Introduction to Protozoa and Fungi in Periodontal Infections

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A Manual of Microbiological Diagnosis and Nonsurgical Treatment

By

Trevor Lyons, BDS (U. Lond.), LDS RCS (Eng.), RM (CCM)

in association with Eleanor Stanfield, B.A.

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Recent reports in the scientific news should cause us to pause and reassess old beliefs. Environmental changes have made many trees more susceptible to infection. Healthy red oak are attacked at their roots by fungi which can kill in as little as sixty days. Dogwood are under siege by both fungi and parasites, the synergism of which causes the trees to die.

Can we afford to ignore the presence of parasites and fungi in our own mouths?

Eleanor Stanfield

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FOREWORD

Foreword to the first edition by Dr. Murray Vimy

"There are many instances in science, and particularly biology, where those closest to the intricacies of the subject have a more highly developed (and ultimately erroneous) sense of its intractability than those at some remove. On the other hand, those at too great a distance may, I am well aware, mistake ignorance for perspective."

Carl Sagan - "The Dragons of Eden"

Periodontal disease, a prevalent disease in man, has occupied the attention of dental and medical researchers with growing frustration. Although both local oral factors (open contacts, crowding, rough restorations etc.) and systemic factors (zinc, vitamin C, diabetes etc.) have been implicated in the periodontal disease process, the overall success in periodontal therapy has often been disappointing. Today, the predominant treatment modality is still oral tissue circumcision. The initial results appear favourable; however, the disappointment of relapse is usually inevitable. Today, the dental profession admonishes the patient for not cleaning his mouth thoroughly, not flossing enough, and not brushing effectively. We have become experts at placing blame for failure solely on the shoulders of the patient, while claiming the responsibility for success for ourselves. Even common sense exposes this obvious fallacy.

From an evolutionary perspective, natural selection dictates that a species' survival depends upon a healthy, disease free mouth. Animals in the wild can not survive with a dental abscess or with a faulty dentition, obtaining food and self-defence is difficult. This must also have been true for the survival of Homo sapiens. How did aboriginal man survive without the "advantage" of floss, tooth brushes or modern dentifrices?

In his anthropological classic, Nutrition and Physical Degeneration, Weston Price develops a strong circumstantial case based on observation for the interplay of the "western industrial diet" and the prevalence of physical degenerative diseases such as skeletal malformations (including mandibular atrophy), caries and tooth loss due to periodontal infections. One might conclude that dentistry is an artifact of man's social evolution, since the incidence of dental diseases is related more to factors of civilization and industrialization then to naturally occurring biological factors.

The nutrition/bacteria link to dental caries is now a scientific fact. Buoyed by this success, researchers have attempted to develop a similar approach for the treatment of periodontal infections by focusing on a bacterial etiology. The current decline in caries rate is directly related to research in the areas of fluoride, sugar metabolism, and improved oral hygiene technologies. The same benefits have not been derived from periodontal research, despite considerable economic investment. Why? What has been overlooked?

The traditional view promotes the general plaque hypothesis. As dental plaque thickens and ages it becomes dominated by anærobic species which utilize amino acids and produce ammonia and urea as metabolic bi-products. This in turn results in hæmostasis, inflammation, immune response and progressive tissue destruction. More recently, attention has been directed toward identifying specific bacterial species which might be prime etiological factors in specific periodontal disease states. Research employing this specific bacteria hypothesis has been disappointing.

Many basic questions remain unanswered.

Why do plaques of similar composition produce rapid destructive periodontal disease in one individual but not another?

Why are some infections generalized, while in other cases periodontal disease is localized to a few sites, even in the absence of specific local factors?

Why does periodontal disease demonstrate a cyclical nature, having periods of remission and exacerbation?

Why do some apparently systemically healthy individuals with excellent oral hygiene still demonstrate signs and symptoms of slowly advancing periodontal destruction?

This book, Introduction to Protozoa and Fungi in Periodontal Infections, by Dr. Lyons addresses these issues head on. Through careful review of the scientific literature and meticulous documentation of periodontal cases in his own practice for the last ten years, Dr. Lyons methodically uncovers an alternate hypothesis, having been published in dental journals in the early part of this century. Simply put, advancing destructive periodontal disease necessarily involves oral parasites, specifically Entamœba gingivalis, Trichomonas tenax or Candida spp. The hypotheses advanced in these pages adds to the traditional view that periodontal disease is predominantly of bacterial origin.

As the picture of oral ecology unfolds, we become aware of the intimate relationship between these oral parasites and maturing dental plaque. Dental plaque is essential in initiating the primary periodontal lesion. By causing irritation and inflammation the bacteria create the specific anærobic and pH conditions essential for parasitic habitation. Thus, our understanding of the local and systemic factors take on a new perspective. These are predisposing factors, creating the environmental conditions conducive to parasitic habitation and destructive periodontal disease. Once established, the parasite becomes the dominant organism in the periodontal pocket. With no known enemies, it is the lion in the jungle.

If Dr. Lyons' observations and hypotheses are so obvious, how could they have been overlooked by several generations of dental researchers? The answer is simple. World War I and the Great Depression diverted interest in this area of investigation. World War II and the advent of modern antibiotics spawned tremendous interest in microbiology, specifically bacteriology. The world view regarding periodontal disease was transfixed in the contemporary bacterial paradigm and we've been stuck on it ever since.

Weston Price saw degenerative dental effect but could not fully identify the mechanisms. The oral cavity is indeed a barometer of human health. Our modern life styles including stress and over-consumption / under-nutrition predisposes our oral cavities to degeneration. This degeneration prepares the way for parasitic infections which transform gingivitis to periodontitis. Our dense urban lifestyles makes the transmission of these organisms easily accomplished through direct social contact (e.g. kissing), air born particles (e.g. sneezing) and through contaminated cooking utensils. All these factors were not relevant to the aboriginal tribesman eating a basic unrefined evolutionary diet in relative

isolation from each other. In light of the material presented in this volume, we must give serious consideration to the proposition that periodontal disease is a communicable infectious disease who's incidence is a function of our social evolution.

Dr. Lyons must be congratulated for his insight, determination and devotion to his beliefs. I believe that he has re-discovered basic evidence which has awaited a discerning eye. The concepts in this text are extremely effective. I know. I've employed them in my practice in varying degrees for the last three years. I recommend this work to you. Employ the approach with caution, remembering that each patient is unique. However, ALL will benefit from a carefully supervised approach.

Murray J. Vimy B.A., D.M.D., F.A.G.D.
Clinical Assistant Professor
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March 1989

A Foreword by Dr. Brian McLean

In 1980 I first heard Dr. Lyons discuss oral protozoa and their role in periodontal disease on a cassette tape produced by "Dentafacts". I was driving home from the office as I heard him describe how one could successfully and predictably treat periodontal disease using a medical treatment regime borrowed and adapted from the field of gastro-enterology. His arguments were logical and well presented. He clearly knew his subject matter. Intellectually everything was in order but it didn't "feel right". Listening to this Canadian dentist with an English accent was not at all like listening to a southern snake-oil salesman who could "cure pus pockets with his pills and potions"; yet my previous dental school training manifested itself in a sneering skepticism of such magnitude that it might have been produced in response to just such a huckster.

