ )
4610 RETURN
4620 LNO% = LNO% + 10
4630 LHS = STR$( LNO% )
4640 LOKEY$ = MID$( LHS , 2,3 )
4650 IF LN$ < 1000 THEN LOKEY$ = MID$( LHS , 2,2 )
4660 CLOSE #2
4670 RETURN
THE "G" ROUTINE

50 CLEAR 1000: T$=CHR$(27): PRINT T$; "E": R$=T$; "F": U$=T$; "G"

40 PRINT TAB(20); R$: *** G.L.C. VIAl INFORMATION ***; U$

50 PRINT: PRINT

60 PRINT "YOU ARE ABOUT TO 'ADD' OR 'FIND' G.L.C. VIAl STORAGE DATA."

70 PRINT "TYPE 'Q' (QUIT) TO BAIL OUT."

80 PRINT: INPUT "ADD, FIND, OR QUIT <A, F, or Q>"; AN$

90 XC%=0

100 IF AN$="Q" GOTO 1460

110 IF AN$="F" GOTO 850

120 IF AN$="A" GOTO 170

130 GOSUB 1510: IF QF$="Y" THEN 1460 ELSE 30

140

150 THE "ADD" PORTION OF THE "G" ROUTINE

160

170 PRINT: PRINT "PLEASE ONLY ADD G.L.C. VIAl INFORMATION WHEN CARD IS COMPLETE."

180 AN$="": INPUT "IF CARD IS INCOMPLETE, TYPE 'G'"; AN$

190 IF AN$="Q" GOTO 1460

200 OPEN "R",1,"SY1:GLCRAND.DAT"

210 PRINT: PRINT

220 PRINT: INPUT "ENTER CARD NUMBER"; CN$

230 DIM GL(6,6)

240 PRINT: PRINT "TO ENTER DATA, THIS PROGRAM USES 'ROWS' (HORIZONTAL)"

250 PRINT "AND 'COLUMNS' (VERTICAL). "JUR$; PLEASE FOLLOW DIRECTIONS. "JUR$

260 PRINT "FOR EACH 'ROW', ENTER 7 (SEVEN) ISOLATE NUMBERS, ONE PER LINE."

270 PRINT: PRINT

280 PRINT TAB(25); R$: "CARD NO. "CN$: U$

290 FOR I%=0 TO 6

300 PRINT R$; "ROE "; I%+1; U$

310 FOR IB%=0 TO 6

320 INPUT GL(I%,IB%)

330 NEXT IB%

340 NEXT I%

350 PRINT: PRINT: PRINT "INPUT COMPLETE. PLEASE VERIFY ITS ACCURACY."

360 PRINT: PRINT

370 PRINT TAB(25); R$: "CARD NO. "CN$: U$

380 PRINT: PRINT

390 PRINT TAB(10)

400 FOR I%=0 TO 6

410 PRINT R$; "COLM")I%+1; U$" ";

420 NEXT I%

430 FOR I%=0 TO 6

440 PRINT: PRINT

450 PRINT R$; "ROW "; I%+1; U$

460 FOR IB%=0 TO 6

470 PRINT USING "####.##"; GL(I%,IB%)

480 NEXT IB%

490 NEXT I%

500 PRINT: PRINT

510 AN$="": INPUT "ANY CHANGES <Y or N> <Y> "JAN$

520 IF AN$<"N" THEN 530 ELSE 600

530 PRINT: PRINT "ENTER POSITION OF NUMBER TO BE CHANGED AS FOLLOWS:" 

540 AR%=0: E%=0

550 INPUT "ROW, COLUMN (i.e., 4,3). TYPE '0.0' TO BAIL OUT."; A%, B%

560 IF A%<>0 AND B%=0 GOTO 360

570 IF A%<7 AND B%<7 GOTO 590

580 GOSUB 1510: IF QF$="Y" THEN 1460 ELSE 530

590 PRINT: PRINT "ENTER NEW ISOLATE NUMBER ON CARD"; CN$

600 PRINT "AT ROW"; A%; "COLUMN"; B%;
610 INPUT NG
620 NA%=AS-1;NB%=B%-1
630 GL%(NA%,NB%)=NG
640 PRINT:PRINT RUS#" NEW ISOLATE NUMBER ON CARD";CN%="ROW";A%="COLUMN";B%="IS";NG:JUR#
650 AN$="";PRINT:INPUT "O.K. <Y or N> <Y> ";AN$
660 IF AN$="N" GOTO 530
670 GOTO 350
680 IF XC%=1 GOTO 690
690 FOR I%=0 TO 6
700 FOR I1%=0 TO 6
710 ST$=ST$+MKS$ < GL%(I%,I1%)>
720 NEXT I1%
730 NEXT I%
740 ST$="'
750 FIELD #1.,198 AS Z%;LSET
760 PUT #1., LOF(1)+1
770 ST$="'
780 IF RL%="" GOTO 21E
790 CLOSE
800 GOTO 1460
810 " THE "FIND" PORTION OF THE "G" ROUTINE
820 
830 PRINT TS$;"E";PRINT "YOU ARE NOW GOING TO FIND THE BOX, ROW, AND "
840 PRINT "COLUMN LOCATION OF A GIVEN ISOLATE(S)."
850 PRINT:PRINT "PLEASE DO NOT LOOK FOR MORE THAN 100 VIALS AT A TIME.";PRINT
860 IN%:0: I2%=0: FOUND%=0: R%=0
870 DIM VIALS(100),PRG(100),N(6)
880 GOSUB 16180: REM--REQUEST HARD COPY
890 PRINT:PRINT "ENTER ISOLATE NUMBER(S) TO BE LOCATED, ONE ENTRY PER LINE."
900 PRINT "TYPE <CR> WHEN FINISHED."
910 FOR K%=0 TO 100
920 INPUT VIALS(K%):IF VIALS(K%)=0! GOTO 1400
930 IF VIALS(K%)=0! GOTO 970
940 NEXT K%
950 NM%=K%:1:OPEN "R",2,"SV1:GLCRAID.DAT"
960 FIELD #2. 196 AS C$
970 GET #2
990 FOR K%=0 TO NM%
1010 A=INSTR(C$,MKS$(VIALS(K%))
1020 IF A<>0 THEN 1040:PRINT "####"
1030 IF LOC(2)=LOF(2) THEN 1140 ELSE 1130
1040 R%=A-28+1
1050 C%=A-<<R%-1>+28+1>)\4+1
1060 CN%=CN$(RIGHT$(C$,2))
1070 NEXT K%; GOTO 1100
1080 PRINT "ISOLATE "VIALS(K%)" IS IN BOX "JCN%" ROW "R%; COLUMN "J%"
1090 GOTO 1110
1100 PRINT #3."ISOLATE "VIALS(K%)"IS IN BOX "JCN%" ROW "R%; COLUMN "J%"
1110 PRG(FOUND%)=VIALS(K%)
1120 FOUND%=FOUND%+1
1130 NEXT K%;IF LOC(2)<LOF(2) THEN 990
1140 DIM NOFD%(NM%+1-FOUND%):I%=0
1150 FOR I%=0 TO NM%
1160 FOR I1%=0 TO FOUND%-1
1170 IF VIALS(I%)=PRG(I1%): GOTO 1210
1180 NEXT I1%
1190 NOFD%(I%)=VIALS(I%)
1200 Q%=Q%+1
1210 NEXT I%
1220 IF FOUND%=NM%+1 GOTO 1340
1230 IF HC=1! GOTO 1260
1240 PRINT:PRINT"THESE ISOLATES WERE NOT FOUND;"
1250 GOTO 1270
1260 PRINT #3.CHRS$(13):PRINT#3,"THESE ISOLATES WERE NOT FOUND;"
1270 FOR I%=0 TO NM%-FOUND%
1280 IF HC=I GOTO 1310
1290 PRINT USING "#####.##";NOFD(I%)
1300 GOTO 1320
1310 PRINT #3.NOFD(I%)
1320 NEXT I%
1330 GOTO 1370
1340 IF HC=1! GOTO 1360
1350 PRINT:PRINT RUS; "ALL ISOLATES FOUND. ":VR$:GOTO 1370
1360 PRINT #3.:PRINT #3."ALL ISOLATES FOUND":PRINT #3.
1370 PRINT:PRINT:PRINT RU$: "SEARCH FINISHED":RU$
1380 CLOSE
1390 ERASE NOFD
1400 ERASE UIALS.PRG.N
1410 AN$="":PRINT: INPUT "ANY MORE G.L.C. VIALS TO BE FOUND <Y or N> <H> ";JAG$
1420 IF AG$="Y" GOTO 950
1430 THE "Q" PORTION OF THE "G" ROUTINE
1440 
1450 "PRINT" DISPLYING "Q" "PRINT" DISPLYING "Q"
1460 AN$="":PRINT
1470 INPUT "DO YOU WANT TO RETURN <R> TO MAIN PROGRAM OR EXIT <E>";JAH$
1480 IF AN$="R" AND AN$="E" THEN 1480
1490 IF AN$="R" THEN RUN "NANCY.BAS" ELSE PRINT: "E": END
1500 
1510 GF$="N":AX$="N"
1520 PRINT
1530 PRINT CHRS$(7);RUS;" !!!! ILLEGAL ENTRY !!!! PLEASE";
1540 PRINT" TRY AGAIN. "
1550 PRINT:PRINT " ARE YOU TRYING TO QUIT <Y or N> <H> ";JUR$
1560 AX$="":INPUT AX$
1570 IF AX$="Y" GOTO 1590
1580 GF$="Y"
1590 RETURN
1600 REM--REQUEST HARD COPY FLAG SUBROUTINE
1610 AN$="":PRINT:INPUT "DO YOU WANT HARD COPY OF LIST <Y or N> <H> ";JAH$
1620 IF AN$="Y" GOTO 1650
1630 HC=0!
1640 PRINT:RETURN
1650 HC=1!
1660 PRINT:RA$=""
1670 INPUT "ID YOU 'LOAD LP:' PRIOR TO RUNNING THIS PROGRAM <Y or N> <N> ";RH$
1680 IF RA$="Y" GOTO 1720
1690 PRINT:PRINT CHRS$(7);RUS;" SORRY, LINE PRINTER IS NOT AVAILABLE";
1700 PRINT " FOR YOU. ";JUR$
1710 PRINT:GOTO 1630
1720 PRINT:PRINT CHRS$(7);RUS;" PLEASE BE SURE THAT THE 'POWER' AND 'ON-';
1730 PRINT"LINE SWITCHES";
1740 PRINT" ARE PROPERLY SET. ";PRINT " THANKS. ";JUR$
1750 CLOSE #3:OPEN "O":WS."LP:LP.DVD"
1760 PRINT:RETURN
10 CLEAR 1000
20 TS$=CHR$(27);PRINT TS$;CHR$(69)
30 PRINTTAB(20);TS$++;""*** IDENTIFICATION ROUTINE ***"TS$;""Q"
40 PRINT
50 PRINT:PRINT"THIS ROUTINE WILL ALLOW YOU TO PROCESS THE EXISTING DATA ON"
60 PRINT"A GIVEN RANGE OF ISOLATES AND DETERMINE THE GENUS AND/OR"
70 PRINT"SPECIES, IF POSSIBLE."
80 PRINT:PRINT"IF ENOUGH DATA DOES NOT EXIST TO IDENTIFY THE ORGANISM,"
90 PRINT"NO IDENTIFICATION IS MADE BUT ADDITIONAL TESTS ARE SUGGESTED.";
100 AN$="";PRINT:INPUT "DO YOU WISH TO PROCEED <Y or N> <Y> ";AN$
110 IF AN$="Y" THEN 3350
120 GOSUB 3490 :REM--REQUEST HARDCOPY
130 GOSUB 3550 ;REM--REQUEST RANGE
140 GOSUB 3780 :REM--REQUEST RANGE
150 GOSUB 3830 ;REM--OPEN PROPER FILE AND GET #RECORDS
170 FOR J=1TO2:FOR I=1 TO 62:B=UBLKMI[I,J]:IF B= THEN 324
180 IF <LO OR DB>NO-c GOTO J23C
190 := I +62 K J- 1 >- 1 T=', ..... 4: I= I-T ,'Z T > T=T-D: R=-3+
200 IF R15= THE 27E ELSE
210 RE$="GROdTH ON F'LTE": GOSUB 38C: GOTO 323C
220 IF R15= THE 528
230 IF R15= THE 598
240 RE$="C8TSLSSE": GOSUB 38C: GOTO 323C
250 IF R15= THE 53C ELSE 55C
260 LSET ST$="ST8'H'LOCO" LSET $85="s.
270 GOTO 3228
280 IF R15=6.2 THE 578
290 RE$="eH": GOSUB 38CI: GOTO 323C
300 IF R15= THE 5G0
310 IF R15= THE 5G0
320 IF R15= THE 320
330 IF R15= THE 330
340 IF R15= THE 340
350 IF R15= THE 350
