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**Coogan, Maeve M**

**2015-01-15**
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# BACTERIAL STUDIES ON DEBRIS TAKEN FROM THE GINGIVAL SULCUS OF MAN

Maeve M Coogan

A Dissertation submitted to the Faculty of Science
of the University of the Witwatersrand, Johannesburg
(in fulfilment of the requirements) for the degree of
Master of Science

Johannesburg
March 1975

I hereby declare that this dissertation is my own work, and has not been submitted or incorporated in another dissertation or thesis for any other degree.

Maeve Mary Coogan

Maeve Mary Coogan

# A C K N O W L E D G E M E N T S

I wish to thank Professor J. F. van Reenen for his help and encouragement in the preparation of this dissertation; and Mrs Y. van Rensburg for typographical assistance.

# A B S T R A C T

The gingival sulcus microecology of three categories of people was studied. Samples of debris were removed from the gingival sulcus of ten male Bantu subjects with pockets of 1 - 2 mm, ten male Bantu subjects with pockets of 3 - 6 mm and twenty male Caucasian subjects with pockets of 1 - 2 mm. The samples were placed in cooked meat medium which was incubated at 37°C for thirty days. They were plated out daily from the cooked meat onto a variety of media. Organisms cultured were identified to the genus level. Changes in the pH and amino acids in the medium were studied in ten of the samples taken from the Bantu subjects. Organisms isolated were classified as dominants, associates and incidentals. Dominants were stable, had a high incidence and persisted in the medium. They included the aerobic streptococci, actinomyces, $\alpha$-haemolytic streptococci, mitis-type streptococci and the enterococci. Associates were not stable or did not survive well and could be divided into five groups. The first group consisted of the veillonella, lactobacilli, $\gamma$-haemolytic streptococci and the sarcina. They were favoured by deep pockets. The second group was favoured by shallow pockets and the environment of the Bantu and consisted of the anaerobic streptococci, staphylococci, bacteroides, neisseria, selanomonas, fusobacteria, diplococci, clostridia, leptotrichia and polysaccharide-producing streptococci. This group contained a number of pathogens and the significance of this finding is discussed.

iv/...The third/...

The third group of associates consists of the coryne-bacteria which had a higher rate of survival in the Caucasians than in the Bantu. The fourth group included the yeasts and the micrococci both with a very low survival rate. The fifth group appeared to be inhibited by the environment of the Bantu and deep pockets and it consisted of the $\beta$-haemolytic streptococci. The incidentals were indifferent to the activities of the associates and dominants and consisted of the Enterobacteriaceae. On the whole, organisms isolated from the Bantu tended to survive better than organisms isolated from the Caucasians. The anaerobic streptococci, aerobic streptococci, $\alpha$-haemolytic streptococci, mitis-type streptococci and veillonella had a relatively high survival rate, seemed to benefit from an association with each other and tended to inhibit the other organisms. The anaerobic streptococci had the highest rate of survival. Because of their pathogenicity, their preference for a low oxidation-reduction potential and their predominance they are probably the most active microorganisms in the gingival sulcus. Succession or the replacement of an initial population by a second population was also studied. The initial population consisted of Gram-positive cocci with the exception of the veillonella which is a Gram-negative coccus. The subsequent population consisted mainly of Gram-positive rods and Gram-negative organisms. The amino acids found were divided into three groups. The first showed little change in the medium and is possibly non-essential to growth, and included tyrosine, glutamic acid, alanine and proline. The second group showed a rise in incidence and included methionine, cysteine, lysine, glycine and threonine. They probably were released as a result of protein breakdown. The third group showed a drop in incidence followed by a rise. They appeared to be utilized and then excreted. This group included isoleucine, leucine, tryptophane, cystine,

v/...histidine/...

histidine, serine, aspartic acid, arginine and valine. Leucine and tryptophane showed the greatest change and could be considered the most important amino acids for this experimental community. Serine and histidine were utilized but not excreted again in large amounts. They seemed to be incorporated into the organisms irreversibly. The pH dropped slightly from 7,04 and then rose to a final figure of 8,29. This rise may be due to the deamination of amino acids in the medium.

# I N T R O D U C T I O N

It is well known that the microorganisms of the human mouth have well-developed relationships with each other and their host environment. These relationships are complex and difficult to study in vivo. It is for this reason that in vitro studies have been undertaken by a number of microbiologists in order to determine the nature of some of these relationships. The findings of these studies may, of course, not be completely valid in an in vivo situation, but, they are nevertheless useful as a first guide to understanding the complex relationships which exist in nature.

The present investigation was undertaken to determine some of the charactoristics of the microorganisms of the periodontal sulcus. Features such as survival potential, stability, and succession were studied. This made it possible to classify the flora as dominants, associates and incidentals. It is hoped to utilize this information in future studies aimed at controlling the growth of microorganisms in this area.

vii/...Growth/...

Growth of the microorganisms in the culture medium used for the study produced changes in the environment, which presumably, influenced the survival of the microorganisms. Some of these changes included alteration in the pH of the medium and changes in the amino acid content of the broth. These changes were recorded at regular intervals and formed part of the present study.

This study is divided into four chapters. In the first chapter, which forms the major section of the investigation, aspects of the ecology of the sulcus microorganisms are recorded. In the second chapter, changes in the amino acid content of the media are recorded and their significance is discussed. The third chapter records the changes in pH of the media and the last chapter summarises the major findings of the investigation. A list of references has been included at the end of each chapter rather than at the end of the dissertation.

The work for this project was carried out in the Oral Microbiology Laboratory of the Oral and Dental Hospital, University of the Witwatersrand, Johannesburg.

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# CHAPTER I

## A ORAL MICROBIAL ECOLOGY

### A REVIEW OF THE LITERATURE

### 1 FLORA OF THE ORAL CAVITY

The microorganisms which constitute the normal flora of the human body have highly developed ecological relationships with one another and the host (Gould, 1972). It is well known, for instance, that the flora of the oral cavity is distinct from that of other parts of the body (Burnett and Scherp, 1968). This is due to the particular character of the mouth which supports the growth of the microorganisms. The oral environment is suitable for the [illegible] of some bacteria but not for others. The interactions between the microbes on the one hand and host or environmental elements on the other hand, therefore, determine the constitution of the flora. The mutual relationships collectively are described as an ecosystem (Brock, 1966).

2/...The oral/...

The oral cavity could be regarded as a ecosystem consisting of biotic and abiotic components (Brock, 1966). The oral flora constitutes the biotic component. The members of the this flora are capable of producing favourable growth conditions for themselves by combining individual metabolic potentials and requirements (Bisset and Davis, 1960). The abiotic component consists of the elements which make up the environment. In the oral cavity these include the teeth, mucosa, saliva, plaque and food particles.

The ecosystem concept is used mainly as an aid in studying thermodynamic and biochemical cycles in nature which are independent of time. The production and consumption of each element of the system is in balance and this is achieved by either population or environmental control (Brock, 1966). The best example of population control is the continuous bacterial culture device of Novick (1955). The volume and population density in this device remain constant while the nutrients are added and removed at a constant rate. Despite the nutrients being present in excess the population does not increase beyond a certain concentration. Novick (1955) feels that this control is due to unknown factors inherent in the population itself. These factors may also operate in the oral cavity. In fact, MacFarlane and Mason (1972) believe that microbial antagonism is of major importance in the regulation of the oral flora. Studies on microbial antagonism between individual bacterial species were

3/...undertaken/...

undertaken by Rosebury, Gale and Taylor (1954) and they showed that Corynebacterium diptheria, Neisseria catarrhalis and micrococci were inhibited by streptococci or Diplococcus pneumoniæ and that Streptococcus mitis was inhibited by Streptococcus pyogenes and Streptococcus faecalis. They also found that there was mutual inhibition of streptococci by other species of the same genus. Oxford (1944) observed that Streptococcus lactis and Streptococcus cremoris were inhibited by a substance diplococcin which was isolated from the latter species.

As previously mentioned the balance in the ecosystem is achieved by both population and environmental control. An example of an environmentally controlled system in the laboratory is the chemostat (Brock, 1966). In this system the volume remains constant, whereas there is a continuous flow of medium. The inflowing medium has an excess of all nutrients except one, the limiting nutrient (Brock, 1966) which controls the amount of growth of the organisms. A similar situation was observed in the oral cavity. Saxton and Critchley (1970) in their work on dental plaque found that relatively little cell division of coccoid organisms occurred in plaque and that this was partly due to the limitation of certain amino acids in plaque.

The conditions present in the continuous culture device and the chemostat are not the same as those found in nature and these systems therefore do not give a true

4/...reflection/...

reflection of the balance achieved in nature. It is, however, difficult to study a microbial population *in vivo* because fluctuations in both the biotic and abiotic components occur. However, Brock (1966) feels that in all systems it is feasible to ignore these fluctuations over a short period of time and to concentrate on the overall stability of the system. The oral cavity is no exception and Davies (1972) states that despite fluctuations which may occur over short periods of time, the oral flora is stable.

A very important aspect of microbial ecology, therefore, is the control of population density. This control is very often obscured in natural ecosystems (Brock, 1966) and again the oral cavity is no exception.

Many natural occurring systems are heterogeneous but they are still in balance or in a steady state. For example, when a rivulet is studied downstream from a sewage outfall each milepost has a characteristic, environmental flora which remains relatively constant from day to day (Brock, 1966). This system could be considered as being heterogeneous and in balance or in a steady state condition. The oral cavity could be studied along similar lines with a characteristic flora for the teeth, cheek epithelium, tongue and the saliva.

A number of studies have been done on organisms in these sites and Carlsson (1967) found that *Streptococcus*

5/...*sanguis*/...

sanguis and Streptococcus mutans favoured the tooth surface whereas Ikeida and Sandham (1971) in a more detailed study found that Streptococcus mutans was mainly in the pits and fissures of the teeth. In an earlier study on plaque Hemmens, Blayney and Harrison (1941) found that there were twice as many streptococci in non-carious as in carious areas whereas there were proportionately more lactobacilli, micrococci and Gram-negative anaerobic cocci in carious areas. Bacteroides melaninogenicus favours the gingival sulcus rather than plaque and Streptococcus salivarius favours the cheek mucosa (Gibbons, Kapsimalis and Socransky, 1964). Studies on the streptococcal flora of the tongue by Krasse (1954) have shown that Streptococcus salivarius also favours this site and he feels that these organisms in saliva originate from the tongue. The saliva could be compared to the water in the stream which carries the organisms away from their sites of origin to other sites where they are eventually disposed of or destroyed. From these studies it seems clear that the oral cavity could be considered as being heterogeneous. In other words, it consists of a number of communities existing in balance between and within each other.

In complex systems with many organisms it is important to study the interactions between the organisms (Brock, 1966). In the oral cavity a number of antagonistic relationships have been studied, as was mentioned previously. However, Parker (1970) in his work on paired

6/...culture/...

culture interactions using Streptococcus salivarius (two strains), Bacterionema matruchotii, Neisseria perflava, Odontomyces viscosus, Fusiformis nucleatum and Lactobacillus casei studied both antagonistic and stimulatory reactions. He found that the ultimate beneficiary of most reactions was one or both strains of Streptococcus salivarius. Fusiformis nucleatum inhibited Bacterionema matruchotii, Neisseria perflava and Odontomyces viscosus, but no one species stimulated or inhibited all the other species. Parker (1970) feels that these paired cultures do not give an indication of the reactions which could occur between three or more organisms. He also states that these multiple reactions could give a clue to the complex pattern of control which exists in the oral flora.

## 2 INTERACTION BETWEEN MICROORGANISMS

Despite there being a wealth of knowledge available about the interaction between microorganisms, very little of this data has been applied to the study of ecosystems (Brock, 1966). If two organisms interact there has to be a means of communication between them. In microbiological systems this communication may be achieved through the transfer of chemical substances, sub-cellular units, electrical impulses, mechanical forces and thermal movements (Brock, 1966). Conjugation and the transfer of information in sub-cellular units by viruses may be included under transfer by chemical substances.

7/...2.1 Chemical/...

2.1 Chemical Substances

In the oral cavity there are organisms which produce a variety of chemical substances that are able to transfer information. In the laboratory this may be visible as inhibition and stimulation of the growth of other organisms. The antibictic diplococcin which is produced by Streptococcus cremoris was mentioned previously. This substance is attached to the cell wall and diffuses into the medium under acid conditions (Oxford, 1944). Mattick and Hirsch (1947) obtained a similar substance with a greater inhibitory range. They called this substance nisin. It was isolated from culture filtrates of Streptococcus lactis and inhibited Streptococcus cremoris, Streptococcus lactis, streptococci of Groups A, B, E, F, G, H, M and N, Diplococcus pneumoniae, neisseria and corynebacterium species. Studies by Bjornesjo (1950) demonstrated that some organisms were inhibited by whole saliva and incubation of the saliva increased this inhibition. He felt that this was due to the accummulation of bacterial metabolites in the incubated samples.

Stimulation of growth presumably by chemical substances has also been observed among oral organisms. Rosebury, Gale and Taylor (1954) described the stimulatory effect of streptococci, micrococci and Diplococcus pneumoniae on the growth of Neisseria catarrhalis. Candida albicans also stimulated the growth of Escherichia coli.

8/...2.2 Sub-cellular/...

## 2.2 Sub-cellular units

Chemical substances may contain information in a specified sequence of repeating units such as deoxyribonucleic acid (DNA) which stores genetic information (Brock, 1966). DNA may be transferred from one bacterial cell to another in a number of ways. Transfer may take place by transformation, transduction or plasma mediated conjugation (Jawetz, Melnick and Adelberg, 1972).

During transformation, the recipient cell takes up soluble DNA released from the donor cell. In some cases, for example neisseria, this DNA is released spontaneously into t extracellular slime surrounding these organisms. Transf ation has also been observed in Diplococcus pneumoniae (Jawetz, Melnick and Adelberg, 1972). Both these genera occur in the oral cavity and this mechanism could operate there. DNA may also be transferred by transduction. In this mechanism a fragment of donor chromosome is carried to the recipient bacteria by a temperate bacteriophage. This phenomena has been observed in escherichiae, vibrio and staphylococcus species (Jawetz, Melnick and Adelberg, 1972).

Bacteria are hosts to small extrachromosomal genetic elements c lled plastids. There are a number of different kinds of plasmids. Amongst these are Col factors which produce colicins that are lethal to coliform bacteria (Jawetz, Melnick and Adelberg, 1972). Brock, Peacher and Pierson (1963) isolated five types of

9/...bacteriocins/...

bacteriocins from members of the group D streptococci. Plasmids have also been observed in oral streptococci. These organisms have the ability to produce bacteriocins which are able to inhibit Streptococcus pyogenes, enterococci and other related streptococci but not unrelated bacteria (Kelstrup and Gibbons, 1969). Resistance factors are another type of plasmid which enables its bacterial host to resist various antimicrobial agents. Some staphylococci carry a penicillinase plastid which enables these organisms to produce penicillinase, thus rendering them resistant to penicillin. Plasmids may be transferred by conjugation or by phage transduction.

Transfer of DNA by these methods could change a number of characteristics of the bacteria and thus influence their growth pattern and interactions with other bacteria in the community.

2.3 Electrical impulses

Cells may also interact by means of electrical impulses (Brock, 1966). Bacterial cells carry a negative charge but substances from the growth medium and metabolic products may adhere to the surface of these cells and cause a change in electrical charge (Shaw, 1969). The products in the medium therefore and not the bacterial cells themselves are important in this type of interaction.

10/...2.4 Mechanical/...

2.4 Mechanical forces

Communication between organisms may also be achieved by mechanical forces. These forces could be caused by motility and active collision between organisms would certainly lead to an exchange of information. In the oral cavity a number of actively motile organisms have been described. The majority of these are rods and they attain velocities of up to 50 µm per second. These organisms include spirochaetes, Vibrio sputorum, Fusobacterium girans and Selenomonas sputigena (Burnett and Scherp, 1968).

2.5 Thermal movements

The last means of communication, thermal movements, is possibly of the least importance in the oral cavity because the oral parasites have exacting temperature requirements (Bisset and Davis, 1960) and therefore there should be little change in temperature within this environment. Only minor thermal movements, therefore, would occur.

3 EFFECTS OF COMMUNICATION BETWEEN MICROORGANISMS

The transfer of information may affect the members of the ecosystem in a positive or a negative way and if they are not affected transfer has not taken place (Brock, 1966). If the bacteria are affected positively favourable conditions are produced by regulating the environment to

11/...a suitable/...

a suitable pH or Eh or by producing adequate levels of nutrients and essential growth factors. Conversely if they are affected negatively the environment becomes unsuitable for their growth (Hobson, 1969). Thus, transfer of information is concerned not only with the element transferred but also with the response of the recipient. When two units exchange information it may be in a reciprocal fashion or unidirectional. In unidirectional transfer the donor may benefit or it may be unaffected by the transfer (Brock, 1966).

Brock (1966) states that the coupling between two units may be tight or loose, but in microbial ecosystems complete coupling among units is extremely unlikely because of the enormous number of individuals and populations. This statement is also applicable to the oral cavity because of the great numbers and variety of organisms found here. In fact, Burnett and Scherp (1968) list twenty-six genera which occur in the oral cavity and Socransky et al (1963) found that the total microscopic count of debris taken from the gingival crevice was $10^{11}$ microorganisms per gram and the viable count was $10^{10}$ organisms per gram.

When a system is very complex the phenomenon of unitization occurs: that is, some components combine and interact strongly with each other and act as a unit with respect to the remainder of the system (Quastler, 1958). This phenomenon may occur in the oral cavity and in

12/...particular/...

particular with the streptococci because of their predominance in the mouth (Burnett and Scherp, 1968). Therefore, a particular species of streptococcus or a number of streptococcal species may combine to form a unit which will interact with the remainder of the organisms present. Unitization makes it possible to simplify the interactions of populations (Brock, 1966) and thus it makes the study of an ecosystem easier.

## 4 PROPERTIES OF AN ECOSYSTEM

Any system which is tightly coupled and is an ecosystem has certain properties (Brock, 1966). These are homeostasis, evolution, defence, repair and reproduction, the presence of a phase boundary, and the system must be uniform.

### 4.1 Homeostasis

Homeostasis is the ability of a system to maintain itself despite external influences (Brock, 1966). This phenomenon is evident in the oral cavity and according to Davies (1972) studies on this population have shown that despite intermittent changes in the external environment, which can be seen as changes in the number of bacteria during different times of the day, the oral flora is relatively stable.

13/...4.2 Evolution/...

4.2 Evolution

Evolution is similar to homeostasis but it occurs over a longer period of time (Brock, 1966). To support the idea of evolution in the mouth, Burnett and Scherp (1968) state that the microbial population of the mouth changes progressively until maturity. Studies have been done on some of these changes and Carlsson et al (1970) found that the oral cavity is sterile at birth but after twenty-four hours it is populated by streptococci, mainly Streptococcus salivarius. Streptococcus sanguis is not present even though this organism is found in the mouths of the families of these children. Three months after tooth eruption, however, this organism is able to establish itself in the oral cavity. The eruption of the teeth, therefore, caused an evolutionary change in the oral flora.

4.3 Defence and Repair

Defence is the response to more violent external influences and repair results if a portion of the system is removed and it is able to replace itself (Brock, 1966). Extraction of all the teeth could be considered as a violent force which would affect the oral ecosystem. Shklair and Mazzarella (1960) studied the flora of patients before and after extraction of teeth. They found that the lactobacilli and yeasts disappeared initially during the edentulous period before the insertion of a denture. After extraction the incidence

14/...of streptococci/...

of streptococci increased and during the first two weeks of denture wear, they remained at a high level. The lactobacilli and yeasts returned gradually and after three to five weeks the system was back to normal and the streptococci returned to a pre-extraction level. This phenomenon could, therefore, be considered as defence and then repair of the system.

4.4 Reproduction

Reproduction is the complete replacement of one system by another which is similar or identical (Brock, 1966). Luckey (1963) in his work on normal animals placed in a germ-free environment found that their bacterial flora became less complex with time. He came to the conclusion that there are regular changes in the normal microbial population, with some organisms being eliminated whereas others were being added. This phenomenon would occur in the oral cavity as well as the whole animal with micro-organisms continually dying and being replaced by new organisms.

4.5 Phase boundary

An ecosystem must have a phase boundary which separates it from the outside world (Brock, 1966). This boundary may be sharp, for example, in the oral cavity, or it may be ill-defined, as in the open ocean.

15/...4.6 Uniformity/...

4.6 Uniformity

Uniformity is one of the aspects of an ecosystem and in a homogeneous system a small sample taken from any part of the system will be representative of the whole system (Brock, 1966). In nature there are degrees of uniformity but rarely complete homogeneity (Brock, 1966). This is true of the oral cavity which has a variety of organisms which inhabit particular sites, for example, Streptococcus sanguis and Streptococcus mutans favour the tooth surface (Carlsson, 1967); Bacteroides melaninogenicus prefers the gingival sulcus and Streptococcus salivarius the cheek mucosa (Gibbons, Kapsimalis and Socransky, 1964).

The oral cavity has all the properties discussed above and, therefore, it is a tightly coupled system. If a system is tightly coupled, it is difficult to remove part of the system and maintain it in isolation (Brock, 1966). This can be applied to the oral cavity and is illustrated by the difficulty in isolating and maintaining pure cultures of a number of oral organisms, particularly the anaerobic species.

When the biotic components of an ecosystem are studied it is found that certain organisms grow together in the same environment. This may occur because they have similar environmental requirements or they may survive better together and benefit by their association. Organisms which benefit from each other would exist as an association or community (Brock, 1966). A community, therefore, is the

16/...result/...

result of succession in a particular environment.

## 5 SUCCESSION

### 5.1 Definition

When an uncolonized fresh substrate provides an abundance of nutrients, microorganisms which are able to survive in the substrate will grow and multiply. As time passes the activites of the initial population cause changes in the environment, such as, changes in pH and Eh, the depletion of nutrients and the accumulation of toxic metabolic products. The altered environment will be less suitable for the growth of the initial population or organisms and other organisms which were unable to grow previously will replace or succeed the initial population. The second population of organisms will increase rapidly and predominate while the initial population will decrease in numbers. This process is called succession and will continue until an equilibrium is reached between all the populations which have colonized the substrate (Brock, 1966).

### 5.2 Succession in the oral cavity

The mouth is unique in two respects. Firstly, it is the first mucous membrane of the body to develop a microbiota and secondly, its flora is the most varied of the whole body (Rosebury, 1962). Succession may be studied

17/...in the/...

in the oral cavity. It is sterile at birth but becomes colonized with organisms within 6 - 10 hours (Kostečka, 1924). The mouth of the new born infant is highly selective during the first few days (Burnett and Scherp, 1968). For instance, studies on identical twins have shown that each baby carries different strains of staphylococci and coliform bacteria (Hurst, 1956). The initial strains which become established in the mouth after birth tend to persist and are not readily replaced by other strains even of the same species (Hurst, 1956).

McCarthy, Snyder and Parker (1965) found that the predominant bacteria in the mouths of infants less than 24 hours old were streptococci and the species most commonly present was Streptococcus salivarius. However, Carlsson et al (1970) failed to isolate this organism from infants 3 - 10 hours after birth. They found that Streptococcus sanguis and Streptococcus mutans were established after the eruption of teeth. Streptococcus sanguis requires an anaerobic environment for the production of polysaccharide (Hehre and Neill, 1946) and Streptococcus mutans prefers anaerobic conditions for growth (Edwardsson, 1968). The eruption of the teeth provides an anaerobic environment which favours the growth of both of these organisms. Thus, Streptococcus sanguis was found in all the infants three months after tooth eruption. Despite the anaerobic environment and the

18/...presence/...

presence of Streptococcus mutans in the mouths of the families of these children, it is not established in their oral cavities. Other factors, therefore, are required to enable this organism to become established and evidence to substantiate this was provided by Ikeida and Sandham (1971) who found that this organism could be isolated from the pits and fissures of the teeth which are more protected and anaerobic than the tooth surface.

The oral flora of infants continues to develop with the growth of the child and McCarthy, Snyder and Parker (1965) found that by the age of twelve months all the infants carried streptococci, staphylococci, veillonella and neisseria. They were able to isolate actinomyces, lactobacilli, nocardia and fusobacteria from half of their subjects, but leptotrichia, candida and corynebacteria from less than half the infants. They did not isolate spirochaetes or vibrios and it seems, therefore, that those organisms are established in the mouth at a later stage. The incidence of streptococci dropped from 98 per cent at the beginning of the investigation to 70 per cent by the end of the first year. At this stage the bacterial population is relatively well established. Succession will take place from this initial p pulation and the development of the biota of each region depends on changes associated with the growth of the host, for example, the eruption of teeth (Rosebury, 1962). Kostečka (1924) found that in the period preceding the eruption of teeth the

19/...oral flora/...

oral flora is exclusively aerobic. When the teeth erupted the flora expanded and anaerobic forms are acquired such as spirochaetes and fusiforms. When teeth are present in the mouth the flora remains mixed, consisting of aerobes and anaerobes. With complete loss of teeth the flora again returns to aerobic forms. If a few teeth are left in the mouth the mixed flora could be found around these teeth.

Kostečka (1924) felt that the multiplication of spirochaetes and fusiform bacilli depends upon the presence of natural teeth. On the other hand, Berger, Kapcvits and Pfeiffer (1959) state that there is no relationship between tooth eruption and the presence of anaerobes in the oral cavity. The anaerobic organisms they studied, however, did not include the more fastidious fusiforms and spirochaetes and their observations were directed towards the presence of peptostreptococci species and Veillonella alcalescens.

Succession can also be studied in plaque accumulated in the adult mouth. Löe, Theilade and Jensen (1965) found that early plaque consisted of mainly coccoid forms, and after two to four days' accumulation of plaque there was a preponderance of filamentous forms which resembled leptotrichia and fusobacteria. When plaque was allowed to accumulate for between six and ten days, there was an increase in the number of bacteria present and a marked rise in the incidence of vibrios and spirochaetes. On the other hand, the Gram-positive cocci and short rods showed a decrease in incidence from an original figure

20/...of between/...

of between 80 and 100 per cent in most subjects to a final incidence of between 45 and 60 per cent. Theilade and Theilade (1970) found similar but less marked differences over a period of three days.

Howell, Rizzo and Paul (1965) cultured organisms from plaque which was allowed to accumulate for a longer period of time. In their work the incidence of streptococci dropped from an initial incidence of 50 per cent in plaque that was two days old to an incidence of 16,5 per cent in plaque older than ninety days. The neisseria also showed a drop in incidence from 12,4 to 1,7 per cent. On the other hand, Actinomyces naeslundi and Actinomyces israeli both showed a marked increase from 0 and 13 per cent to 9,4 and 26,6 per cent respectively.