When I got home, I replayed the tape and was still left in conflict; my skepticism was at odds with my curiosity and excitement that perhaps there was something valuable here. My scientific dental school training left no doubt that periodontal disease was solely a bacterial phenomenon. My scientific pre-dental school training however stressed the value of an open mind. Fortunately scientific attitude defeated scientific dogma, and I sent in some plaque samples for testing. Results were promising but not predictable at first. Then I bought a microscope. Since then, the great majority of the people who come to me for care and who are treated for periodontal disease are successful in eliminating or controlling the infection. Now I have almost a decade of thankful and enthusiastic people who are no longer threatened with periodontal disease and whose periodontal tissues have never met a scalpel.

It is interesting that the therapies taught by many North American dental schools have evolved over the last decade into ones which recognize the limited long-term effectiveness of surgery for pocket reduction. How long will it be before researchers in dental schools look beyond the bacterial components of the plaque for possible pathogens? In the meantime, Dr. Lyons has provided us with a sound rationale and an extremely effective treatment regime.

Brian D. McLean, B.Sc. D.D.S.
Mississauga, Ontario

March, 1989

A Foreword by Dr. Richard Christie

Newtonian Physics theorized for 175 years that time was absolute - that it moved forward perpetually and at a uniform rate. Einstein came along and said this theory was wrong - that time was relative and that the speed of light was absolute, thus revolutionizing our understanding of the universe. So too, theories in medicine and dentistry endure, rightly or wrongly, until progressive thinkers appear to theorize anew.

The treatment of periodontal disease is desperate for new thought, for a new treatment direction that is not invasive. The modality of treatment

described herein breaks through the traditional mystique of periodontal disease that has frustrated dentist and patient alike in their quest for optimum dental health.

Trevor Lyons' treatment is an exciting adventure that involves the dentist-hygienist-patient in a true team effort that allows the patient to keep their teeth for a lifetime.

Richard Christie, B.Sc., D.D.S.
Ottawa, Ontario

March 1989

A Foreword by Dr. John F. Coombs

In the few years that I have had the privilege of collaborating with Dr. Lyons on the mutual care of patients, I have been astounded at the ability of gingival infections to cause constitutional symptoms. This seems to occur frequently, even in infections that would not be apparent to the untrained observer. Though such symptoms are usually not dramatic enough to be of concern to someone such as an emergency room physician, they are nevertheless debilitating to the patient, and they should be of concern to the general physician. It is unfortunate then, that the gingivæ are such a sadly neglected part of the body, with all but the most severe infections going untreated, and at times even unnoticed. Physicians need to be better trained in the recognition of gingival infection, and both physicians and dentists need to be reminded of the significant role such infections can play in the general medical condition of patients.

The most frequent medical problems in which I have seen gingival treatment be of benefit are: chronic fatigue, anxiety, depression, panic disorder and rheumatic symptoms. In such patients I now refer for microscopic examination of plaque at the slightest indication of gingival infection. Patients with enteric infection with Candida albicans frequently have accompanying gingival involvement as well, and will never be permanently freed of their infection unless the gingival component of their infection is treated concurrently. I now routinely

screen such patients for oral candidiasis, using a gingival swab plated onto Nickerson's medium.

The ready accessibility of Entamœba gingivalis for examination makes it an ideal specimen for detailed research, and much of what may be learned about E.gingivalis will be of use in studying amœbæ harboured elsewhere in the body. My preliminary impression from very closely monitored cases of intestinal infection with so-called 'nonpathogenic' amœbæ, is that they are as capable of causing generalized constitutional symptoms as is E.gingivalis. We have much to learn from continued research on the oral amœbæ.

It is my hope that this book by Dr. Lyons will generate greater interest amongst physicians and dentists alike in the medical treatment of gingival infection.

John F. Coombs, B.Sc., M.D.
Lanark, Ontario

March 1989

A Foreword by Dr. I. M. Warrack

I know that this publication will challenge the traditional attitudes to oral infections. Approaching the topic with an open mind and following the the recommendations regarding the various treatments will, I am sure, have positive results for dentists, physicians and, of course, patients.

I. M. Warrack, M.B., Ch.B., C.C.F.P.
Ottawa, Ontario

March 1989

PREFACE

ORAL AMŒBIASIS

From 1913 to 1930, published investigations demonstrated a correlation between Entamœba gingivalis and destructive periodontal disease. However, there was no safe medication available, at that time, to effectively treat amœbic infections of the mouth. Despite rapid developments in antibiotic and antiprotozoal therapies during and after World War II, the post war boom saw the concept of Oral Amœbiasis apparently forgotten. During this period many texts, without substantive evidence, simply dismissed E.gingivalis as a nonpathogen and an organism of little importance. This conclusion then became so entrenched in the conventional wisdom of the day, that many scientists became wary of suggesting a re-evaluation of the role of E.gingivalis in human disease. As a dentist, concerned with the well being of patients harbouring this organism, I was not hindered by tunnel vision.

After eighteen years in general dental practice I had found that traditional periodontal care was not always effective. My quest for knowledge led me to investigate the microbiology of the plaque of my regular dental patients. What I found, sent me in search of answers. I found some of these by spending extra time looking down the microscope. Other answers were to be found in the literature of dental and other scientific fields. Some answers were found by conferring and collaborating with microbiologists. In the course of the last eleven years I have discovered overwhelming circumstantial evidence to lead to the conclusion that Entamœba gingivalis is indeed, tissue invasive.

This text has been especially prepared as a response to those who have requested more information relative to the concepts of Oral Amœbiasis in the diagnosis and treatment of periodontal diseases. An historical perspective regarding the oral protozoa, particularly E.gingivalis, is included and typical case histories are described. The concept of Oral Candidosis is also covered.

General concepts of diagnosis and case management, as currently used by the author in his practice, are dealt with in separate chapters in a step by step fashion. It should be stressed that the diagnosis and therapies presented are based on the medical approach to the diagnosis and treatment of lumen dwelling parasites. These methods have been adapted by the author to better suit the oral environment. Similarly, the laboratory procedures described have been modified from those routinely used in medical laboratories. The development of these modified tests was necessitated by the minute samples in dental specimens. The ease of direct phase contrast microscopic examination of oral material allows for rapid diagnosis without the delay usually encountered with submission of samples to a laboratory for processing and diagnosis. Direct examination of samples also allows the clinician to weed out probable negative results before material is submitted to a laboratory, if diagnostic confirmation is needed.

Although it might seem that the use of a microscope, antibiotics and antiseptics is the whole therapy, this is far from the truth as the discerning reader will quickly learn. Basic dental services must also be provided in order to improve the oral environment. The extent of these will depend on the presenting condition as well as the response to initial therapy. Significantly, the use of the microscope to monitor infection will allow the timing of routine dental treatment to be optimised.