360 IF R15= THE 360
370 GOSUB 360: GOTO 3230
380 IF HC=I! GOTO 410
390 PRINT RH$;"NO I.D. POSSIBLE DUE TO INCOMPLETE": ";RE$
400 RETURN
410 PRINT #3, RH$;"NO I.D. POSSIBLE DUE TO INCOMPLETE": ";RE$
420 RETURN
430 GRM POSITIVE COCCI -- FACULTATIVE
440 GRM POSITIVE COCCI -- FACULTATIVE
450 GRM POSITIVE COCCI -- FACULTATIVE
460 IF S1$="+" THEN 490
470 IF S1$="-" THEN 690
480 RE$="AEROBIC GROWTH":GOSUB 360:GOTO 3230
490 IF R0$="+" THEN 520
500 IF R0$="-" THEN 590
510 RE$="CATAHLASE":GOSUB 360:GOTO 3230
520 IF P5<=6.2 AND P50$: THEN 530 ELSE 550
530 LSET S7$="ST8'H'LOCO":LSET S$="sp.
540 GOTO 3320
550 IF P5=6.2 THEN 570
560 RE$="H":GOSUB 360:GOTO 3320
570 LSET S7$="MICROCOCCI":LSET S$="sp.
580 GOTO 3220
590 IF R7#="+" THEN 620
600 IF R7#="-" THEN 640
610 R$="ESCUF":gosub 380:goto 3230
620 let s7#="STREPTOCOCC":let S8#="MUTK"SANGS"
630 goto 3220
640 let s7#="STREPTOCOCC":let S8#="MUNIUR"
650 goto 3220
660 /
670 GRAM POSITIVE COCCI -- ANAEROBIC
680 /
690 IF P5>6.2 then 710
700 IF P5>0! THEN 840 ELSE 560
710 IF R9#="+" THEN 740
720 IF R9#="-" THEN 800
730 R$="INDO":gosub 380:goto 3230
740 IF R7#="-" AND R8#="-" AND R9#="-" THEN 750 ELSE 770
750 let s7#="PEPTOCOCC":let S8#="ASAC.
760 goto 3220
770 IF R7#<"" AND R8#<"" AND R9#<"" THEN 780 ELSE 790
780 let S7#="G+AN CO HF":goto 3220
790 R$="ESC., CAT., OR TEXT.":gosub 380:goto 3220
800 IF R7#="-" AND R8#="-" AND R9#="-" THEN 810 ELSE 830
810 let s7#="PEPTOSTREP":let S8#="MICRO"
820 goto 3220
830 IF R7#<"" AND R8#<"" AND R9#<"" THEN 840 ELSE 860
840 IF R7#="-" THEN 880
850 IF R7#="-" THEN 910 ELSE 610
860 IF R8#<"-" AND R8#<"-" THEN 970 ELSE 880
870 let s7#="PEPTOCOCC":let S8#="MICRO":goto 3220
880 IF R9#<"" AND R9#<"" AND R9#<"" THEN 900 ELSE 890
890 R$="IND. IND., OR CAT.":gosub 320:goto 3220
900 let s7#="G+AN CO FE":goto 3220
910 IF LEFT$(R9#1)="1" THEN 940
920 IF R9#="-" AND R8#="-" AND R9#="-" THEN 950 ELSE 880
930 let s7#="PEPTOSTREP":let S8#="ANABES":goto 3220
940 IF R9#="-" AND R8#="-" AND R9#="-" THEN 950 ELSE 880
950 let s7#="PEPTOCOCC":let S8#="MICRO":goto 3220
960 /
970 GRAM POSITIVE RODS -- FACULTATIVE
980 /
990 IF S1#="+" THEN 1010
1000 IF S1#="-" THEN 1130 ELSE 480
1010 IF R0#="+" THEN 1030
1020 IF R0#="-" THEN 1080 ELSE 510
1030 if r7#<"6.2 AND p5<6.2 AND P5>0! AND R9#="-" THEN 1070
1040 if r7#<"" AND R8#<"" AND R9#<"" AND P5>0! THEN 1060
1050 R$="IND., NIT., OR IND.":gosub 380:goto 3220
1060 let s7#="G+AN ROD":goto 3220
1070 let s7#="ACTINOMYCE":let S8#="VISNOCUS":goto 3220
1080 IF R7#="-" AND R8#="+" AND P5<6.2 AND P5>0! AND R9#="-" THEN 1090 ELSE 1040
1090 let s7#="ACTINOMYCE":let S8#="NAGSEbind":goto 3220
1100 /
1110 GRAM POSITIVE RODS -- ANAEROBIC
1120 /
1130 IF R0#="+" THEN 1150
1140 IF R0#="-" THEN 1190 ELSE 510
1150 IF R7#="-" AND R9#="+" AND P5<6.2 AND P5>0! AND R9#="-" THEN 1120
1160 IF R7#<"" AND R8#<"" AND P5>0! AND R8#<"" THEN 1170 ELSE 1050
1170 let s7#="G+AN ROD":goto 3220
1180 let s7#="PROPIONIB":let S8#="ACNES":goto 3220
1190 IF LEFT$(RD$,1)="1" THEN 1520
1200 IF R2$="7" THEN 1230
1210 IF R9$="=" THEN 1230
1220 IF R9$<>" " THEN 1170 ELSE 730
1230 IF LEFT$(S2$,1)<"N" THEN 1250 ELSE 1240
1240 LSET S2$="N":RE$="G.L.C":GOSUB 380:GOTO 1170
1250 IF LEFT$(S2$,1)="H" GOTO 1240
1260 IF INSTR$(S2$,"A")<0 AND INSTR$(S2$,"L")<0 AND INSTR$(S2$,"F")<0 AND INSTR$(S2$,"B")<0 THEN 1350
1270 IF R2$="7" THEN 1170
1280 IF INSTR$(S2$,"A")<0 AND INSTR$(S2$,"P")<0 THEN 1310
1290 IF INSTR$(S2$,"A")<0 AND INSTR$(S2$,"L")<0 AND INSTR$(S2$,"S")<0 THEN 1330 AND INSTR$(S2$,"B")=0 THEN 1330
1300 GOTO 1160
1310 IF R7$="=" AND P5<6.2 AND P5<0 AND R8$="=" THEN 1320 ELSE 1160
1320 LSET S7$="ARACHNIA":LSET S8$="PROPIONICA":GOTO 3220
1330 IF R7$="=" AND P5<6.2 AND P5<0 AND R8$="=" THEN 1240 ELSE 1160
1340 LSET S7$="ACTINOMYCE":LSET S8$="ISRAELII":GOTO 3220
1350 IF R2$="7" THEN 1510
1360 IF P5=0 THEN 560
1370 IF P5<6.2 THEN 1350 ELSE 1420
1380 IF R7$="=" AND R8$="=" THEN 1390 ELSE 1400
1390 LSET S7$="EUABACTERIU":LSET S8$="LENTUM":GOTO 3220
1400 IF R7$<>" " AND R8$<>" " AND R9$<>" " THEN 1170
1410 RE$="ESC.,NIT. OR IND.";GOSUB 380:GOTO 3230
1420 IF R7$="=" THEN 1440
1430 IF R7$="=" THEN 1490 ELSE 610
1440 IF R6$="=" THEN 1450 ELSE 1460
1450 LSET S7$="EUABACTERIU":LSET S8$="LIMOSUM":GOTO 3220
1460 IF R9$<>" " AND R8$<>" " AND R9$<>" " THEN 1480
1470 RE$="IND. AND NIT.";GOSUB 380:GOTO 3230
1480 LSET S7$="EUABACTERIU":LSET S8$="":GOTO 3220
1490 IF R8$="=" THEN 1500 ELSE 1460
1500 LSET S7$="EUABACTERIU":LSET S8$="ALACTOLYTI":GOTO 3220
1510 LSET S7$="CLOSTRIDIU":LSET S8$="":GOTO 3220
1520 IF R7$="=" AND R9$="=" AND R8$="=" AND P5<6.2 AND P5<0 THEN 1530 ELSE 1160
1530 LSET S7$="ACTINOMYCE":LSET S8$="ODHONTYTI":GOTO 3220
1540 GR$="NEGRTIUE COCCI -- FACULTATIVE"
1550 GR$="NEGRTIUE COCCI -- FACULTATIVE"
1560 GR$="NEGRTIUE COCCI -- FACULTATIVE"
1570 IF S1$="=" THEN 1590
1580 IF S1$="=" THEN 1660 ELSE 480
1590 IF R0$="=" THEN 1610
1600 IF R0$="=" THEN 1620 ELSE 510
1610 LSET S7$="NEISSERIA":LSET S8$="":GOTO 3220
1620 LSET S7$="G. AER COC":GOTO 3220
1630 GR$="NEGRTIUE COCCI -- ANAEROBIC"
1640 GR$="NEGRTIUE COCCI -- ANAEROBIC"
1650 GR$="NEGRTIUE COCCI -- ANAEROBIC"
1660 IF R0$="=" THEN 1680
1670 IF R0$="=" THEN 1720 ELSE 510
1680 IF P5<6.2 AND R8$="=" AND R9$="=" THEN 1700
1690 IF R7$<>" " AND R8$<>" " AND R9$<>" " AND P5<>0 THEN 1710 ELSE 1050
1700 LSET S7$="UEILLONELL":LSET S8$="PARULA":GOTO 3220
1710 LSET S7$="G. AER COC":GOTO 3220
1720 IF R7$="=" AND R8$="=" AND R9$="=" AND P5<6.2 THEN 1730 ELSE 1690
1730 LSET S7$="UEILLONELL":LSET S8$="ALCALESCE":GOTO 3220
1740 GR$="NEGRTIUE RODS -- FACULTATIVE"
1750 GR$="NEGRTIUE RODS -- FACULTATIVE"
1760 GR$="NEGRTIUE RODS -- FACULTATIVE"
1770 IF $1$"="+" THEN 1790
1780 IF $1$"="-" THEN 2250 ELSE 480
1790 IF R5="=" THEN 1830
1800 IF R8="="+" THEN 1880
1810 IF R8="="-" THEN 2190
1820 RE$="NITRATE";gosub 380;goto 3230
1830 IF R$="=" AND R9="="-" AND R8$="="-" AND P5>=6.2 THEN 1870
1840 IF R$="="+" AND R9$="="-" AND P5>=6.2 THEN 1860
1850 RE$="ESC", IND., OR CAT.";gosub 380;goto 3230
1860 LSET S$="G- FAC ROD";goto 3220
1870 LSET S$="CAMPYLOBAC";LSET S$="SPUTORUM";goto 3220
1880 IF P5<6.2 AND P5>0! THEN 1900
1890 IF P5>=6.2 THEN 2030 ELSE 560
1900 IF R7$="="-" THEN 1920
1910 IF R7$="="+" THEN 1990 ELSE 618
1920 IF R0$="=" THEN 1940
1930 IF R0$="="+" THEN 1970 ELSE 480
1940 IF R9$="="-" THEN 1960
1950 IF R9$="="+" THEN 1950
1960 LSET S$="AHEMOPHILU";LSET S$="APHROPHILU";goto 3220
1970 IF R9$="="-" THEN 1980 ELSE 1950
1980 LSET S$="ACTINOBAC";goto 3220
1990 IF R9$="="-" AND R0$="="-" AND MID$(R5;2;1)="1" THEN 2020
2000 IF R9$="="+" AND R0$="="-" THEN 1860
2010 RE$="IND., OR CAT.";gosub 380;goto 3230
2020 LSET S$="CAPNOCYTOP";LSET S$="SPUTIGENA";goto 3220
2030 IF R$="="-" AND R9$="="-" AND P5>0! THEN 2040 ELSE 1840
2040 IF S$="="+" AND S$="="-" AND S$="="-" AND S$="="-" THEN 2120
2050 IF S$="=" THEN LSET S$="H"
2060 IF S$="=" THEN LSET S$="H"
2070 RE$="UREASE AND OXIDASE"
2080 IF S$="=" THEN RE$="OXIDASE"
2090 IF S$="=" THEN RE$="UREASE"
2100 GOSUB 380
2110 GOTO 1860
2120 IF S$="="-" AND S$="="+" THEN 2130 ELSE 1860
2130 LSET S$="EIKENELLA";LSET S$="COPODENES";goto 3220
2140 IF S$="="+" AND S$="=" THEN 2170
2150 RE$="AMMONIAC";gosub 380