In all these studies there was a decrease in the number of aerobic and facultative organisms, with a corresponding increase in the quality and quantity of anaerobic organisms. These changes, therefore, could be due to the development of an anaerobic environment. In fact, Kenney and Ash (1969) measured an overall decrease of 395 millivolts in plaque which was allowed to accumulate over a period of four days. Similar but lesser changes in the oxidation reduction potential could occur in the mouths of the infants. This smaller change would account for the inability of the very strict anaerobes to establish themselves. Another factor which could

21/...contribute/...

contribute towards this shift in populations would be competition between the initial microorganisms and the later populations. The availability of nutrients could play an important role because the spirochaetes and oral vibrios or selenomonads require very exacting conditions for growth (Rosebury et al, 1951; Bisset and Davis, 1960).

# CHAPTER I

## B MATERIALS AND METHODS

### PRESENT STUDY

#### INTRODUCTION

The oral flora is subjected to intermittent changes in its external environment. This can be seen as changes in cell populations at different times of the day. The effect is not the same in all parts of the mouth and varies from site to site (Burnett and Scherp, 1968). Davies (1972) states that despite these variations the flora has an overall stability. The widespread prevalence of dental disease, however, indicates that the balance between the commersal organisms and the host easily can be disturbed. The mechanisms of this control are poorly understood and this necessitates the study of mixed microbial populations. This possibly could lead to a better understanding of the different roles that bacteria play in the aetiology of dental caries and periodontal disease (Davies, 1972).

23/...It has been/...

It has been suggested by Carlsson and Egelberg (1965) that plaque should be regarded as a naturally occurring microbial ecosystem. In most ecosystems successional changes cannot be seen and the mature system is already present (Brock, 1966). It is, therefore, difficult to study succession in vivo, which makes an in vitro study valuable. If two or more organisms are found growing together, they may benefit by their association and thus form a community or they may merely have similar environmental requirements (Brock, 1966). This relationship can only be determined by means of experiment.

It is possible to differentiate between three main groups of organisms in an ecosystem. Firstly, dominants or those organisms which possess the greatest activity and have a controlling influence on the other organisms in the system. Secondly, associates or the organisms which are dependent upon the activities of the dominants for their development. Thirdly, incidentals or those organisms which are indifferent to the activities of the dominants or associates (Brock, 1966).

In the study of bacterial populations investigations can be undertaken on a number of levels. Thus, mixed cultures can be studied in complex media and then pure cultures in chemically defined media (Woods, 1953). Van Niel (1955) states, that however useful pure cultures are, they are not as constant as earlier workers believed. The continuous appearance of mutants and the process of

24/...natural/...

natural selection which takes place in these cultures leads to great variations. However, despite their limitations pure cultures do help in the characterization of the biochemical activities and physiological properties of bacteria (Krasse, 1970).

In the enrichment culture technique a chemically and physically defined environment is used (Van Niel, 1955). The medium is inoculated with test material in sufficient quantities to ensure that most microbial types from the original environment are present. Regular observations are then made on the microflora which develops in the course of time. The outcome of the experiment demonstrates which organisms are able to survive in competition with others (Van Niel, 1955). This experiment is essentially the application on a microscale of the principles of natural selection (Stanier, Doudoroff and Adelberg, 1971).

The purpose of the present study was to determine some of the relationships which exist in the microbial community of the gingival crevice of man. The experiments might give an indication of the competition which exists amongst the microbes and therefore may demonstrate the natural selection which takes place in this environment.

## 1 MATERIALS AND METHODS

Samples of debris were taken from the gingival sulcus of twenty Caucasian and twenty Bantu male subjects

25/...selected/...

selected at random. Pocket depths were determined at the site where the debris was removed. The specimens were taken from the buccal sulcus of the most posterior upper tooth on the right side (molar or premolar), by means of a wire loop and inoculated into 20 ml of cooked meat medium (Oxoid) in McCartney bottles. The bottles were incubated at 37°C for 30 days. A loopful of broth was removed at daily intervals and plated on the following media: nutrient agar (Oxoid) containing 5 per cent sterile citrated horse blood; tomato juice agar (Davis, Bisset and Hale, 1955); mitis salivarius agar (Difco) as described by Chapman (1946); veillonella medium (Rogosa, 1956); fusiform medium (Baird-Parker, 1957); and leptotrichia medium (Baird-Parker and Davis, 1958).

The tomato juice, blood and mitis salivarius agar plates were incubated aerobically at 37°C for 48 hours. A second blood and mitis salivarius agar as well as the veillonella, fusiform and leptotrichia plates were incubated anaerobically at 37°C for a minimum of 4 days in a Baird and Tatlock anaerobic jar filled with 20 per cent carbon dioxide and 80 per cent hydrogen.

The cultures obtained were studied macroscopically and microscopically using Grams method of staining. Previous experience in the culture and identification of oral microorganisms facilitated their classification to the genus level using colonial morphology, texture, colour

26/...and the odour/...

and the odour produced. The following criteria were used in the identification of the genera studied.

## 2 CRITERIA FOR CLASSIFICATION OF MICROORGANISMS

### 2.1 Gram-positive organisms

#### 2.1.i The aerobic streptococci

These organisms are Gram-positive, spherical or ovoid cells occurring in pairs or short chains. No pigments are produced on agar plates with a few exceptions. They produce small colonies, usually less than 1 mm in diameter. Colony variants may be rough to smooth (matt or glossy) or mucoid. The cytochrome systems are absent and the organisms are catalase negative. The organisms are facultative with respect to oxygen (Breed, Murray and Smith, 1957). Skerman (1959) states that the colonies on blood agar are surrounded by a zone in which the blood pigments have altered. This enables a classification of the streptococci into three groups. The β-haemolytic streptococci produce a water clear zone around the colonies. The blood pigments and cells have completely disappeared from this zone. The α-haemolytic streptococci have a greenish zone around the colonies. The red blood cells remain intact but there is an alteration in the haematin pigments. The γ-haemolytic streptococci cause no change in the blood agar.

When the streptococci are grown on mitis salivarius agar

27/...they can/...

they can be divided into the following groups:-

2.1.i (a) Polysaccharide-producing streptococci

Mitis salivarius agar contains sucrose which enables certain streptococci to produce extracellular polysaccharides visible as large gum drop colonies on this medium (Chapman, 1946). These species include Streptococcus sanguis, Streptococcus salivarius, Streptococcus bovis (Breed et al, 1957), and Streptococcus mutans (Clark, 1924).

2.1.i (b) Enterococci

On mitis salivarius agar these organisms are visible as small dark blue or black colonies about 1 mm in diameter (Chapman, 1946). Species in this group are Streptococcus faecalis, Streptococcus facaelis var. liquifaciens, Streptococcus faecalis var. zymogenes, and Streptococcus durans (Breed et al, 1957).

2.1.i (c) Mitis-type streptococci

Organisms belonging to this group can be recognised as small, light colonies on mitis salivarius agar and species include Streptococcus mitis, Streptococcus acidominimus, Streptococcus equinus, Streptococcus thermophilus, Streptococcus lactis, Streptococcus cremoris, and the non-polysaccharide-producing $\beta$-haemolytic streptococci (Breed et al. 1957).

28/...2.1.ii The/...

2.1.ii The anaerobic streptococci

Under this group are included the strictly anaerobic chain-forming cocci. Their cells are usually smaller than the facultative streptococci and the colonial forms vary with some species producing coal-black colonies and others are round, convex, translucent and grayish white. Many species disintegrate fibrin and blood, cause a black or green discolouration and produce foetid gasses (Burnett and Scherp, 1968).

2.1.iii Diplococci

Diplococcus pneumoniae is a monotypic species (Breed et al, 1957). Wilson and Miles (1957), however, place it under the streptococci. It differs from the streptococci with its characteristic morphology and possession of a polysaccharide capsule. Diplococci appear as capsulated lanceolate cocci grouped in pairs, the blunt ends of the cells being adjacent and the capsule surrounding each pair. Colonies tend to be of the "draughtsman" type; they have a smooth flat surface with steeply shelving sides and the edges may be raised to form a circumferential ring above the rest of the colony. They are surrounded by a zone of $\alpha$-haemolysis with the usual green colouration (Wilson and Miles, 1957).

2.1.iv Staphylococci

They are spherical or ovoid, non-motile, Gram-positive

29/...cells/...

cells, arranged in grapelike clusters on solid medium. They are strongly catalase positive (Wilson and Miles, 1957) and facultative as regards oxygen requirements (Breed et al, 1957). On solid media the colonies are circular, entire, convex, smooth, shining, opaque, butyrous, and easily emulsifiable. The colour of the colonies varies from gold to white or yellow (Wilson and Miles, 1957).

2.1.v Sarcina

Sarcina are Gram-positive, spherical, unicellular organisms which divide in three perpendicular planes to produce cubical packets of eight cells each. This genus includes aerobic and anaerobic species (Skerman, 1959).

2.1.vi Lactobacilli

Lactobacilli are Gram-positive rods which are often long and slender and non-motile (Breed et al, 1957). These organisms are non-sporing and sometimes pleomorphic and they divide in one plane only without branching (Burnett and Scherp, 1968). Pigment production is rare and when present yellow, orange, rust or brick-red. The organisms are catalase negative and surface growth is poor (Breed et al, 1957) because the organisms are facultative anaerobes or micro-aerophilic (Wilson and Miles, 1957). Some are strict anaerobes (Wilson and Miles, 1957) but none of the oral

30/...species/...

species are included in this category (Davis, 1956). Colonies are usually smooth and domed with a texture resembling orange peel (Burnett and Scherp, 1968). Some species have the typical colonies with a "Medusa head" appearance as described by Davis, Bisset and Hale (1955). Most oral lactobacilli grow best in a medium with a surface-tension-reducing agent and an adequate carbohydrate supply (Burnett and Scherp, 1968). Growth on tomato juice agar (Davis, Bisset and Hale, 1955) is satisfactory because of the complex nature of the medium and the presence of Tween 80, a surface-tension reducing agent. This medium, therefore, facilitates a quick identification of these organisms.

2.1.vii Clostridia

They are Gram-positive, rod-shaped, anaerobic, catalase negative, motile or non-motile organisms which produce endospores. The endospores are oval, cyclindrical or spherical and occur in a central, subterminal or terminal position. The rods are swollen to form spindle-, club-, racquet- or spoon-shaped cells. Colonies vary with the species and some are minute and colourless while others are large and spreading (Skerman, 1959).

2.1.viii Corynebacteria

They are non-sporing, rod-shaped organisms with irregularly stained segments and granules. The organisms

31/...are Gram-positive/...

are Gram-positive and often the cells loose the stain but the granules invariably stain Gram-positive. The organisms frequently show club-shaped swellings and are non-motile except for plant pathogens (Breed et al, 1957). Characteristically the organisms appear in smears in palisade or Chinese letter forms. The colonies vary with species but usually exceed 1 mm in diameter and are circular, entire, butyrous, grayish white or pigmented cream, yellow or red (Skerman, 1959).

2.1.ix Actinomyces

Actinomyces are anaerobic Gram-positive organisms which in young cultures produce a well defined mycelium which fragments into simple, unbranched and branched rods as the culture ages (Bisset and Davis, 1960). These organisms are catalase negative (Prevot, 1966) and are non-sporing but show swellings which look like spores (Wilson and Miles, 1957). Colonies are small, smooth and pale in colour not unlike streptococci. On blood agar Actinomyces odontolyticus develops a dark red haemin-like colour which renders them distinctive (Bisset and Davis, 1960).

2.1.x Leptotrichia

There are two species in this genera which differ regarding several characteristics. Leptotrichia buccalis

32/...is a paired/...

is a paired bacillus with the free ends slightly tapered. They are large up to 200$\mu$ in length. Young cultures are usually strongly Gram-positive. Catalase is not produced. Colonies are of the wreathed "Medusa head" variety and do not adhere to the medium (Bisset and Davis, 1960).

Leptotrichia dentium also consists of very large filaments - several 100 microns long. They are strongly Gram-positive and branch very freely to form the characteristic "whip hand..." filaments (Bisset and Davis, 1960).

2.1.xi Yeasts

The true fungi that occur as regular members of the oral flora are ovoid and reproduce by budding. These organisms are strongly Gram-positive and are known as the yeasts (Bisset and Davis, 1960).

2.2 Gram-negative organisms

2.2.i Neisseriae

Neisseria are Gram-negative, aerobic, kidney-shaped organisms occurring in pairs with the flat sides adjacent (Skerman, 1959). The colonial appearance is subject to considerable variation and the colour varies from greenish-yellow to bright yellow to grayish white (Wilson and Miles, 1957).

33/...2.2.ii Veillonella/...

2.2.ii Veillonella

Veillonellae are Gram-negative, spherical organisms arranged in pairs or clusters (Skerman, 1959). They are obligatory anaerobes, non-motile and non-sporing. Colonies vary from 1 - 3 mm in diameter and are smooth, entire, lens-, diamond- or heart-shaped. They are opaque, grayish-white and butyrous or soft in consistency (Burnett and Scherp, 1968). On Rogosa's medium they appear as large creamy or small transparent conical colonies.

2.2.iii Enterobacteriaceae

The Enterobacteriaceae are Gram-negative motile or non-motile rods which do not form spores. They are aerobic or facultatively anaerobic and frequently occur in the alimentary tract of vertebrates (Breed et al, 1957). Cultures can often be recognised by the production of a faecal odour on blood agar.

2.2.iv Fusobacteria

Fusobacteria are straight or curved rods usually with tapering ends, occurring singly, in pairs and sometimes in short chains. Filaments are common (Breed et al, 1957). The bacilli have pointed ends and are often divided centrally by obvious septa (Bisset and Davis, 1960). Motile and non-motile species occur. The organisms are Gram-negative and stain with more or less

34/...distinctive/...

distinctive granules. They possess fastidious requirements for growth and are anaerobic to microaerophilic. Surface colonies are butyrous, round and entire (Breed, et al, 1957). Rough and more irregular deeply veined forms occur (Bisset and Davis, 1960) and these have a star-shaped appearance. Two species of oral fusobacteria occur, Fusobacterium nucleatum and Fusobacterium polymorphum, which would account for the smooth and rough types respectively (Bisset and Davis, 196C).

2.2.v Bacteroids

Bacteroids are Gram-negative rods with rounded ends which are sometimes pleomorphic. Bacteroides melaninogenicus, the organism studied in this investigation, is an anaerobic organism which is non-motile, not capsulated and requires serum or ascitic fluid for growth. It produces a characteristic black pigment on blood agar (Breed et al, 1957).

2.2.vi Selenomonads

Selenomonads are Gram-negative, anaerobic, curved, rod-shaped organisms which are motile by means of a bunch of flagella inserted in the concave side of the cell. Only one species has been isolated from the buccal cavity of man, Selenomonas sputigena (Breed et al, 1957).

35/...2.3 Statistical/...

2.3 Statistical analyses

Statistical methods were used to test for differences between the incidence of microorganisms in the Caucasians with pockets of 1 - 2 mm, in the Bantu with pockets of 1 - 2 mm, and in the Bantu with pockets of 3 - 6 mm. The sign test (Siegel, 1956) and the student t test (Wetherill, 1967) were used for the testing of pair differences. The sign test was determined by the following formula:-

$$\frac{B = x - .5 - \frac{N}{2}}{\frac{1}{2}\sqrt{N}} \text{ if } x > y \text{ and } \frac{B = y - .5 - \frac{N}{2}}{\frac{1}{2}\sqrt{N}} \text{ if } y > x$$

N = number of positives or negatives

x = number of positives

y = number of negatives

Tables of Normal Distribution were consulted for significance levels. This formula was used for values of $n > 20$. For values of $n \leq 20$ exact probabilities based on the binomial distribution were used.

It depends upon the null hypothesis i.e. that the differences do not on the average differ from zero and that there is the same number of differences smaller and larger than zero (Siegel, 1956). The student t test was determined by the following formula:-

36/...,$s^2$=/...

$$s^2 = \frac{1}{n-1}\left\{\sum d_\lambda^2 - \bar{d}\sum d_\lambda\right\}$$

$$t = \frac{\bar{d}}{\sqrt{\frac{s^2}{n}}}$$

where $n$ = no. of observations

$B$ = daily incidence in Bantu

$C$ = daily incidence in Caucasians

$d$ = $B - C$

$\sum d_\lambda$ = sum of the d's

$\bar{d}$ = $\frac{\sum d_\lambda}{n}$

$\sum d_\lambda^2$ = sum of the squares of d's

The results were compared with t-distribution tables with $n - 1$ degrees of freedom.

The student t test depends upon the assumption that the population is normally distributed and any results outside this limit are considered significant (Wetherill, 1967). In the present investigation the populations studied need not necessarily fulfil this criteria.

## 2.4 Plaque index

A plaque index was determined for the two ethnic groups according to the methods described by Lemmer (19 4). Each tooth has a possible score of 2 - only the buccal and the lingual surfaces were examined. Subjects with at least 24 teeth were used in the present experiment. The plaque index was expressed as a fraction of 64.

37/...Chapter I C/...

# CHAPTER I

## C RESULTS

### GENERAL

The results of this study are summarised in Tables I, II and III. The average percentage incidence on days 1 and 2; 3 and 4; 5 and 6; etc., was determined for each organism. This was worked out for the twenty Bantu and twenty Caucasians separately as well as for the total of forty patients.

### 1 PERCENTAGE INCIDENCE OF THE STREPTOCOCCI

Table I shows the percentage incidence for the streptococci. Diplococcus pneumoniae was included in this table because of its similarity to the streptococci (Wilson and Miles, 1957).

The anaerobic streptococci had the highest percentage incidence and persisted for the longest period of time.

38/...Their/...

Their incidence was 100 per cent on days 1 and 2; it dropped slightly to 97,5 per cent by days 7 and 8, and remained above 50 per cent up until the sixteenth day. After this it dropped gradually to below 10 per cent by the twenty fifth to the twenty sixth day. There was a difference between the incidence of these organisms in the samples from the two population groups; the values generally were higher for the Bantu than the Caucasians during the duration of the experiment.

The aerobic streptococci had the second highest percentage incidence which started at 100 per cent and dropped below 50 per cent after the twelfth day. There was no marked difference between the incidence of the aerobic streptococci in the Caucasian and Bantu samples, but they did persist for a longer period in the case of the latter.

The $\alpha$-haemolytic streptococci were the next group and were present in more than 50 per cent of the samples for 10 days. Their initial incidence, however, was 81,2 per cent and dropped to zero after 18 days. There was very little difference between the incidence of these organisms in the Bantu and Caucasians.

The mitis-type streptococci had an initial incidence of 88,7 per cent which was higher than the $\alpha$-haemolytic streptococci but their incidence dropped below 50 per cent after 8 days. On the other hand, the incidence of these organisms was higher among the Caucasians than the

39/...Bantu/...

Bantu samples.

The enterococci were the next most numerous group among the streptococci. Their initial incidence, however, was only 51,2 per cent. This figure dropped slowly to zero on days 13 and 14, but rose again to 1,2 per cent on days 15 and 16. Initially the incidence of the enterococci was higher among the Bantu than the Caucasians but from days 10 and 11 the opposite was true.

The polysaccharide-producing streptococci had an initial incidence of 60 per cent which dropped dramatically to zero after 4 days. The figures for the Bantu and Caucasians were similar.

Diplococcus pneumoniae had an initial incidence of 33,7 per cent which dropped gradually and persisted for 16 days. It only persisted for 8 days in the Caucasians but was present for double that period in the Bantu.

The $\gamma$-haemolytic streptococci were present in only 10 percent of the samples initially but this incidence rose to 33,7 per cent on days 7 and 8. They disappeared from the sample after the twenty first day. The incidence of these organisms was higher and persisted for a longer period of time among the Bantu than the Caucasians.

The percentage incidence of the $\beta$-haemolytic streptococci never rose above 10 per cent and tapered off after 18 days.

40/...2 Percentage/...

2 PERCENTAGE INCIDENCE OF THE REMAINDER OF THE GRAM-POSITIVE ORGANISMS

Table II consists of the results for the rest of the Gram-positive organisms which were isolated in this experiment. Staphylococcus had a low incidence initially but they persisted for a long period. Initially the incidence was 20 per cent and this remained at about the same level throughout the experiment and on days 29 and 30 the incidence was still 12,5 per cent. The incidence of the staphylococci was higher among the Caucasians than the Bantu for the first 8 days but from day 9 onwards the incidence was higher among the Bantu.

The actinomyces had an initial incidence of 12,5 per cent which was low. They increased, however, to 45 per cent by day 10 and then decreased gradually to 1,2 per cent on the last day of the experiment. These organisms persisted for a longer period of time in the Bantu than in the Caucasians.

Lacto[illegible] ad an initial incidence of 27,5 per cent which [illegible] rise similar to the actinomyces but their incidence was lower. They also did not persist for the same time as the actinomyces and disappeared after the twenty fourth day. In both the Bantu and Caucasian samples there was a slight rise and then a drop in incidence followed by a second and greater rise. The organisms were absent after the fourteenth day in the samples from Cuacasians but persisted until the twenty fourth day in the Bantu.

41/...Initially/...

Initially the corynebacteria were present in three times as many samples from Caucasians than from the Bantu, but after the sixth day the incidence was about 12 per cent in both these groups. Overall the initial incidence of 27,5 per cent rose to 38,6 per cent and dropped to zero after the twelfth day.

The sarcina were only present for the first 6 days of the experiment with an initial incidence of 12,5 per cent. There was a drop in incidence in the Bantu with the initial incidence of 22,5 per cent dropping gradually until it reached zero on the seventh day. The corynebacteria were only present in 2,5 per cent of the samples from Caucasians and they disappeared after the fourth day.

The total incidence of the leptotrichia never rose above 10 per cent. In the samples from the Bantu, however, the initial incidence was 15 per cent which dropped to 5 per cent and rose again to 12,5 per cent by the sixth day. These organisms persisted until the fourteenth day when the final incidence was 2,5 per cent. In the samples from Caucasian patients the incidence never rose above 7,5 per cent. Micrococci, clostridia and the yeasts were never present in more than 10 per cent of samples. They were present either within the first 8 days or after the eighteenth day. Micrococci were not isolated from the samples from Bantu patients, whereas the clostridia were present in more of the samples from the Bantu than the Caucasians. On the other hand, yeasts were found more often in samples from the Caucasian patients.

42/...3 Percentage/...

3 PERCENTAGE INCIDENCE OF THE GRAM-NEGATIVE ORGANISMS

Table III consists of the results for the Gram-negative organisms. The veillonella were the most prominent in this table and were present in 95 per cent of the samples on the first 2 days. This figure remained above 50 per cent for the first 8 days and stayed above 30 per cent until the twelfth day. The incidence dropped gradually until the thirtieth day. Generally the incidence of the veillonella was higher in the Bantu than the Caucasians and they persisted for a longer period of time.

The initial incidence of the neisseria was 62,5 per cent which fell below 50 per cent after 4 days. It dropped still further until they disappeared after the sixteenth day. The incidence was higher among the samples from the Bantu than the Caucasians but they persisted for a shorter period of time.

Bacteriodes melaninogenicus was an organism which persisted for a long period of time - 12 days with a percentage incidence above 20 per cent. The incidence among the Bantu was much higher than that among the Caucasians throughout the duration of the experiment. Initially these organisms were absent in samples from the Caucasians and present in 40 per cent of samples from the Bantu. Within 4 days their incidence rose to 72,5 per cent and only 3 per cent in the Caucasians. By the sixth day these figures had changed to 40 per cent and 10 per cent respectively. The bacteroids disappeare

43/...from the/...

from the Caucasian samples after 12 days but persisted for 24 days in the Bantu samples.

The fusobacteria were present in a larger number of samples than the bacteroides initially but their incidence fell below 20 per cent within 8 days and disappeared after 16 days.

The incidence of the Enterobacteriaceae never rose above 5 per cent during the entire experiment. They were present in 5 per cent of samples and remained until the end of the experiment in the case of the Bantu but disappeared after the twenty second day in samples from Caucasians.

The total incidence of selenomonas in all the samples never rose above 8,7 per cent. Their incidence, however, was higher among the Bantu than the Caucasians, in fact, as high as 15 per cent and they persisted for 10 days in the samples from Caucasian patients and more than twice that time in the Bantu samples.

4 MEAN INCIDENCE OF MICROORGANISMS FROM THE BANTU, CAUCASIANS AND THE TOTAL SAMPLE

The figures for these observations are tabulated in Tables IV, V and VI. The organisms have been arranged in order of incidence. The organisms which had the highest incidence and persisted for the longest period were placed first and those with the lowest incidence last. It was

44/...possible/...

possible to divide the organisms into three categories according to their incidence:-

i) Those above 50 per cent
ii) Those between 20 and 49 and
iii) Those below 20 per cent.

4.1 Organisms with an incidence above 50 per cent were considered to have a high incidence. In the total this group consisted mainly of the streptococci. The group included the anaerobic, aerobic, $\alpha$-haemolytic, mitis-type and polysaccharide-producing streptococci as well as the enterococci, neisseria and veillonella. In the Bantu this group consisted of the anaerobic, aerobic, $\alpha$-haemolytic, $\gamma$-haemolytic, mitis type and polysaccharide-producing streptococci as well as the enterococci, veillonella, neisseria and bacteroids. In the Caucasians this group was not as well represented as in the Bantu and consisted of the aerobic, anaerobic, $\alpha$-haemolytic, polysaccharide-producing and mitis-type streptococci as well as the actinomyces and veillonella.

4.2 Organisms with an incidence between 20 and 49 per cent were considered to form an intermediate group. In the total this group consisted of actinomyces, corynebacteria, bacteroids, $\gamma$-haemolytic streptococci, Diplococcus pneumoniae, lactobacilli, fusobacteria, and staphylococci. The group was smaller in the Bantu and consisted of the actinomyces, Diplococcus pneumoniae, lactobacilli, fusobacteria,

45/...staphylococci/...

staphylococci and sarcina. In the Caucasians this group was larger and consisted of neisseria, Diplococcus pneumoniae, enterococci, corynebacteria, lactobacilli, staphylococci, fusobacteria and the ɣ-haemolytic streptococci.