By using a phase contrast microscope, with attached TV camera and monitor, the patient is afforded the opportunity to observe the microbiology associated with pathology. This stimulus can be effective in better patient compliance to the prescribed treatment. Just as good equipment is essential to accurate diagnosis, so good compliance is essential to a favourable response in treatment.

The purposes of this book are to make it easier for a dentist to integrate a phase contrast microscope into dental practice, to help identify what the dentist should look for in a microscope and to explain the basics of its construction, function and use. The neophyte should be able to learn from my experience without having to "re-invent the wheel". No excuse is made for the use of the first person, where such use adds to the impact, nor is any made for the use of everyday language where such use may help to explain technical matters with which the reader may not be familiar.

I attended a seminar by Bob Barkley in 1972 during which he stated that no dental office was complete without a phase contrast microscope.

Although I saw no validity in his statement, my curiosity was aroused. I decided to try one in my office and sought out a local supplier who would provide a microscope on a free trial basis. My plan was merely to see living bacteria, then return the instrument to the supplier as being of no benefit in dental practice.

When I contacted the local representative of a manufacturer of quality microscopes, as used in hospitals and medical laboratories, I discovered that there was a wide range of models and choice of accessories. Since I was merely curious and not seriously contemplating a purchase, I ordered the best available phase contrast microscope with binocular eyepieces, complete with all accessories.

When the instrument arrived I was surprised that slides and cover slips were not supplied with it. On questioning the company I was informed that they supplied microscopes, not slides, cover slips nor any other ancillaries such as mounting media or other laboratory equipment. They did, of course, supply the appropriate oil for the high power oil immersion lens, provide an instructional booklet on assembly and adjustment and offer repair service.

I had a few microscope slides and cover slips, left over from my student days, but no mounting media. Barkley had suggested that, in order to be scientific and avoid introducing unknown variables, plaque be mounted in sterile broth or normal saline. On contacting local suppliers in search of these products, I ran into a supply problem. Normal saline, available in various quantities, was backordered a week or so. Sterile broth was available on special order and supplied in litre containers. It was relatively expensive at $18 a litre. (The cost of gas in Canada was still less than 11 cents a litre.) Deciding to order the broth, I mentioned to the representative that it should last me a year or two. "No!" he replied, "It has a two week shelf life". Then he went on to say that it would be two to three weeks before delivery could be made. Since I was to have the use of the microscope for less than a week "on free trial", I decided against ordering.

I was now ready to examine my first plaque sample, but had no mounting medium, so I used the sublingual saliva of the patient as an expedient. It later transpired that this was a significant decision. Amœbæ are adjusted to the environment within the host, not that provided by saline or broth, even if care is taken to adjust the pH and temperature to a constant value. The host values may not be constant but differ between

hosts. Keeping the amœbæ in their own environment makes it easier for the natural historian of dental plaque to make accurate observations.

Unfortunately, instead of examining plaque from an infected site, I followed Barkley's suggestion at first and selected a "standard site". From the outset it became obvious that the numbers of motile bacilli present in plaque, particularly smaller bacilli, correlated to known caries incidence of regular dental patients. The microscope was purchased. No correlation between the bacteriology of plaque and periodontal disease could be established during the next seven years. However, when I started to examine plaque from periodontally involved sites I was able to make a correlation between protozoa and destructive periodontal disease.

In subsequent conversation with Dr Paul Mashimo, State University of New York, he told me of his early investigations into the protozoa. He, too, had used sublingual saliva, but it had been ingeniously collected from the gland so that it could not be contaminated with oral micro-organisms. His research showed the sublingual saliva taken directly from the gland was free of micro-organisms. In particular, it contained no protozoa. My own experience confirmed that saliva taken from the floor of the mouth appeared to be sterile. I randomly selected patients for examination of saliva from the area adjacent to the orifice of the sublingual salivary duct. Although the selected patients had protozoa in plaque, none had any micro-organisms, particularly protozoa, present in the saliva.

At the outset, since no sterile broth nor normal saline was available, each patient's own saliva was used as a mounting medium. This protocol is still in use since it has been found that other liquids cause distortion of the protozoa which make them unrecognizable. Following my experience with the initial case, plaque has always been taken from a diseased site, rather than some arbitrary standard site.

Explanatory note: quantitative assessments from minimum to maximum, which are used in this text are: <o+, o+, +, ++, +++.

Breakthrough or Unique Case Report?

The initial observations were made in February, 1978. A female recall patient, in her mid thirties, complained that her gums felt "itchy" and that one of her front teeth had moved. It was noted that the upper right central incisor had started to drift labially and although the gingival condition was poor, her oral hygiene was good. Subgingival scaling was uneventful and the patient was re-instructed in oral hygiene. No variations in the conditions previously charted were noted. These

conditions included minimal subgingival calculus; stain and plaque were both minimal (o+) and the pocket depths were less than 2mm. Two weeks later the patient returned complaining that the condition had worsened. Re-examination confirmed this. The tissue was now mauve and flaccid and pocket depth had increased to 5mm palatal to the affected tooth. There was no calculus detectable but the tooth had over-erupted. Perplexed at this apparent failure of both conventional dental treatment and home care, the plaque from this site was compared with plaque from the "standard" site, mesial to the lower left first permanent molar. The latter was not periodontally involved and the obvious difference was the presence of Entamœba gingivalis and Trichomonas tenax in the pocket around the affected incisor. Neither of these protozoan parasites were present in the standard site. With phase contrast examination of the plaque, no significant difference in bacterial populations of the two sites could be detected. The advice of physicians was sought in order to formulate a suitable regime of medication which would eliminate the protozoa from the oral cavity. The regime adopted was modelled on conventional therapy to eliminate lumen dwelling (protozoan) parasites.

After one ten-day course of antiparasitic medication it was observed that the plaque no longer harboured protozoa, the gingival condition had improved and the pocket depth had reduced to 2mm without a matching recession. Traditional remedies were then implemented to return the tooth to its position in the arch. The tooth has remained stable following the removal of temporary splints (January 1989, a period of almost eleven years.)

During the balance of 1978 the plaque of those patients with periodontal problems was re-assessed using the site of infection instead of an arbitrary standard site as source. In each case oral parasites were found and a clinical improvement obtained by eliminating them. Entamœba gingivalis, being the most frequent protozoan, was found thirteen times more frequently than Trichomonas tenax.

Subsequently, attention was turned to those patients in whose mouths Entamœba gingivalis had been previously recorded. Even those patients whose mouths had initially appeared healthy now exhibited a periodontal decline. Elimination of oral parasites was invariably followed by improved periodontal health.

The initial cases treated were all regular dental patients in the recall programme. In spite of conscientious efforts of patients at home and

routine periodontal care in the office, these patients had shown inexplicable periodontal deterioration.