2160 LSET S$="N";LSET S$="CAPNOCYTOP";LSET S$="";goto 3220
2170 IF S$="=" THEN 2210
2180 IF S$="=" THEN 2200 ELSE 2150
2190 IF R$="="+" AND P5="="-" AND P5<6.2 AND P5>0! AND R9$="="-" AND MID$(R5;2;1)="1" THEN 2140 ELSE 1840
2200 LSET S$="CAPNOCYTOP";LSET S$="OCHPACEUS";goto 3220
2210 LSET S$="CAPNOCYTOP";LSET S$="GINGIVALIS";goto 3220
2220 / GRAM NEGATIVE RODS -- ANAEROBIC AND NON-FIgMENTED
2240 / G095 IF LEFT$(R5;1)="1" THEN 2980
2260 IF R$="="+" THEN 2810
2270 IF R$="="-" THEN 2280 ELSE 610
2280 IF R$="=" THEN 2300
2290 IF R$="="+" THEN 2770 ELSE 730
2300 IF P5<=6.2 THEN 2320
2310 IF P5<6.2 AND P5>0! THEN 2640 ELSE 560
2320 IF R8$="=" THEN 2440
2330 IF R8$="=" THEN 2340 ELSE 2380
2340 IF R8$="="+" THEN 2430
2350 IF R8$="="-" THEN 510
2360 IF $65="N" OR $65="" THEN 2400
2370 IF $65="N" THEN 1870 ELSE 2430
2380 IF $65="N" THEN 2390 ELSE 1820
2390 IF $65="N" OR $65="" THEN 2400 ELSE 2410
2400 RE$="MOTILITY" LSET $65="N" GOSUB 3001 GOTO 2430
2410 IF $65="N" THEN 2420 ELSE 2360
2420 LSET $75="VIBRIONS-AN" GOTO 3220
2430 LSET $75="G- AND R05" GOTO 3220
2440 IF R05="-" THEN 2450 ELSE 3110
2450 IF $65="N" OR $65="" OR S55="N" OR S55="" OR S4S="N" OR S4S="" THEN 2410
2460 IF $65="N" OR S55="" THEN LSET S65="N"
2470 IF S55="" THEN LSET S55="N"
2480 IF S4S="" THEN LSET S4S="N"
2490 IF $65="N" AND S5S="N" AND S4S="N" THEN RE$="MOTILITY URE, AND ORI."
2500 IF $65="N" AND S5S="N" AND S4S="N" THEN RE$="MOTILITY AND ORI."
2510 IF $65="N" AND S5S="N" AND S4S="N" THEN RE$="MOTILITY AND ORI."
2520 IF $65="N" AND S5S="N" AND S4S="N" THEN RE$="MOTILITY AND ORI."
2530 IF $65="N" AND S5S="N" AND S4S="N" THEN RE$="MOTILITY AND ORI."
2540 IF $65="N" AND S5S="N" AND S4S="N" THEN RE$="MOTILITY AND ORI."
2550 IF $65="N" AND S5S="N" AND S4S="N" THEN RE$="MOTILITY AND ORI."
2560 GOSUB 3000: GOTO 2430
2570 IF $65="-" THEN 2590
2580 IF $65="" THEN 2620 ELSE 2480
2590 IF S5S="" THEN S5S="N"
2600 GOTO 2430
2610 LSET $75="BACTERIOIDEI" LSET S55="CORPIDENS" GOTO 3220
2620 IF S5S="" THEN 2630 ELSE 2430
2630 LSET S75="VIBRIONS" LSET S55="SUCCINOV" GOTO 3220
2640 IF R05="-" THEN 2660
2650 IF R05="-" AND R05="-" THEN 2430 ELSE 2750
2660 IF $65="N" OR $65="" OR S5S="N" OR S5S="" OR S4S="N" OR S4S="" THEN 2760 ELSE 2740
2670 RE$="MOTILITY AND G.L.C."
2680 IF $65="-" OR S65="" THEN RE$="G.L.C."
2690 IF LEFT$(S2$., 1)="N" AND LEFT$(S2$., 1)="N" THEN RE$="MOTILITY"
2700 GOSUB 3000
2710 IF LEFT$(S2$., 1)="N" THEN LSET S2$="N"
2720 IF S65="N" THEN LSET S65="N"
2730 GOTO 2430
2740 IF $6S="-" AND INSTR(S2$, "A")=0 AND INSTR(S2$, "S")=0 THEN 2760 ELSE 2430
2750 RE$="NIT. OR CAT." GOSUB 3001: GOTO 3220
2760 LSET S75="SUCCINIV" GOTO 3220
2770 IF R05="" AND R65="" AND P5<6.2 THEN 2800
2780 IF R05="" AND R65="" AND P5<6.2 THEN 2430
2790 RE$="CAT., NIT., OR PH" GOSUB 3001: GOTO 3220
2800 LSET S75="FUSOBACTER" LSET S55="NUCLEATUM" GOTO 3220
2810 IF R05="" AND R65="" AND R05="" AND P5<6.2 AND P5<6.2 THEN 2820 ELSE 2800
2820 IF S65="N" AND S65="N" AND LEFT$(S2$, 1)="N" AND LEFT$(S2$, 1)="N" THEN 2860 ELSE 2850
2830 IF R05="" AND R65="" AND P5<6.2 AND R65="" THEN 2430
2840 RE$="NIT., IND., PH., OR CAT." GOSUB 3001: GOTO 3220
2850 GOTO 2670
2860 IF $6S="-" THEN 2880
2870 IF $6S="-" THEN 2920 ELSE 2480
2880 IF INSTR(S2$, "$")=0 THEN 2910
2890 IF INSTR(S2$, "$")=0 THEN 2920 ELSE 2480
2900 LSET S75="BUTYRIVIB" GOTO 3220
2910 IF INSTR(S2$, "$")=0 THEN 2920 ELSE 2480
2920 LSET S75="SELENOMON" LSET S85="SPLITGENA" GOTO 3220
2930 IF INSTR(S2$, "S")>0 AND INSTR(S2$, "A")>0 THEN 2940 ELSE 2430
2940 LSET S7$="BACTEROIDE"; LSET S8$="ORALIS"; GOTO 3220
2950 '$
2960 ' GRAM NEGATIVE RODS -- ANAEROBIC AND PIGMENTED
2970 '$
2980 IF LEFT$(S2$, 1)="N" OR LEFT$(S2$, 1)="" THEN LSET S2$="N" ELSE 3000
2990 RE$="G.L.C."; GOSUB 380: GOTO 2430
3000 IF INSTR(S2$, "Y")>0 THEN 3030
3010 IF R9$="+" AND R7$="-" AND R8$="-" AND INSTR(S2$, "A")>0 AND @
3020 INSTR(S2$, "B")>0 AND INSTR(S2$, "I")>0 THEN 3020 ELSE 2430
3030 LSET S7$="BACTEROIDE"; LSET S8$="ASAC(H-OR)"; GOTO 3220
3040 IF R9$="+" THEN 3050
3050 IF R9$="-" THEN 3180 ELSE 730
3060 IF R5<6.2 AND R5>0 THEN 3140 ELSE 560
3070 IF R7$="-" AND R8$="-" AND INSTR(S2$, "A")>0 AND INSTR(S2$, "B")>0 AND @
3080 INSTR(S2$, "I")>0 THEN 3090
3090 IF R9$="+" AND R0$="" AND R0$="" THEN 2430 ELSE 2430
3100 IF R0$="+" THEN 3120
3110 IF R0$="-" THEN 3150
3120 LSET S7$="BACTEROIDE"; LSET S8$="ASAC<AND>"; GOTO 3220
3130 LSET S7$="BACTEROIDE"; LSET S8$="ASAC<H-NO)>; GOTO 3220
3140 IF R7$="-" AND R8$="-" AND R0$="-" AND INSTR(S2$, "A")>0 AND @
3150 IF R7$="-" AND R8$="-" AND R0$="-" AND INSTR(S2$, "S")>0 THEN 3170
3160 IF R7$="-" AND R8$="-" AND R0$="-" THEN 2430
3170 LSET S7$="BACTEROIDE"; LSET S8$="MEL. INT."; GOTO 3220
3180 IF R7$="+" AND R8$="-" AND R0$="-" AND R5<6.2 AND R5>0 AND @
3190 IF R7$="+" AND R8$="-" AND R0$="-" AND R5<6.2 AND R5>0 THEN 3170
3200 RE$="ESC. HIT. OR CAT."; GOSUB 380: GOTO 3220
3210 LSET S7$="BACTEROIDE"; LSET S8$="MEL. MEL.";
3220 PUT #2, RH
3230 NEXT J
3240 NEXT I
3250 X=LEFT$(HNN$, 3)
3260 IF VAL(HNN$)<1000 THEN HNN$=LEFT$(HNN$, 2)
3270 IF VAL(HOKEY$)>VAL(HIKEY$) THEN HIKEY$=HOKEY$; GOTO 3290
3280 GOSUB 380: GOTO 160
3290 PRINT:PRINTS$="" IDENTIFICATIONS COMPLETE ";TS$=""
3300 IF HC=1! THEN PRINT #3, "$;
3310 IF HC=1! THEN PRINT #3, "IDENTIFICATIONS COMPLETE"
3320 CLOSE
3330 AN=""; PRINT: PRINT "ANY MORE IDENTIFICATIONS <Y or N> <N> ";HNN$
3340 IF AN$="Y" GOTO 20
3350 PRINT: PRINT "DO YOU WISH TO RETURN <R> TO MAIN PROGRAM OR EXIT <E?>";BE$;
3360 IF BE$="R" THEN RUN "NANCY.BAS"
3370 IF BE$="E" THEN END ELSE 3350
3380 REM -- ERROR MESSAGE SUBROUTINE
3390 OF$=""; PRINT "HANZ"; *HANZ*;
3400 PRINT "YES, CHECK TS$;" HANZ; " YES, CHECK TS$;" HANZ;
3410 PRINT "CHECK TS$;" HANZ; " YES, CHECK TS$;" HANZ;
3420 PRINT "CHECK TS$;" HANZ; " YES, CHECK TS$;" HANZ;
3430 PRINT "CHECK TS$;" HANZ; " YES, CHECK TS$;" HANZ;
3440 PRINT "CHECK TS$;" HANZ; " YES, CHECK TS$;" HANZ;
3450 PRINT "CHECK TS$;" HANZ; " YES, CHECK TS$;" HANZ;
3460 PRINT "CHECK TS$;" HANZ; " YES, CHECK TS$;" HANZ;
3470 RETURN
3480 REM -- REQUEST HARD COPY FLAG SUBROUTINE
DO YOU WANT HARD COPY OF LIST <Y or N> <Y> \n3500 IF ANS="Y" GOTO 3530
3510 HC=0!
3520 PRINT:RETURN
3530 HC=1!
3540 PRINT:AN$=""
3550 INPUT "DID YOU 'LOAD LP' PRIOR TO RUNNING THIS PROGRAM <Y or N> <Y> \n3560 IF ANS="Y" GOTO 3500
3570 PRINT:PRINT CHR$(7);STS$;"SORRY, LINE PRINTER IS NOT AVAILABLE";
3580 PRINT "FOR YOU. ";STS$=""
3590 PRINT:GOTO 3510
3600 RETURN:PRINTCHR$(7);STS$;"PLEASE BE SURE THAT THE 'POWER' @ " \n" AND 'ON' @ ";STS$=""
3610 PRINT"LINE'SWITCHES";
3620 PRINT ARE PROPERLY SET. ";PRINT "THANKS. ";STS$=""
3630 CLOSE #3:OPEN "O",#3,"LP:LP.DVD"
3640 PRINT:RETURN
3650 PRINT:PRINT"ENTER THE ";STS$;"" LOWEST ";STS$;" SAMPLE" @ " \n" NUMBER OF THE RANGE TO BE SEARCHED"
3660 INPUT "<NO DECIMAL PLEASE>";LN$;LO=VAL(LN$)
3670 PRINT:PRINT"ENTER THE ";STS$;"" HIGHEST ";STS$;" SAMPLE" @ " \n" NUMBER OF THE RANGE TO BE SEARCHED"
3680 INPUT "<NO DECIMAL PLEASE>";HN$;
3690 IF VAL(LN$)<=VAL(HN$) GOTO 3710
3700 GOSUB 3380:IF QF="Y" THEN 3920 ELSE 3650
3710 IF VAL(LN$)<100 OR VAL(HN$)>100000 THEN GOSUB 3380 ELSE 3720
3720 IF QF="Y" THEN 3920 ELSE 3650
3730 LOKEY$=LEFT$(LN$,3)
3740 IF VAL(LN$)<100 THEN LOKEY$=LEFT$(LN$,2)