4.3 Organisms with an incidence below 20 per cent were considered to have a low incidence. In the total this group consisted of sarcina, leptotrichia, Enterobacteriaceae, selenomonas, β-haemolytic streptococci, micrococci, yeasts and clostridia. This group was small in the Bantu and included the corynebacteria, leptotrichia, selenomonas, β-haemolytic streptococci, Enterobacteriaceae, clostridia and yeasts. It was larger in the Caucasians and included the β-haemolytic streptococci, micrococci, bacteroids, selenomonas, leptotrichia, Enterobacteriaceae, clostridia, sarcina and yeasts.

These table show that some organisms are able to survive in the medium for longer periods of time than others. The ability of an organism to survive may be expressed by a survival index.

## SURVIVAL INDEX

The survival of an organism in a medium is dependent upon its ability to live in the medium for a particular period of time. In the present experiment some organisms could

46/...not be/...

not be subcultured until day 20. They, therefore, had the ability to survive in low numbers in this environment for the first 20 days and when conditions were favourable they multiplied noticeably. Their survival rate therefore was high. On the other hand other organisms, present in large numbers for the first few days, seemed to disappear and their survival rate was relatively low.

The survival of organisms depends upon a number of factors which includes their ability to grow in the medium, their incidence in the initial inoculum and their relationship to other organisms in the population. The survival rate may be expressed as an index where weight is given to the duration of survival.

Thus the survival index may be expressed by the following formula:-

$$\sum \text{percentage incidence} \times \text{time}$$

Survival indices were prepared and are shown in Tables VII, VIII and IX.

Table VII gives the combined survival index for the Bantu and Caucasians. The organisms in this table could be divided into 3 groups:-

1 ORGANISMS WITH A SURVIVAL INDEX GREATER THAN 1799

These organisms could be called good survivors and form a major fraction of the flora in the medium. This group

47/...included/...

included staphylococci, veillonella, actinomyces and the anaerobic, aerobic and α-haemolytic streptococci.

2 ORGANISMS WITH A SURVIVAL INDEX BETWEEN 900 and 1799

Their ability to survive was intermediate and the group consisted of γ-haemolytic streptococci, mitis-type streptococci, enterococci, bacteroids, lactobacilli, neisseria and the Enterobacteriaceae.

3 ORGANISMS WITH A SURVIVAL INDEX OF LESS THAN 900

The organisms in this group could, therefore, be considered poor survivors. This group was the largest in the Table but the organisms belonging to it form a minor fraction of the flora in the medium. Organisms in this group included the fusobacteria, Diplococcus pneumoniae, corynebacteria, β-haemolytic streptococci, selenomonas, clostridia, leptotrichia, polysaccharide-producing streptococci, sarcina, micrococci and yeasts.

Table VIII shows the survival indices for specimens from Bantu subjects. The first group of good survivors is much larger than in the combined index and consists of anaerobic streptococci, staphylococci, veillonella, actinomyces, aerobic streptococci, α-haemolytic streptococci, bacteroids and γ-haemolytic streptococci.

The second group of intermediate survivors consists of lactobacilli, neisseria, Enterobacteriaceae, mitis-type

48/...streptococci/...

streptococci, enterococci and fusobacteria.

The third group of poor survivors consists of Diplococcus pneumoniae, selenomonas, corynebacteria, clostridia, leptotrichia, β-haemolytic streptococci, polysaccharide-producing streptococci, sarcina and yeasts.

Table IX shows the survival indices for the Caucasians. The first group of good survivors consisted of the anaerobic streptococci, the aerobic streptococci, the α-haemolytic streptococci, actinomyces, veillonella, mitis-type streptococci and the staphylococci. There was only one organism in the intermediate group, namely, the enterococci. The third group of poor survivors was large and consisted of fusobacteria, corynebacteria, neisseria, Enterobacteriaceae, lactobacilli, β-haemolytic streptococci, γ-haemolytic streptococci, Diplococcus pneumoniae, bacteroids, polysaccharide-producing streptococci, clostridia, micrococci, leptotrichia, selenomonas, yeasts and sarcina.

EFFECT OF POCKET DEPTH ON FLORA

The organisms from the Bantu could be divided into two groups with ten subjects in each group. Firstly, those microorganisms isolated from pockets with a depth of 1 - 2 mm. Secondly, those organisms isolated from pockets with a depth of between 3 - 6 mm.

49/...Survival/...

Survival indices were calculated for these organisms and are shown in Tables X and XI respectively.

In Table X, which consists of the survival indices for microorganisms isolated from pockets of 1 - 2 mm, there are three groups. These groups are similar to those used for the survival indices previously.

The first group consisted of organisms with a survival index greater than 1800. This group comprises the anaerobic streptococci, staphylococci, aerobic streptococci, actinomyces, veillonella, $\alpha$-haemolytic streptococci and bacteroids. These organisms all have a high survival index.

The second group of organisms with a survival index between 900 and 1800 consisted of $\gamma$-haemolytic streptococci, neisseria, lactobacilli, fusobacteria, mitis-type streptococci, enterococci and diplococci. These organisms have an intermediate survival index.

The third group with a survival index below 900 consists of selenomonads, clostridia, leptotrichia, corynebacteria, $\beta$-haemolytic streptococci, polysaccharide-producing streptococci and sarcina. These organisms have a low survival index.

Table XI, which shows the survival indices for microorganisms isolated from pockets of 3 - 6 mm, can also be divided into three groups.

50/...Firstly/...

Firstly, those with an index above 1800. This group was larger than the similar group in Table X and consisted of the anaerobic streptococci, staphylococci, veillonella, actinomyces, aerobic streptococci, $\gamma$-haemolytic streptococci, Enterobacteriaceae, $\alpha$-haemolytic streptococci, lactobacilli and bacteroids. These organisms all had a high survival index.

Secondly, those with a survival index between 900 and 1800 or an intermediate index. This group was relatively small and consisted of the neisseria, mitis-type streptococci and the enterococci.

The third group with an index of less than 900 consisted of diplococci, fusobacteria, selenomonas, corynebacteria, leptotrichia, sarcina, polysaccharide-producing streptococci, clostridia, $\beta$-haemolytic streptococci and yeasts. These organisms all had a low survival index.

## CLASSIFICATION OF ORGANISMS IN THE ECOSYSTEM STUDIED

As pointed out above it is possible to define three types of organisms in a microecosystem, namely, dominants, associates and incidentals. Dominants are those organisms which possess the greatest activity and have a controlling influence on other organisms in the community (Brock, 1966). Because dominants have these characteristics they should have the highest incidence and be the most

51/...persistent/...

persistent of all the organisms in the population. Both these criteria are incorporated in the survival index. Organisms that are able to demonstrate these characteristics under different environmental conditions may be referred to as stable. Stability is another feature of an ecosystem (Brock, 1966) and dominants which have a controlling influence over the population would be the most stable of the biotic components. Changes in the environment therefore should not influence their incidence.

The associates which Brock (1966) points out depend upon the activities of the dominants for their development, would not be as active or as stable as the dominants.

The incidentals, or those organisms which are indifferent to the activities of the dominants or associates (Brock, 1966), would be foreign to the population and would therefore either be completely suppressed by it or conversely would suppress it once the environment has been changed, as happens, for example, when a population from the gingival sulcus is placed in a bottle of cooked meat medium.

In the present experiment, microbial populations from the gingival sulci of Caucasians with pockets of 1 - 2 mm and two groups of Bantu, one with pockets of 1 - 2 mm and the other with pockets of 3 - 6 mm, were placed in a similar environment, namely, cooked meat medium, which

52/...was incubated/...

was incubated at 37°C for 30 days. Differences between these experimental categories, particularly regarding the stability and survival of the organisms would give some indication of the differences which existed in the original populations. Those organisms which were stable, had a high incidence and were able to persist in the three experimental categories described above were classified as dominants. Conversely those which were not stable or did not survive well were regarded as associates.

Survival is expressed in the survival indices which are shown in Tables VII to XI. Microorganisms with a survival index above 1000 were regarded as potential dominants. These organisms were tested by statistical analyses for stability using the sign test (Siegel, 1956) and the student t test (Wetherill, 1967) for paired samples. Differences in daily incidence in the three categories were compared; when there was no difference in at least two of the three categories, they were classified as dominants. The results of this analysis are summarized in Table XII. When there was a difference between the two statistical tests, the sign test was accepted as being more meaningful and exact than the student t test.

Microorganisms which were classified as dominants were the aerobic streptococci, actinomyces, $\alpha$-haemolytic streptococci, mitis type streptococci and enterococci. Those organisms which did not fulfil these criteria were

53/...classified/...

classified as associates. These were divided into two groups: major associates and minor associates. The major associates which included the anaerobic streptococci, staphylococcocci, veillonella, $\gamma$-haemolytic streptococci, bacteroids and the lactobacilli had a survival index greater than 1000, but there was a significant difference between at least two of the three categories studied. On the other hand, the minor associates which included the neisseria, fusobacteria, diplococci, corynebacteria, $\beta$-haemolytic streptococci, selenomonas, clostridia, leptotrichia, polysaccharide-producing streptococci, sarcina, micrococci and yeasts, some of which were more stable, had a survival index of less than 1000. It was not possible to classify incidentals from this table. The Enterobacteriaceae showed very little variation in incidence (Table III). In the Bantu their incidence remained at 5 per cent for the entire duration of the experiment. The value was similar in the Caucasians but they apparently disappeared after the twenty first day. They, therefore, had a relatively constant incidence and this was interpreted as indicating that they were relatively indifferent to the activities of the other organisms. They were, therefore, classified as incidentals.

Table XIII is similar to Table XII in that it lists the combined survival indices for each organism studied, but it lists the highest percentage incidence and the percentage difference in survival index for the three categories investigated. Thus, the aerobic streptococci

54/...had the/...

had the highest percentage incidence, namely, 100 per cent. The percentage difference in survival index between the total Bantu and the total Caucasian samples amounted to -5,9, which indicates that the survival index was higher in the Caucasians than in the Bantu. Statistical analyses showed that this difference was not significant (Table XII). The percentage difference between the Bantu with pockets of 1 - 2 mm and the total Caucasians was 13,1. Statistical analyses showed that this difference was also not significant (Table XII). Lastly, the percentage difference in survival index between the Bantu with pockets of 1 - 2 mm and the Bantu with pockets of 3 - 6 mm was 49,5. This difference was significant at the one per cent level according to the student t test and significant at the 0,1 per cent level according to the sign test (Table XII).

The analyses in Table XIII were useful in dividing the associates into five groups. The first group ($A_1$) consisted of organisms with a higher survival index in the Bantu than the Caucasians and in the Bantu with pockets of 3 - 6 mm than in the Bantu with pockets of 1 - 2 mm. This group included the veillonella, $\gamma$-haemolytic streptococci, lactobacilli, and the sarcina. The second group of associates ($A_2$) consisted of organisms with a higher survival index in the total Bantu and the Bantu with pockets of 1 - 2 mm than in the Caucasians. The survival indices for these organisms were higher in the Bantu with pockets of 1 - 2 mm than in the Bantu with

55/...pockets/...

pockets of 3 - 6 mm. This group included the anaerobic streptococci, staphylococci, bacteroides, neisseria, fusobacteria, diplococci, selenomonas, clostridia, leptotrichia and polysaccharide-producing streptococci. The third group of associates ($A_3$) consisted of the corynebacteria with a higher survival index in the Caucasians than the total Bantu. Their index was higher in the Bantu with pockets of 1 - 2 mm than in the Caucasians. Their survival index was also higher in the Bantu with pockets of 1 - 2 mm than in the Bantu with pockets of 3 - 6 mm. The fourth group of associates ($A_4$) consisted of the micrococci and the yeasts. These organisms were absent in some of the categories (Tables II and X), and thus it was not possible to compare their survival indices. The last group of associates ($A_5$) consisted of the $\beta$-haemolytic streptococci with a survival index which was higher in the Caucasians than the total Bantu or in the Bantu with pockets of 1 - 2 mm. Their index was also higher in the Bantu with pockets of 1 - 2 mm than in the Bantu with pockets of 3 - 6 mm.

## PLAQUE INDICES

The average plaque index for the Caucasians was $\frac{19}{64}$, whereas the plaque index for the Bantu was $\frac{55}{64}$. The average index for the Bantu subjects, therefore, was more than twice the value of the index for the Caucasians.

56/...Chapter I D/...

# CHAPTER I

## D DISCUSSION

### 1 ANAEROBIC STREPTOCOCCI

The anaerobic streptococci had the highest combined mean incidence as well as the highest mean incidence for the Bantu and Caucasians (Table I). In fact this value was initially 100 per cent in all three categories listed. They were included in the first group of organisms in Table IV together with the other organisms which had a mean incidence greater than 50 per cent. Moreover, they were cultured for 28 of the 30 days. In Table I their combined mean incidence remained at 100 per cent for the first 6 days and then dropped to 97,5 per cent but remained above 50 per cent for 16 days. This value dropped to zero on days 27 and 28 but rose again to 2,5 per cent on the last day.

These organisms, therefore, persisted for almost the entire duration of the experiment even though they had a low incidence after the 24th day.

57/...The ability/...

The ability of the anaerobic streptococci to survive is reflected in their survival index which was the highest for all the organisms studied (Table VII). The value of the combined index was 8153 which is more than four times the value required for placing them in the category of organisms with a good survival index. This value was even higher in the Bantu group which had an index value of 9405 (Table VIII). In the Caucasians it was lower and amounted to 6910 (Table IX). The difference in value between the two ethnic groups amounted to 36,1 per cent (Table XIII). However, because there was a significant difference in all three categories (Table XII) they were classified as associates. The difference for the other streptococci who were classified as dominants in Table XII, is less significant.

The difference in incidence of anaerobic streptococci in the two ethnic groups could be due to a number of factors. These include the state of oral hygiene, diet and other sociological factors. Of these, oral hygiene was probably the most important factor involved. The Caucasians were undergraduate dental students at the University of the Witwatersrand, who were practising a regime of good oral hygiene, and had an average plaque index of $\frac{19}{64}$, whereas the Bantu were patients attending the extraction surgery at the Dental Hospital, were not conscious of oral hygiene and had an average plaque index of $\frac{55}{64}$. Poor oral hygiene promotes an accumulation

58/...of plaque/...

of plaque and this in turn increases the number of organisms in the particular oral environment. It also creates an anaerobic environment which would favour the growth of the anaerobic streptococci.

Pocket depth also affects the incidence of these organisms. In Table X their survival index for pockets 1 - 2 mm was 10,600, whereas for pockets 3 - 6 mm it was 8,210 (Table XI). This difference was significant (Table XII) and amounted to 29 per cent (Table XIII). The pocket depths of the Caucasians varied from 1 - 2 mm. However, half of the Bantu subjects had a pocket depth of 1 - 2 mm and the other half 3 - 6 mm. When the survival index for the anaerobic streptococci was compared in the two ethnic groups with a pocket depth of 1 - 2 mm, the Bantu had a higher index which was significantly different (Table XII) and amounted to a 53 per cent difference in index (Table XIII).

An increase in pocket depth in the Bantu group was associated with a lower survival index for the anaerobic streptococci. An accumulation of plaque, therefore, seemed to favour the growth of the anaerobic streptococci and an increase in pocket depth appeared to have the *opposite effect*.

Kenney and Ash (1969) measured the decrease in oxidation reduction potential in plaque which was allowed to accumulate for 4 days and found a decrease of 395 millivolts. In another group of patients with periodontal

59 /...pockets/...

pockets a comparison of the oxidation reduction potential of the normal gingival sulci and pockets showed a mean decrease of 122,6 millivolts. The average oxidation reduction potential in the accumulated plaque was -126 millivolts, the lowest reading being -141 millivolts. In the periodontal pockets the average oxidation-reduction-potential was only -47 millivolts, but the lowest reading was -157 millivolts.

The general trend in oxidation reduction potentials, therefore, shows that plaque is more anaerobic than periodontal pockets and the decrease in oxidation-reduction-potential would favour the growth of the more fastidious anaerobic organisms. Readings taken in individual pockets however do not support this observation and it seems that other factors also influence the growth of anaerobic organisms.

From the observations made in the present study it appears that the low oxidation reduction potential in periodontal pockets and plaque affects the balance of organisms which form an ecosystem in these environments. When this community is transferred to an artificial environment this effect is still apparent and can be expressed as a survival index. The anaerobic streptococci are one of the prominent groups of organisms in this community and their growth in the experimental environment was probably influenced by their status in the original environment.

60/...The anaerobic/...

The anaerobic streptococci also possess characteristics which would favour their growth in pockets. According to Breed, Murray and Smith (1957) they are known as the peptostreptococci and have the ability to ferment protein decomposition products. They are often found in septic conditions. One species is very peptolytic and has the ability to invade tissues. Others are highly proteolytic. These organisms may demonstrate aggressive activities in pockets where tissue elements and tissue breakdown products are readily available. However, in deep pockets there appear to be influences present that inhibit the growth of the anaerobic streptococci.

2 DOMINANTS

There are 5 organisms in the present study which are classified as dominants (Table XII). They are the aerobic streptococci, actinomyces, the $\alpha$-haemolytic streptococci, mitis-type streptococci and the enterococci. Table XIII shows that the aerobic streptococci obtained the highest percentage incidences of 100 per cent. They also had the second highest combined survival index (4146), a value which is almost half that obtained for the anaerobic streptococci; therefore they were not able to survive in this environment as well as the anaerobic streptococci. The incidence of the aerobic streptococci was slightly lower in the Bantu than in the Caucasians, namely, 5,9 per cent (Table XIII), with no significant difference between these two categories (Table XII),

61/...but when/...

but when these incidences were compared in the two ethnic groups with the same pocket depth, they were 13,1 per cent higher in the Bantu (Table XIII), but the difference was still not significant (Table XII).

There was a difference in incidence in the two pocket depths in the Bantu. This difference amounted to a 49,5 per cent higher value in shallow pockets (Table XIII). This difference in incidence was highly significant (Table XII). Pocket depth, therefore, had a greater effect and a difference in race a lesser effect on survival.

The actinomyces are classified as dominants because of their great stability with no significant difference in incidence in any of the three categories of subjects (Table XII). Their highest percentage incidence was 45 per cent (Table XIII) but their survival index was relatively high (3046). They can, therefore, be considered as dominants in the artificial environment of the present experiment and possibly also as dominants in the mouth.

The $\alpha$-haemolytic streptococci were similar to the aerobic streptococci but had a slightly higher incidence in the total Bantu than the [illegible]ans with pockets of 1 - 2 mm (Table XIII). This d[illegible]e was not significant (Table XII). The difference in incidence in the Bantu with different pocket depths was significant (Table XII) with a higher value in shallower pockets (Table XIII). They, therefore, were favoured by shallow pockets and poor oral hygiene.

62/...The/...

The mitis-type streptococci were classified as dominants because there was no significant difference between the incidence of these organisms in the Bantu and Caucasians with similar pocket depths and between the different pocket depths in the Bantu, even though the difference was highly significant between the total Bantu and Caucasians (Table XII). They had a combined survival index which was over 1000, i.e. 1706, but it was less than half the value of the index for the aerobic streptococci and, therefore, they seem to have less influence on the population (Table XII).

The enterococci had a survival index which was just above 1000 and the overall percentage difference was 9,9 per cent, which was not significant (Table XII), the value being higher in the Bantu than in the Caucasians (Table XIII). If, however, the pockets of 1 - 2 mm are compared in these two ethnic groups, the difference was 26,8 per cent which was not significant (Table XII); the incidence also was higher in the Bantu. The difference in the Bantu between pockets of 1 - 2 mm and 3 - 6 mm was 36,1 per cent higher in the shallow pockets (Table XIII) which was significant at the one per cent level (Table XII). The enterococci, like the aerobic streptococci, therefore were favoured by shallow pockets and patients with poor oral hygiene.

3 ASSOCIATES

The associates can be divided into a number of groups with similar characteristics (Table XIII).

63/... The first/...

The first group consists of the veillonella, lactobacilli, ɣ-haemolytic streptococci and the sarcina. In this group the survival index increases, with a higher incidence in pockets of 3 - 6 mm than in pockets of 1 - 2 mm. Veillonella had the lowest percentage difference, namely, 14,3 per cent (Table XIII). The difference in index in the sarcina was double this value, namely, 33,3 per cent (Table XIII). The sign test, however, shows that these differences are not significant (Table XII). The organisms with the next highest percentage difference were the lactobacilli with a value of 47,9 per cent (significant at the one per cent level (Table XII)), followed by the ɣ-haemolytic streptococci with a difference of 61,8 per cent which was highly significant (Tables XII and XIII). The ɣ-haemolytic streptococci, therefore, were organisms most favoured by deep pockets. They also were favoured by the environment in the Bantu rather than that of the Caucasians. The index was 354,2 per cent higher in the Bantu with pockets of 1 - 2 mm and this was significant at the 0,2 per cent level (Tables XII and XIII). The percentage difference between the total Bantu and Caucasian group amounted to 468,4 per cent which was highly significant (Tables XII and XIII). The percentage difference in the survival index for the sarcina was even greater in the Bantu with pockets of 1 - 2 mm and the Caucasians, but the sign test shows that the difference was not significant (Table XII). The difference observed between the two ethnic groups, however, was significant (Table XII). There was no significant difference between the Bantu and Caucasians with pockets of 1 - 2 mm in the

64/...case of/...

case of the lactobacilli (Table XII). However, when the two ethnic groups were compared, the difference was significant (Table XII). Veillonella showed the least difference in index in the Bantu when values for pockets of 1 - 2 mm and 3 - 6 mm were compared. Both statistical tests show that the differences were not significant (Table XII). A comparison of the two ethnic categories, however, showed that the observed differences were significant (Table XII).

In this group of organisms, therefore, the $\alpha$-haemolytic streptococci had the greatest difference in index with increase in pocket depth followed by the lactobacilli, then the sarcina and finally the veillonella. Similarly, the environment of the Bantu favoured, firstly, veillonella and $\alpha$-haemolytic streptococci, followed by the lactobacilli, and lastly the sarcina (Table XIII). The veillonella had the highest survival index (3205) in this group followed by the $\alpha$-haemolytic streptococci (1719) and then the lactobacilli (1175), and lastly the sarcina with an index of 77 (Table XIII). In this environment, therefore, the veillonella have the greatest ability to survive and would possibly have a greater influence on the population than the sarcina who have an index which is almost 300 times smaller than the veillonella.

The second group of associate organisms (Table XIII) consists of the anaerobic streptococci, staphylococci,

65/...bacteroids/...

bacteroids, neisseria, selenomonas, fusobacteria, diplococci, clostridia, leptotrichia and polysaccharide-producing streptococci. These organisms had an index which was higher in shallow pockets than in deep pockets in the Bantu and was higher in the Bantu than in the Caucasians. These organisms, therefore, were favoured by the environment of the Bantu.

The anaerobic streptococci had the greatest variation in incidence which was highly significant in all the three categories studied (Table XII). The neisseria showed a similar trend, the difference between the total Bantu and Caucasians was significant at the one per cent level (Table XII). In the case of the staphylococci this trend was less marked and was significant at the one per cent level when the total Bantu and Caucasians, and the Bantu with 1 - 2 mm pockets and the Caucasians were compared with each other (Table XII). A comparison of the two categories of the Bantu with different pocket depths (Table XII) shows that the difference in incidence was significant at the 5 per cent level. Statistical tests showed that the difference in incidence of the bacteroides observed between the total Bantu and Caucasians, and the Bantu with pockets of 1 - 2 mm and the Caucasians was highly significant. The difference in the Bantu with different pocket depths was not significant. With the fusobacteria there was no significant difference in the incidence between the total Bantu and the Caucasians, but there was a difference between the Bantu with pockets of 1 - 2 mm and the

66/...Caucasians/...

Caucasians, and in the Bantu between pockets of 1 - 2 mm and 3 - 6 mm. In the case of the selenomonas the difference in incidence was highly significant between the total Bantu and the Caucasians whereas there was no significant difference between the other two categories. The last 4 associates belonging to this group, i.e. the diplococci, clostridia, leptotrichia and polysaccharide-producing streptococci had insignificant differences between all the categories studied. A comparison between the Caucasians on the one hand, and the total Bantu as well as the Bantu group with pockets of 1 - 2 mm on the other hand, showed that firstly, the incidence of the bacteroids, anaerobic streptococci and neisseria is higher in Caucasians and secondly, that this difference is significant according to the sign test or the student t test. In other words, these organisms were stimulated by the environment of the Bantu. On the other hand, the diplococci, clostridia, leptotrichia and polysaccharide-producing streptococci were unaffected. The staphylococci, fusobacteria, and selenomonas were moderately affected. This phenomena could be due to an increased anaerobic environment in plaque. Anaerobic streptococci, bacteroides, fusobacteria and selenomonas are anaerobic organisms which would benefit from a reduction in oxidation-reduction potential. They would therefore be stimulated by this environment. Staphylococci are facultative anaerobes and neisseria are aerobic organisms. They would, therefore, not be stimulated by a decrease in

67/...oxidation/...

oxidation-reduction potential. This suggests that there are other factors which could cause these differences in incidence.

This group of associate organisms is probably under the control of the dominant species. If this control is disturbed they could increase in numbers and become infective. In fact a number of these organisms are potential pathogens and, therefore, are able to become invasive under certain conditions. It is well known that the anaerobic streptococci include peptolytic and proteolytic species (Breed, Murray and Smith, 1957), that Staphylococcus aureus produces coagulase, lipase, esterase and toxins and is considered an opportunistic pathogen (Cruickshank et al, 1973) and that Bacteroides melaninogenicus produces aggressive substances such as collagenase (Gibbons and McDonald, 1961), fibrinolysin (Weiss, 1937) and heparinase (Gesner and Jenkin, 1961), even though it occurs as a normal commensal in the mouth and gastro-intestinal tract (Bisset and Davis, 1960). The fusobacteria have often been credited with a pathological role in parts of the body in addition to their association with Vincent's angina (Bisset and Davis, 1960). The pneumococci produce a haemolysin and a leucocidin but generally only infect when the host's resistance is lowered (Cruickshank et al, 1973). Clostridia consist of mainly saprophytic forms but a few species are opportunistic pathogens which cause gasgangrene, tetanus and botulism (Cruickshank et al, 1973). The polysaccharide-

68/...producing/...

producing streptococci include Streptococcus sanguis and Streptococcus salivarius which are implicated in bacterial endocarditis (Cruickshank et al, 1973).

The pathogenicity of these organisms and their tendency to invade when the resistance of the host is lowered would support the idea that these organisms are controlled or suppressed by the dominant species under normal conditions. The observation that these organisms are generally inhibited in deep pockets would also tend to support this suggestion.