It became obvious to me that the therapeutic differences between the treatment of parasitic and bacterial diseases were necessitated by the differences in the natures of the infecting organisms. Parasites just do not behave like bacteria, cannot be regarded as if they are bacteria, and it was going to become necessary to learn more about the oral protozoa and parasites in general.

Author's note:

I would like to thank all those, who, by the sharing of knowledge or lending of expertise helped me to a better understanding of the nature of the infections herein described. To my many friends, patients and colleagues, who encouraged me to prepare this manuscript I would also say "thankyou". Without their positive reinforcement this work might never have been collated. This is a book written by a practicing dentist for other practicing dentists. It is about the role of protozoan parasites, particularly Entamœba gingivalis, in oral disease.

CHAPTER 1

INTRODUCTION TO PARASITOLOGY

THE CINDERELLA OF THE HEALTH SCIENCES

For most health professionals, including dentists, the field of parasitology is an unknown entity. Common misconceptions about the rarity of parasite infections lead most of us to assume that "we couldn't become infected with a parasite because parasites are third world problems." "Parasite infections only happen when inadequate hygiene practices are observed, etc.". A brief survey of public health literature reveals that protozoan infections are far from rare in North America. As our knowledge about parasites increases opinions about pathogenicity have also changed.

LENINGRAD

Prior to the great Canada-Soviet hockey matches of the early 1970's, a small flagellate called Giardia lamblia was dismissed as being non-pathogenic. People returning from Leningrad with symptoms of gastro-intestinal disturbance were found to harbour this organism. Resolution of signs and symptoms normally accompanied the elimination of this parasite. This sudden peak of infection, coupled with a ten year data base gathered by public health services, was instrumental in prompting a change in thinking. Giardia is now considered to be a pathogen and its presence requires notification of the medical officer of health in some jurisdictions.

PARASITES and PATHOGENS

One frequently hears the following questions asked: What is a parasite? What is the difference between a parasite and a pathogen? Are all parasites pathogens? Are there any good parasites? The answers to these questions will help to give a clearer understanding of the nature of Oral Amœbiasis.

The Medical Dictionary defines a parasite is an organism which "lives upon or within another living organism, at whose expense it obtains some advantage." A pathogen is an organism that produces disease. From the definition of parasite it should be immediately apparent that there can be no nonpathogenic parasite. All parasites are to some extent pathogenic. What remains to be determined is the degree of pathogenicity of any particular parasite. Thus, by definition, there can be no "good" parasites. Parasitism and symbiosis should not be confused. A symbiotic relationship is one which is mutually beneficial to both organisms without detriment to either. Where there is a benefit for one organism without detriment to the other, then the state is referred to as commensalism. The medical literature describes Entamœba gingivalis as a parasite. The purpose of this book is to demonstrate the degree of pathogenicity of this parasite.

OBLIGATE and OPPORTUNISTIC PARASITES

There are two basic types of parasite: obligate and opportunistic. An obligate parasite is one which cannot live freely in the environment, but must depend on a host for survival. E.gingivalis fits this category. On the other hand an opportunistic parasite can live freely in the environment without a host. However, if it finds itself in a host it can continue to survive. Some free living amœbæ (e.g. of the genus Nægleria) fall within this category. Normally found in stagnant non-salt water, they can enter the nose of a swimmer, track along the olfactory nerve to the brain with ensuing (usually fatal) encephalitis. The state of parasitism thus created is relatively short-lived.

HOST RESPONSE

If a parasite causes a severe host reaction, the host may die. Frequent and premature host death may result in extinction of the host. If the host reaction is so severe that the parasite always dies before completing its life cycle, then the parasite becomes extinct. Although there is archæological evidence of extinct vertebrates, invertebrate parasites may pass from the face of the earth with little or no evidence of their previous existence. It is therefore not surprising to find that, in the case of successful parasites, the associated diseases are chronic and debilitating. Generally, the host does not die until the parasite has completed that part of its life cycle which is host dependent. To complete the cycle the parasite must undergo maturation and/or reproduction before being released into the environment to search out a new host.

SECONDARY HOST

With many parasites, particularly those which may cause the death of the host, an intermediate (or secondary) host is required. The primary host harbours the parasite while it matures and reproduces. To ensure survival of the parasite species, a further host must be infected. This can be achieved in many ways. A resistant form, such as eggs or cysts, may pass into the environment where they lie dormant until entering a new host to continue the cycle.

CYSTS and EGGS

Most parasites are able to ensure survival by having a resistant form, either cysts (e.g Giardia lamblia, a protozoan flagellate) or eggs (e.g. Ascares lumbricoides, the large intestinal roundworm). Cysts remain in the environment until passed back to a primary or to a secondary host (e.g Giardiasis). Cysts ingested by man, dogs or aquatic mammals such as beaver, cause enteric infection and repetition of the cycle. Eggs from intestinal worms may pass into the environment and give rise to an infection of a secondary host (e.g. pork tapeworm). The re-infection of the primary host occurs when the secondary host (pig) is eaten by the primary host (man).

GIARDIA

Giardia lamblia, a protozoan flagellate, is a parasite of the intestinal tract of animals and man. Giardia looks like a microscopic version of a manta ray which attaches to the intestinal wall by a "sucker". Hundreds of thousands of these tiny creatures form a plaque lining the bowel, absorbing the nutrients intended for the host while eliminating protozoan excrements which are absorbed into the blood stream of the host. Giardia forms cysts which drop off the plaque and pass with the stool into the environment. Here the cysts lie dormant until washed into the water supply. Cysts in water, drunk by the next host, set up infection and the cycle is complete. Human symptoms of Giardia include fatigue and Malabsorption Syndrome.

ROUND WORMS

Nematodes, such as Ascares lumbricoides, do not have an intermediary host, neither can they complete their cycle if accidentally ingested by a nonhuman host. Within the human host, however, they have a complex

cycle. Outside the body of the host the egg matures provided it is not desiccated. If a mature egg is swallowed it hatches to become a larva which penetrates the duodenal wall and gains access to the blood or lymphatic drainage. It is then carried to the heart or liver and finally via the pulmonary circulation to the lung. The larvæ lodge in the capillaries and break out into the alveoli where they grow and moult for about ten days. Migrating along the bronchi they enter the œsophagus to return again to the small intestine to mature and mate. Three months after the host has ingested an ovum (egg) the females start laying her own eggs. A mature female may produce as many as 200,000 a day.

TAPEWORMS

Some intestinal worms, such as pork tapeworm, a member of genus Tænia, not only pass eggs into the environment, but also infect muscle or other organs of the host. Immature "baby" worms (larva migrans) burrow through the intestinal wall of the pig and migrate to a distant site. Here the "embryo" worm curls up and protects itself inside a capsule. If insufficiently cooked pork, containing encysted larvæ (cysticerci), is eaten by Man, the new host becomes infected when the larva is released and grows into an adult worm in the digestive tract. The adult segmented worm in the digestive tract gains nutrients intended for the host. The head of the worm attaches to the intestinal wall. Distal to this head the segments of the worm mature. The terminal segments, containing eggs, drop off the worm and become embedded in the stool of the host. Another secondary host (pig) must ingest the eggs from this worm segment to become infected, whereupon the cycle repeats. The natural history for beef tapeworm, which by contrast with pork tapeworm is frequently encountered in North America, (Markel and Voge, 1976), is similar.