3750 LNO%=VAL(LN$)
3760 HNO%=VAL(HN$)+1
3770 RETURN
3780 IF HC=1 GOTO 3810
3790 PRINT TS$=CHR$(69)
3800 PRINT"THE SELECTED RANGE IS FROM ";LN$ TO ";HN$;PRINT GOTO 3820
3810 PRINT #3, ;PRINT #3, "THE SELECTED RANGE IS FROM ";LN$ TO ";HN$; PRINT #3.
3820 RETURN
3830 FILNAM$="SV1:EK"+LOKEY$+".DAT"
3840 OPEN "R",2,FILNAM$
3850 RETURN
3860 LNO%=LNO%+10
3870 LN$=STR$(LNO%)
3880 LOKEY$=MID$(LN$,2,3)
3890 IF LN$<1000 THEN LOKEY$=MID$(LN$,2,2)
3900 CLOSE #2
3910 RETURN
3920 RUN "NANCY.BAS"
10 CLEAR 1004: CLOSE: TS$=CHR$(27): PRINT TS$;"E"
20 PRINT: PRINT "DATA AND STATISTICS": PRINT;
30 PRINT: PRINT "THIS ROUTINE WILL ALLOW YOU TO CREATE A
40 PRINT: PRINT "DATA SUMMARY AND STATISTICS"
50 PRINT "AS INDICATED.
60 PRINT: PRINT "DO YOU WISH TO PROCEED <Y> OR <N> <Y>";: AN$="" : INPUT AN$
70 IF AN$="N" THEN 120
80 PRINT: INPUT "DO YOU WANT TO RETURN <R> TO MAIN PROGRAM OR EXIT <E>"; BE$
90 IF BE$="R" THEN RUN "NANCY.BAS"
100 IF BE$="E" THEN END
110 GOTO 80
120 PRINT
130 PRINT: PRINT "THIS PROGRAM ASSUMES THAT YOU HAVE PREVIOUSLY LOADED LP: "
140 PRINTTS$="a;"
150 NS$=#0: PRINT: PRINT "HOW MANY SAMPLES ARE BEING ANALYZED <TYPE '0' TO QUIT>
160 IF NS$=#0 THEN 10
170 NS%=#1: PRINT: PRINT "THE MANAGEABLE SAMPLES ARE BEING ANALYZED (TYPE '0' TO QUIT)"
180 IF NS%=#1 THEN 20
190 IF NS%=#1 THEN R$="I": GOTO 240
200 PRINT: PRINT "DO YOU WANT THESE SAMPLES ANALYZED: "
210 PRINT "I" - INDIVIDUALLY"
220 PRINT: PRINT "PLEASE ENTER CHOICE <I, T, OR B> "; BR$
230 IF R$="I" AND R$="B" AND R$="T" THEN 200
240 CC$=#0: "PROPONIIARACHNES" ;CC$=#1: "ARACHNIA PROPIONICA"
250 CC$=#2: "ACTINOMYEISRAELII" ;CC$=#3: "ACTINOMYCEODONTOLYTIC"
260 CC$=#4: "EUBACTERIUM" ;CC$=#5: "EUBACTERIUMSIMILIS"
270 CC$=#6: "EUBACTERIUM" ;CC$=#7: "EUBACTERIUM" ;CC$=#8: "CLOSTRIDIU".
280 CC$=#9: "ACTINOMYCEAEASUM" ;CC$=#10: "ACTINOMYCEUROSUS"
290 CC$=#11: "PEPTOSTREPMICROS"
300 CC$=#12: "PEPTOSTREPAHAEROBIUS" ;CC$=#13: "PEPTOCCOCCUSACCC"
310 CC$=#14: "PEPTOCCOCCUS" ;CC$=#15: "PEPTOCCOCCUS"
320 CC$=#16: "MICROCOCCUS" ;CC$=#17: "STAPHYLOCOCCUS"
330 CC$=#18: "STREPTOCOCCUS" ;CC$=#19: "STREPTOCOCCUSACCC"
340 CC$=#20: "VEILLONELLOPARUU" ;CC$=#21: "VEILLONELLOPARUU"
350 CC$=#22: "HESSERECIA" ;CC$=#23: "CAMPYLOBACTERSUTERUM"
360 CC$=#24: "HAEMOPHILUM" ;CC$=#25: "HAEMOPHILUM"
370 CC$=#26: "EIKENELLA" ;CC$=#27: "EIKENELLA"
380 CC$=#28: "CAPNOCYTOSPUTERGENA"
390 CC$=#29: "CAPNOCYTOSPUTERGENA"
400 CC$=#30: "VIBRIO" ;CC$=#31: "VIBRIO"
410 CC$=#32: "BACTEROIDESACCANII" ;CC$=#33: "BACTEROIDESACCANII"
420 CC$=#34: "BACTEROIDESACCC" ;CC$=#35: "BACTEROIDESACCC"
430 CC$=#36: "FUSOBACTERIUM" ;CC$=#37: "FUSOBACTERIUM"
440 CC$=#38: "BUTYRIVIBIUS" ;CC$=#39: "BUTYRIVIBIUS"
450 CC$=#40: "BACTEROIDESALIS" ;CC$=#41: "BACTEROIDESALIS"
460 CC$=#42: "BACTEROIDESALIS" ;CC$=#43: "BACTEROIDESALIS"
470 PRINT: PRINT "PLEASE ENTER SAMPLE(S) TO ANALYZE (ONE PER LINE)"
480 PRINT "TYPE <CR> IF DONE EARLY"
490 FOR J=0 TO NS%
500 SM$(J)="": INPUT SM$(J)
510 IF SM$(J)="" THEN 540
520 GOTO 550
530 FOR J=0 TO NS%
540 NS%=J+1
550 END FOR J=0 TO NS%
560 NS%=0
570 KEYS=LEFT$(SM$(J),2)
1150 \[ P(J,0) = AC(J,2) + AC(J,3) ; P(J,1) = AC(J,0) + AC(J,1) ; P(J,2) = AC(J,4) + AC(J,5) ; \]
\[ P(J,3) = AC(J,6) + AC(J,7) \]
1160 IF \( R = *T* \) THEN 1290
1170 OPEN "0",3,LP:LP,DUD:"PRINT#3, TAB(30);"DATA SUMMARY"
1180 PRINT#3, ,"SAMPLE NUMBER: ";SM$(J);
1190 PRINT#3, ,"SOURCE: ";MJ$(J);
1200 AR=0:AS=0
1220 FOR P=0 TO 6 STEP 2
1240 AR=AR+AC(J,P)
1240 AS=AS+AC(J,P+1)
1250 NEXT P
1260 IF AS=0 THEN AS=1E-06
1270 AF=INT(AR/AS+10+.5)/10
1280 PRINT#3, ,"ANAEROBE/FACULTATIVE RATIO:";AF;";"
1290 CB$=RIGHT$(M6$,2)
1310 CT=(VAL(C$)+VAL(CB$))/2
1320 IF M4$="STO" THEN 1350
1340 IF M4$="1/2" THEN CT=CT/2.31+1000
1350 IF M4$="2/2" THEN CT=CT/4.62+1000
1360 IF LEFT$(M5$,3)="III" THEN CT=CT/1.31+1000
1370 IF LEFT$(M5$,2)="IV" THEN CT=CT/6.1+1000
1380 TC(J)=INT(CT*10+5)/10
1390 IF TC(J)>=INT(CF;+10+.5) THEN 10
1500 ADD B. ASACC TO EBP ASACC, ADD B. MEL. MEL. AND INT. TO EBP SACC
1410 IF AC(J,52) = AC(J,52) + AC(J,41) + AC(J,40) + AC(J,39) THEN
1430 IF R="T" THEN 2220
1440 PRINT#3, ,TAB(50);"C.F.U./ml.";TC(J);
1450 PRINT#3, ,"PERCENT NO GROWTH:";AC(J,8);"%"
1460 PRINT#3, ,"PERCENT OF TOTAL COLONIES"
1470 PRINT#3, ,"-------------"
1480 IF J>NS% THEN PRINT#3, ,"(MEAN +/- S.D.)"
1490 IF J>NS% THEN P(J,0)=P(10)
1500 PRINT#3, ,"GRAM POSITIVE COCCI";TAB(50);P(J,0);"A=0:GOSUB 2690"
1510 PRINT#3, ,"-------------------"
1520 PRINT#3, ,"ANAEROBIC";TAB(60);AC(J,2);"L=-7:GOSUB2650"
1530 PRINT#3, ,"-------------"
1540 FOR L=11 TO 15
1550 IF AC(J,L)=0 THEN 1570
1560 PRINT#3, ,TAB(6)=LEFT$(CC$(L),10);"RIGHT$(CC$(L),10);TAB(64);AC(J,L+9);0 GOSUB 2650"
1570 NEXT L
1580 PRINT#3, ,"FACULTATIVE";TAB(60);AC(J,3);"L=-6:GOSUB 2650"
1590 PRINT#3, ,"-------------"
1600 FOR L=16 TO 19
1610 IF AC(J,L)=0 THEN 1630
1620 PRINT#3, ,TAB(6)=LEFT$(CC$(L),10);"RIGHT$(CC$(L),10);TAB(64);AC(J,L+9);0 GOSUB 2650"
1630 NEXT L
1640 IF J>NS% THEN P(J,1)=P(1)
1650 PRINT#3, ,"GRAM POSITIVE RODS";TAB(50);P(J,1);"A=1:GOSUB 2690"
1660 PRINT#3, ,"-------------------"
1670 PRINT#3, ,"ANAEROBIC";TAB(60);AC(J,0);"L=-9:GOSUB 2650"
1680 PRINT#3, ,"-------------"
1690 FOR L=0 TO 8
1700 IF AC(J,L)=0 THEN 1720
1710 PRINT#3, TAB(6)*LEFT$(CC$(L),10);"";RIGHT$(CC$(L),10);TAB(64);AC(J,L+9);GOSUB 2650
1720 NEXT L
1730 PRINT#3, "FACULTATIVE";TAB(60);AC(J,1);L=-8:GOSUB 2650
1740 PRINT#3, "";
1750 FOR L=9 TO 10
1760 IF AC(J,L+9)=0! THEN 1780
1770 PRINT#3, TAB(6)*LEFT$(CC$(L),10);"";RIGHT$(CC$(L),10);TAB(64);AC(J,L+9);GOSUB 2650
1780 NEXT L
1790 IF J=NS% THEN P(J,2)=P1(2)
1800 PRINT#3, :PRINT#3, "GRAM NEGATIVE COCCI";TAB(50);P(J,2);A=2:GOSUB 2690
1810 PRINT#3, "";
1820 PRINT#3, "ANAEROBIC";TAB(60);AC(J,4);L=-5:GOSUB 2650
1830 PRINT#3, "";
1840 FOR L=20 TO 21
1850 IF AC(J,L+9)=0! THEN 1870
1860 PRINT#3, TAB(6)*LEFT$(CC$(L),10);"";RIGHT$(CC$(L),10);TAB(64);AC(J,L+9);GOSUB 2650
1870 NEXT L
1880 PRINT#3, "FACULTATIVE";TAB(50);AC(J,5);L=-4:GOSUB 2650
1890 PRINT#3, "";
1900 IF AC(J,31)=0! THEN 1920
1910 PRINT#3, TAB(6)*LEFT$(CC$(22),10);"";RIGHT$(CC$(L),10);TAB(64);AC(J,31);GOSUB 2650
1920 IF J=NS% THEN P(J,3)=P1(3)
1930 PRINT#3, :PRINT#3, "GRAM NEGATIVE RODS";TAB(50);P(J,3);A=3:GOSUB 2690
1940 PRINT#3, "";
1950 PRINT#3, "ANAEROBIC";TAB(50);AC(J,6);L=-3:GOSUB 2650
1960 PRINT#3, "";
1970 FOR L=30 TO 42
1980 IF AC(J,L+9)=0! THEN 2000
1990 PRINT#3, TAB(6)*LEFT$(CC$(L),10);"";RIGHT$(CC$(L),10);TAB(64);AC(J,L+9);GOSUB 2650
2000 NEXT L
2010 PRINT#3, "FACULTATIVE";TAB(50);AC(J,7);L=-2:GOSUB 2650
2020 PRINT#3, "";
2030 FOR L=23 TO 29
2040 IF AC(J,L+9)=0! THEN 2060
2050 PRINT#3, TAB(6)*LEFT$(CC$(L),10);"";RIGHT$(CC$(L),10);TAB(64);AC(J,L+9);GOSUB 2650
2060 NEXT L
2070 IF AC(J,54)=0! THEN 2090
2080 PRINT#3, TAB(6);"TOTAL CAPNOCV/TOPHAGA";TAB(63);AC(J,54);GOSUB 2650
2090 IF AC(J,55)=0! THEN 2160
2100 PRINT#3, :PRINT#3, "TOTAL BLACK PIGMENTED BACTEROIDES";L
2110 TAB(50);AC(J,55);GOSUB 2650
2120 PRINT#3, "";
2130 FOR L=43 TO 44
2140 PRINT#3, "ACC$(L);TAB(60);AC(J,L+9);GOSUB 2650
2150 NEXT L
2160 IF J=NS%+1 THEN 2210
2170 IF MA=1 THEN 2190
2180 PRINTCHR$(27);"";"" SORRY, I COULD NOT FIND SAMPLE ";";SM$(J);CHR$(27);"";"" PATIENCE, PLEASE. CIPHER IN THEN S.D."";}}}
"TAKES ME A FEW SECONDS...";TS";"q"