If the survival indices of these organisms are considered, the anaerobic streptococci had the highest index (8153), followed by the staphylococci (3871), bacteroides (1246), neisseria (952), fusobacteria (862), diplococci (580), selenomonas (349), clostridia (234), leptotrichia (195) and finally, the polysaccharide-producing streptococci (154). The anaerobic streptococci and the staphylococci thus had the greatest ability to survive in the experimental environment and the polysaccharide-producing streptococci the least ability.

The third group of associates consists of one organism, the corynebacteria (Table XIII). It is similar to the aerobic streptococci because it has a higher survival index in the Caucasians than in the Bantu. There was no significant difference, however, between the incidence of this organism in any of the three categories studied (Table XII). The accumulation of plaque and differences

69/...in pocket/...

in pocket depth, therefore, had no influence on this organism. The survival index of the corynebacteria was relatively low (494), thus it is assumed that it had very little influence on the microbial population in the present experiment.

The fourth group of associates consists of the micrococci and yeasts (Table XIII). These microorganisms had a very low survival index - less than 70. In fact, the micrococci did not occur in the Bantu at all and the yeasts were not present in the Bantu pockets of 1 - 2 mm (Tables II and X). Both these organisms, therefore, had a low survival rate in the present experiment and probably also in plaque and pockets.

The $\beta$-haemolytic streptococci have not been included in any of these groups. They are similar to the corynebacteria because they have an index which is 78 per cent higher in Caucasians when the whole sample is considered (Table XIII). The difference in incidence is significant (Table XII). They were also higher in the Caucasians when pockets of 1 - 2 mm were studied, which differed from the corynobacteria who had a lowered index. The difference, however, was not significant. The $\beta$-haemolytic streptococci, therefore, are favoured by the Caucasians and possibly by a reduction in plaque. This could be due to their inhibition by other organisms in plaque and pockets which would result in a low survival rate.

70/...Incidentals/...

4 INCIDENTALS

The Enterobacteriaceae have been classified as incidentals because their incidence does not rise above 5 per cent and they persisted for the entire duration of the experiment (Table III). Their persistence for a long time results in a relatively high survival index (901) (Table XIII). It is not possible to compare their survival indices in the different ethnic groups and pocket depths because of a low percentage incidence.

5 FLORA OF THE ORAL CAVITY

Gould (1972) stated that the microorganisms of the normal flora of the body have highly developed relationships with each other and their host. Aspects of these relationships were observed in the present study (Table XIII). The genera of the organisms studied differ so much as regards percentage incidence, combined survival index and survival index in the different ethnic groups and pockets depths, that it is not possible to group them into clear-cut units.

Burnett and Scherp (1968) feel that the flora of the oral cavity is distinct from other parts of the body. It is possible to state from the present experiment that the flora in each subject is distinct, a particular flora developing in deep pockets, another when good and yet another when poor oral hygiene is practiced.

71/...This may be/...

This may be due to the particular character of the mouth which supports the growth of microorganisms but it may also be due to the properties of each organism which makes up this flora. Even though the communities in the present experiment were placed in a similar environment they maintained their identity and were different for each one of the subjects. This confirms Brock's (1966) statement that the interaction between the microbes on the one hand and the host on the other determines the constitution of the flora. The flora in the gingival sulcus, therefore, forms an ecosystem.

The oral cavity could be regarded as an ecosystem, consisting of biotic and abiotic components (Brock, 1966). The oral flora is the biotic component and only this component was studied in the present experiment. However, the effects of the abiotic component were still present and accounted for some of the differences observed in each group of subjects. For example, the accumulation of plaque appeared to affect the results obtained from subjects with good and poor oral hygiene. Changes in the concentration of hormone in the female periodic cycle could also be considered an abiotic factor which could affect the oral flora. For this reason only male subjects were used in the present experiment in order to reduce the number of variables.

Novick (1955) found in his continuous culture device that despite nutrients being present in excess, the

72 /...population/...

population did not increase beyond a certain concentration. He felt that this was due to inherent factors in the population. In the present experiment, the nutrients were present in limited amounts. Despite this limitation, under certain circumstances growth was more prolific than under other circumstances. For example, the incidence of the bacteroides and veillonella was significantly higher in the Bantu than in the Caucasians (Tables XII and XIII). Conversely, the incidence of mitis-type streptococci was significantly higher in the Caucasians than in the Bantu (Tables XII and XIII).

Studies by Rosebury, Gale and Taylor (1954) have shown that Corynebacterium diptheria, Neisseria catarrhalis and micrococci were inhibited by streptococci. In the present experiment the combined survival index for the aerobic streptococci (4146) and the anaerobic streptococci (8153) was much higher than the neisseria (952), corynebacteria (494) and the micrococci (68) (Table XII). This demonstrates that these organisms were inhibited by the streptococci and supports their findings. The inhibition of one species of streptococcus by another observed by the same authors and Oxford (1944) could not be studied in the present experiment because groups of organisms rather than particular species were used. The inhibition, however, of the polysaccharide-producing streptococci is clear. They have a survival index of 154 which is very much lower than the mitis-type

73./...streptococci/...

streptococci (1706) or the enterococci (1058) (Table XII).

Davies (1972) states that despite fluctuations which may occur over short periods of time, the oral flora is stable. This can be substantiated by the observation in the present experiment that the communities cultured from different ethnic groups and pocket depths differ in their constitution (Tables XII and XIII). If these communities were not stable they would have been influenced by a new environment and would have given similar results in a similar environment.

The control of population density is a very important aspect of microbial ecology and this control is often obscured (Brock, 1966). Population control does exist in the oral cavity to a certain extent. With no oral hygiene the amount of plaque increases. If this plaque (which consists mainly of microorganisms) is allowed to accumulate, uncontrolled gingivitis and eventually periodontitis follows. This could simply be due to an increase in the number of organisms or it could be caused by the introduction or presence and proliferation of certain aggressive strains or organisms in the gingival sulcus. The very high survival index of Bacteroides melaninogenicus in the Bantu as compared to the Caucasians (Tables VIII and IX), as well as the significant difference in incidence (Table XII) could be an example of this phenomenon. An increase in plaque, therefore, could

74/...enable/...

enable this organism to take over a population and thus nullify the controlling influence of other members in this population. Once the aggressive strains have increased in sufficient numbers disease will result. Bacteroides melaninogenicus does produce aggressive substances such as collagenase (Gibbons and McDonald, 1961), fibrinolysin (Weiss, 1937) and heparinase (Gesner and Jenkin, 1961). These substances would enable them to invade tissue. This characteristic together with their high survival index in the Bantu (who have poor oral hygiene and have accumulated plaque) would make these organisms important in periodontal disease.

Studies have been done on the characteristic flora of certain areas of the mouth. Carlsson (1967) found that Streptococcus sanguis and Streptococcus mutans favoured the tooth surface. In the present experiment the polysaccharide-producing streptococci (which includes these two species) had a low survival index (154) and thus they were suppressed by the other organisms (Table XIII). The tooth surface would not have the abundance of organisms present in the sulcus or plaque and, therefore, it would be a suitable environment for the growth of these sensitive organisms.

Similarly Gibbons, Kapsimalis and Socransky (1964) found that Streptococcus salivarius favoured the cheek mucosa. This area is also not as densely populated as plaque and the results of the present experiment suggest that this is a reason why this environment would be

75/...more favourable/...

more favourable for their growth.

Ikeida and Sandham (1971) found that Streptococcus mutans favoured the pits and fissures of teeth. These areas are possibly more protected and anaerobic than the smooth tooth surface and would, therefore, also be more favourable for the growth of these sensitive organisms which prefer an anaerobic environment (Edwardsson, 1968).

Gibbons, Kapsimalis and Socransky (1964) found that Bacteroides melaninogenicus favours the gingival sulcus rather than plaque. This would seem contrary to the findings of the present experiment. The material studied in my experiment, however, was mainly from the gingival sulcus and not plaque. The bacteroids in the present experiment had a survival index which was more than 1000 per cent higher in the Bantu than in the Caucasians - irrespective of pocket depth - and an incidence which was significantly different in both cases (Tables XII and XIII). It would seem that the sulci of the Bantu are conducive to the establishment and persistence of certain strains of these organisms. Possibly the accumulation of plaque helps to form an anaerobic environment in the sulcus which would enable this organism which is very oxygen sensitive (Bisset and Davis, 1960) to flourish under these circumstances.

Studies on the incidence of certain organisms in particular sites, show that there are a number of communities

76/...in the oral/...

in the oral cavity. The present experiment also shows that the same site supports different populations when environmental conditions are different. Thus, the flora of the gingival sulcus of the Caucasians and the Bantu were found to be different. With poor oral hygiene the Bantu have organisms with a total survival index which is more than one and a half times higher than in the Caucasians (Tables VIII and IX). There seemed to be factors present in the Bantu which were selective for certain organisms in the bacterial communities studied.

When different pockets depths were studied in the Bantu, the anaerobic streptococci, fusobacteria, $\alpha$ -haemolytic streptococci and neisseria had an incidence which was significantly higher in shallow pockets than in deep pockets (Tables XII and XIII). These organisms, therefore, were suppressed by the community or environment of the deeper pocket. A difference in race or oral hygiene and pocket depth had an effect on the characterization of a community.

## 6 INTERACTION BETWEEN MICROORGANISMS

There seems to be an interaction of microorganisms in different situations which determines the dominance of certain genera. Parker (1970) worked on paired culture techniques and found that Streptococcus salivarius was the ultimate beneficiary of most reactions. These

77/...results/...

results are almost contrary to the findings of the present experiment because the polysaccharide-producing streptococci which include Streptococcus salivarius had a very low survival index (154) (Table XIII). The anaerobic streptococci had the highest combined survival index (8153) followed by the aerobic streptococci (4146) (Table XIII). It seems probable that the polysaccharide-producing streptococci were suppressed by these other streptococci which were not used in his experiment.

Parker (1970) states that paired cultures do not give a true indication of the reactions which could occur between three or more organisms. This statement is borne out in the present experiment where the survival indices of fusobacteria (862) and neisseria (952) were similar (Table XIII) despite Parker's discovery that Fusiformis nucleatum inhibited the growth of Neisseria perflava.

Reference has already been made to the communication between organisms of the oral cavity. This can be studied on a number of levels:-

6.1 Chemical substances

These substances may include antibiotics and can have a stimulatory or inhibitory effect. Oxford (1944) and Mattick and Hirsch (1947) found that Streptococcus cremoris and Streptococcus lactis (which would be

78/...included/...

included under mitis-type streptococci in the present experiment) produced an antibiotic which inhibited the Groups A, B, E, F, G, H, M and N streptococci, (these are $\beta$ -haemolytic streptococci except for Group E according to Breed, Murray and Smith (1957)), Diplococcus pneumoniae, neisseria and corynebacteria. The present experiment supports these findings. The mitis-type streptococci had a survival index of 1706 which was higher than the index for the $\beta$ -haemolytic streptococci (352), Diplococcus pneumoniae (580), corynebacteria (494) and neisseria (952) (Table XII). The survival index for the anaerobic streptococci, however, was almost 5 times higher than the index for the mitis-type streptococci (Table XII). These organisms, therefore, seemed to produce an antibiotic in the present experiment which is more powerful than the abiotic effect of all the other streptococci.

Organisms can also have a stimulatory effect upon each other. Rosebury, Gale and Taylor (1954) found that Neisseria catarrhalis was stimulated by streptococci, micrococci and Diplococcus pneumoniae. If an organism is stimulated by another microorganism it would either be able to grow better in the presence of the stimuli or it would be able to survive for a longer period of time. The ability to survive is one of the criteria which has been measured in the present experiment. This information is contained in the survival index. A comparison of survival indices for the streptococci,

79/..micrococci/...

micrococci, Diplococcus pneumoniae and neisseria in the Bantu and Caucasians with similar pocket depths showed that the indices for the streptococci, Diplococcus pneumoniae and neisseria were higher in the Bantu (Table XIII). An increase of index in one organism, therefore, could possibly result in a similar increase in other organisms and this could be interpreted as stimulation. On the other hand, the micrococci were inhibited (Table II). In the Bantu this could be due to an increased inhibitory effect of the organisms which have a higher survival potential.

Stimulation and inhibition, therefore, did not seem to be absolute criteria for a particular species but seemed to vary from strain to strain and community to community.

6.2 Subcellular units

Communication can take place by means of subcellular units. Brock, Peacher and Pierson (1963) isolated bacteriocins from the Group D streptococci or enterococci. These are plasmids which enabled the host organism to inhibit Streptococcus pyogenes and other streptococci. In the present experiment the survival index for the enterococci or Group D streptococci was more than 6 times higher than the index for the $\beta$-haemolytic streptococci (which would include Streptococcus pyogenes) (Table XIII). The inhibition of the $\beta$-haemolytic streptococci could, therefore, have been due to

80/...bacteriocins/...

bacteriocins produced by these plasmids. The survival index for the enterococci in the present experiment is much lower than the index for most of the other streptococci (Table XIII). The production of bacteriocins by the enterococci against these organisms did not seem to have occurred, but the possibility cannot be excluded.

6.3 Effects of communication between organisms

Brock (1966) states that the transfer of information may affect the members of an ecosystem in a positive or a negative way. Hobson (1969) enlarges on this statement and shows that when bacteria are affected positively, favourable conditions are produced by regulating the environment to a suitable pH, Eh, or by the production of adequate nutrients and essential growth factors. A comparison of the total survival index in the Bantu and Caucasians in the present experiment (Tables VIII and IX) showed that the index for the Bantu was more than one and a half times the value of the index for the Caucasians. This indicated that the organisms in the Bantu were able to survive better than in the Caucasians. It, therefore, seems feasible to suggest that the gingival sulcus ecosystem is affected positively by the environment of the Bantu.

On the other hand, organisms may be affected negatively and the environment becomes unsuitable for their

81/...growth/...

growth (Hobson, 1969). For instance, in the present experiment an increase in pocket depth in the Bantu affected growth of the microbes. The total survival index for microorganisms in sulci of 1 - 3 mm was 48,920, and for pockets of 3 - 6 mm, 41,300 (Tables X and XI). Thus the index was lower in the deeper pockets which indicated the presence of a negative effect with increased pocket depth.

Quastler (1958) states that unitization occurs in a very complex system. This means that some components combine and interact strongly and act as a unit with regard to the rest of the system. This phenomena seemed to occur in the present experiment. The incidence of the anaerobic streptococci, aerobic streptococci, $\alpha$-haemolytic streptococci, mitis-type streptococci and veillonella remained above 50 per cent for the first 8 days (Table IV). These organisms did not seem to inhibit each other but appeared to benefit from the association. On the other hand, the incidence of neisseria and enterococci dropped below 50 per cent after 4 days and the polysaccharide-producing streptococci after 2 days (Table IV). Even though these organisms were present in large numbers in the initial population, they did not benefit from an association with the first group but were inhibited. Other organisms, for example actinomyces, selenomonas and the $\beta$-haemolytic streptococci were even more suppressed and only increased in incidence after the first week (Table IV), once the

82 /...incidence/...

incidence of the anaerobic, aerobic, ∝-haemolytic and mitis-type streptococci as well as the veillonella had begun to decline. These latter organisms seemed to act as a unit regarding mutual stimulation and inhibition.

## 7 PROPERTIES OF AN ECOSYSTEM

### 7.1 Homeostasis

An ecosystem has the ability to maintain itself despite external influences - this phenomena is called homeostasis (Brock, 1966). Evidence for the stability of the organisms in the present experiment was the finding that there was a similarity in their sequence and survival in cultures of debris from the different pocket depths and racial groups, despite the fact that the communities were placed in a similar environment Table XIII). Each habitat in the mouth, therefore, seems to develop a different ecosystem under different conditions and once it is established it remains relatively stable.

### 7.2 Evolution

Brock (1966) defines evolution as being similar to homeostasis but it occurs over a longer period of time. Evolution occurs in the flora of the oral cavity and the results can be seen in the present experiment. The differences in survival index for the organisms found

83 /...in the Bantu/...

in the Bantu and Caucasians in Tables VIII, IX and XIII are probably due to a slow process of evolution which produces different types of populations under the varying conditions of poor and good oral hygiene.

7.3 Defence and repair

This could occur with the extraction of teeth and insertion of dentures. Shklair and Mazzarella (1960) found in their studies on patients before and after extraction that the lactobacilli and yeasts were completely suppressed by the streptococci before the insertion of dentures. Similar results were obtained from the present experiment. The aerobic streptococci, $\alpha$-haemolytic streptococci, mitis-type streptococci and the $\gamma$-haemolytic streptococci had survival indices which were one and a half to three and a half times greater than the survival index for the lactobacilli and about 40 to 100 times greater than the yeasts (Table XIII). These organisms, therefore, seemed to suppress the lactobacilli and the yeasts. Shklair and Mazzarella (1960) also found after the insertion of dentures that the streptococci increased and remained at a high level for two weeks. This could be due to the creation of an anaerobic environment which would enable the anaerobic streptococci to flourish. The present experiment would support this suggestion because the anaerobic streptococci had a survival index which was almost twice the index of the aerobic streptococci and they would, therefore, flourish because of

84/...their greater/...

their greater ability to survive as well as the creation of an anaerobic environment. The survival index for the anaerobic streptococci was almost 7 times higher than the index for the lactobacilli and more than 200 times higher than the yeasts (Table XIII). These organisms, therefore, would seem to be even more suppressive than the aerobic organisms. Shklair and Mazzarella (1960) found that the system returned to normal 3 - 5 weeks after the insertion of dentures. This could be due to the establishment of other members (particularly anaerobic) of the oral flora and the eventual attainment of a state of equilibrium.

## 7.4 Reproduction

Brock (1966) defines reproduction as the complete replacement of one system by another which is similar or identical. In the present experiment efforts were made to reproduce a system similar to the ecosystem found in the gingival sulcus. The conditions of the experimental system were not identical to the in vivo situation but this study possibly reflects mechanisms of control present in the oral cavity. The variation in incidence and survival of different organisms from different population groups and pocket depths (Table XIII) demonstrated that despite enormous differences between the normal and experimental environment, there were intrinsic properties in each population which enabled them to develop in different ways and thus facilitate the

85/...establishment/...

establishment of new populations despite the similarity of the experimental conditions. This seems to have indicated that the population or ecosystem from the gingival sulcus reproduced itself.

7.5 Phase boundary

Brock (1966) states that an ecosystem must have a phase boundary. The whole mouth could be considered as an ecosystem with a sharply defined phase boundary. In the present experiment, however, different results were obtained for the two population groups and pocket depths. This could indicate that the gingival sulcus constitutes an ecosystem of its own and thus the phase boundary would not be sharply defined. It could also indicate that other ecosystems in the rest of the mouth could influence the development of the population in the gingival sulcus. For example, the accumulation of plaque in subjects with poor oral hygiene could be the cause of the different results obtained for the two ethnic groups (Table XIII).

7.6 Uniformity

Brock (1966) states that an ecosystem is uniform and that a small sample taken from any part will represent the whole system. He also feels that there are degrees of uniformity but rarely complete homogeneity. These criteria seem to be applicable to the oral cavity. In the present experiment samples taken from a similar

86/...type of/...

type of environment in the Bantu gave consistent results, for example, the high survival rate of bacteroids and staphylococci (Table VIII). This is indicative of a degree of uniformity but a true assessment can only be made if samples were taken from the same gingival sulcus at different time intervals.

The organisms from the gingival sulcus form an ecosystem and thus they are tightly coupled. According to Brock (1966) a system which is tightly coupled is difficult to separate into individual species and maintain in isolation. If a pure culture of organisms is inoculated into a bottle of cooked meat medium it will undergo a short lag period and once it has adapted to the new environment it will increase in size until all the available nutrients are exhausted. The anaerobic streptococci, aerobic streptococci, $\alpha$- haemolytic streptococci, veillonella and mitis-type streptococci followed this growth pattern in the present experiment (Table IV). Other organisms increased after a longer period of time, for example, the actinomyces (Tables (IV). They seemed to benefit from the breakdown products of the first group of organisms and were able to survive for a longer period in the Bantu than the Caucasians (Tables V and VI), possibly because of the higher survival rate of the other organisms present. Their development seemed to depend upon the development of the other members of the population. The incidence of the bacteroids was even more remarkable. It never increased above 10 per

87/...cent in/...

cent in the Caucasians, and they were present for about 8 days (Table VI). On the other hand, they were present for 24 days in the Bantu, their incidence went up to 72 per cent on the third and fourth days and it was 40 per cent or higher for at least 12 days (Table V). These organisms seemed to depend upon the development of the microbial population of the Bantu. Their increased growth or survival was fourteen times greater in the Bantu than in the Caucasians (Tables VIII and IX). Similarly, the total increase in index for all the organisms was one and a half times greater. These organisms definitely benefit from their presence in the Bantu which could have been due to the presence and excretion of vital nutrients by the other organisms in the population.

Organisms may grow together in the same environment because they have the same requirements or they may benefit from their association (Brock, 1966). In the present experiment the bacteroides benefited greatly from their association with the other organisms from the Bantu, whereas this benefit was much smaller in the Caucasians. This can be observed as the high survival index in the Bantu (2280) and the low index (162) in the Caucasians (Tables VIII and IX). These organisms do form an association or community which is probably the result of years of succession in a particular environment which is more beneficial in the Bantu than in the Caucasians.

88/.../Succession/...

8 SUCCESSION

If an uncolonized substrate has an abundance of nutrients, microorganisms which are able to grow will multiply (Brock, 1966). In the present experiment a mixed inoculum from the gingival sulcus was inoculated into an uncolonized substrate (cooked meat medium). In samples from the Caucasians (Table VI) the anaerobic streptococci, aerobic streptococci, veillonella, ∝-haemolytic streptococci, polysaccharide-producing streptococci and Diplococcus pneumoniae had a high incidence initially and were able to grow well in the original environment. In samples from the Bantu (Table V) the initial organisms which were cultured were the anaerobic streptococci, aerobic streptococci, ∝-haemolytic streptococci, mitis-type streptococci, enterococci, polysassharide-producing streptococci, sarcina and leptotrichia. As time passes this initial population causes changes in the environment which will make it less suitable for the growth of the original population and other organisms which were unable to grow previously will replace the initial population (Brock, 1966). In the Caucasians (Table VI) the environment changed and after the second day the incidence of the polysaccharide-producing streptococci and the Diplococcus pneumoniae dropped sharply. The anaerobic streptococci, aerobic streptococci, veillonella and ∝-haemolytic streptococci were not as sensitive to the change and their incidence dropped less sharply.

89/...The majority/...

The majority of organisms in the Caucasians, for example actinomyces, neisseria, lactobacilli and micrococci increased in incidence on the third and fourth days, once the substrate had been changed slightly. Other organisms were more sensitive to change and increased after a longer period, for example the staphylococci, fusobacteria and bacteroids with their highest incidence on the fifth and sixth days, the $\gamma$-haemolytic streptococci and selenomonas on the seventh and eighth days and the $\beta$-haemolytic streptococci on the ninth and tenth days. The clostridia with a very low overall incidence appeared after the twentieth day once most of the organisms had disappeared. The situation was similar in the Bantu. In this group the survival of organisms was much higher; the total survival index was one and a half times larger in them than in the Caucasians (Tables VIII and IX). In addition, there were more organisms cultured which belonged to the initial population. The incidence of the anaerobic streptococci, aerobic streptococci and $\alpha$-haemolytic streptococci declined slowly; the anaerobic streptococci remained at 100 per cent for the first 10 days (Table V). The enterococci started to decline in incidence after the fourth day, whereas the mitis-type streptococci, polysaccharide-producing streptococci, sarcina and leptotrichia had a sharp decrease in incidence after 2 days (Table V). The environment also changed in the Bantu. All these organisms were sensitive to this change in the medium to a greater or

90/...lesser/...

lesser degree. The veillonella, neisseria, bacteroides, fusobacteria and corynebacteria increased in incidence after the second day; the value was highest on the third and fourth days (Table V). The incidence of the ϑ -haemolytic streptococci and Diplococcus pneumoniae was highest on the fifth and sixth days, the staphylococci and actinomyces on the ninth and tenth days and the lactobacilli on the eleventh and twelfth days (Table V). The incidence of the clostridia was similar in samples from the Caucasians and the Bantu and they increased after the twentieth day (Table V).

Under normal conditions this second population of organisms will increase rapidly and predominate with the initial population decreasing in numbers (Brock, 1966). In the present experiment, however, conditions were not ideal as regards the supply of nutrients which were limited and the toxic products of organisms which had accumulated in the medium. This second population, therefore, was not able to develop fully but went into a state of decline. Normally the process of succession continues until an equilibrium is reached (Brock, 1966). It was not possible to follow this process to completion in the present experiment but it was possible to study the initial stages of the development of an ecosystem.

Succession has been studied in the oral cavity. McCarthy, Snyder and Parker (1965) found that the predominant bacteria in mouths of infants less than 24 hours old

91/...was/...

was streptococci, and that Streptococcus salivarius was the most common organism. Carlsson et al (1970) found that Streptococcus sanguis and Streptococcus mutans appeared after the eruption of the teeth. This could be due to the introduction of an anaerobic environment. All these streptococci, however, are polysaccharide-producing streptococci and were classified as minor associates in the present experiment (Table XII). They were suppressed by the other oral organisms as was demonstrated by their low survival index (154) in Table XII. It seems, therefore, that these organisms would be favoured by the uncolonized environment of a new born infant as well as the smooth surfaces of teeth where there are fewer organisms than in plaque and the gingival sulcus.

McCarthy, Snyder and Parker (1965) found that by the age of 12 months all the infants they studied carried streptococci, veillonella, neisseria and staphylococci. The incidence of the streptococci had dropped from 98 to 70 per cent after 12 months. In the present experiment the first group of organisms in the Caucasians and Bantu (Table IV) consisted of streptococci, veillonella and neisseria. They had an incidence above 50 per cent and persisted for a long period of time. Their dominance and persistence in the present experiment seem to enable them to predominate in the mouths of infants. The drop in incidence of the streptococci was also similar to the change in the infants. The

92...staphylococci/...

staphylococci were classified as major associates (Table XII) because of their high survival index and the significant difference in all the three categories studied (Table XII). They had a relatively high incidence of about 25 per cent in the Bantu for the entire duration of the experiment (Table V). It seems that they would be able to establish themselves easily on a new substrate because of their indifference to the inhibitory effect of the other oral organisms. This resistance could possibly be overcome over a long period of time and this could explain why they do not predominate in the normal adult mouth with good oral hygiene.