BILHARZIA

Other more complex life cycles exist where passage of the resistant form into the environment results in a short free living stage which infects the secondary host. Here the parasite completes a phase of its life cycle and is once again released into the environment. This second free living form then infects another primary host. An example would be Bilharzia, an African parasite disease, also called schistosomiasis. With some parasite diseases (e.g. filariasis and also malaria) the passage from primary to secondary host is direct; a blood sucking insect vectors the disease.

THE PERFECT PARASITE?

E.gingivalis is an obligate parasite. In fact, it cannot be maintained in pure culture for any length of time. It has achieved, by evolution, because it requires no intermediate host. It is able to complete its cycle in the primary host and pass directly to another primary host without causing death. It may be able to spread rapidly through a community, causing minimal disturbance to the health of the hosts, with whom it lives in a state of balance, though not necessarily harmony. The problem arises when this state of equilibrium is upset by external factors. Then, the parasite may gain ascendancy over the host, rapidly proliferate and cause pathosis. Although host response may be minimal, this response may still be unnecessarily debilitating.

SPECIES DEPENDENCE and IMMUNITY AVOIDANCE MECHANISMS

Host specificity, whereby the parasite of one species is unable to parasitize a different species, together with complexities in life cycles of parasites, complicates research. If the parasite is to be successful, it must be able to control or sidestep the host's immune response. There are a number of ways parasites have evolved such mechanisms, some of them quite complex and host specific. To appreciate how E.gingivalis has adapted to become an obligate parasite, one must first understand host-parasite interactions. Some of the mechanisms of immune system avoidance commonly employed by parasites include:

Camouflage,

Hiding,

Antigenic variation,

Counter defense.

Camouflage

This system is demonstrated in Bilharzia, (schistosomiasis, a disease caused by an African parasite,) as well as in other diseases caused by filaria. These worms survive within blood vessels and in tissue of the host. On entering the host the parasite develops a protective coating or simply coats itself with host protein (antigen). For example, within three days of schistosomes (Bilharzia) infecting the host, the organism simply becomes invisible to the host's immune system by developing a protective coating of host antigen. Within the veins and tissue the parasites live and breed for 7-10 years. Finally the female parasites lay vast numbers of

eggs to which there is a strong host immune response. It is the patient's immune response to this new generation of parasites within the liver and bladder which is so damaging, often resulting in the death of the host. Meantime, the eggs of the parasite pass into the environment. On entering water, eggs hatch, becoming free living larvæ. These infect snails, complete a life cycle stage, re-enter the water again as a free living baby worm and find another (human) host, burrow through the skin and restart the whole cycle.

Hiding

Some parasites take up residence inside host cells. As an intracellular parasite it is protected from the immune response and can complete part of its life cycle. With the death of the host cell, the parasite is released into the host system causing vigorous host response. The parasite then takes refuge inside a new cell and the cycle repeats. An example of this is Malaria, where parasites multiply within erythrocytes. An even more dramatic example is Leishmaniasis, where the parasite goes within the host macrophage. The macrophage, which should be destroying the parasite, is then unable to respond leaving the immune system of the host unable to deal with the invader.

Antigenic Variation

Having invaded the host, some parasites, such as African trypanosomes, keep changing their surface antigen. The result is that the host never has enough time to develop antibodies in sufficient quantity to eliminate the parasite. It is speculated that the production of host antibody may actually stimulate mutation of the parasite's surface antigen. As the surface antigen of the parasite changes, the host's immune response lags about a week behind. If the antigenic variation could be halted, the host antibodies would be able to eliminate the parasite.

Counter Defense

Some parasites may seek to make their environment less hostile by producing anti-inflammatory agents that counteract the host response. The parasite may even produce enzymes which destroy the host antibody. By disabling the host's immune response the parasite is free to complete its life cycle without interference.

CO-INFECTION, INTERDEPENDENCE and SYNERGISM

In addition to these four main mechanisms, it should also be remembered that co-infecting organisms may play a significant role in the progress of disease. For example, although guinea pigs are susceptible to infection with Entamœba histolytica, the parasite is unable to infect germ free guinea pigs, (Grollman and Grollman, 1970). There may be interdependence and synergism of the micro-organisms. The total load on the immune system in multiple infections may cause the immune system to break down. If infected with only one organism from such a group, the host may well be able to cope. When the organism is present, but no disease is observed, then it might be reasonably concluded that the disease includes an incubation period. However the potential for disease remains, particularly if the load of infecting organisms increases. If we find a particular organism consistently present in a disease state; if that organism is readily identifiable, and if elimination of that organism brings about resolution and healing, then it seems obvious that the organism must be a key.

The use of a phase contrast microscope in dental practice has revealed an almost invariable correlation between the oral protozoa and actively destructive periodontal lesions. This prompted research of the literature relative to Entamœba gingivalis and Trichomonas tenax.

CHAPTER II

THE CASE FOR PATHOGENICITY OF THE ORAL PROTOZOA

PREAMBLE

Grouping signs and symptoms together to identify a "disease" has been the foundation of diagnosis. If the cause of an illness is known, both the organism and the disease which it causes may be commonly referred to by essentially the same name, such as "Malaria" - "malarial parasite". Early descriptions of disease simply tied a group of signs and symptoms together. Often the ætiology was unknown, for example, the "flu", the "dropsy", a "cough". Sometimes the causative organism was identified, and the name of the disease would change to reflect this discovery, for example, "consumption" became tuberculosis. In some cases the disease and its cause were identified so closely together that the cause and the disease took similar names, for example the outbreak of "Legionaires' Disease" was soon linked to "Legionella pneumonphila". This chapter will outline some recent observations on the nature of destructive periodontal disease, certain organisms invariably found in the plaque of diseased sites and a review of the literature in the context of current evidence.

INTRODUCTION and DEFINITIONS

Oral Amœbiasis is an infection of the oral cavity with Entamœba gingivalis. This protozoan parasite is described by Markel and Voge (1976) as a lumen dweller. It is sometimes found elsewhere and has been reported in pulmonary and tonsillar suppuration, Faust, Russel and Jung (1970); Lapierre and Rousset (1973); Markel and Voge (1976); Sutliffe, Green and Suter (1951). Westphal (1941) and Dao (1985) both noted that confusion over the identification of either organism may arise because of the similarity of the morphology of E.gingivalis to the pathogen E.histolytica. Both Dao (1985) and de Moraes-Ruehsen (1980) reported

the presence of E.gingivalis in cervical and uterine smears taken from infections associated with intra-uterine devices. Removal of these devices resulted in remission of signs and symptoms of disease and "prompt disappearance of the organisms".