2240 FOR KK=0 TO 55
2250 FOR LL=0 TO NS%
2260 AC(NS%+2, KK)=AC(NS%+2, KK)+AC(LL, KK)+AC(LL, KK)
2270 AC(NS%+1, KK)=AC(NS%+1, KK)+AC(LL, KK)
2280 NEXT LL
2290 AC(NS%+3, KK)=INT(SQR((AC(NS%+2, KK)-AC(NS%+1, KK)/2/(NS%+1))/NS%)+10+5)/10
2300 AC(NS%+1, KK)=INT((AC(NS%+1, KK)/(NS%+1))+10+5))/10
2310 NEXT KK
2320 MT=0; MM=0
2330 FOR B=0 TO NS%
2340 MT=MT+TC(B); MM=MM+TC(B); TC(B) ; NEXT B
2350 MT=INT(MT/(NS%+1)/NS%+10+5)/10
2360 MT=MT+TC/(NS%+1)*10+5)/10
2370 FOR A=0 TO 3
2380 P1(A)=0; P2(A)=0; P3(A)=0
2390 FOR B=0 TO NS%
2400 P1(A)=P1(A)+P(B, A)
2410 P2(A)=P2(A)+P(B, A)+P(B, A)
2420 NEXT B
2430 P3(A)=INT(SQR((P3(A)=P1(A)+P1(A)/(NS%+1)/NS%+10+5)/10
2440 P1(A)=INT(P1(A)/(NS%+1)*10+5)/10
2450 NEXT A
2460 CLOSE #3; OPEN "0", 3; "LP:LP.DUM"
2470 PRINT#3,TAB(25); "GROUP SUMMARY DATA"
2480 PRINT#3, TAB(25); "-------------------"
2490 PRINT#3, :PRINT#3; "SAMPLE NUMBER"; TAB(50); "SOURCE"
2500 PRINT#3, "-------------------"; TAB(50); "------"
2510 FOR I=0 TO NS%
2520 PRINT#3, TAB(4); SM#(I); TAB(50); NS#(I)
2530 NEXT I
2540 AR=0; AS=0
2550 FOR M=0 TO NS%
2560 FOR P=0 TO 6 STEP 2
2570 AR=AR+AC(M, P)
2580 AS=AS+AC(M, P+1)
2590 NEXT P; NEXT M
2600 IF AS=0 THEN AS=1E-06
2610 AF=INT(AR/AS+10+5)/10
2620 PRINT#3, :PRINT#3; "COMBINED ANAEROBE/FACULTATIVE RATIO"; AF; 
2630 PRINT#3, :PRINT#3; "MEAN C.F.U./ml. (S.D.) = ";MT; "+/-"; SD
2640 J=NS%+1: GOTO 1640
2650 IF J<=NS% THEN 2660 ELSE 2670
2660 PRINT #3, :RETURN
2670 PRINT#3, "+/-"; AC(NS%+3, L+9)
2680 RETURN
2690 IF J<=NS% THEN 2700 ELSE 2710
2700 PRINT #3, :RETURN
2710 PRINT #3, "+/-"; P3(A)
2720 RETURN