McCarthy, Snyder and Parker (1965) also found that they could isolate actinomyces, lactobacilli and fusiforms from half their subjects. These belong to the second group of organisms in Table IV, that is, they had an incidence between 20 and 49 per cent. This incidence was lower than the first group of organisms and could explain why they were found in only half the subjects. McCarthy, Snyder and Parker (1965) also demonstrated the presence of leptotrichia, candida and corynebacteria in less than half their samples. Leptotrichia and candida or yeasts belonged to the third group of organisms in the present experiment (Table IV) with a low incidence below 20 per cent. This would correlate with their low incidence in the mouths of infants. In the Bantu the corynebacteria belonged to the third group of

93/...organisms/...

organisms (Table V) and in the Caucasians to the second group (Table VI). These organisms seemed to be sensitive to poor oral hygiene and an accumulation of plaque found in the Bantu, whereas they were able to survive much better in the mouths of Caucasians with good oral hygiene and less plaque accumulation.

Studies have been done on successions of organisms in plaque with changes from mainly coccoid to filamentous forms after 2 to 4 days (Loë, Theilade and Jensen, 1965). After 6 to 10 days the incidence of the streptococci dropped from between 80 and 100 per cent to between 45 and 60 per cent. Similar results were obtained for the incidence of the aerobic streptococci in the present experiment. Their incidence was 100 per cent on the first two days and dropped to 50 per cent after the [illegible]th day (Table IV). The incidence of the anaerobic streptococci remained at 100 per cent for the first six days and dropped to 60 per cent on the fifteenth and sixteenth days. There was a rise in incidence of the vibrios and spirochaetes on the sixth and tenth days (Loë, Theilade and Jensen, 1965). The incidence of the selenomonads (which include the vibrios) was also high on the ninth and tenth days in the present experiment (Table IV).

Howell, Rizzo and Paul (1965) did similar studies on plaque over a longer period of time. They found that the incidence of streptococci dropped from 50 per cent

94/...on the third/...

on the third day to 16,5 per cent in plaque older than 90 days. This is not unlike the results obtained for the streptococci in the present experiment (Table IV) but, because there was no external source of nutrients to support their growth, their incidence eventually dropped to zero. Similarly there was a drop in incidence of the neisseria in plaque (Howell, Rizzo and Paul, 1965) and in the present experiment (Table IV).

The actinomyces showed an increase in incidence in plaque from an average of 6 per cent initially to 18 per cent after 90 days (Howell, Rizzo and Paul, 1965). In the present experiment the incidence of these organisms was highest (45 per cent) on the ninth and tenth days (Table IV). Their incidence did not remain high for long but dropped possibly because of the limiting conditions of the experiment.

From the studies on infants, plaque and the present experiment it appears that the shift in populations could be due to competition between organisms and the antagonistic or abiotic effect of certain organisms upon others. Environmental factors like Eh and pH could also play a role but the biotic factors seem to be more important.

95/...References/....

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101/...Chapter 2/...

# CHAPTER II

## CHANGES IN AMINO ACIDS IN MEDIUM STUDIED

### 1 INTRODUCTION

There are considerable differences in the growth requirements of microorganisms. For instance, some of them can utilize carbon dioxide as a source of carbon; on the other hand, the pathogens and those organisms which are resident in the body are nutritionally far more exacting. They require amino acids, purines, pyrimidines and vitamins (Davis et al, 1969). The oral parasites are similar to the pathogens in this respect and many of them require particular amino acids and vitamin-like substrates (Bisset and Davis, 1960). These compounds are known as essential metabolites.

Both mammalian and microbial cells consist of proteins and other substances which have to be synthesized from the basic units (Davis et al, 1969). The fundamental structural units of proteins are amino acids (Conn and

102/...Stumpf/...

Stumpf, 1964], and these must be present in media suitable for the culture of pathogenic microbes.

## 2 CLASSIFICATION OF AMINO ACIDS

The naturally occurring amino acids obtained on hydrolysis of proteins may be classified according to their structure, or biosynthetic origin. In the case of the former they may be divided into three groups, namely, the aliphatic, aromatic and heterocyclic amino acids (Conn and Stumpf, 1964).

### 2.1 Classification according to chemical structure

#### 2.1.a Aliphatic amino acids

(i) Monoamino monocarboxylic amino acids

- Glycine
- Alanine
- Valine
- Leucine
- Isoleucine
- Serine
- Threonine

(ii) Sulphur containing amino acids

- Cysteine
- Cystine
- Methionine

(iii) Dicarboxylic amino acids

- Glutamic acid
- Aspartic acid

(iv) Basic amino acids

- Lysine
- Arginine
- Histidine

(weakly basic)

103/...2.1.b Aromatic/...

2.1.b Aromatic amino acids

Phenylalanine
Tyrosine

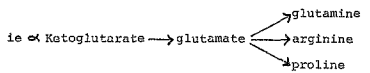
2.1.c Heterocyclic amino acids

Tryotophane
Proline
Hydroxyproline
Histidine

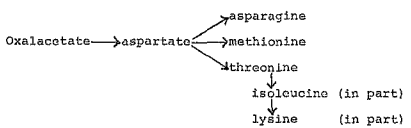
2.2 Classification according to biosynthetic origins

The classification of amino acids according to their biosynthetic origins admits five basic families (Stainer, Doudoroff and Adelberg, 1971).

2.2.a The glutamate family

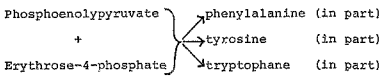



2.2.b The asparate family

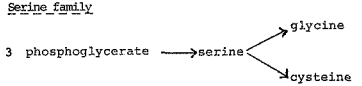



104/...2.2.c Aromatic/...

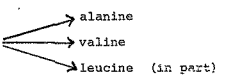
2.2.c Aromatic family




2.2.d Serine family




2.2.e Pyruvate family




The synthesis of histidine has a separate origin, namely, it requires phosphoriboxyl pyrophosphate + ATP.

Almost all the amino acids that occur naturally in proteins have the L configuration (Conn and Stumpf, 1964). Bacteria, however, have the ability to racemise D amino acids to the L form (Davis et al, 1969) and can, therefore, utilize L and D forms.

## 3 AMINO ACIDS IN SALIVA

Saliva is considered an unsatisfactory culture medium. Burnett and Scherp (1968) found that the growth of lactobacilli was poor unless a carbohydrate source was added

105/...to saliva/...

to saliva. In fact Battistone and Burnett (1961) indicated that whole saliva is generally deficient in amino acids particularly tryptophane which is necessary for the growth of Lactobacillus acidophilus (Weisberger, 1946). Williams and Powlen (1959) used parotid saliva as a culture medium for microorganisms. They found it could support the growth of Staphylococcus aureus, enterococci, yeast and bacillus species but none of the lactobacilli, viridans streptococci or Corynebacterium diptheria. These findings seem to suggest that saliva is a poor culture medium.

Studies have been done on the amino acid content of whole saliva, and saliva from the parotid gland, sublingual and submaxillary-submandibular glands. Battistone and Burnett (1961) have shown that whole saliva contains seventeen amino acids excluding tryptophane, cystine and cysteine. Kirsh et al (1947) showed that there were only sixteen amino acids present in whole saliva. On the other hand, recent work by Critchley (1969) who used a Technicon amino acid analyser showed that whole saliva contained only fifteen amino acids. He did not isolate cystine, cysteine, tryptophane or phenylalanine from whole saliva. Battistone and Burnett (1961) were not able to demonstrate more than trace amounts of phenylalanine in whole saliva and tryptophane in only one patient. When they studied saliva from the parotid gland it was found to be poor in amino acids and, in fact, contained only seven in very low concentrations.

106 /...Work by/...

Work by Critchley (1969) has shown that sublingual saliva contains more amino acids than whole saliva except for tyrosine and proline which were more concentrated in whole saliva. On the other hand, submaxillary-submandibular saliva, according to Battistone and Burnett (1961), contains fewer amino acids than whole saliva. It does not contain tyrosine, arginine or histidine. It seems feasible to suggest from the evidence produced by Battistone and Burnett (1961) and Critchley (1969) that some amino acids in whole saliva are the excretory products of microorganisms. The proteolytic enzymes in saliva described by Critchley (1969) could be bacterial in origin and these could produce amino acids as degradation by-products.

4 AMINO ACIDS IN PLAQUE

Socransky et al (1963) have established that dental plaque consists largely of bacteria; in fact, in the same concentration as a centrifuged culture of streptococci. The products in plaque, therefore, would be mainly bacterial in origin. Critchley (1969) in his studies on plaque showed that the same amino acids are present in plaque and saliva. However, the concentrations of proline, glycine and glutamic acid are lower in plaque than in saliva, but plaque contains more alanine than either saliva or epithelial cells. On the other hand, Singer and Kleinberg (1973) in their studies on free amino acid pools in plaque found that glutamic acid

107/...constituted/...

constituted about 50 per cent of the total amino acids present. These differences could be explained by the utilization and excretion of amino acids, by variations in the composition of plaque bacteria, as well as by the type and intake of food which contribute to plaque formation.

The facultative streptococci, according to Handleman *and Mills* (1965), form the largest group of microbes in the mouth. They constitute half the viable count of saliva and dorsum of tongue, and a quarter of the flora of plaque and gingival sulcus. The anaerobic streptococci form another 4 - 13 per cent of the viable count (Burnett and Scherp, 1968).

The diet of the host influences the constitution of the oral flora and the availability of nutrients plays a part in maintaining the ecological balance. Competition *for nutrients is important when* their supply is limited. This factor is applicable to the streptococci because the species differ in their nutritional requirements (Inward, Upstone and van Houte, 1970).

Studies on saliva and plaque have shown that certain amino acids such as methionine and histidine are present in small amounts and others, such as cystine and tryptophane, are absent. Microorganisms, and the streptococci in particular, compete for these nutrients and to quote Darwin (1859): "As the species of the same genus usually have, though by no means invariably, much similarity in

108/...habits and/...

habits and constitution, the struggle will generally be more severe between them, if they come into competition with each other, than between the species of distinct genera." The streptococcal species, in fact, do differ in their nutritional requirements and this means that there is less competition between them which leads to a state of balance between them.

5 AMINO ACID REQUIREMENTS OF MICROORGANISMS

Amino acid requirements of a number of streptococcal species have been studied. Barnes, Seeley and van de Mark (1961) found that most Streptococcus bovis strains could utilize ammonium chloride as a sole nitrogen source although earlier work by Smiley, Niven and Sherman (1943) showed that this species is more exacting. The enterococci, according to the same authors, requires tryptophane for growth. Mergenhagen and Scherp (1957) in their studies on the anaerobic streptococci found that oral strains required aspartic acid, histidine and isoleucine but that vaginal strains were more exacting in this respect. They felt that they resembled Streptococcus salivarius which, according to Smiley et al (1943), requires seven amino acids, namely, arginine, cystine or methionine, glutamic acid, isoleucine, leucine, lysine and tyrosine. Inward et al (1970) in a later study, found that Streptococcus salivarius required an additional four amino acids: cysteine, histidine, threonine and valine. All authors agreed

109 /...that glutamic/...

that glutamic acid was more essential for growth of this species than the other amino acids. Inward et al (1970) showed that Streptococcus mutans differs in this respect from Streptococcus salivarius. It has no special requirements for a single amino acid provided that the other nineteen amino acids are present. However, in a minimal medium Streptococcus mutans requires the same eleven amino acids as Streptococcus salivarius, Streptococcus sanguis and Streptococcus mitis to sustain growth. On the other hand, Berman and Gibbons (1966) found that Streptococcus mitis needed only six amino acids, and this demonstrates that there is some variation amongst strains within a species regarding nutritional requirements. It seems, however, that the pathogenic streptococci belonging to Lancefield Group A require more amino acids for satisfactory growth (Mickelson, 1964) than other streptococci.

These studies, therefore, have shown that Streptococcus salivarius and Streptococcus sanguis require methionine or cystine and histidine for growth. These amino acids are present in limited concentrations in saliva and plaque, and the growth of these organisms in plaque or saliva would be poor. Streptococcus mitis requires tryptophane for growth and this amino acid is absent in saliva and plaque. This species, therefore, is unable to grow in plaque or saliva. However, despite the limited concentrations of amino acids available to these organisms they survive and flourish in the oral cavity. This suggests that there is an interaction between

110/...microorganisms/...

microorganisms which makes the essential amino acids available to all members of the community.

However, Streptococcus bovis, which does not require any amino acids, would have an advantage over Streptococcus pyogenes, which requires all the amino acids. On the other hand, if enough nutrients are available for the satisfactory growth of Streptococcus bovis, it produces an excess of amino acids which could be utilized by Streptococcus pyogenes. This demonstrates one way in which these two organisms could live in equilibrium with each other. In contrast, Streptococcus salivarius and Streptococcus sanguis have the same requirements and would compete with each other for the same amino acids. If the competition is too strict they will both die out. If other organisms are present, such as Streptococcus bovis and Streptococcus pyogenes competition will be reduced and all the species could live in equilibrium. Streptococcus bovis, however, would be the controlling organism because it is able to synthesize all the amino acids from a simple nitrogen source.

The streptococci, therefore, form a continuous series regarding their amino acid requirements. As far as their dependence on amino acids, some of them are completely dependent and others independent on amino acids for growth. This observation could be extended to include their biochemical properties. Morris (1954) showed that the oral streptococci form a continuous series in this regard.

111/...6 Present/...

6 PRESENT STUDY

The gingival sulcus of man supports a mixed population of microbes consisting of streptococci and other organisms which together form a community. These organisms are implicated in periodontal disease and the breakdown of the supporting tissues of the teeth (Brandtzaeg, 1966). The community of microbes produces aggressive substances which are responsible for the breakdown of these tissues (Burnett and Scherp, 1958). It is difficult to study these processes in vivo and for this reason it was decided to investigate the amino acid metabolism of this community in vitro. It was hoped that this would give some indication of the degradation of proteins by the community of microbes as well as their amino acid metabolism.

Cooked meat medium was used in this study because it contains pieces of tissue. The medium consists of pieces of heart muscle and peptone water (Lepper and Martin, 1929). Muscle is a rich source of protein and the broth contains a readily available source of carbon, nitrogen and amino acids.

7 MATERIALS AND METHODS

Samples taken from the gingival sulcus of ten Bantu males with pockets 3 - 6 mm were inoculated into 100 ml of cooked meat medium (Oxoid) which was incubated at 37°C for 30 days. Samples of 1 ml of the broth were removed from the medium at the beginning of the experiment and every second day.

112/...for 30 days/...

for 30 days. These samples were lyophilized and stored at ~20°C to prevent protein hydrolysis.

The lyophilized specimens were resuspended in 0,2 ml distilled water. Proteins in the samples were precipitated with 0,5 M perchloric acid. This suspension was centrifuged and the supernatant was neutralized with 0,5 M sodium hydroxide to give a final pH of 7.0 using Merck neutralit indicator paper. This gave a final volume of approximately 0,5 ml.

Thin layer chromatography was used for amino acid analysis using the methods described by Smith (1969). Plates for the chromatogram were obtained from E Merck, Darmstadt, Germany. They were thin sheets of glass 20 x 20 cm square which were precoated with a thin layer of cellulose.

Pencil marks 2 cm apart were made in a line parallel to, and 2 cm from the edge of the plate. Six drops of sample were placed on each mark using a microhaemocrit tube with a diameter of 1,4 mm. The spots were kept small by drying them with cold air from a hair dryer. The plates were placed in a tank containing a solvent consisting of butanol, acetic acid and water in the ratio 12:3:5, with the line of spots arranged just above the solvent level. The tank was sealed and the solvent was allowed to ascend until it reached the top of the plates. These were removed and dried immediately with cold air from a hair dryer. They were sprayed with a solution of ninhydrin (Merck) and placed in an oven at 105°C to allow the

113/...colour to/...

colour to develop. Rf values were measured and determined by the formula

$$Rf = \frac{\text{distance substance has travelled from the origins x 100}}{\text{distance solvent front has travelled from the origins}}$$

Standard solutions of 19 amino acids were prepared in the same way. A 0,1 per cent solution of amino acid in 10 per cent isopropanol was used. It was necessary to place one standard solution of amino acid on each plate used in the experiment to standardize the results. Leucine was chosen because it has the greatest Rf value under the conditions of this experiment. Amino acids were identified by their Rf values and their colour reaction with ninhydrin.

8 RESULTS

The results are summarized in Figure 1 and Table XIV.

In Figure 1 the percentage incidence at the beginning of the experiment, the average percentage incidence and the final incidence are shown. The amino acids were divided into three groups. Group 1 consisted of amino acids which showed no change in incidence during the experiment. Tyrosine and proline belong to this group. Tyrosine had a 90 per cent incidence and proline occurred in 10 per cent of the samples.

The second group consisted of methionine, cystaine, glutamic acid, lysine, glycine, threonine, alanine

and isoleucine. This group showed a rise in incidence and the final incidence was higher than the initial or average incidence with the exception of glutamic acid and alanine. Here the final incidence was slightly lower than the average incidence. Methionine had an initial incidence of 30 per cent and an average and final incidence of 90 per cent, that is, a 60 per cent overall increase in incidence. Cysteine had an initial incidence of 60 per cent which also rose to a final incidence of 90 per cent. Lysine, glycine, threonine and isoleucine were low in incidence and all showed a 20 per cent increase in incidence during the experiment.

In the third group the average incidence was lower than the initial or final incidence. This group consisted of arginine, valine, leucine, tryptophane, cystine, serine and aspartic acid. Leucine and tryptophane showed the greatest overall change in incidence, namely, 90 and 60 per cent respectively. The incidence of leucine dropped 65 per cent and rose by 25 per cent to a final incidence of 50 per cent. Tryptophane dropped by 50 per cent and rose again by 10 per cent to a final incidence of 40 per cent. Cystine dropped by 25 per cent and rose again by 25 per cent showing a gross change of 50 per cent. Aspartic acid showed a similar change, but only by 20 per cent. Serine dropped by 20 per cent and rose again only slightly.

Histidine showed a 10 per cent drop in incidence and could not be placed into any of these three groups.

115/... In Table/...

In Table XIV the incidence in the 10 samples is given for every fourth day. As before the amino acids were again divided into three groups. The first group consisted of amino acids which showed very little overall change; during the experiment a slight rise was followed by a slight drop in incidence. Tyrosine, glutamic acid, alanine and proline belong to this group. The range in incidence for tyrosine and proline varied by only 10 per cent, alanine by 20 per cent and glutamic acid by 30 per cent. The overall change, nevertheless, remained minimal.

The second group showed a rise in incidence. Methionine, cysteine, lysine, glycine and threonine belong to this group. Methionine showed a rapid rise from an initial incidence of 30 per cent to an incidence of 90 per cent for the rest of the duration of the experiment. Cysteine showed a more gradual increase from 60 per cent to 90 per cent. Lysine, glycine and threonine also increased gradually with an overall increase of 20 per cent.

The third group of amino acids showed a drop in incidence followed by a rise. Leucine showed the greatest change. The initial incidence was 90 per cent which dropped to 0 per cent and rose again to 50 per cent. With tryptophane the initial incidence was 80 per cent which dropped to 10 per cent and rose again to 40 per cent. Cystine dropped from 50 per cent incidence to 0 per cent and rose back to 50 per cent. The other amino acids serine, isoleucine, aspartic acid, arginine and valine showed

116/...far less/...

far less change in incidence.

9 DISCUSSION

Despite the use of only one batch of cooked meat medium, the original amino acid content varied from bottle to bottle. This is possibly due to the preparation of the medium. In the method described by Lepper and Martin (1929) bullock heart is boiled in $N/_{20}$ NaOH. The concentration is very low and according to Conn and Stumph (1964) 2N NaOH is necessary for the hydrolysis of protein. This high concentration of alkali does destroy cystine, cysteine, serine, threonine and arginine. The low concentration, however, would not hydrolize the protein completely. Some amino acids would be released and depending on the conditions of preparation these could vary from batch to batch and within a batch. When the media is dehydrated different amino acids could occur in different parts of the media. If this media is subdivided and placed in separate bottles on rehydration it would give media with different amino acid concentrations. Certain amino acids may be present in such low concentrations in the initial medium with the result that they are not present in particular bottles.

If an organism is not supplied with an amino acid and is unable to synthesize the amino acid, it obtains that particular amino acid from an external source rather than break down its own proteins. It has been shown by

117/...Hagness/...

Hogness, Cohn and Monad (1955) and Rotman and Spiegelman (1954) that less than one per cent of newly formed enzyme is synthesized at the expense of cellular protein. The organisms in this study would, therefore, of necessity have to degrade proteins in the medium once the available nutrients are exhausted. A number of people have studied this protein breakdown by organisms. According to Wilson and Miles (1957) the hydrolytic breakdown of protein is due to the action of specialized enzymes. These are produced in adequate amounts when the bacteria are supplied with food material which can be assimilated. Nutrients were available in the medium and, therefore, the organisms in this experiment would be able to produce sufficient enzymes to initiate protein breakdown and thereby increase the amount of amino acids available. The proteolytic activity of bacteria is confined to some species many of which are anaerobic (Wilson and Miles, 1957). Cooked meat which was used in this experiment is an anaerobic medium and would support the growth of these organisms. They would, therefore, be able to degrade proteins.

The initial stage of the liquifaction of protein gels is brought about by the opening of peptide linkages to reduce the protein to polypeptides and dipeptides. Many of the enzymes necessary for this hydrolysis are intracellular and are excreted by intact cells. Further breakdown into amino acids is brought about by polypeptidases. Peptidases appear to be of intracellular

118/...origin/...

origin, are found in older cultures and are presumably liberated by autolysis of bacterial cells (van Heyningen, 1940; Gorini and Fromageot, 1950). Therefore, the amino acids which were released into the medium, especially during the latter part of the experiment, could have been released by bacterial cells which were already dead and had excreted their enzymes into the medium.

The division of the amino acids into three groups (Figure 1) is based on their participation in the metabolism of the community of microbes studied. The first group consisting of tyrosine and proline showed no change in incidence and these amino acids, therefore, probably are not metabolised by the community of microorganisms. They possibly are not essential for the growth of these organisms.

The second group consisting of methionine, cysteine, glutamic acid, lysine, glycine, threonine, alanine and isoleucine was excreted by the community of microorganisms (Figure 1). Methionine, a sulphur-containing amino acid which had a low incidence initially was found in 9 of the 10 samples on the fourth day (Table XIV). This increase could be the result of the breakdown of proteins in the medium with the subsequent release of methionine. The excretion of this amino acid by organisms in the medium seems less probable because the incidence of methionine did not change after 4 days. It, therefore, does not seem to play an active role in the growth of these organisms.

119/...Cysteine/...

Cysteine which is also a sulphur-containing amino acid had a relatively high incidence initially which increased further with time until it reached the same level as methionine (Table XIV). The increase was far more gradual than in the case of methionine. This could be explained by the gradual increase of organisms in the medium which would lead to a gradual accumulation of their metabolic products in the medium. Amino acids would be amongst these products if they are produced in excess. The other amino acids in this group were similar in their activities, but to a lesser extent. Thus, the incidence of alanine and glutamic acid was similar to methionine and the incidence of lysine, glycine and threonine was similar to cysteine (Table (XIV). Isoleucine was also similar to cysteine but it accumulated at a later stage (Table XIV).

The third group (Figure 1) is the most active group and possibly the most important in this community. These amino acids appeared to be utilized and then excreted. Leucine had the greatest change in incidence and could be regarded as the most active amino acid in this experiment. One explanation for this observation could be that there was a free source of this amino acid present in the medium initially. The community may require this amino acid for growth and, therefore, it is ulitized until the supply is exhausted. The amino acid builds up again in the medium once the organisms which required it have died. Tryptophane was the next most active amino acid in this group. It was only slightly less active than leucine and together

120/...these could/...

these could be the most important amino acids for this community. Serine was the only amino acid in this group which was utilized and not excreted again. It seems to be incorporated into the microorganism in a form which cannot be released again. Serine is also a precursor of cysteine and glycine (Davis et al, 1969). It could be used in the biosynthesis of these amino acids which both showed an increase in incidence during the experiment. The other amino acids in this group were arginine, valine, cystine and aspartic acid. These amino acids were less active than leucine and tryptophane but, nevertheless, they were utilized and excreted by the community.

Histidine which was not grouped with any of these amino acids, showed a drop in incidence (Figure 1). It was most similar to serine because it was utilized and did not increase in incidence.

In Table XIV there were also three groups of amino acids. The percentage incidence on every fourth day was used instead of an average incidence. This analysis had the effect of demonstrating in more detail the changes in incidence of the amino acids. The 4-day analysis shows some changes in the grouping of the amino acids. Tyrosine and proline were still in the first group. Alanine and glutamic acid were added to this group. Both these amino acids were placed in the second group in the first analysis because they showed a rise in incidence. They were the only two amino acids in the

121/...group with/...

group with a final incidence lower than the mean incidence. In this respect they were not typical members of the group.

The 4-day analysis left only 5 amino acids in the second group, namely, methionine, cysteine, lysine, glycine and threonine. All these showed a rise in incidence.

The third group consisted of arginine, valine, cystine, leucine, tryptophane, serine and aspartic acid. Isoleucine and histidine both fitted into this group because they all had a drop in incidence initially which increased again on about the twentieth day.

The classification of amino acids into three groups in this experiment does not correlate with the classification according to their chemical structure or biosynthetic origins. This is not surprising because the metabolism of the community is very complex and all the organisms in the community would not have the same amino acid requirements. They would, therefore, not metabolize one group or a particular family of amino acids.

According to Weisberger (1946) tryptophane is necessary for the growth of Lactobacillus acidophilus. This experiment showed that it was also necessary for the community studied. Tryptophane was present in 8 of the 10 samples initially and after 2 days it was found in only one sample. Tryptophane was, therefore, utilized right from the beginning of the experiment.

Critchley (1969) observed that whole saliva contains more tyrosine and proline than sublingual saliva. Battistone

122/...and Burnett/...

and Burnett (1961) also found that submaxillary-submandibular saliva does not contain tyrosine, arginine or histidine, whereas whole saliva contains it. The possibility arises that these amino acids could be excreted by organisms in the saliva. The results of the present experiment did not support this suggestion because none of these amino acids were excreted by the community. On the other hand, the amino acids that were excreted such as cysteine, are not found in whole saliva. These findings suggest that the major fraction of amino acids in saliva are excreted by the salivary glands. The origin of the rest is not known.

Studies on plaque and saliva by Critchley (1969) have shown that the concentrations of proline, glycine and glutamic acid are lower in plaque than in saliva. This suggests that they are utilized by the microbes in plaque. In the present experiment proline showed very little change in incidence (Table XIV). This amino acid, therefore, did not appear to be utilized or excreted by the community. Glycine was present in lower concentrations in plaque than in saliva (Critchley, 1969). In this study, however, it appeared to be excreted by the community (Table XIV).