Dao (1985) noted that E.gingivalis was found only in association with Actinomyces species, which are a known to cause inflammation and necrosis in the female genital tract. He suggests a symbiotic relationship between the two organisms and possible involvement of E.gingivalis in infectious processes. Keyes (1982, 1983) also noted these two organisms, together with cocci which colonise the surface of the Actinomyces filaments, were invariably to be found in close proximity in the plaque at periodontally diseased sites.

As previously discussed, a parasite is an organism which lives at the expense of its host. By definition there can be no nonpathogenic parasite. Only the degree of pathogenicity might be questioned. Confusion over pathogenic potential is well illustrated by Entamœba histolytica. This pathogen may remain dormant in an apparently symptomless host for a long time before severe illness threatens the life of the patient, (Markel and Voge, 1976). Disregarding an apparently benign organism which may have pathogenic potential is inadvisable. References in the literature regarding the presence of E.gingivalis in relation to various states of disease may indicate that infections with this parasite may exhibit a latency period before signs and symptoms of disease develop.

HISTORICAL BACKGROUND

Gros (1849) published the first descriptions of "Endamœba gingivalis". Little attention seems to have been paid to it for over half a century. Then, in 1914, an important discovery was made. Barrett (July), Chiavaro (August) then Bass and Johns (September) independently reported the presence of E.gingivalis in "Pyorrhea Alveolaris". Both Barrett (1914) and Keyes (1982) reported 100% correlation between E.gingivalis and destructive periodontal lesions. Bass and Johns (1914) reported E.gingivalis present in eighty six cases of destructive periodontal disease. They repeated their investigations with 300 cases the following year with the same results. Significantly, the reports from 1914 indicated beneficial results in treatment of the disease with Emetine Hydrochloride, a derivative of Ipecacuanha. Kofoid et al (August 1929) reported their own research at Berkeley and reviewed the literature. Unfortunately, neither Kofoid nor his co-workers were dentists.

Dr Paul Mashimo of the State University of New York, related a typical example of this approach (personal communication 1980). Shortly after World War II a patient in Osaka, Japan, who had received two years of conventional periodontal treatment to little avail, was found to harbour E.gingivalis. The oral parasites were eliminated with topical applications of Emetine Hydrochloride. Following the complete eradication of the protozoa, the periodontal condition of the patient immediately improved and stabilised.

Reports of the successful treatment of periodontal disease with Emetine Hydrochloride have great significance when one considers its pharmacology. This potent alkaloid irreversibly inhibits protein synthesis in mammalian, protozoan and yeast cells, but does not affect bacterial metabolism. Although Emetine Hydrochloride is toxic to both protozoa and yeasts, but not bacteria, Grollman (1970), the significance of this in relation to the ætiology of destructive periodontal disease has inexplicably been ignored or dismissed as insignificant.

Empirically treating periodontal disease with a potentially lethal drug, without accurate microbiological data for each patient, led to rapid abandonment of this form of therapy due to the sometimes severe complications encountered. With recent advances in pharmacology the use of Emetine Hydrochloride is now inadvisable, since it may cause a complete cardiovascular collapse even with topical application.

Dr Paul Keyes (1983), former head of dental research at the United States National Institute of Health, reported the almost invariable relationship between oral protozoa and periodontal deterioration. Dr Jason Tanzer, University of Connecticut, also recorded the presence of E.gingivalis in what he termed "aggressive osteolytic periodontal disease." (Personal Communication 1979.)

CLINICAL REVIEW

Since 1972 the author has routinely used a phase contrast microscope to evaluate plaque quality versus known clinical history of patients in general dental practice. In 1978, E.gingivalis was observed in the plaque from a destructive periodontal lesion. The plaque from a nondiseased site in the mouth of this patient was bacteriologically found to be essentially the same. The significant difference was the presence of protozoa only in the diseased site. This led to the investigation of the relationship between the oral protozoa and destructive periodontal lesions. In the ensuing ten years over 25,000 plaque examinations of clinical patients

have been made. The results, reported in this chapter, suggest a strong relationship between the incidence of destructive periodontal disease, the deterioration of the patient's general health and the occurrence of amœbæ in plaque.

Since amœbæ, when present, were consistently found at or near the base of the pocket, plaque samples were collected only from that area. Experience had shown that significantly fewer amœbæ could be found if samples were taken more coronally. E.gingivalis could seldom be recovered from sites less than 3mm deep.

Research at the University of Münster, West Germany, has shown that, in destructive periodontal lesions, organisms recovered from the base of the pocket are anærobic. It is only from the base of the pocket that significant numbers of oral protozoa are found (Prof D.E. Lange et al, personal communications 1983-1988). Further evidence to support E.gingivalis being anærobic is found in the research of Clayton and Ball, (1954). E.gingivalis was unaffected by anærobic conditions, even in the presence of bacteriostatic concentrations of Penicillin.

MATERIALS and METHODS

The mouth was examined in order to identify possible sites of disease. Plaque from a suspect site was examined by phase contrast microscope. (For comparison, plaque from apparently healthy sites were also examined.) A drop of the patient's saliva was taken from the sublingual area and deposited on a clean microscope slide. Plaque from an appropriate site was taken with a thin explorer from the base of the pocket. Care was taken to avoid taking supragingival plaque, food debris and other detritus. Care was taken to avoid promoting bleeding. The plaque was then quickly deposited in the saliva on the slide and detached from the probe with a second one. Care was taken to avoid agitating, teasing out or otherwise disturbing the sample. A cover slip was then dropped into position. The material was spread by squeegee pressure on the the cover slip to produce a thin film. Other liquids, such as broth or saline, when used as a mounting medium, temporarily distorted the amœbæ and made them unrecognizable during the time that a slide would normally be examined.

For laboratory examination, plaque from several pockets could be preserved in SAF fixative which was developed by Yang and Scholten (1977). Palmer and Scholten (1981) developed a new technique for processing dental plaque into modified iron hæmatoxylin smears. SAF is

well suited to the preservation of oral specimens since it is relatively nontoxic. It is unlikely to cause corrosion of dental instruments and allows for long periods of storage without deterioration of the specimen. This technique facilitates submission of samples to distant laboratories for primary diagnosis, or when confirmation of diagnosis is deemed necessary.

Statistical Relevance

After the initial case of Oral Amœbiasis was identified (Lyons, 1980) 200 previously uninfected patients were examined during the following winter. E.gingivalis was found in 62.5% of these patients, while Trichomonas tenax was found in only 4.5%. Information exchange with other dental offices confirmed that the percentage of patients with oral protozoa closely reflected the incidence of destructive periodontal disease, however, the ratio between the two infecting species of protozoa varied both by geographical location and time. It was noted that locations in more southern latitudes seemed to favour a higher incidence of the flagellate, T.tenax.

The plaque of those patients with destructive periodontal lesions was re-assessed. Although many authors, e.g. Socransky (1977) or Cambon (1979) used a standard site from which to take plaque, an infected site was now selected instead of an arbitrary standard site. In each case, oral parasites were only found in diseased sites. A clinical improvement, above that which could be obtained by routine home and office care, was then obtained by eliminating the protozoa. Protozoa were only found in "standard sites" if they were sites of disease.