<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1-2</th>
<th>4-6</th>
<th>8-11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>% 0-1</td>
<td>% 2-3</td>
<td>$\bar{x}$</td>
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<tr>
<td>P.I.</td>
<td>1.6</td>
<td>38</td>
<td>63</td>
<td>2.0</td>
</tr>
<tr>
<td>G.I.</td>
<td>1.6</td>
<td>38</td>
<td>63</td>
<td>2.8</td>
</tr>
<tr>
<td>pocket depth (mm ± S.D.)</td>
<td>2.2±0.4</td>
<td>3.0±0.8</td>
<td>3.7±0.8\textsuperscript{NCF}</td>
<td>3.7±0.7\textsuperscript{NCF}</td>
</tr>
</tbody>
</table>

Table 1. Clinical parameters of ligated sites. Statistically significant differences designated as follows: $F$=different from first value in row; $N$=different from non-ligated sites; $C$=different from control sites. See Table 2 for number of sites sampled.
<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1-2</th>
<th>4-6</th>
<th>8-11</th>
</tr>
</thead>
<tbody>
<tr>
<td># Samples</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>AN:FAC Ratio</td>
<td>1.4</td>
<td>1.2</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>CFU (x 10^6)^a</td>
<td>7.4±3.9</td>
<td>12.1±8.6</td>
<td>9.1±8.3</td>
<td>28.2±17.1N</td>
</tr>
<tr>
<td>GRAM + COCCI b</td>
<td>23.4±8.4</td>
<td>14.8±10.4</td>
<td>3.4±4.6 FNC</td>
<td>7.6±7.1 FC</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>6.3±9.7</td>
<td>3.6±5.0</td>
<td>1.8±3.3</td>
<td>7.6±7.1</td>
</tr>
<tr>
<td>Facultative</td>
<td>17.1±13.3</td>
<td>5.2±6.5</td>
<td>1.5±4.0 FN</td>
<td>0N</td>
</tr>
<tr>
<td>GRAM + RODS</td>
<td>9.6±9.2</td>
<td>13.2±9.8</td>
<td>4.8±6.2 C</td>
<td>15.9±15.3</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>9.6±9.2</td>
<td>4.5±9.0</td>
<td>3.6±6.2</td>
<td>8.5±9.5</td>
</tr>
<tr>
<td>Facultative</td>
<td>0</td>
<td>8.8±11.0F</td>
<td>1.2±3.1C</td>
<td>7.4±10.6</td>
</tr>
<tr>
<td>GRAM – COCCI</td>
<td>6.7±6.0</td>
<td>4.8±9.7</td>
<td>7.4±9.9</td>
<td>10.2±15.8</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>3.9±5.6</td>
<td>4.8±9.7</td>
<td>7.4±9.9</td>
<td>0</td>
</tr>
<tr>
<td>Facultative</td>
<td>2.8±4.4</td>
<td>0</td>
<td>0</td>
<td>10.2±15.8</td>
</tr>
<tr>
<td>GRAM – RODS</td>
<td>62.8±11.5</td>
<td>66.6±15.6 C</td>
<td>78.5±12.8 FNC</td>
<td>54.7±18.5</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>47.5±16.0</td>
<td>41.7±19.6</td>
<td>58.1±25.6</td>
<td>40.0±14.9</td>
</tr>
<tr>
<td>B.P.B.</td>
<td>22.0±7.2</td>
<td>17.9±5.4 C</td>
<td>25.4±13.2N</td>
<td>21.3±7.3NC</td>
</tr>
<tr>
<td>B. ging.</td>
<td>2.4±4.5</td>
<td>4.0±8.0</td>
<td>23.4±11.0 FNC</td>
<td>18.4±11.3 FNC</td>
</tr>
<tr>
<td>B. mel. int.</td>
<td>16.9±11.1</td>
<td>13.9±10.6</td>
<td>2.0±5.4 F</td>
<td>3.9±5.3 F</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>9.0±8.1</td>
<td>9.5±2.7</td>
<td>16.5±24.5</td>
<td>11.7±6.0</td>
</tr>
<tr>
<td>Facultative</td>
<td>15.3±10.0</td>
<td>25.1±7.8 NC</td>
<td>20.4±18.7 C</td>
<td>14.7±13.8</td>
</tr>
<tr>
<td>STB + motile rods</td>
<td>4.8±4.2</td>
<td>20.8±2.0 FNC</td>
<td>14.2±21.6</td>
<td>16.7±6.3 F</td>
</tr>
<tr>
<td>SPIROCHETES C</td>
<td>14.3±8.3</td>
<td>31.4±9.1 F</td>
<td>25.5±14.8 N</td>
<td>28.5±20.5</td>
</tr>
</tbody>
</table>

Table 2. Subgingival microflora of ligated sites. a=mean ± standard deviation of CFU/ml. b=mean percentage ± standard deviation of TCF. c=mean percentage ± standard deviation of TMC. Statistically significant differences designated as follows: F=different from first value in row; N=different from non-ligated sites; C=different from control sites.
<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1-2</th>
<th>4-6</th>
<th>8-11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>0-1</td>
<td>2-3</td>
<td>0-1</td>
</tr>
<tr>
<td>P1.I.</td>
<td>1.8</td>
<td>19</td>
<td>81</td>
<td>1.7</td>
</tr>
<tr>
<td>G.I.</td>
<td>2.0</td>
<td>0</td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>pocket depth (mm ± S.D.)</td>
<td>2.3±0.5</td>
<td>2.6±0.5</td>
<td>2.5±0.5</td>
<td>(\bar{L})</td>
</tr>
</tbody>
</table>

Table 3. Clinical parameters of non-ligated sites. \(\bar{L}\)=statistically different from ligated sites. See Table 4 for number of sites sampled.
<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1-2</th>
<th>4-6</th>
<th>8-11</th>
</tr>
</thead>
<tbody>
<tr>
<td># Samples</td>
<td>14</td>
<td>7</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>AN: FAC Ratio</td>
<td>1.3</td>
<td>1.4</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>CFU (x 10^6)^a</td>
<td>4.4±3.6</td>
<td>7.8±4.3</td>
<td>5.9±3.8</td>
<td>4.9±3.1^L</td>
</tr>
<tr>
<td>GRAM + COCCI^b</td>
<td>14.8±13.1</td>
<td>19.9±13.3</td>
<td>12.5±10.6^L</td>
<td>8.2±6.3^C</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>5.1±8.6</td>
<td>7.9±13.7</td>
<td>0.9±2.8</td>
<td>2.2±6.2</td>
</tr>
<tr>
<td>Faculative</td>
<td>9.7±13.6</td>
<td>12.0±10.7</td>
<td>11.6±9.5^L</td>
<td>6.0±5.6^L</td>
</tr>
<tr>
<td>GRAM + RODS</td>
<td>15.1±5.8</td>
<td>8.9±6.9</td>
<td>7.9±8.2</td>
<td>9.0±4.7</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>12.7±5.2</td>
<td>2.3±4.0</td>
<td>5.4±8.3</td>
<td>6.2±4.4</td>
</tr>
<tr>
<td>Faculative</td>
<td>2.3±4.6</td>
<td>6.6±6.5</td>
<td>2.4±4.4^C</td>
<td>2.9±5.3</td>
</tr>
<tr>
<td>GRAM – COCCI</td>
<td>10.0±10.8</td>
<td>7.0±6.9</td>
<td>4.2±5.2</td>
<td>6.4±6.6</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>3.6±4.3</td>
<td>6.1±7.4</td>
<td>2.1±4.2</td>
<td>3.8±5.6</td>
</tr>
<tr>
<td>Faculative</td>
<td>6.5±9.4</td>
<td>0.9±2.4</td>
<td>2.0±4.1</td>
<td>2.5±5.9</td>
</tr>
<tr>
<td>GRAM – RODS</td>
<td>50.1±16.8</td>
<td>54.9±7.4^C</td>
<td>55.4±17.0^L</td>
<td>62.6±19.2</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>44.2±15.6</td>
<td>42.1±4.8</td>
<td>39.8±16.5</td>
<td>39.7±19.5</td>
</tr>
<tr>
<td>B. P. B.</td>
<td>14.9±9.1</td>
<td>12.8±10.7</td>
<td>13.2±9.5^L</td>
<td>8.3±10.0^L</td>
</tr>
<tr>
<td>B. ging.</td>
<td>2.1±4.4</td>
<td>2.9±6.0</td>
<td>1.2±3.0^L</td>
<td>0.7±1.9^L</td>
</tr>
<tr>
<td>B. mel. int.</td>
<td>12.6±10.5</td>
<td>9.9±5.6</td>
<td>8.5±10.3</td>
<td>6.1±8.2</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>9.4±7.2</td>
<td>14.6±10.2</td>
<td>8.9±9.0</td>
<td>10.1±9.8</td>
</tr>
<tr>
<td>Faculative</td>
<td>8.5±5.8</td>
<td>12.8±7.3^FC</td>
<td>15.4±10.6^F</td>
<td>22.9±15.3^FC</td>
</tr>
<tr>
<td>STB + motile rods</td>
<td>4.6±5.2</td>
<td>7.0±8.9^L</td>
<td>13.7±6.2^FC</td>
<td>20.3±10.6^FC</td>
</tr>
<tr>
<td>SPIROCHETES^C</td>
<td>18.3±6.1</td>
<td>12.4±19.9</td>
<td>14.8±5.7^L</td>
<td>26.0±10.4^F</td>
</tr>
</tbody>
</table>