Glutamic acid and alanine, on the other hand are found in high concentrations in plaque (Singer and Kleinberg, 1973; Critchley, 1969) and were excreted in the initial stages of this experiment (Table XIV).

The community of microbes in plaque is in it's natural environment and is in a dynamic state of growth. The

123/...community/...

community of the experiment was growing in a limiting environment and tended to be in a state of decline. The metabolism of these two communities, therefore, would differ and possibly different organisms would dominate in each community. This could explain why the amino acids in plaque and saliva differ from those obtained in the present experiment.

The streptococci according to Smiley et al (1943); Mergenhagen and Scherp (1957); Barnes et al (1961); Mickelson (1964); Berman and Gibbons (1966) and Inward et al (1970), vary in their acid amino requirements. Some species do not require any amino acids for growth and others require twenty amino acids. Streptococcus bovis according to Barnes et al (1961) has no amino acid requirements and the enterococci only require tryptophane (Smiley et al, 1943). Streptococcus bovis, therefore, would be unaffected by the availability of amino acids, but the enterococci would depend upon the presence of tryptophane. This amino acid was present in 80 per cent of the samples initially, and after 2 days it had dropped to an incidence of 10 per cent. It was utilized by the community as a whole and this change could be due partly to the enterococci.

The anaerobic streptococci require three amino acids for growth: histidine, isoleucine and aspartic acid (Mergenhagen and Scherp, 1957). In this experiment all these amino acids dropped initially and then increased in incidence. They, therefore, were utilized and then excreted

124/...by the/...

by the community. They could contribute to the metabolism of these organisms, but their effect would not be very great, because they were present in less than 40 per cent of samples.

Of the six amino acids required by Streptococcus mitis, serine and tryptophane were the only two that showed a drop in incidence; the other four amino acids showed a slight increase. Serine and tryptophane also could be metabolized by this species as it is a member of the community.

Inward et al (1970) found that four species of streptococci, Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguis and Streptococcus mitis all required the same eleven amino acids. Some of the amino acids were utilized and others excreted in the present experiment. For example, in Figure 1, tyrosine and glutamic acid showed very little change in incidence, but cysteine, lysine and threonine were excreted by the community. Arginine, histidine, valine, cystine and leucine were all utilized by the community, cystine and leucine to a greater extent and the rest to a lesser extent. Cystine was present in 50 per cent of the samples initially, but leucine was present in 90 per cent. The incidence of leucine dropped to zero after 4 days and a further 10 per cent 2 days later (Table XIV). This amino acid seems to be important in the metabolism of this community, and possibly this group of streptococci had a marked effect on the incidence of this amino acid in the experiment.

125/...From the/...

From the foregoing discussion it seems possible that changes in the incidence of tryptophane and leucine probably indicate that they are required by certain streptococci in the community. Serine and cysteine showed similar changes but not to the same extent. The other changes in incidence of amino acids do not seem to correlate with the growth requirements of the streptococci. The community in this experiment after all consists of other species besides the streptococci and they would also have an influence on the amino acid content of the medium.

Studies on plaque and saliva have shown that certain amino acids which are essential for the growth of streptococci are absent, for example, tryptophane, whereas others are present in very small amounts, for example, cystine and histidine. Despite these observations most streptococcal species are able to flourish in plaque. In the present experiment there were some amino acids which were present in only a small number of samples, for example, isoleucine, aspartic acid and threonine (Table XIV). Even though these amino acids, which are necesssary for the growth of streptococci, are absent the community which consists of streptococci and other species, was able to survive and flourish. This supports the idea that certain organisms will produce substances which other organisms can utilize. These three amino acids are all derived from the same precursor. The sequence in the biosynthetic chain is as follows:-

oxalacetate ⟶ aspartate ⟶ threonine ⟶ isoleucine

126/...(Stanier/...

(Stanier, Doudoroff and Adelberg, 1971). Possibly the enzyme necessary for the conversion of oxalacetate to aspartate is not present in the streptococci which require these amino acids. Oxalacetate or a precursor would accumulate in the cell and be excreted into the medium. Other bacteria or bacterial enzymes could convert these products to aspartate, threonine and isoleucine and thus supply these necessary amino acids to the streptococci. A further possibility is that these amino acids could be changed into peptides by competent bacteria. These products could then be made available to organisms which are unable to synthesize the necessary amino acids which make up these peptides.

127/...References/...

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# CHAPTER III

## CHANGES IN pH IN THE MEDIUM STUDIED

### 1 INTRODUCTION

The pH of a medium generally changes after it has been inoculated with microorganisms. The change depends upon the major constituent of the growth medium. For instance, if peptone is the principle nutrient the pH rises due to the formation of ammonia by the deamination of amino acids. If fermentable sugars are present the pH falls due to the formation of acids. Often, however, the pH rises in sugar media even though the sugar is fermented. This is the result of alkali produced by the deamination of amino acids. The amount of acid produced also depends upon the manner in which the sugar is utilized. Some species, for example, Escherichia coli metabolize sugar anaerobically to produce organic acids, whereas under aerobic conditions these acids are broken down to water and carbon dioxide which escape from the medium. The amount of acid produced, therefore, is less under aerobic

131/...than/...

than anaerobic conditions (Meynell and Meynell, 1970).

2 EFFECT OF SINGLE ORGANISMS ON pH OF GROWTH MEDIUM

Studies have been done to determine the effect of individual organisms on the pH of a medium. Stephan and Hemmens (1947) used a medium consisting of glucose and a buffer. They found that staphylococci caused an initial drop in pH from 7,0 to 5,5 within 30 - 60 minutes. After about 150 minutes the pH returned to 7,0. Neisseria had a similar action. Sarcina also gave a similar curve but it was lower. In the case of the lactobacilli the pH dropped to 4,0 and never returned above pH 5,0, even after 150 minutes. Studies on the streptococci gave variable results. One strain of $\alpha$-haemolytic streptococci produced a rapid drop in pH to 4,5 followed by a gradual return to pH 7,0, whereas another strain produced no change at all. A $\gamma$-haemoltyic streptococcus produced changes which were intermediate between these two results. Actinomyces and neisseria were similar to the first $\alpha$-haemolytic streptococcus. The corynebacteria gave completely different results because they did not change the medium at all during the first 2 hours, but gradually increased the alkalinity until the pH reach a value of 8,5. These investigations have shown that the pH of a medium changes when it is inoculated with microorganisms and that the response depends on the metabolic activities of a particular species.

132/...3 Effect/...

3 EFFECT OF TWO ORGANISMS ON pH OF GROWTH MEDIUM

Similar studies were undertaken on the effect of two organisms on the pH of the medium. If a strongly acidogenic lactobacillus is mixed with a weakly acidogenic streptococcus the pH curve is intermediate between these two species, but closer to the lactobacillus. On the other hand, if a strongly acidogenic streptococcus is used the curve is intermediate between the two species (Stephan and Hemmens, 1947). A mixture of organisms, therefore, can have different effects on pH depending upon the characteristics of the strain used. The general trend, however, is a return to a pH of 7,0.

4 EFFECT OF ORGANISMS ON pH OF PLAQUE

Mixed populations of oral microorganisms in plaque and saliva have also been studied. Kleinberg and Jenkins (1964) investigated the pH in plaque in fasting subjects, in subjects consuming food, and in saliva. They found that the pH of plaque in fasting subjects varied in different sites in the mouth. The mean ranged from pH 7,39 - pH 8,10 with a minimum value of 5,86. In the fasting state meals with little or no carbohydrate had very little effect on plaque pH. On the other hand, meals with carbohydrate lowered the pH level for up to 3½ hours after mealtime. The pH of saliva was lower than in plaque and varied from pH 6,77 - pH 7,10. Generally it was lower than in the adjacent plaque. Saliva, therefore, did not seem to have a great influence on the pH of the plaque.

133/...Kleinberg/...

Kleinberg and Jenkins (1964) feel that plaque bacteria close to the enamel surface utilize saliva and dietary sugars which results in acid formation. This produces a drop in pH and ultimately caries. On the other hand, plaque in contact with the gingival tissues will utilize protein. This will result in the accumulation of proteolytic products (a rise in pH) and periodontal disease.

In the present experiment microorganisms from the gingival sulcus were studied. Cooked meat medium which consists of pieces of meat and peptone water was used as a culture medium. This could be considered similar to the substrate in the gingival sulcus. The effect of the metabolism of the whole microbial population from the gingival sulcus on the pH of the medium, therefore, could be studied. This would give some indication of the potential proteolytic or acidogenic properties of the population as a whole.

5 MATERIALS AND METHODS

Samples taken from the gingival sulcus of ten Bantu males with pockets 3 - 6 mm were inoculated into bottles containing 100 ml of cooked meat medium (Oxoid) which were then incubated at 37°C for 30 days. These samples were the same as those used for the amino acid analyses. Samples of 1 ml of the broth were removed at daily intervals. The pH was measured by means of a Beckman Zeromatic SS-3 pH meter with a combination electrode containing an internal element of silver, silver chloride.

134/... 6 Results/...

6 RESULTS

The results are summarized in Table XV and Figure 2.

In Table XV the daily mean pH of the ten samples is given. At the beginning of the experiment the mean pH of the cooked meat media was 7,04. Once the media had been cultured for 24 hours, the pH dropped to 6,7. After this period the pH rose and fell intermittently with a general upward trend. The maximum pH recorded was 8,29 on day 24. After this the pH tended to decrease. The greatest change in pH occurred on the third to fifth days with an increase of almost 0,7 pH units from 7,05 to 7,69.

In Figure 2 the results from Table XV are presented in graph form.

7 DISCUSSION

The initial drop in pH from 7,04 to 6,71 within the first 24 hours was probably due to the fermentation of small amounts of carbohydrate (mainly glucose and glycogen) present in the meat medium. This was similar to the conditions observed by Stephan and Hemmens (1947) who found that the pH dropped from 7,0 to between 4,0 and 5,5, within 30 minutes. After about 2 hours, however, the pH generally returned to 7,0. In the present experiment the observed pH was not as low as pH 5,0, but it remained at its lowest level for at least 24 hours. This could be due to the cumulative effect of a

135/...number of/...

number of organisms and possibly their inhibition of proteolytic enzymes in the medium which would tend to enable the pH to return to 7,0 and then become alkaline. Organisms which could possibly have this effect would be those organisms with an initial high incidence followed by a sudden decrease in incidence after the first 24 hours. These organisms are mainly streptococci (Table V) and include the mitis-type, $\alpha$-haemolytic and polysaccharide-producing streptococci. Drucker (1970) found the pH optimum for Streptococcus mutans was 6,2, whereas the optimum for the other streptococci varied from 6,6 - 7,8. This suggests that the polysaccharide-producing streptococci (which include Streptococcus mutans) would have more effect on the pH than the other streptococci. The drop in incidence of these three species would also tend to support this idea. The incidence of the polysaccharide-producing streptococci decreased from 60 per cent to 10 per cent which amounted to a decrease of 50 per cent, whereas the mitis-type streptococci and $\alpha$-haemolytic streptococci only decreased by 30 per cent (Table V). The low optimum pH for Streptococcus mutans would tend to make the medium of the present experiment inhibitory for this organism. This is probably true because the polysaccharide-producing streptococci disappeared completely from the medium after the third day once the pH was above 7,0 (Table XV).

Davis et al (1969) state that a culture growing on a limited amount of sugar often exhibits a fall in pH followed by a rise as the acid accumulates and then is utilized.

136/...This/...

This phenomenon seemed to occur in the present experiment, but after the third day the pH rose above 7,0 to 7,7, which seems to indicate that proteolytic enzymes were being produced as well, which would result in the production of ammonia and an increase in pH (Meynell and Meynell, 1970).

There are a number of organisms in the present experiment that increased markedly on the third and fourth days. These include the bacteroides, $\delta$-haemolytic streptococci and the actinomyces (Table V). The bacteroides are considered slightly proteolytic by Prévot (1966), whereas Gibbons and Macdonald (1961) demonstrated the production of a collagenase by these organisms and Weiss (1937) a fibrinolysin. The only members of the streptococci that are considered proteolytic are the enterococci (Breed et al, 1957). Some $\delta$-haemolytic streptococci could be included in this group, but the enterococci did not increase after the second day. On the contrary, they declined in incidence after the fourth day (Table V). The streptococci, therefore, did not seem to be involved in this change in pH. The actinomyces have no proteolytic activity (Breed et al, 1957) and would, therefore, also not be involved in the pH change. From these observations it seems probable that the bacteroides were one of the important proteolytic microorganisms at this stage in the experiment. They prefer to grow in mixed culture (Breed et al, 1957) and possibly under these conditions they are more proteolytic than in pure culture.

137/...Other/...

Other organisms in the microbial population of the present experiment may also contribute towards the observed increase in pH. They may switch from a saccharolytic to a proteolytic type of metabolism once the available sugars have been exhausted or the proteolytic species may be inhibited or masked by the saccharolytic species, and once the sugar has been exhausted they become prominent. Organisms which may be responsible for this increase in pH could include the anaerobic streptococci with proteolytic and peptolytic properties (Breed et al, 1957), the staphylococci which produce ammonia from peptone water and liquify gelatin and thus are proteolytic (Breed et al, 1957), as well as the veillonella which would include Veillonella alcalescens a non-saccharolytic and alkali forming microorganism (Prévot, 1965).

The observed increase in pH supports the findings of Mühleman and De Boever (1970) who stated that peak acid production occurs in three-day-old plaque and that less acid could be detected in older plaque. They felt that this could be due to the accumulation of phosphate buffers in plaque. In the present experiment the media was very much more limiting than in plaque which is exposed to dietary nutrients in saliva. The sequence of events, therefore, would take place more rapidly than in plaque and the subsequent rise in pH or buffering action would also be accelerated. From the present experiment, therefore, it seemed that organisms from the gingival sulcus and plaque produced a variety of alkali buffering agents.

138/...These/...

These would counteract a drop in pH in plaque and maintain a proteolytic type of environment.

After the fifth day the pH tended to fluctuate until it reached a peak i.e. pH 8,29 on day 24 (Table XV). This rise and fall is probably due to the production and utilization of acids as described by Davis et al (1969). This continual but slow increase in pH tends to support the observations of Stanier et al (1971) who state that serious difficulties are encountered in controlling the pH of slightly alkaline media in which basic substances are produced as a result of bacterial growth. Despite this phenomenon the pH started to drop after the twenty fourth day.

An increase in amino acids in a medium could be a measure of proteolytic activity. A number of amino acids did increase in the medium after an initial drop in incidence. These include arginine, valine, cystine, leucine, tryptophane, isoleucine and histidine (Table XIV). Histidine, unlike the other amino acids, showed a drop in incidence after the twenty fourth day. Other amino acids had a rise in incidence, for example, methionine, cysteine, lysine, glycine and threonine (Table XIV). The proteolytic activity does tend to continue during the entire duration of the experiment with the production of certain amino acids and the loss of others.

These amino acids could be one of the buffering agent systems in the medium which would tend to cause a drop

139/...in pH/...

in pH. According to Conn and Stumpf (1964) amino acids have a "Zwitterion" structure. When alkaline is added to a neutral solution of amino acids, protons on the $NH_3^+$ group are titrated. When acid is added the dissociated carboxyl group accepts the proton to form the protonated amino acid. Each amino acid, therefore, has at least 2 pKa values. Some amino acids have other groups which are capable of dissociating protons. Thus the sulphydryl group of cystoine dissociates with a pKa of 10,8 and the phenolic hydroxyl group of tyrosine has a pKa of 10,1. In the present experiment the medium is basic, therefore the alkaline pKa values are important. If amino acids are released into this medium they will tend to neutralise the basic groups in the medium. This would account for the fluctuation in the pH in the present experiment as well as the apparent return to a neutral pH after the twenty fourth day.

According to Meynell and Meynell (1970), if peptone water is the principle nutrient in a culture medium, ammonia is produced and the pH rises. If fermentable sugars are present acid is produced. Often, however, the pH rises in sugar media as the result of alkali produced by the deamination of amino acids. In the present experiment, after the initial drop in pH on the first day, the pH rose (Table XV). This could be due to the production of ammonia from peptone water and the deamination of amino acids. There are a number of amino acids which decrease in incidence after the first day. These include arginine,

140/...valine/...

valine, cystine, leucine, tryptophane and serine (Table XIV). The deamination of amino acids, therefore, seems to be more important than the production of ammonia from peptone water.

Stephan and Hemmens (1947) inoculated a sugar medium and found that the staphylococci, neisseria, actinomyces and $\alpha$- and $\gamma$-haemolytic streptococci produced an initial drop in pH followed by a return to a pH of 7,0. This phenomenon is similar to the changes observed in the pH for the first 4 days in the present experiment (Table XV). These organisms were found in the medium during this period. The neisseria had a high incidence initially (80 - 85 per cent) during the first 4 days and then they declined in incidence (Table V). The $\alpha$-haemolytic streptococci were similar. The $\gamma$-haemolytic streptococci, on the other hand, increased after the fourth day (Table V). The staphylococci and the actinomyces increased gradually with a maximum incidence on the tenth day (Table V). These organisms, therefore, could have been responsible for the changes in pH observed and possibly the neisseria and the $\alpha$-haemolytic streptococci were more important than the $\gamma$-haemolytic streptococci, actinomyces or the staphylococci in bringing about this change.

In their study in 1947 Stephan and Hemmens also used lactobacilli. They found that the pH dropped to 4,0 and never rose above pH 5,0, even after 2 hours. In this experiment lactobacilli were present in at least 20 per cent of

141/. .the samples/...

the samples for 4 days (Table V). Despite their presence the pH never dropped below 6,71 (Table XV). Their effect, therefore, seemed to be counteracted by other organisms in the media. In fact, they appeared to be inhibited by other organisms and were found in only about 25 per cent of the samples, whereas they were generally present in the mouth but formed a minor fraction of the flora (Burnett and Scherp, 1968).

Stephan and Hemmens (1947) found that the corynebacteria did not change the pH of the medium initially but gradually increased the alkalinity until the pH reach a value of 8,5. During this experiment the corynebacteria were present in only 12 per cent of the cultures and disappeared after the tenth day (Table V). They could have contributed to the rise in pH but it is more than likely that their influence was minimal.

Similarly, Stephan and Hemmens (1947) studying the effect of two organisms on the pH of a culture medium found that the pH is usually intermediate between the pH observed for the strains separately. In this study there were at least 23 genera or species isolated (Table V). The resultant pH would be intermediate, the acid-forming bacteria balancing the alkali-forming bacteria.

Despite this possible balancing effect, the pH rose, which suggests that in the present experiment the population was ultimately proteolytic. On the twenty fourth day, however, a peak was reached (Table XV), and it seems

142/...probable/...

probable that the pH would have returned to neutrality. If, however, fresh nutrients were available, the pH could fall and rise again, the conditions probably present in plaque. Kleinberg and Jenkins (1964) found that the pH of plaque fell with the intake of carbohydrate and then returned again to pH 7,39 and eventually to pH 8,10. The pH of plaque, therefore, became alkaline. The observation by Mühleman and De Boever (1970) that old plaque produced less acid than three-day-old plaque suggests that the ultimate pH in the present experiment would be alkaline rather than neutral. The population studied, therefore, appears to be proteolytic.

143/...References/...

# CHAPTER III

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144/...contd/...

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# CHAPTER IV

## SUMMARY

The gingival sulcus microecology of three categories of people were studied. Samples of debris were removed from the gingival sulcus of ten male Bantu subjects with pockets of 1 - 2 mm, ten male Bantu subjects with pockets of 3 - 6 mm and twenty male Caucasian subjects with pockets of 1 - 2 mm. The samples were placed in cooked meat medium which was incubated at 37°C for 30 days. They were plated out daily from the cooked meat onto a variety of media. Organisms cultured were identified to the genus level. Changes in the pH and amino acids in the medium were studied in ten of the samples taken from the Bantu. Observations made in this study included the following:-

1. The anaerobic streptococci had the highest mean incidence in all the categories studied. They also had the highest survival index i.e. an index which is determined by adding together the product of the percentage incidence and the

146/...number/...

number of days' survival. Therefore, they had the greatest ability to survive in the present experiment. Because of their prominence, proteolytic nature and preference for a low oxidation reduction potential, they are probably the most active microorganisms in pockets.

2. The organisms in the present study were classified as:

(a) Dominants,
(b) Associates,
(c) Incidentals,

according to their position in the ecosystem.

(a) Dominants were defined as those organisms which were stable, had a high incidence and were able to persist in the medium. They included the aerobic streptococci, actinomyces, $\alpha$-haemolytic streptococci, mitis-type streptococci and the enterococci.

(b) Associates were those organisms which were not stable, or did not survive well and could be divided into 5 groups. The first group consisted of the veillonella, lactobacilli, $\gamma$-haemolytic streptococci and the sarcina all with a higher incidence in pockets of 3 - 6 mm than in pockets of 1 - 2 mm. These organisms were favoured by deep pockets. The veillonella had the greatest ability to survive and possibly had a greater influence on the population than the other

147/...organisms/...

organisms in this group.

The second group consisted of organisms with a higher survival index in pockets of 1 - 2 mm than in 3 - 6 mm. Their index was also higher in the Bantu than in the Caucasians. These organisms, therefore, were favoured by shallow pockets, and the environment of the Bantu. This group included the anaerobic streptococci, staphylococci, bacteroides, neisseria, selenomonas, fusobacteria, diplococci, clostridia, leptotrichia and the polysaccharide-producing streptococci. The bacteroides, anaerobic streptococci and neisseria were stimulated most by the environment of the Bantu. The staphylococci, fusobacteria and selenomonas were moderately affected. On the other hand, the diplococci, clostridia, leptotrichia and polysaccharide-producing streptococci were unaffected. It has been suggested that this group of organisms, which includes a number of pathogenic species, probably is under the control of the dominants. If this control is upset they could become invasive. The pathogenicity of these organisms and their tendency to invade when the resistance of the host is lowered, supports the idea that these organisms are controlled or suppressed by the dominants under normal conditions. The inhibition of these organisms in deep pockets also supports this suggestion.

148/...The third/...

The third group contained one organism, the corynebacteria which had a higher survival index in the Caucasians than in the Bantu. There was no significant difference between the indices in any of the three categories studied.

The fourth group consisted of the micrococci and the yeasts. They had a very low survival index. In fact, the micrococci were absent in the Bantu and the yeasts did not occur in the Bantu with pockets of 1 - 2 mm.

The fifth group had only one organism, the $\beta$-haemolytic streptococci with a survival index which was higher in the Caucasians than in the Bantu. These organisms, therefore, were inhibited by the environment of the Bantu and deep pockets.

(c) The incidentals were those organisms which were indifferent to the activities of the dominants or associates. They showed very little change in incidence and consisted of only one group of organisms, the Enterobacteriaceae. These organisms probably have no effect on the community in the oral cavity.

3. Organisms isolated from the Bantu subjects generally were able to survive better than organisms from the Caucasians.

149/...4. The/...

4. The bacteroides had a high survival index in the Bantu subjects and statistical analyses showed that there was a significant difference between their incidence in the two ethnic groups. This suggested that they benefited greatly from their association with the other organisms in the Bantu environment. Pocket depth in this environment did not influence their incidence significantly.

5. Taking the ability to survive into consideration, the anaerobic streptococci, aerobic streptococci, ∝-haemolytic streptococci, mitis-type streptococci and veillonella had relatively high survival indices, seemed to benefit from an association with each other and tended to inhibit the other organisms.

6. Succession (replacement of an initial population by a second population) was also studied. The initial population in the Caucasian subjects consisted of the anaerobic streptococci, aerobic streptococci, veillonella, ∝-haemolytic streptococci, polysaccharide-producing streptococci, and Diplococcus pneumoniae. In the Bantu this group included the anaerobic streptococci, ∝-haemolytic streptococci, aerobic streptococcocci, mitis-type streptococci, enterococci, polysaccharide-producing streptococci, sarcina and leptotrichia. With the growth of these organisms, the substrate

150/...changed/...

changed and their incidence declined. Then the second group of organisms increased. In the Caucasians this group included the actinomyces, neisseria, lactobacilli, micrococci, staphylococci, fusobacteria, bacteroides, ɣ-haemolytic streptococci, selenomonas, β-haemolytic streptococci and clostridia. This group was smaller in the Bantu and consisted of the veillonella, neisseria, bacteroides, fusobacteria, corynebacteria, ɣ-haemolytic streptococci, Diplococcus pneumoniae, staphylococci, actinomyces, lactobacilli and clostridia.

7. The amino acids were divided into three groups according to their change in incidence. The first group consisted of amino acids which showed little change and consisted of tyrosine, glutamic acid, alanine and proline. These amino acids possibly were not essential for the growth of the community. The second group showed a rise in incidence and included methionine, cysteine, lysine, glycine and threonine. Their increase in incidence possibly was due to the breakdown of proteins, with their release into the medium. They were not utilized and, therefore, do not seem to play an active role in the growth ofthe community. The third group showed a drop in incidence followed by a rise and included isoleucine, leucine, tryptophane, cystine, serine, aspartic acid, arginine and valine.

151/...This

This was the most active group and appeared to be utilized and then excreted.

8. Leucine and tryptophane showed the greatest drop in incidence initially and could be considered the most important amino acids for this community.

9. Serine and histidine were utilized but not excreted again and seemed to be incorporated into the microorganisms irreversibly.

10. The mean pH of the medium dropped from 7,04 to 6,70 within 24 hours. This probably was due to a sacchrolytic type of metabolism. After this period it fluctuated with a general upward trend until it reached a maximum pH of 8,29 on the twenty fourth day. After this the pH tended to drop but still remained above 7,0. This alkaline pH suggested that the majority of organisms which were active in the present study were in a proteolytic phase of metabolism. This was not unexpected in view of the fact that the cooked meat and broth, which formed the chief source of nutrients, were protein in nature.

11. In the present experiment the population tended to be proteolytic.

12. The rise in pH seems to be due to the deamination of amino acids rather than the production of

152/...ammonia/...

ammonia from peptone water because there was a large number of amino acids with a decrease in incidence in the present experiment.

153/...Tables/...