Having identified a suspected site, plaque was examined by direct phase contrast microscope to identify all organisms, including protozoa. Where necessary, laboratory examination of fixed plaque samples was used to confirm diagnosis (Lyons, Palmer and Scholten, IAPM, 1981). In some cases only one of the two methods might be employed. The recommended protocol for the elimination of a potentially tissue invasive lumen dwelling parasite, the concurrent use of systemic and topical amœbacides, Grollman and Grollman (1970), was used for treatment of the infection. After completion of therapy patients were retested to ensure that therapy had succeeded. They were periodically retested and retreated if necessary. Routine periodontal treatment was initiated at the appropriate time in order to maximize the response.

Diagnosis of E.gingivalis is not difficult. However, to avoid false negatives, it is essential that the sampling be done meticulously. False negatives frequently resulted from:

Mishandling the plaque,
Using liquids other than the patient's own saliva,
Using fixatives other than SAF,
Using alternative staining techniques,
Taking plaque other than from the extreme base of the pocket,
The influence of recent medication,
The influence of recent hygiene,
Consuming some types of food or,
Consuming some types of beverage.

CLINICAL RESULTS

The signs and symptoms, associated with an infection of the oral cavity by E.gingivalis, frequently included:

1) apparent difficulty in maintaining a clean mouth;
2) heavy plaque formation which rapidly regenerated after removal;
3) an unpleasant taste;
4) an awareness of the gums,
5) gingival bleeding;
6) ulcerations;
7) a garlic-like halitosis;
8) sore, dry or itchy eyes
9) a history of generalized malaise,
10) fatigue,
11) frequent headache.
12) Protracted or repetitive influenza-like symptoms were frequent (if the infection had been recently contracted).

Systemic disturbance was not generally observed in patients in whom T.tenax was the only parasite found.

If E.gingivalis was recovered from apparently healthy gingival tissue and not eliminated, subsequent re-examination, almost invariably revealed a periodontal decline. Typically, initial infections might be accompanied by little or no transient soreness. Following an asymptomatic period, which may represent an incubation stage, is an influenza like illness which was typically severe or repetitive. Rather than returning to normal health, patients seemed to acclimatize to a diminished state of health which was frequently manifested by undue fatigue and more frequent headaches. At this stage periodontal deterioration was observed to occur, typified by the onset of bleeding and heavier plaque accumulation.

This phase of the infection ran a variable course over a number of years, during which patients usually experienced good general health. However, despite the best efforts of the dentist and patient, the periodontal condition slowly worsened. Pockets gradually deepened, there was apical migration of the periodontal attachment and loss of bone.

Nearing the terminal phase of periodontal disease, more alveolar bone is lost, the teeth loosen and periodontal abscesses may occur. It is at this phase that the general health of the patient also starts to decline. Some authorities hold that periodontal breakdown is symptomatic of a general decline in health.

Since E.gingivalis might be implicated in periodontal disease, patients' records were reviewed. Analysis suggested that infection with E.gingivalis preceded the oral and systemic declines. Treatment to eliminate the parasite was usually followed by reversal of signs and symptoms of both oral and general disease. In some instances, where there had been irreversible disease, although it did not worsen, elimination of infection only resulted in a state of stability. This indicated that some of the serious disturbances of the health of periodontal patients might be due to periodontal disease rather than vice-versa.

Incubation and Immune Response

Experimental evidence suggests that there could be an incubation stage, Kofoid (1929), and is also supported by the experience of King (Stones, 1954) who only succeeded in infecting himself with acute ulceromembranous gingivitis (synonyms: Vincent's Infection; ANUG) after a number of attempts. The ultimate successful attempt was preceded by a series of severe colds. Lehner (1967) reported prolonged elevation of IgM class antibody in recurrent ulceromembranous gingivitis which he stated would be consistent with a protozoal ætiology. Although ANUG

patients have been infrequent in this writer's practice, examination of plaque from typical ANUG lesions has always been positive for protozoa.

Periodontal deterioration in patients with oral parasites did not respond favourably to routine treatment unless covered by appropriate antibiotics. Without the latter, such treatment often worsened the patient's general or dental health. A higher success rate on the first course of medication, (judged by clinical improvement and absence of protozoa,) could be obtained by delaying most routine dental treatment until after the infection was controlled or eliminated. The duration of therapy, as well as the antiprotozoal drugs employed, varied with the severity and past history of the disease.

Clinical experience suggests that some infections are refractory and require more than one course of medication. Most of these, however, were cases where the patients did not follow prescription or home care instructions. Some cases were immediate re-infections during the healing or convalescent phase. Of those that appeared to have been genuinely refractory, the patients had other systemic problems. For example, long term use of antibiotics by patients with acne may have produced tetracycline resistant infections. Re-infections seemed to be largely related to a patient's social habits, for example, the sharing of food. However both direct and indirect mouth-to-mouth contact must be considered. These conclusions concur with earlier writers (Chandler, 1958; and Lapierre et Rousset, 1973) who stated that reinfection is to be expected until patients are prepared to make changes in their lifestyles and habits. Some apparent failures were found to have been due to superinfections with nonsusceptible organisms, such as Candida species, which were initially present and which flourished when the bacteria and protozoa were eliminated.

Experience gained from 1978 to the present upheld and confirmed the initial observations that deterioration of both the oral and systemic health was associated with Oral Amœbiasis.

DISCUSSION

Over the years there has been continuing controversy whether the oral protozoa are pathogens. Howitt (1926) reported that E.gingivalis ingested both erythrocytes and leucocytes in addition to the nuclei of leucocytes. Infection with E.gingivalis could be termed a disease in which the patients leucocytes are being consumed by the disease. Howitt (1926) states that the partly digested remains of leucocytes are very often

seen to almost fill the body of the amœba. In this state it often resembles a multinucleated giant cell (personal observations.)

Chandler (1958) succinctly summarised the potential pathogenicity of E.gingivalis:

> *"since this amœba ingests both red corpuscles and leucocytes, and can dissolve tissues, the burden of proof falls on those who believe in its innocence."*

So while medical graduates at that time may well have been aware of the evidence implicating E.gingivalis as a pathogen, their dental confreres remained blissfully unaware. He further went on to describe the habitat in which the amœba can be found:

> *"The amebas often cluster about on the filamentous bacteria which are involved in the formation of tartar, and prey upon the nuclei of the swarming leucocytes, without invading the adjacent gum tissue."*

He concludes:

> *"Whether the formation of pus pockets is initiated by the amebas is doubtful, but E.gingivalis is nearly always, perhaps always, present in the lesions, and at the very bottom of them, OFTEN BURIED IN THE INFLAMED TISSUES....."*

(Emphasis not in original text.)