Table 4. Subgingival microflora of non-ligated sites. a=mean ± standard deviation CFU/ml. b=mean percentage ± standard deviation of TCF for all non-spirochetal species. c= mean percentage ± standard deviation of TMC. Statistically significant differences designated as follows: F=different from first value in row; C=different from control sites; L=different from ligated sites.
Table 5. Clinical parameters of control sites. L=statistically different from ligated sites. See Table 6 for number of sites sampled.
<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$#$ Samples</strong></td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>AN:FAC Ratio</td>
<td>1.5</td>
<td>1.1</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>CFU ($x 10^6$)(^a)</td>
<td>4.4±1.6</td>
<td>9.4±6.8</td>
<td>6.2±2.7</td>
<td>9.9±7.9</td>
</tr>
<tr>
<td><strong>GRAM + COCCI</strong>(^b)</td>
<td>11.6±13.8</td>
<td>15.2±15.5</td>
<td>15.8±8.3(^L)</td>
<td>23.4±2.3(^NL)</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>5.8±10.2</td>
<td>0</td>
<td>5.8±10.0</td>
<td>9.1±11.2</td>
</tr>
<tr>
<td>Facultative</td>
<td>5.8±6.5</td>
<td>15.2±15.5</td>
<td>10.1±12.0</td>
<td>14.3±15.1</td>
</tr>
<tr>
<td><strong>GRAM + RODS</strong></td>
<td>18.3±9.4</td>
<td>15.1±11.6</td>
<td>15.7±8.3(^L)</td>
<td>15.5±10.0</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>8.8±9.0</td>
<td>9.4±10.4</td>
<td>5.7±8.3</td>
<td>7.3±7.2</td>
</tr>
<tr>
<td>Facultative</td>
<td>9.5±11.7</td>
<td>5.7±4.7</td>
<td>10.0±9.1(^NL)</td>
<td>8.2±14.1</td>
</tr>
<tr>
<td><strong>GRAM – COCCI</strong></td>
<td>4.3±6.6</td>
<td>6.4±4.4</td>
<td>0</td>
<td>7.3±12.0</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>3.2±5.4</td>
<td>2.5±4.9</td>
<td>0</td>
<td>7.3±12.0</td>
</tr>
<tr>
<td>Facultative</td>
<td>1.1±2.6</td>
<td>4.0±4.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>GRAM – RODS</strong></td>
<td>53.6±20.1</td>
<td>40.3±11.6(^NL)</td>
<td>47.3±2.5(^L)</td>
<td>42.0±5.7</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>42.7±20.4</td>
<td>40.3±11.6</td>
<td>43.4±4.9</td>
<td>39.6±7.0</td>
</tr>
<tr>
<td>B. P. B.</td>
<td>12.8±9.8</td>
<td>4.2±3.6(^L)</td>
<td>11.6±8.3</td>
<td>6.1±4.1(^L)</td>
</tr>
<tr>
<td>B. ging.</td>
<td>1.9±3.0</td>
<td>0</td>
<td>0(^L)</td>
<td>0(^L)</td>
</tr>
<tr>
<td>B. mel. int.</td>
<td>9.3±12.0</td>
<td>4.2±3.6</td>
<td>11.6±8.3</td>
<td>6.1±4.1</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>14.2±10.7</td>
<td>15.8±11.7</td>
<td>20.6±9.3</td>
<td>6.0±7.4</td>
</tr>
<tr>
<td>Facultative</td>
<td>10.9±10.4</td>
<td>0(^NL)</td>
<td>3.9±5.4(^L)</td>
<td>2.4±1.9(^N)</td>
</tr>
<tr>
<td>STB + motile rods</td>
<td>3.7±5.8</td>
<td>3.0±2.2(^L)</td>
<td>4.6±8.0(^N)</td>
<td>6.0±7.4(^N)</td>
</tr>
<tr>
<td><strong>SPIROCHETES</strong>(^c)</td>
<td>18.3±13.1</td>
<td>24.8±3.6</td>
<td>20.3±11.5</td>
<td>29.0±14.0</td>
</tr>
</tbody>
</table>

Table 6. Subgingival microflora of control sites. \(^a\)=mean ± standard deviation CFU/ml. \(^b\)=mean percentage ± standard deviation of TCF for all non-spirochetal species. \(^c\)=mean percentage ± standard deviation of TMC. Statistically significant differences designated as follows: F=different from first value in row; N=different from non-ligated sites; L=different from ligated sites.
Table 7. Clinical parameters of infected non-ligated sites. Non-ligated sites were infected with either subgingival plaque from a ligated site (one site) or pure cultures of *B. gingivalis* (two sites) three and four weeks prior to sampling as described in Materials and Methods.

<table>
<thead>
<tr>
<th>Week</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>% 0-1</td>
</tr>
<tr>
<td>Pl.I.</td>
<td>2.0</td>
<td>33</td>
</tr>
<tr>
<td>G.I.</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>pocket depth (mm ± S.D.)</td>
<td>2.3±0.6</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>8</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Week</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong># Samples</strong></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>AN:FAC Ratio</strong></td>
<td>1.3</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>CFU (x 10^6)^a</strong></td>
<td>6.0±4.2</td>
<td>5.4±0.9</td>
</tr>
<tr>
<td><strong>GRAM + COCCI</strong>^b</td>
<td>11.0±13.7</td>
<td>5.7±6.8^c</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>6.1±10.6</td>
<td>0</td>
</tr>
<tr>
<td>Facultative</td>
<td>4.9±4.3</td>
<td>5.7±6.8</td>
</tr>
<tr>
<td><strong>GRAM + RODS</strong></td>
<td>11.9±4.1</td>
<td>6.7±6.1</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>3.3±5.3</td>
<td>6.7±6.1</td>
</tr>
<tr>
<td>Facultative</td>
<td>8.6±9.6</td>
<td>0</td>
</tr>
<tr>
<td><strong>GRAM – COCCI</strong></td>
<td>1.8±1.6</td>
<td>3.0±5.3</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0.9±1.5</td>
<td>0</td>
</tr>
<tr>
<td>Facultative</td>
<td>1.7±1.5^L</td>
<td>3.0±5.3</td>
</tr>
<tr>
<td><strong>GRAM – RODS</strong></td>
<td>57.7±12.8^L</td>
<td>67.8±19.6</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>46.2±13.0</td>
<td>67.8±19.6^C</td>
</tr>
<tr>
<td>B.P.B.</td>
<td>22.6±9.6</td>
<td>25.8±4.2^NC</td>
</tr>
<tr>
<td>B. ging.</td>
<td>3.7±6.4^L</td>
<td>23.8±3.2^NC</td>
</tr>
<tr>
<td>B. mel. int.</td>
<td>18.9±11.7^L</td>
<td>0.9±1.5</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>18.4±8.9</td>
<td>10.3±11.7</td>
</tr>
<tr>
<td>Facultative</td>
<td>11.4±10.7</td>
<td>0^N</td>
</tr>
<tr>
<td>STB + motile rods</td>
<td>3.2±2.8^N</td>
<td>3.2±5.5^NL</td>
</tr>
<tr>
<td>SPIROCHETES^c</td>
<td>23.0±2.8^N</td>
<td>20.5±17.7</td>
</tr>
</tbody>
</table>

Table 8. Subgingival microflora of infected non-ligated sites. See Tables 2 and 4 for explanation of superscripts.
<table>
<thead>
<tr>
<th>Week</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>%0-1</td>
</tr>
<tr>
<td>Pl.I.</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>G.I.</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>pocket depth (mm ± S.D.)</td>
<td>2.5±0.7</td>
<td>3.0±0.0</td>
</tr>
</tbody>
</table>

Table 9. Clinical parameters of sham-infected non-ligated sites. None of these parameters were different from the non-infected non-ligated sites in these animals. See Table 10 for number of samples.
Table 10. Subgingival microflora of sham-infected non-ligated sites. For explanation of superscripts see Table 2. None of these values were significantly different from the non-infected non-ligated values.
Table 11. Clinical parameters of infected control sites. Sites were infected at weeks 0 and 1 or weeks 4 and 5 and then monitored four weeks later. None of these parameters were significantly different from sham-infected or non-infected control site values. See Table 12 for number of sites sampled.