TABLE I

PERCENTAGE INCIDENCE OF MICROORGANISMS

GRAM POSITIVE STREPTOCOCCI

| Gram-positive streptococci | | days | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 + 2 | 3 + 4 | 5 + 6 | 7 + 8 | 9 + 10 | 11 + 12 | 13 + 14 | 15 + 16 | 17 + 18 | 19 + 20 | 21 + 22 | 23 + 24 | 25 + 26 | 27 + 28 | 29 + 30 |
| Anaerobic | A | 100 | 100 | 100 | 97,5 | 97,5 | 87,5 | 78,7 | 60 | 40 | 20 | 21,2 | 13,7 | 3,7 | 0 | 2,5 |
| | B | 100 | 100 | 100 | 100 | 90 | 92,5 | 77,5 | 65 | 50 | 27,5 | 32,5 | 27,5 | 7,5 | 0 | 5 |
| | C | 100 | 100 | 100 | 95 | 95 | 82,5 | 80 | 55 | 30 | 12,5 | 10 | | | | |
| Aerobic | A | 100 | 96,2 | 93,7 | 95 | 82,5 | 50 | 30 | 16,2 | 6,2 | 1,2 | | | | | |
| | B | 100 | 95 | 92,5 | 90 | 72,5 | 50 | 27,5 | 17,5 | 7,5 | 2,5 | | | | | |
| | C | 100 | 97,5 | 95 | 100 | 52,5 | 50 | 32,5 | 15 | 5 | | | | | | |
| α-Haemolytic | A | 81,2 | 65 | 67,5 | 68,7 | 61,2 | 37,5 | 22,5 | 13,7 | 1,2 | | | | | | |
| | B | 92,5 | 62,5 | 70 | 75 | 57,5 | 35 | 25 | 12,5 | 2,5 | | | | | | |
| | C | 70 | 67,5 | 65 | 62,5 | 65 | 40 | 20 | 15 | | | | | | | |
| Mitis-type | A | 88,7 | 76,2 | 56,2 | 61,2 | 33,7 | 5 | | | | | | | | | |
| | B | 87,5 | 55 | 30 | 42,5 | 27,5 | | | | | | | | | | |
| | C | 90 | 97,5 | 82,5 | 80 | 40 | 10 | | | | | | | | | |
| Enterococci | A | 31,2 | 56,2 | 37,5 | 30 | 18,7 | 7,5 | 0 | 1,2 | | | | | | | |
| | B | 70 | 70 | 35 | 37,5 | 15 | 2,5 | | | | | | | | | |
| | C | 32,5 | 42,5 | 30 | 22,5 | 22,5 | 12,5 | 0 | 2,5 | | | | | | | |
| Pyo- genes | A | 60 | 3,7 | | | | | | | | | | | | | |
| | B | 60 | 10 | | | | | | | | | | | | | |
| | C | 60 | 7,5 | | | | | | | | | | | | | |
| Diplococcus pneumoniae | A | 33,7 | 18,7 | 10,2 | 21,2 | 8,7 | 2,5 | 2,5 | 1,2 | | | | | | | |
| | B | 20 | 15 | 32,5 | 30 | 17,5 | 5 | 5 | 2,5 | | | | | | | |
| | C | 47,5 | 22,5 | 0 | 12,5 | | | | | | | | | | | |
| β-Haemolytic | A | 10 | 21,2 | 28,2 | 33,7 | 32,5 | 20 | 2,5 | 1,2 | 3,7 | 2,5 | | | | | |
| | B | 10 | 37,5 | 42,5 | 47,5 | 50 | 37,5 | 5 | 2,5 | 7,5 | 5 | | | | | |
| | C | 10 | 5 | 0 | 20 | 15 | 2,5 | | | | | | | | | |
| γ-Haemolytic | A | 3,7 | 5 | 3,7 | 3 | 0 | 6,2 | 1,2 | 2,5 | 2,5 | | | | | | |
| | B | 0 | 0 | 2,5 | 5 | 2,5 | 7,5 | 0 | 2,5 | 2,5 | | | | | | |
| | C | 7,5 | 10 | 5 | 5 | 15 | 5 | 2,5 | 2,5 | 2,5 | | | | | | |

A = mean incidence for both groups

B = mean incidence of microorganisms for Bantu subjects

C = mean incidence of microorganisms for Caucasian subjects

TABLE II PERCENTAGE INCIDENCE OF MICROORGANISMS

REMAINDER [illegible] POSITIVE MICROORGANISMS

| Gram-positive microorganisms | | d a y e | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 + 2 | 3 + 4 | 5 + n | 7 + 8 | 9 + 10 | 11 + 12 | 13 + 14 | 15 + 16 | 17 + 18 | 19 + 20 | 21 + 22 | 23 + 24 | 25 + 26 | 27 + 28 | 29 + 30 |
| Staphylococci | A | 20 | 25 | 28,7 | 25 | 26,2 | 22,5 | 20 | 16,2 | 16,2 | 17,5 | 16,2 | 10 | 12,5 | 12,5 | 12,5 |
| | B | 15 | 17,5 | 22,5 | 22,5 | 32,5 | 30 | 30 | 22,5 | 22,5 | 25 | 25 | 20 | 25 | 25 | 25 |
| | C | 25 | 32,5 | 35 | 27,5 | 20 | 15 | 10 | 10 | 10 | 10 | 7,5 | | | | |
| Actinomyces | A | 12,5 | 42,5 | 26,2 | 27,5 | 45 | 38,7 | 27,5 | 13,7 | 20 | 1,2 | 8,7 | 8,7 | 2,5 | 2,5 | 1,2 |
| | B | 7,5 | 35 | 15 | 17,5 | 47,5 | 42,5 | 32,5 | 12,5 | 32,5 | 0 | 15 | 17,5 | 5 | 5 | 2,5 |
| | C | 17,5 | 50 | 37,5 | 37,5 | 42,5 | 35 | 22,5 | 15 | 7,5 | 2,5 | 2,5 | | | | |
| Lactobacilli | A | 27,5 | 11,2 | 13,7 | 10 | 25 | 23,7 | 7,5 | 8,7 | 0 | 0 | 1,2 | 1,2 | | | |
| | B | 20 | 25 | 12,5 | 17,5 | 32,5 | 41,5 | 12,5 | 17,5 | 0 | 0 | 2,5 | 2,5 | | | |
| | C | 35 | 37,5 | 15 | 2,5 | 17,5 | 5 | 2,5 | | | | | | | | |
| Corynebacteria | A | 27,5 | 38,8 | 8,7 | 12,2 | 7,5 | 5 | | | | | | | | | |
| | B | 12,5 | 17,2 | 12,5 | 12,5 | 5 | | | | | | | | | | |
| | C | 42,5 | 60 | 5 | 12 | 10 | 10 | | | | | | | | | |
| Sarcina | A | 12,5 | 7,5 | 3,7 | | | | | | | | | | | | |
| | B | 22,5 | 12,5 | 7,5 | | | | | | | | | | | | |
| | C | 2,5 | 2,5 | | | | | | | | | | | | | |
| Leptotrichia | A | 8,7 | 6,2 | 8,7 | 3,7 | 2,5 | 2,5 | 1,2 | | | | | | | | |
| | B | 15 | 5 | 12,5 | 5 | 2,5 | 5 | 2,5 | | | | | | | | |
| | C | 2,5 | 7,5 | 5 | 2,5 | 2,5 | | | | | | | | | | |
| Micrococci | A | 3,7 | 5 | 1,2 | 1,2 | 0 | 0 | 0 | 0 | 0 | 1,2 | | | | | |
| | B | | | | | | | | | | | | | | | |
| | C | 7,5 | 10 | 2,5 | 2,5 | 0 | 0 | 0 | 0 | 0 | 2,5 | | | | | |
| Clostridia | A | 1,2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,5 | 1,2 | 3,7 | 1,2 | 0 | 1,2 |
| | B | 2,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,5 | 7,5 | 2,5 | | |
| | C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 2,5 |
| Yeasts | A | 2,5 | 0 | 1,2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1,2 | | | | |
| | B | 2,5 | | | | | | | | | | | | | | |
| | C | 2,5 | 0 | 2,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,5 | | | | |

A = mean incidence for both groups

B = mean incidence of microorganisms for Bantu subjects

C = mean incidence of microorganisms for Caucasian subjects

TABLE III PERCENTAGE INCIDENCE OF MICROORGANISMS

GRAM-NEGATIVE MICROORGANISMS

| Gram-negative microorganisms | | day | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 + 2 | 3 + 4 | 5 + 6 | 7 + 8 | 9 + 10 | 11 + 12 | 13 + 14 | 15 + 16 | 17 + 18 | 19 + 20 | 21 + 22 | 23 + 24 | 25 + 26 | 27 + 28 | 29 + 30 |
| Veillonella | A | 95 | 87,5 | 81,2 | 68,7 | 43,7 | 33,7 | 21,2 | 11,2 | 3,5 | 0 | 3,7 | 6,2 | 0 | 0 | 1,2 |
| | B | 92,5 | 95 | 87,5 | 77,5 | 60 | 50 | 32,5 | 15 | 2,5 | 0 | 7,5 | 12,5 | 0 | 0 | 2,5 |
| | C | 97,5 | 80 | 75 | 60 | 27,5 | 17,5 | 10 | 7,5 | 2,5 | | | | | | |
| Neisseria | A | 62,5 | 66,2 | 36,2 | 26,2 | 8,7 | 2,5 | 0 | 1,2 | | | | | | | |
| | B | 80 | 85 | 52,5 | 37,5 | 10 | 5 | | | | | | | | | |
| | C | 45 | 47,5 | 20 | 15 | 7,5 | 0 | 0 | 2 5 | | | | | | | |
| Bacteroides | A | 29 | 37,7 | 25 | 23 | 21,5 | 21,7 | 5 | 2,5 | 1,2 | 0 | 1,2 | 2,5 | | | |
| | B | 40 | 72,5 | 40 | 40 | 40 | 42,5 | 10 | 5 | 2,5 | 0 | 2,5 | 5 | | | |
| | C | 0 | 3 | 10 | 6 | 3 | 1 | | | | | | | | | |
| Fusobacteria | A | 23,7 | 22,5 | 30 | 21,2 | 15 | 12,5 | 2,5 | 2,5 | | | | | | | |
| | B | 27,5 | 30 | 25 | 20 | 15 | 17,5 | 5 | 5 | | | | | | | |
| | C | 20 | 15 | 35 | 22,5 | 15 | 7,5 | | | | | | | | | |
| Enterobacteria sp. | A | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 7 | 2,5 | 2,5 | 2,5 | 2,5 |
| | B | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | C | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 2,5 | | | | |
| Selenomonas | A | 3,7 | 1,7 | 2,5 | 7,5 | 8,7 | 6,2 | 1,2 | 1,2 | 0 | 0 | 1,2 | 1,2 | | | |
| | B | 7,5 | 5 | 5 | 10 | 15 | 12,5 | 2,5 | 2,5 | 0 | 0 | 2,5 | 2,5 | | | |
| | C | 0 | 2,5 | 0 | 5 | 2,5 | | | | | | | | | | |

A - mean incidence for both groups

B - mean incidence of microorganisms for Bantu subjects

C - mean incidence of microorganisms for Caucasian subjects

TABLE IV MEAN INCIDENCE OF MICROORGANISMS (SANTO AND CALLASIANS)

| Microorganisms | days 1 + 2 | 3 + 4 | 5 + 6 | 7 + 8 | 9 + 10 | 11 + 12 | 13 + 14 | 15 + 16 | 17 + 18 | 19 + 20 | 21 + 22 | 23 + 24 | 25 + 26 | 27 + 28 | 29 + 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP I | Incidence over 50 per cent | | | | | | | | | | | | | | |
| Anaerobic streptococci | 100 | 100 | 100 | 97,5 | 97,5 | 87,5 | 78,7 | 60 | 40 | 20 | 21,2 | 17,7 | 9,7 | 0 | 2,5 |
| Aerobic streptococci | 100 | 96,2 | 91,7 | 95 | 87,5 | 50 | 30 | 16,2 | 6,2 | 1,2 | | | | | |
| $\alpha$ -Haemolytic streptococci | 81,2 | 86 | 87,5 | 66,7 | 61,2 | 37,5 | 22,5 | 13,7 | 1,2 | | | | | | |
| Veillonella | 75 | 81,5 | 81,2 | 68,7 | 43,7 | 33,7 | 21,2 | 11,2 | 2,5 | 0 | 3,7 | 6,2 | 0 | 0 | 1,2 |
| Mitis-type streptococci | 86,7 | 78,2 | 56,2 | 61,2 | 31,7 | 5 | | | | | | | | | |
| Neisseria | 62,5 | 60,2 | 38,2 | 26,2 | 8,7 | 2,5 | 0 | 1,2 | | | | | | | |
| Enterococci | 51,2 | 56,2 | 41,5 | 30 | 18,7 | 7,5 | 0 | 1,2 | | | | | | | |
| Polysaccharide-producing streptococci | 90 | 8,7 | | | | | | | | | | | | | |
| GROUP II | Incidence 20 - 49 per cent | | | | | | | | | | | | | | |
| Actinomyces | 12,5 | 42,5 | 26,2 | 27,5 | 45 | 38,7 | 27,5 | 13,7 | 20 | 1,2 | 8,7 | 8,7 | 2,5 | 2,5 | 1,2 |
| Corynebacteria | 27,5 | 38,6 | 8,7 | 12,2 | 7,5 | 5 | | | | | | | | | |
| Bacteroides | 20 | 37,7 | 25 | 23 | 21,5 | 21,7 | 5 | 2,5 | 1,2 | 0 | 2,5 | 2,5 | | | |
| $\delta$ -Haemolytic streptococci | 10 | 21,2 | 26,2 | 23,7 | 32,5 | 20 | 2,5 | 1,2 | 5,7 | 2,5 | | | | | |
| Diplococci | 33,7 | 18,7 | 16,2 | 21,2 | 8,7 | 2,5 | 2,5 | 1,2 | | | | | | | |
| Lactobacilli | 27,5 | 31,2 | 13,7 | 10 | 25 | 23,7 | 7,5 | 8,7 | 0 | 0 | 1,2 | 1,2 | | | |
| Fusobacteria | 23,7 | 22,5 | 30 | 21,2 | 15 | 12,5 | 7,5 | 2,5 | | | | | | | |
| Staphylococci | 20 | 25 | 25,7 | 25 | 25,7 | 22,5 | 20 | 16,2 | 16,2 | 17,5 | 16,2 | 10 | 12,5 | 12,5 | 12,5 |
| GROUP III | Incidence less than 20 per cent | | | | | | | | | | | | | | |
| Sarcina | 12,5 | 7,5 | 3,7 | | | | | | | | | | | | |
| Leptotrichia | 8,7 | 6,2 | 8,7 | 3,7 | 2,5 | 7,5 | 1,2 | | | | | | | | |
| Enterobacteriaceae | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3,7 | 2,5 | 2,5 | 2,5 | 2,5 |
| Selenomonas | 3,7 | 3,7 | 2,5 | 7,5 | 8,7 | 6,2 | 1,2 | 1,2 | 0 | 0 | 1,2 | 1,2 | | | |
| $\beta$ -Haemolytic streptococci | 3,7 | 5 | 3,7 | 5 | 8,7 | 6,2 | 1,2 | 2,5 | 2,5 | | | | | | |
| Micrococci | 3,7 | 5 | 1,2 | 1,2 | 0 | 0 | 0 | 0 | 0 | 1,2 | | | | | |
| Yeasts | 2,5 | 0 | 1,2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1,2 | | | | |
| Clostridia | 1,2 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,5 | 1,2 | 3,7 | 1,2 | 0 | 1,2 |

TABLE V MEAN INCIDENCE OF MICROORGANISMS FROM MANIP

| Microorganisms | Day 1 + 2 | 3 + 4 | 5 + 6 | 7 + 8 | 9 + 10 | 11 + 12 | 13 + 14 | 15 + 16 | 17 + 18 | 19 + 20 | 21 + 22 | 23 + 24 | 25 + 26 | 27 + 28 | 29 + 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP I | incidence over 50 per cent | | | | | | | | | | | | | | |
| Anaerobic streptococci | 100 | 100 | 100 | 100 | 100 | 92,5 | 77,5 | 65 | 50 | 27,5 | 32,5 | 77,5 | 7,5 | 0 | 5 |
| Aerobic streptococci | 100 | 95 | 92,5 | 90 | 72,5 | 50 | 27,5 | 17,5 | 7,5 | 2,5 | | | | | |
| α-Haemolytic streptococci | 92,5 | 62,5 | 70 | 75 | 57,5 | 35 | 25 | 12,5 | 2,5 | | | | | | |
| *Veillonella* | 92,5 | 95 | 87,5 | 77,5 | 60 | 50 | 32,5 | 15 | 2,5 | 0 | 7,5 | 12,5 | 0 | 0 | 2,5 |
| Mitis-type streptococci | 87,5 | 55 | 30 | 42,5 | 27,5 | | | | | | | | | | |
| Neisseria | 80 | 85 | 52,5 | 37,5 | 10 | 5 | | | | | | | | | |
| Enterococci | 70 | 70 | 35 | 37,5 | 15 | 2,5 | | | | | | | | | |
| Polysaccharide-producing streptococci | 60 | 10 | | | | | | | | | | | | | |
| Bacteroides | 40 | 72,5 | 40 | 40 | 40 | 42,5 | 10 | 5 | 2,5 | 0 | 2,5 | 5 | | | |
| γ-Haemolytic streptococci | 10 | 37,5 | 52,5 | 47,5 | 50 | 37,5 | 5 | 2,5 | 7,5 | 5 | | | | | |
| GROUP II | incidence 20 - 49 per cent | | | | | | | | | | | | | | |
| Staphylococci | 15 | 17,5 | 22,5 | 22,5 | 32,5 | 30 | 30 | 22,5 | 22,5 | 25 | 25 | 20 | 25 | 25 | 25 |
| Actinomyces | 7,5 | 35 | 15 | 17,5 | 47,5 | 42 | 32,5 | 12,5 | 32,5 | 0 | 15 | 17,5 | 5 | 5 | 2,5 |
| Lactobacilli | 20 | 25 | 12,5 | 17,5 | 32,5 | 42,5 | 12,5 | 17,5 | 0 | 0 | 2,5 | 2,5 | | | |
| Diplococcus pneumoniae | 20 | 15 | 32,5 | 20 | 17,5 | 5 | 5 | 2,5 | | | | | | | |
| Fusobacteria | 27,5 | 30 | 25 | 20 | 15 | 12,5 | 5 | 5 | | | | | | | |
| Bacillus | 22,5 | 12,5 | 7,5 | | | | | | | | | | | | |
| GROUP III | incidence less than 20 per cent | | | | | | | | | | | | | | |
| Corynebacteria | 12,5 | 17,2 | 12,5 | 12,5 | 5 | | | | | | | | | | |
| Leptotrichia | 15 | 5 | 12,5 | 5 | 2,5 | 5 | 2,5 | | | | | | | | |
| Selenomonas | 7,5 | 5 | 5 | 10 | 15 | 12,5 | 2,5 | 2,5 | 0 | 0 | 2,5 | 2,5 | | | |
| β-Haemolytic streptococci | 0 | 0 | 2,5 | 5 | 2,5 | 7,5 | 0 | 2,5 | 2,5 | | | | | | |
| Clostridia | 2,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,5 | 7,5 | 2,5 | | |
| Enterobacteriaceae | 5 | 5 | 5 | 5 | 5 | 2 | 2 | 5 | 5 | 5 | 5 | 5 | 5 | 2 | 5 |
| Yeasts | 2,5 | | | | | | | | | | | | | | |

TABLE VI MEAN INCIDENCE OF MICROORGANISMS FROM CAUCASIANS

| Microorganism | day 1 + 2 | 3 + 4 | 5 + 6 | 7 + 8 | 9 + 10 | 11 + 12 | 13 + 14 | 15 + 16 | 17 + 18 | 19 + 20 | 21 + 22 | 23 + 24 | 25 + 26 | 27 + 28 | 29 + 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP I | incidence over 50 per cent | | | | | | | | | | | | | | |
| Anaerobic streptococci | 100 | 100 | 100 | 95 | 95 | 82,5 | 80 | 55 | 40 | 12,5 | 10 | | | | |
| Aerobic streptococci | 100 | 97,5 | 95 | 100 | 92,5 | 90 | 32,5 | 15 | 5 | | | | | | |
| Veillonella | 97,5 | 80 | 75 | 60 | 27,5 | 17,5 | 10 | 7,5 | 2,5 | | | | | | |
| Mitis-type streptococci | 90 | 97,5 | 82,5 | 80 | 40 | 10 | | | | | | | | | |
| α-Haemolytic streptococci | 70 | 67,5 | 65 | 62,5 | 65 | 40 | 20 | 15 | | | | | | | |
| Polysaccharide-producing streptococci | 60 | 7,5 | | | | | | | | | | | | | |
| Actinomyces | 17,5 | 50 | 37,5 | 37,5 | 42,5 | 55 | 22,5 | 15 | 7,5 | 2,5 | 2,5 | | | | |
| GROUP II | incidence 20 - 49 per cent | | | | | | | | | | | | | | |
| Neisseria | 45 | 47,5 | 20 | 15 | 7,5 | 0 | 0 | 2,5 | | | | | | | |
| Diplococcus pneumoniae | 47,5 | 22,5 | 0 | 12,5 | | | | | | | | | | | |
| Enterococci | 32,5 | 42,5 | 30 | 22,5 | 22,5 | 12,5 | 0 | 2,5 | | | | | | | |
| Corynobacteria | 42,5 | 60 | 5 | 12 | 10 | 10 | | | | | | | | | |
| Lactobacilli | 35 | 37,5 | 15 | 2,5 | 17,5 | 5 | 2,5 | | | | | | | | |
| Staphylococci | 25 | 32,5 | 35 | 27,5 | 20 | 15 | 10 | 10 | 10 | 10 | 7,5 | | | | |
| Fusobacteria | 20 | 15 | 35 | 22,5 | 15 | 7,5 | | | | | | | | | |
| γ-Haemolytic streptococci | 10 | 5 | 0 | 20 | 15 | 2,5 | | | | | | | | | |
| GROUP III | incidence less than 20 per cent | | | | | | | | | | | | | | |
| β - Haemolytic streptococci | 7,5 | 10 | 5 | 5 | 15 | 5 | 2,5 | 2,5 | 2,5 | | | | | | |
| Micrococci | 7,5 | 10 | 2,5 | 2,5 | 0 | 0 | 0 | 0 | 0 | 2,5 | | | | | |
| Bacteroides | 0 | 5 | 10 | 6 | 5 | 1 | | | | | | | | | |
| Selenomonas | 0 | 2,5 | 0 | 5 | 2,5 | | | | | | | | | | |
| Leptotrichia | 2,5 | 7,5 | 5 | 2,5 | 2,5 | | | | | | | | | | |
| Enterobacteriaceae | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 2,5 | | | | |
| Clostridia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 2,5 |
| Sarcina | 2,5 | 2,5 | | | | | | | | | | | | | |
| Yeasts | 2,5 | 0 | 2,5 | | | | | | | | 2,5 | | | | |

TABLE VII COMBINED SURVIVAL INDICES

| | days | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Microorganisms | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | TOTAL |
| Anaerobic streptococci | 200 | 400 | 600 | 760 | 975 | 1050 | 1101,8 | 960 | 720 | 400 | 466,4 | 328,8 | 96,2 | 0 | 75 | 8153,2 |
| Aerobic streptococci | 200 | 384,8 | 562,2 | 740 | 825 | 600 | 420 | 259,2 | 111,6 | 76 | | | | | | 4146,8 |
| Staphylococci | 40 | 100 | 172,2 | 200 | 262 | 270 | 280 | 259,2 | 231,6 | 350 | 356,4 | 240 | 325 | 350 | 375 | 3871,4 |
| Veillonella | 190 | 350 | 487,2 | 549,6 | 437 | 464,4 | 296,8 | 179,2 | 45 | 0 | 81,4 | 148,8 | 0 | 0 | 36 | 3205,4 |
| Actinomyces | 25 | 170 | 157,2 | 220 | 450 | 464,4 | 385 | 219,2 | 360 | 25 | 191,4 | 208,8 | 65 | 70 | 36 | [illegible] |
| α-Haemolytic streptococci | 162,4 | 260 | 405 | 549,6 | 612 | 450 | 315 | 219,2 | 21,6 | | | | | | | 2994,8 |
| γ-Haemolytic streptococci | 20 | 84,8 | 157,2 | 269,6 | 327 | 390 | 280 | 40 | 21,6 | 74 | 55 | | | | | 1719,2 |
| Mitis-type streptococci | 177,4 | 304,8 | 337,2 | 489,6 | 337 | 60 | | | | | | | | | | 17[illegible] |
| Bacteroides | 40 | 150,8 | 150 | 184 | 215 | 260,4 | 70 | 40 | 21,6 | 0 | 55 | 60 | | | | 12[illegible] |
| Lactobacilli | 55 | 124,8 | 82,2 | 80 | 250 | 284,6 | 105 | 139,2 | 0 | 0 | 26,4 | 28,8 | | | | 1[illegible],8 |
| Enterococci | 102,4 | 224,8 | 195 | 240 | 187 | 90 | 0 | 19,2 | | | | | | | | 1058,4 |
| Neisseria | 125 | 264,8 | 217,2 | 209,6 | 87 | 30 | 0 | 19,2 | | | | | | | | 952,8 |
| Entero-bacteriaceae | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 81,4 | 60 | 65 | 70 | 75 | 901,4 |
| Fusobacteria | 47,4 | 90 | 180 | 169,6 | 150 | 150 | 35 | 40 | | | | | | | | 862 |
| Diplococci | 6[illegible],4 | 74,8 | 97,2 | 169,6 | 87 | 30 | 35 | 19,2 | | | | | | | | 580,2 |
| Corynebacteria | 55 | 154,4 | 52,2 | 97,6 | 75 | 60 | | | | | | | | | | 494,2 |
| β-Haemolytic streptococci | 7,4 | 20 | 22,2 | 40 | 87 | 74,4 | 16,8 | 40 | 45 | | | | | | | 352,8 |
| Selenomonas | 7,4 | 14,8 | 15 | 60 | 87 | 74,4 | 16,8 | 19,2 | 0 | 0 | 26,4 | 28,8 | | | | 349,8 |
| Clostridia | 2,4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 26,4 | 88,8 | 31,2 | 0 | | 2[illegible],6 |
| Leptotrichia | 17,4 | 24,8 | 52,2 | 29,6 | 25 | 30 | 16,8 | | | | | | | | | 195,8 |
| Polysaccharide-producing streptococci | 120 | 34,8 | | | | | | | | | | | | | | 154,8 |
| Sarcina | 25 | 30 | 22,2 | | | | | | | | | | | | | 77,2 |
| Micrococci | 7,4 | 20 | 7,2 | 9,6 | 0 | 0 | 0 | 0 | 0 | 24 | | | | | | 68,2 |
| Yeasts | 5 | 0 | 7,2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26,4 | | | | | 38,6 |