<table>
<thead>
<tr>
<th>Week</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} )</td>
<td>%</td>
</tr>
<tr>
<td>Pl.I.</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>G.I.</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>Pocket depth (mm ± S.D.)</td>
<td>2.0±0.0</td>
<td></td>
</tr>
<tr>
<td>Week</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td># Samples</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>AN:IFAC Ratio</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>CFU (x 10^6)^a</td>
<td>6.5± 3.7</td>
<td>13.6± 8.4</td>
</tr>
<tr>
<td>GRAM + COCCI</td>
<td>12.1± 7.4</td>
<td>23.2± 3.4</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>8.7±12.2</td>
<td>15.7± 7.3</td>
</tr>
<tr>
<td>Facultative</td>
<td>3.5± 4.9</td>
<td>7.6±10.6</td>
</tr>
<tr>
<td>GRAM + RODS</td>
<td>20.2± 9.2</td>
<td>19.0±14.4</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>10.2± 4.2</td>
<td>4.4± 6.2</td>
</tr>
<tr>
<td>Facultative</td>
<td>10.0±14.7</td>
<td>14.6±20.7</td>
</tr>
<tr>
<td>GRAM – COCCI</td>
<td>0</td>
<td>2.1± 3.0</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0</td>
<td>2.1± 3.0</td>
</tr>
<tr>
<td>Facultative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GRAM – RODS</td>
<td>47.3± 1.5</td>
<td>39.3± 3.4</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>43.4± 6.9</td>
<td>36.3± 1.7</td>
</tr>
<tr>
<td>B.P.B.</td>
<td>14.1±10.1</td>
<td>8.6± 0.4</td>
</tr>
<tr>
<td>B. ging.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. mel. int.</td>
<td>14.1±10.1</td>
<td>8.6± 0.4</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>24.2± 9.8</td>
<td>1.8± 2.5</td>
</tr>
<tr>
<td>Facultative</td>
<td>3.9± 5.4</td>
<td>3.0± 1.7</td>
</tr>
<tr>
<td>STB + motile rods</td>
<td>6.9± 9.8</td>
<td>4.8± 0.8</td>
</tr>
<tr>
<td>SPIROCHETES</td>
<td>26.0±15.6</td>
<td>28.0±15.6</td>
</tr>
</tbody>
</table>

Table 12. Subgingival microflora of infected control sites. Sites were infected at weeks 0 and 1 or weeks 4 and 5 and then monitored four weeks later. None of these values were different from the sham-infected or non-infected control site values. For explanation of superscripts see Table 2.
Table 13. Clinical parameters of sham-infected control sites. Sites were "infected" with a sterile curette at week 0 and 1 or week 4 and 5 and then were monitored four weeks later. None of these parameters were different from the infected or non-infected control site values. See Table 14 for number of sites sampled.

<table>
<thead>
<tr>
<th>Week</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>% 0-1</td>
</tr>
<tr>
<td>Pl.I.</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>G.I.</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>Pocket depth (mm ± S.D.)</td>
<td>2.5±0.4</td>
<td>2.0±0.0</td>
</tr>
</tbody>
</table>
Table 14. Subgingival microflora of sham-infected control sites. Sites were "infected" with a sterile curette at weeks 0 and 1 or weeks 4 and 5 and then were monitored four weeks later. None of these values were different from the infected or non-infected control site values. For explanation of the superscripts, see Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Week 4</th>
<th>Week 8</th>
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</thead>
<tbody>
<tr>
<td><strong># Samples</strong></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>AN:FAC Ratio</strong></td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>CFU (x 10^6)</strong></td>
<td>5.7±6.3</td>
<td>7.8±2.4</td>
</tr>
<tr>
<td><strong>GRAM + COCCI</strong></td>
<td>22.4±2.3</td>
<td>18.5±7.6</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>8.9±13.1</td>
<td>10.2±5.9</td>
</tr>
<tr>
<td>Facultative</td>
<td>13.5±8.0</td>
<td>8.3±1.7</td>
</tr>
<tr>
<td><strong>GRAM + RODS</strong></td>
<td>6.0±8.5</td>
<td>11.2±2.4</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0</td>
<td>4.8±6.7</td>
</tr>
<tr>
<td>Facultative</td>
<td>6.0±8.5</td>
<td>6.5±9.1</td>
</tr>
<tr>
<td><strong>GRAM – COCCI</strong></td>
<td>4.0±5.7</td>
<td>1.5±2.1</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0</td>
<td>1.5±2.1</td>
</tr>
<tr>
<td>Facultative</td>
<td>4.0±5.7</td>
<td>0</td>
</tr>
<tr>
<td><strong>GRAM – RODS</strong></td>
<td>56.2±8.7</td>
<td>49.3±10.5</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>46.2±20.8</td>
<td>44.0±18.0</td>
</tr>
<tr>
<td>B.P.B.</td>
<td>14.6±3.7</td>
<td>14.0±6.3</td>
</tr>
<tr>
<td>B. ging.</td>
<td>3.5±2.1</td>
<td>0</td>
</tr>
<tr>
<td>B. mel. int.</td>
<td>9.2±1.6</td>
<td>14.0±6.3</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>8.9±6.9</td>
<td>16.1±10.2</td>
</tr>
<tr>
<td>Facultative</td>
<td>10.0±8.1</td>
<td>5.3±7.4</td>
</tr>
<tr>
<td>STB + motile rods</td>
<td>12.0±17.0</td>
<td>5.8±5.2</td>
</tr>
<tr>
<td><strong>SPIROCHETES</strong></td>
<td>21.0±7.0</td>
<td>24.5±10.6</td>
</tr>
</tbody>
</table>

Table 14. Subgingival microflora of sham-infected control sites. Sites were "infected" with a sterile curette at weeks 0 and 1 or weeks 4 and 5 and then were monitored four weeks later. None of these values were different from the infected or non-infected control site values. For explanation of the superscripts, see Table 2.
<table>
<thead>
<tr>
<th>Week</th>
<th>4-6 R</th>
<th></th>
<th></th>
<th></th>
<th>4-6 S</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>0-1</td>
<td>2-3</td>
<td>$\bar{x}$</td>
<td>0-1</td>
<td>2-3</td>
<td></td>
</tr>
<tr>
<td>P.I.</td>
<td>1.5</td>
<td>25</td>
<td>75</td>
<td>2.0</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>G.I.</td>
<td>1.8</td>
<td>25</td>
<td>75</td>
<td>2.0</td>
<td>25</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>pocket depth (mm $\pm$ S.D.)</td>
<td>2.5$\pm$0.6</td>
<td></td>
<td></td>
<td>2.5$\pm$0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 15. Clinical parameters of the effect of sampling. Several sites in experimental animals were sampled either repeatedly (R) or only once (S) as described in Materials and Methods. No differences were observed between the two groups. See Table 16 for number of sites sampled.
Table 16. Subgingival microflora related to the effect of sampling. Several sites in experimental animals were sampled either repeatedly or only once as described in Materials and Methods. No differences were observed between the two groups. For explanation of superscripts, see Table 2.
Figure 1. Experimental protocol - Part I.
Figure 2. Experimental protocol - Part II.
Figure 3. Identification scheme for Gram-positive cocci.
Figure 4. Identification scheme for Gram-negative cocci.
Figure 5. Identification scheme for Gram-positive rods.
Figure 6. Identification scheme for Gram-negative facultative rods.
Figure 7. Identification scheme for non-pigmented Gram-negative anaerobic rods.
Figure 8. Identification scheme for pigmented Gram-negative anaerobic rods.
Figure 9. Radiographs of ligated sites. Standardized bitewing radiographs of mandibular right first molar with one mm. grid aligned at cementoenamel junctions. Top, week 0 (pre-ligation). Bottom, week 4 (4 weeks post-ligation).
Figure 10. Subgingival microflora of ligated sites. G+ = Gram-positive rods and cocci. G- = Gram-negative rods and cocci. B. ginvialis.
Figure 11. Radiographs of non-ligated sites. Standardized bite-wing radiographs of mandibular left first molar with one mm. grid aligned at cementoenamel junctions. Top, week 0. Bottom, week 8.
Figure 12. Stage I subgingival microflora of non-ligated sites. Size of sections represent approximate percentage of TMC. U=unidentified organisms that could not be subcultured following primary isolation. Fuso=Fusobacterium nucleatum. B. ging.=B. gingivalis.
Figure 13. Stage III subgingival microflora of non-ligated sites. Size of sections represent approximate percentage of TMC. U=unidentified organisms that could not be subcultured following primary isolation. Fuso=Fusobacterium nucleatum. B. ging.=B. gingivalis.
Figure 14. Stage IV subgingival microflora of non-ligated sites. Size of sections represent approximate percentage of TMC. U=unidentified organisms that could not be subcultured following primary isolation. Fuso=Fusobacterium nucleatum. B. ging.=B. gingivalis.
Figure 15. Changes in *B. gingivalis* and STB in non-ligated sites following infection at week 0 and 1. Arrow indicates time of initial infection. ◯-◯, mean percentage *B. gingivalis*. ○-○, mean percentage Gram-negative STB.
Figure 16. Changes in *B. gingivalis* and STB in non-ligated sites following infection at week 4 and 5. Arrow indicates time of initial infection. ---, mean percentage *B. gingivalis*. --, mean percentage Gram-negative STB.
Figure 17. Major changes in subgingival species in non-ligated and control sites; 

- B. gingivalis levels in control sites; ---, Gram-negative STB levels in control sites; -- , BPB levels in non-ligated sites; 

----, Gram-negative STB levels in non-ligated sites.
Figure 18. Example of CABIS output: A hypothetical CABIS output demonstrating the capabilities of the program to identify organisms. Genus and species assignment were made based on entered biochemical characteristics.
### DATA SUMMARY

**SAMPLE NUMBER:** 7010  
**ANAEROBE/FACULTATIVE RATIO:** 2.9 : 1

**SOURCE:** MONKEY 25  
**C.F.U./m1:** 8.83976E+06  
**PERCENT NO GROWTH:** 0 %

**PERCENT OF TOTAL COLONIES**

<table>
<thead>
<tr>
<th>Gram Positive Cocci</th>
<th>Anaerobic</th>
<th>Facultative</th>
<th>Strepococ Mutn/Sangs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptostrep Micros</td>
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<td>7.3</td>
<td>7.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gram Positive Rods</th>
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<th>Facultative</th>
<th>Actinomyce Viscosus</th>
</tr>
</thead>
<tbody>
<tr>
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<td>10.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gram Negative Cocci</th>
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<th>Veillonell Alectescen</th>
<th>Facultative</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3.6</td>
<td></td>
<td>3.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gram Negative Rods</th>
<th>Anaerobic</th>
<th>Bacteroid Asac(H-Or)</th>
<th>Bacteroid Mel. Int.</th>
<th>Fusobacter Nucleatum</th>
<th>Selenomona Sputigena</th>
<th>Facultative</th>
<th>Campylobac Sputorum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>29.1</td>
<td>10.9</td>
<td>14.5</td>
<td>3.6</td>
<td>7.3</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**Total Black Pigmented Bacteroides**  

<table>
<thead>
<tr>
<th>BPB</th>
<th>Asacc</th>
<th>SACCC</th>
<th>FACCC</th>
<th>MEGCC</th>
<th>MICCC</th>
<th>VACC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29.1</td>
<td>10.9</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Figure 19. Example of CABIS data summary. An example of data summarizing capabilities of CABIS based on hypothetical sample in Figure 18.