TABLE VIII

SERVIVAL INDICES FOR MICROORGANISMS FROM BANTU

| Microorganisms | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Anaerobic streptococci | 200 | 500 | 600 | 800 | 1000 | 1110 | 1085 | 1040 | 900 | 550 | 715 | 660 | 195 | 0 | 150 | 9605 |
| Staphylococci | 30 | 70 | 135 | 190 | 325 | 360 | 470 | 360 | 405 | 560 | 550 | 480 | 650 | 700 | 750 | 5915 |
| Veillonella | 185 | 580 | 525 | 620 | 600 | 600 | 455 | 240 | 45 | 0 | 165 | 300 | 0 | 0 | 75 | 4190 |
| Actinomyces | 15 | 140 | 90 | 140 | 475 | 510 | 455 | 200 | 585 | 0 | 330 | 470 | 130 | 140 | 75 | 3765 |
| Aerobic streptococci | 200 | 380 | 555 | 770 | 725 | 600 | 385 | 280 | 135 | 50 | | | | | | 4030 |
| $\alpha$ -Haemolytic streptococci | 185 | 290 | 420 | 600 | 575 | 620 | 350 | 200 | 45 | | | | | | | 3635 |
| Bacteroides | 80 | 290 | 240 | 320 | 400 | 310 | 140 | 80 | 45 | 0 | 55 | 120 | | | | 2280 |
| $\gamma$ -Haemolytic streptococci | 20 | 150 | 315 | 380 | 500 | 450 | 70 | 40 | 135 | 100 | | | | | | 2160 |
| Lactobacilli | 50 | 100 | 75 | 140 | 325 | 510 | 175 | 280 | 0 | 0 | 55 | 60 | | | | 1760 |
| Neisseria | 100 | 160 | 315 | 300 | 100 | 80 | | | | | | | | | | 1255 |
| Lateve-bacteriaceae | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 1200 |
| Mitis-type streptococci | 175 | 250 | 180 | 340 | 295 | | | | | | | | | | | 1190 |
| Enterococci | 140 | 280 | 210 | 400 | 150 | 30 | | | | | | | | | | 1110 |
| Fusobacteria | 95 | 120 | 150 | 160 | 190 | 210 | 70 | 80 | | | | | | | | 995 |
| Diplococcus pneumoniae | 40 | 80 | 195 | 240 | 175 | 60 | 70 | 40 | | | | | | | | 880 |
| Selenomonas | 10 | 20 | 30 | 80 | 150 | 150 | 35 | 40 | 0 | 0 | 55 | 60 | | | | 635 |
| Corynobacteria | 10 | 30 | 75 | 100 | 50 | | | | | | | | | | | 319 |
| Clostridia | | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 180 | 65 | | | 305 |
| Leptotrichia | | | | 0 | 25 | 60 | 35 | | | | | | | | | 120 |
| $\beta$ -Haemolytic streptococci | 0 | | 5 | 40 | 25 | 90 | 0 | 40 | 45 | | | | | | | 255 |
| Polysaccharide-producing streptococci | 120 | 40 | | | | | | | | | | | | | | 160 |
| Sarcina | 15 | 90 | 45 | | | | | | | | | | | | | 150 |
| Yeasts | 5 | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | TOTAL | 45,244 |

TABLE IX SURVIVAL INDICES FOR MICROORGANISMS

FROM CAUCASIANS

| Microorganisms | d a y s | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | TOTAL |
| Anaerobic streptococci | 200 | 400 | 600 | 760 | 950 | 990 | 1120 | 880 | 540 | 250 | 220 | | | | | 6,910 |
| Aerobic streptococci | 200 | 390 | 570 | 800 | 925 | 600 | 455 | 240 | 90 | | | | | | | 4,270 |
| α-Haemolytic streptococci | 140 | 270 | 380 | 500 | 450 | 480 | 280 | 240 | | | | | | | | 2,950 |
| Actinomyces | 35 | 200 | 225 | 300 | 425 | 420 | 315 | 240 | 135 | 50 | 55 | | | | | 2,400 |
| Veillonella | 195 | 320 | 450 | 480 | 275 | 210 | 140 | 120 | 45 | | | | | | | 2,235 |
| Mitis-type streptococci | 180 | 390 | 495 | 640 | 400 | 170 | | | | | | | | | | 2,225 |
| Staphylococci | 50 | 130 | 210 | 220 | 200 | 180 | 140 | 160 | 180 | 200 | 165 | | | | | 1,835 |
| Enterococci | 65 | 170 | 180 | 180 | 225 | 150 | 0 | 40 | | | | | | | | 1,010 |
| Fusobacteria | 40 | 60 | 210 | 180 | 150 | 90 | | | | | | | | | | 730 |
| Corynebacteria | 85 | 240 | 30 | 90 | 100 | 120 | | | | | | | | | | 671 |
| Neisseria | 90 | 190 | 120 | 120 | 75 | 0 | 0 | 40 | | | | | | | | 635 |
| Entero-bacteriaceae | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 55 | | | | | 605 |
| Lactobacilli | 70 | 150 | 90 | 20 | 175 | 60 | 35 | | | | | | | | | 600 |
| β-Haemolytic streptococci | 15 | 40 | 30 | 40 | 150 | 60 | 35 | 40 | 45 | | | | | | | 455 |
| γ-Haemolytic streptococci | 20 | 20 | 0 | 160 | [illegible] | 30 | | | | | | | | | | 380 |
| Diplococcus pneumoniae | 95 | 90 | 0 | 100 | | | | | | | | | | | | 285 |
| Clostridia | | | | | | | | | | 100 | | | | | 75 | 175 |
| Bacteroides | 0 | 12 | 60 | 48 | 30 | 12 | | | | | | | | | | 162 |
| Polysaccharide-producing streptococci | 120 | 30 | | | | | | | | | | | | | | 150 |
| Micrococci | 15 | 40 | 15 | 20 | 0 | 0 | 0 | 0 | 0 | 50 | | | | | | 140 |
| Leptotrichia | 5 | 30 | 30 | 20 | 25 | | | | | | | | | | | 110 |
| Selenomonas | 0 | 10 | 0 | 40 | 25 | | | | | | | | | | | 75 |
| Yeasts | 5 | 0 | 15 | | | | | | | 55 | | | | | | 75 |
| Sarcina | 5 | 10 | | | | | | | | | | | | | | 15 |
| | | | | | | | | | | | | | | | TOTAL | 29,098 |

TABLE X

SURVIVAL INDICES FOR MICROORGANISMS ISOLATED FROM BANTU POCKETS OF 1 - 2 mm

| Microorganisms | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Anaerobic streptococci | 200 | 400 | 800 | 800 | 1000 | 1140 | 1190 | 1120 | 990 | 800 | 1100 | 720 | 190 | 0 | 150 | 10,600 |
| Staphylococci | 30 | 60 | 120 | 280 | 450 | 420 | 420 | 400 | 360 | 500 | 660 | 720 | 910 | 840 | 900 | 7,070 |
| Anaerobic streptococci | 200 | 400 | 600 | 800 | 850 | 720 | 490 | 400 | 270 | 100 | | | | | | 4,800 |
| Actinomyces | 10 | 180 | 120 | 140 | 150 | 420 | 420 | 80 | 630 | 0 | 350 | 490 | 130 | 280 | 150 | 4,150 |
| Veillonella | 190 | 400 | 540 | 640 | 700 | 540 | 350 | 240 | 0 | 0 | 0 | 240 | | | | 3,840 |
| $\alpha$ -Haemolytic streptococci | 170 | 260 | 480 | 750 | 850 | 660 | 490 | 320 | 90 | | | | | | | 3,840 |
| Bacteroides | 60 | 300 | 270 | 320 | 350 | 560 | 70 | 160 | 90 | 0 | 110 | 240 | | | | 2,510 |
| $\gamma$ -Haemolytic streptococci | 30 | 140 | 300 | 280 | 450 | 360 | 0 | 0 | 90 | 200 | | | | | | 1,650 |
| Neisseria | 180 | 360 | 360 | 360 | 140 | 60 | | | | | | | | | | 1,470 |
| Lactobacilli | 30 | 80 | 30 | 80 | 250 | 420 | 210 | 320 | | | | | | | | 1,420 |
| Fusobacteria | 60 | 140 | 210 | 280 | 150 | 300 | 140 | 80 | | | | | | | | 1,360 |
| Mitis-type streptococci | 190 | 260 | 270 | 360 | 250 | | | | | | | | | | | 1,330 |
| Enterococci | 170 | 340 | 260 | 330 | 140 | 60 | | | | | | | | | | 1,280 |
| Diplococcus pneumoniae | 40 | 60 | 210 | 240 | 150 | 120 | 140 | 80 | | | | | | | | 1,040 |
| Selenomonas | 20 | 20 | 30 | 0 | 50 | 300 | 0 | 80 | 0 | 0 | 110 | | | | | 610 |
| Clostridia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 110 | 240 | 130 | | | 480 |
| Corynebacteria | 30 | 80 | 120 | 120 | 50 | | | | | | | | | | | 400 |
| $\beta$ -Haemolytic streptococci | 0 | 0 | 90 | 80 | 0 | 120 | 0 | 80 | 90 | | | | | | | 400 |
| Leptotrichia | 30 | 20 | 90 | 80 | 50 | 60 | | | | | | | | | | 330 |
| Polysaccharide-producing streptococci | 120 | 80 | | | | | | | | | | | | | | 140 |
| Sarcina | 50 | 40 | 30 | | | | | | | | | | | | | 120 |
| | | | | | | | | | | | | | | | TOTAL | 48,920 |

TABLE XI SURVIVAL INDICES FOR MICROORGANISMS ISOLATED FROM BANU POCKETS OF 3 - 6 mm

| | d | | | | | a | | | y | | | p | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Microorganisms | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | TOTAL |
| Anaerobic streptococci | 200 | 400 | 600 | 800 | 1000 | 1080 | 980 | 960 | 810 | 300 | 330 | 600 | 0 | 0 | 150 | 8,210 |
| Staphylococci | 30 | 80 | 150 | 20 | 200 | 300 | 420 | 320 | 450 | 500 | 440 | 240 | 390 | 560 | 600 | 4,760 |
| Veillonella | 180 | 360 | 510 | 600 | 900 | 660 | 560 | 240 | 90 | 0 | 330 | 360 | | | | 4,390 |
| Actinomyces | 20 | 100 | 60 | 120 | 400 | 600 | 490 | 320 | 540 | 0 | 110 | 360 | 130 | | | 3,450 |
| Aerobic streptococci | 200 | 360 | 510 | 640 | 600 | 480 | 280 | 160 | | | | | | | | 3,230 |
| $\gamma$ -Haemolytic streptococci | 10 | 160 | 330 | 480 | 650 | 540 | 140 | 80 | 180 | 100 | | | | | | 2,670 |
| Entero bacteriaceae | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 2,400 |
| $\alpha$ -Haemolytic streptococci | 200 | 240 | 360 | 480 | 900 | 180 | 210 | 80 | | | | | | | | 2,250 |
| Lactobacilli | 50 | 120 | 120 | 200 | 400 | 600 | 140 | 240 | 0 | 0 | 110 | 120 | | | | 2,100 |
| Bacteroides | 100 | 280 | 210 | 320 | 450 | 480 | 210 | | | | | | | | | 2,050 |
| Neisseria | 140 | 320 | 270 | 240 | 50 | 60 | | | | | | | | | | 1,080 |
| Mitis-type streptococci | 160 | 180 | 90 | 320 | 300 | | | | | | | | | | | 1,050 |
| Enterococci | 110 | 220 | 180 | 280 | 150 | | | | | | | | | | | 940 |
| Diplococcus pneumoniae | 40 | 60 | 180 | 240 | 200 | | | | | | | | | | | 720 |
| Pseudobacteria | 50 | 100 | 90 | 40 | 150 | 120 | 0 | 80 | | | | | | | | 630 |
| Selenomonas | 10 | 20 | 30 | 160 | 250 | 0 | 70 | | | | | | | | | 540 |
| Corynebacteria | 20 | 60 | 30 | 80 | 50 | | | | | | | | | | | 240 |
| Leptotrichia | 30 | 30 | 60 | 0 | 0 | 60 | 70 | | | | | | | | | 240 |
| Sarcina | 40 | 60 | 60 | | | | | | | | | | | | | 160 |
| Polysaccharide-producing streptococci | 120 | 20 | | | | | | | | | | | | | | 110 |
| Clostridia | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 120 | | | | 130 |
| $\beta$ -Haemolytic streptococci | 0 | 0 | 0 | 0 | 50 | 60 | | | | | | | | | | 110 |
| Yeasts | 10 | | | | | | | | | | | | | | | 10 |
| | | | | | | | | | | | | | | | TOTAL | 41,300 |

TABLE XII CLASSIFICATION OF MICROORGANISMS ACCORDING TO COMBINED SURVIVAL INDICES AND STATISTICAL TESTS

| Microorganisms | Combined Survival Index | Tests for significance between categories | | | | | | | | | | Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\Sigma B - \Sigma C$ | | | | $B_{(1-2mm)} - \Sigma C$ | | | | $B_{(1-2mm)} - B_{(3-6mm)}$ | | |
| | | Sign | | t | | Sign | | t | | Sign | | t | | |
| | | n | | n | | n | | n | | n | | n | | |
| Aerobic streptococci | 4140 | 14 | * | 19 | * | 14 | * | 19 | * | 19 | <,001 | 19 | ,01 | Dominant |
| Actinomyces | 3046 | 27 | * | 28 | * | 27 | * | 27 | * | 23 | * | 26 | * | Dominant |
| α-Hemolytic streptococci | 2995 | 17 | * | 17 | * | 17 | * | 17 | ,05 | 11 | ,02 | 17 | ,01 | Dominant |
| Mitis-type streptococci | 1706 | 10 | ,002 | 12 | ,001 | 11 | * | 11 | ,02 | 9 | * | 10 | ,05 | Dominant |
| Enterococci | 1056 | 11 | * | 12 | * | 12 | * | 12 | ,05 | 7 | ,01 | 11 | ,01 | Dominant |
| Anaerobic streptococci | 8153 | 20 | <,001 | 28 | ,001 | 20 | <,001 | 27 | <,001 | 16 | <,001 | 28 | ,04 | Associate (Major) |
| Staphylococci | 3871 | 30 | ,01 | 30 | ,001 | 30 | ,01 | 30 | ,001 | 24 | ,05 | 30 | ,01 | Associate (Major) |
| Veillonella | 3205 | 22 | <,001 | 22 | ,001 | 18 | ,003 | 19 | ,001 | 17 | * | 22 | * | Associate (Major) |
| γ-Hemolytic streptococci | 1719 | 18 | <,001 | 18 | ,001 | 16 | ,002 | 16 | ,001 | 16 | ,001 | 18 | ,001 | Associate (Major) |
| Bacteroides | 1256 | 19 | <,001 | 19 | ,001 | 18 | <,001 | 18 | ,001 | 16 | * | 19 | * | Associate (Major) |
| Lactobacilli | 1175 | 18 | ,008 | 18 | ,05 | 14 | * | 15 | * | 14 | ,01 | 18 | ,01 | Associate (Major) |
| Neisseria | 952 | 11 | ,01 | 12 | ,001 | 12 | ,006 | 12 | ,001 | 10 | ,023 | 11 | ,001 | Associate (Minor) |
| Pasteurella | 867 | 13 | * | 14 | * | 12 | ,03 | 14 | ,01 | 12 | ,006 | 14 | ,001 | Associate (Minor) |
| Diplococci | 580 | 14 | * | 14 | * | 14 | * | 14 | * | 10 | * | 14 | * | Associate (Minor) |
| Corynebacteria | 494 | 10 | * | 12 | ,05 | 10 | * | 12 | * | 9 | * | 10 | * | Associate (Minor) |
| β-Hemolytic streptococci | 352 | 10 | ,02 | 14 | ,05 | 14 | * | 15 | * | 5 | * | 5 | ,05 | Associate (Minor) |
| Selenomonas | 349 | 13 | ,004 | 14 | ,001 | 9 | * | 10 | ,02 | 11 | * | 13 | * | Associate (Minor) |
| Clostridia | 234 | 8 | * | 8 | * | 6 | * | 6 | * | 4 | – | 5 | * | Associate (Minor) |
| Leptotrichia | 193 | 11 | * | 13 | * | 10 | * | 11 | ,05 | 7 | * | 10 | * | Associate (Minor) |
| Polysaccharide-producing streptococci | 154 | 5 | * | 7 | * | 5 | * | 7 | * | 4 | – | 4 | * | Associate (Minor) |
| Sarcina | 77 | 6 | ,03 | 6 | ,01 | 5 | * | 5 | ,02 | 3 | – | 6 | * | Associate (Minor) |
| Micrococci | 68 | 7 | ,01 | 7 | ,001 | 7 | ,01 | – | ,001 | – | – | – | – | Associate (Minor) |
| Yeasts | 18 | 4 | – | 4 | * | 4 | – | 4 | * | 2 | – | 2 | * | Associate (Minor) |
| Anaero-bacteriaceae | 901 | | | | | | | | | | | | | Incidental |

$\Sigma B$ – Total Banta
$\Sigma C$ – Total Gnaccalians with pockets 1 – 2 mm
$B_{(1-2mm)}$ – Banta with pockets 1 – 2 mm
$B_{(3-6mm)}$ – Banta with pockets 3 – 6 mm
Sign – Sign test
t – Student t test
* – No significant difference
n – number of observations
– – not sufficient number of observations for test

TABLE XIII

HIGHEST PERCENTAGE INCIDENCE, COMBINED SURVIVAL INDEX AND PERCENTAGE DIFFERENCE IN SURVIVAL INDEX OF THE THREE CATEGORIES, FOR EACH MICROORGANISM STUDIED

| Group | Microorganisms | Highest Percentage Incidence | Combined Survival Index | Percentage difference in survival index | | | Classification |
|---|---|---|---|---|---|---|---|
| | | | | $\Sigma B - \Sigma C$ | $B_{(1-2\ mm)} - \Sigma C$ | $B_{(1-2\ mm)} - B_{(3-6\ mm)}$ | |
| D | Aerobic Streptococci | 100 | 4146 | -5,9 | 13,1 | 49,5 | Dominant |
| | Actinomyces | 45 | 3046 | -4,4 | 73,1 | 28 | Dominant |
| | $\alpha$-Haemolytic streptococci | 81,2 | 2995 | 3,2 | 40,2 | 70,6 | Dominant |
| | Mitis-type streptococci | 88,7 | 1706 | -87 | 67,3 | 36,7 | Dominant |
| | Enterococci | 50,2 | 1058 | 9,9 | 28,8 | 36,1 | Dominant |
| $A_1$ | Veillonella | 95 | 3205 | 87,5 | 71,8 | -14,3 | Associate (Major) |
| | $\gamma$-Haemolytic streptococci | 31,7 | 1719 | 468,4 | 334,2 | -61,8 | Associate (Major) |
| | Lactobacilli | 31,2 | 1175 | 193,3 | 138 | -47,9 | Associate (Major) |
| | Sarcina | 12,5 | 77 | 833,1 | 700 | -31,3 | Associate (Minor) |
| $A_2$ | Anaerobic streptococci | 100 | 8153 | 36,1 | 53,4 | 29,1 | Associate (Major) |
| | Staphylococci | 26,2 | 3871 | 222,3 | 285,4 | 48,6 | Associate (Major) |
| | Bacteroides | 37,7 | 1246 | 1307 | 1449 | 22,4 | Associate (Major) |
| | Neisseria | 86,2 | 952 | 100,7 | 131,5 | 36,1 | Associate (Minor) |
| | Fusobacteria | 30 | 862 | 36,1 | 86,3 | 15,8 | Associate (Minor) |
| | Diplococci | 33,7 | 580 | 208,8 | 265 | 44,4 | Associate (Minor) |
| | Selenomonas | 8,7 | 349 | 706,7 | 713 | 12,9 | Associate (Minor) |
| | Clostridia | 3,7 | 234 | 74,1 | 174 | 269,1 | Associate (Minor) |
| | Leptotrichia | 8,7 | 195 | 159 | 200 | 37,5 | Associate (Minor) |
| $A_3$ | Polysaccharide-producing streptococci | 60 | 154 | 6,6 | 20 | 28,5 | Associate (Minor) |
| | Corynebacteria | 38,6 | 494 | -110,3 | 67 | 86,6 | Associate (Minor) |
| $A_4$ | Micrococci | 5 | 68 | - | - | - | Associate (Minor) |
| | Yeasts | 25 | 38 | -1400 | - | - | Associate (Minor) |
| $A_5$ | $\beta$-Haemolytic streptococci | 8,7 | 352 | -76,4 | -13,7 | 263,6 | Associate (Minor) |
| I | Enterobacteriods | 3,7 | 901 | 98,4 | - | - | Incidental |

$\Sigma B$ = Total Bantu

$\Sigma C$ = Total Caucasians with pockets 1 - 2 mm

$B_{(1-2mm)}$ = Bantu with pockets 1 - 2 mm

$B_{(3-6mm)}$ = Bantu with pockets 3 - 6 mm

TABLE XIV INCIDENCE OF AMINO ACIDS IN TEN SAMPLES FROM BANTU WITH 4 - 6 mm POCKETS

| | | days | | | | | | | | Total P |
|---|---|---|---|---|---|---|---|---|---|---|
| | Amino acid | 0 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 30 | |
| | Tyrosine | 9 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 9 | 94,3 |
| GROUP I | Glutamic acid | 3 | 4 | 6 | 4 | 4 | 4 | 3 | 4 | 4 | 40 |
| | Alanine | 1 | 1 | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 20 |
| | Proline | 1 | 1 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 11 |
| | Methionine | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 83 |
| | Cysteine | 6 | 7 | 8 | 9 | 9 | 9 | 9 | 9 | 9 | 82 |
| GROUP II | Lysine | 3 | 3 | 4 | 2 | 3 | 4 | 5 | 4 | 5 | 36,5 |
| | Glycine | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 28,8 |
| | Threonine | 1 | 2 | 3 | 2 | 3 | 3 | 3 | 2 | 3 | 24,5 |
| | Arginine | 8 | 5 | 7 | 6 | 5 | 5 | 5 | 7 | 7 | 58 |
| | Histidine | 4 | 4 | 4 | 2 | 2 | 4 | 4 | 4 | 3 | 34,5 |
| | Valine | 5 | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 4 | 34,5 |
| | Cystine | 5 | 2 | 3 | 1 | 0 | 1 | 3 | 5 | 5 | 30 |
| GROUP III | Leucine | 9 | 0 | 0 | 0 | 0 | 4 | 4 | 5 | 5 | 30 |
| | Tryptophane | 8 | 1 | 1 | 2 | 2 | 2 | 2 | 4 | 4 | 29 |
| | Serine | 4 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 20 |
| | Isoleucine | 1 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 13,6 |
| | Aspartic acid | 2 | 1 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 12,2 |

GROUP I Slight rise and then drop in incidence
GROUP II Rise in incidence
GROUP III Drop and then rise in incidence

TABLE XV MEAN pH VALUES FOR TEN SAMPLES FROM BANTU WITH 3 - 6 mm POCKETS

| Day | pH | Day | pH | Day | pH |
|---|---|---|---|---|---|
| 0 | 7,04 | | | | |
| 1 | 6,71 | 11 | 7,54 | 21 | 8,08 |
| 2 | 7,09 | 12 | 7,52 | 22 | 8,01 |
| 3 | 7,05 | 13 | 7,69 | 23 | 8,05 |
| 4 | 7,15 | 14 | 7,76 | 24 | 8,29 |
| 5 | 7,69 | 15 | 7,59 | 25 | 8,1 |
| 6 | 7,61 | 16 | 7,99 | 26 | 7,93 |
| 7 | 7,36 | 17 | 7,84 | 27 | 7,85 |
| 8 | 7,73 | 18 | 7,84 | 28 | 8,12 |
| 9 | 7,67 | 19 | 7,89 | 29 | 7,63 |
| 10 | 7,44 | 20 | 8,03 | 30 | 8,03 |

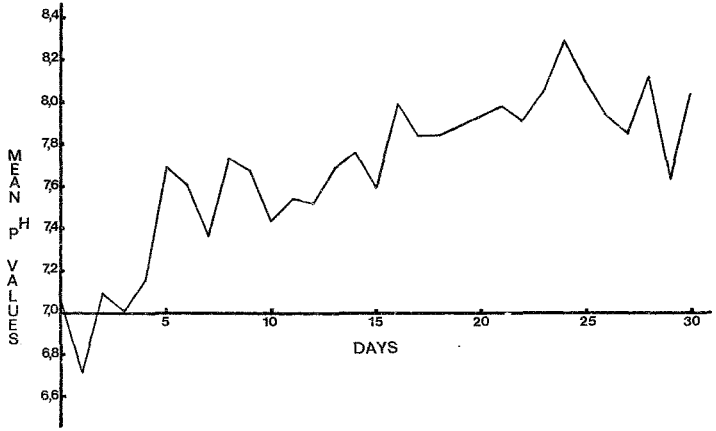


FIGURE 7 CHANGES IN pH IN TEN SAMPLES FROM BANTU WITH 3 - 6 mm POCKETS

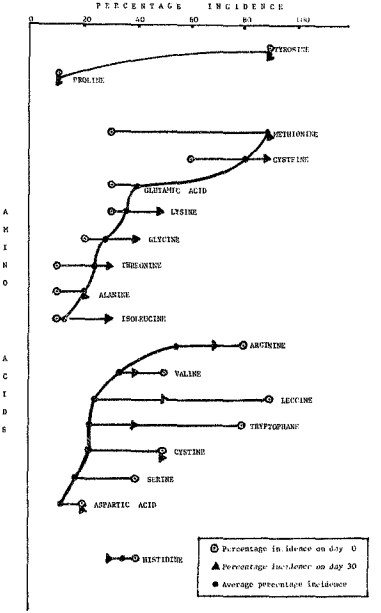


FIGURE 1 CHANGES IN PERCENTAGE INCIDENCE OF AMINO ACIDS IN TEN SAMPLES FROM BARTO WITH 3 - 6 mm POCKETS

**Author** Coogan Maeve Mary
**Name of thesis** Bacterial studies on debris taken from the gingival sulcus of man. 1975

*PUBLISHER:*
**University of the Witwatersrand, Johannesburg**
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