> *".....The host reacts to the stimulus of this combination of bacteria, amebas and tartar by an active and continuous accumulation of leucocytes and resulting flow of pus. Even if the amebas do not actually initiate the ulcerations but merely find a pleasant field of activity in them ..... one must be very generous to absolve them from complicity in their extension."*

It seems incredible that such observations are not found in dental texts. Had this knowledge been transferred from the fields of medicine, parasitology and zoology to dentistry, research might have been more broadly based and not restricted to the bacteriology of dental plaque.

Rysky (1977) reported on the ultrastructure of E.gingivalis. He stated:

> *"the endocytic organelles together with multivesicular bodies and phagosomes indicate that these parasites are sufficiently pathogenic to maintain a stage of chronic irritation and to encourage the multiplication of other pathogenic organisms."*

It may be observed that these findings are comparable to typical periodontal deterioration.

Flagellates

Trichomonas tenax, whilst seen less frequently, and having less apparent effect on the general health of those patients in whom it was observed, also deserves much closer attention. Kazakova, Riogas and Teras (1977) isolated T.tenax not only from the mouths of patients, but also from the bronchial tubes. Working with Ryigas and Trapido, Kazakova (1976) also reported the presence of T.tenax in chronic lung diseases. Mussaev (1976) found patients with Paradontosis to be infected with T.tenax. Treatment to eliminate the organism cured the condition. Their statistics in relation to several thousand case reports were similar to those previously reported by Lyons et al (1980, 1982, 1983) on the incidence, cure and reinfection rate of patients with oral protozoa associated with destructive periodontal disease.

Trichomonas tenax should not be confused with either Trichomonas vaginalis, a pathogen of the reproductive system, nor Trichomonas hominis which may be found in infection of the bowel. Both Westphal (1936) and Stabler and Feo, (1942) who applied to human subjects the preliminary work of Bonestal (1936), achieved results similar to Karnaky (1934); namely that all three species of trichomonad found in humans are site specific.

T.tenax has been isolated from tonsillar suppuration and chronic purulent pulmonary disease, which might indicate that its habitat is the mouth and related structures in the respiratory tree. Hersh (1984) reviewed pulmonary trichomoniasis and reported two specific antibodies to T.tenax. This would lend credence to a pathogenic role. He notes that Trichomonas species, generally, have variable genetically determined pathogenicity. Some normally benign strains may have their pathogenicity enhanced by a DNA-RNA mediated virulence transformation. The

possibility exists that host antibody might prompt antigenic variation with this species.

Protozoa and Oral Hygiene and Environmental Factors

There have been many investigators of the oral protozoa who, like Westphal (1942), found that the the incidence of oral protozoa was not related to oral hygiene. The writer particularly notes that despite good oral hygiene the presence of E.gingivalis seemed associated with periodontal deterioration and venous stasis. E.gingivalis was associated with pockets which were typically 3mm or more in depth. When oral hygiene was poor, this deterioration was often masked by gingival inflammation. The degree of inflammation seemed to correlate with the numbers of motile bacteria seen on phase contrast microscopy. This inflammation decreased with improved oral hygiene but the apical migration of the epithelial attachment of the periodontal membrane, though slowed, was not arrested unless the protozoa were eliminated.

Perhaps a mutual dependence, even a synergism, might exist between the oral protozoa and other plaque organisms. Attempts to grow E.gingivalis in pure culture in Münster showed that no matter what antibiotic was used to eliminate the bacteria, antibiotics inevitably resulted in the death of the amœba culture. It may be tentatively concluded that almost any antibiotic might be of clinical value. The key is the right dosage and the appropriate duration of antibiotic therapy. By using a phase contrast microscope at regular intervals for immediate examination of the plaque, the clinician need no longer guess at the duration or effectiveness of therapy.

Clayton et al (1954) reported that E.gingivalis grew well at pH 7.0-7.5, and survived down to pH 5.5. Caries is commonly held to occur when the pH drops to 5.4 or less. In mixed cultures of E.gingivalis with bacteria the pH returned to a point close to neutrality within 24 hours, no matter at which end of the scale it started. This finding is compatible with the clinical observation that caries and periodontal destruction are seldom active at the same site and time. Moore (1988) reports that S.mutans (bacteria associated with cariogenic activity) are negatively associated with sites of periodontal breakdown.

Soil Amœbæ and Systemic Infection

Even if the oral protozoa, particularly E.gingivalis, prove to be non-pathogenic, their role in transmission of other micro-organisms, such as

virus and viroid particles, requires careful evaluation. Elsdon-Dew (1976) found subcellular organisms in Entamœba histolytica. Schuster and Dunnebacke (1974) reported virus like particles in a free living amœba of the genus Nægleria. Armstrong and Pereira (1967) demonstrated that the infamous "Ryans Virus" variant of the Poliomyelitis virus was an amœba of the genus Hartmanella. The virus particle so completely filled the body of the amœba that it appeared to be a giant virus under the electron microscope. A normal stained slide examined by light microscopy revealed the truth.

Rowbotham (1980) at Leeds Public Health Laboratory, reported that two types of common free living amœbæ (Acanthamœba and Nægleria) were "infected" with and might vector Legionella bacteria. Once inside the amœba, the bacteria now protected from the environment, could be transported in water droplets. If inhaled, the cytoplasm packet (the amœba) could rupture releasing a high concentration of bacteria into a single site. A single amœba can vector more than enough bacteria to cause an infection. Thus, one amœba, if inhaled, can rupture and release into one lobe of the lung enough bacteria to cause pneumonia. Typically, the disease is lobar in distribution. If only one droplet containing one soil amœba can initiate infection, the time the patient spends in the "risk area" would not appear to be a significant factor in contracting Legionaires' Disease. Wright et al (1988), at the University of Calgary, artificially produced aggregates of Legionella which they introduced into the lungs of Guinea pigs. The animals showed higher morbidity and mortality than animals infected with an equal number of bacteria introduced as single cells.

Vectoring and Spread of Infection

E.gingivalis could be the critical factor in vectoring other less easily identifiable organisms. There may even be an essential symbiotic relationship between E.gingivalis and these organisms, as suggested by Dao (1985). In either case, the possibility of an association between protozoa and bacteria at infected sites would be significant. Many of the oral bacteria, even those which have the potential for pathogenesis, are difficult to readily identify, whereas E.gingivalis is relatively easy to identify. For this reason, E.gingivalis would still remain significant as a target organism even if it were proven to be a nonpathogen.

A common finding (Lyons et al, 1980), is the relationship between a new oral infection with E.gingivalis and the development of general malaise or influenza like symptoms. These systemic phenomena may be

indicative of a sudden release of virus and/or other antigenic material. Systemic disturbance, which may be loosely described as influenza, is sometimes noted following routine dental procedures. This was previously held to be just a coincidence (Royal Dental Hospital, London, UK ca 1958). However, clinical experience and microbiological data of such cases suggest that only those patients already infected with E.gingivalis reported such disturbances. It might be concluded that dental procedures, such as the use of high speed water-cooled instruments, produce an infective ærosol spray which the patient could inhale with unfortunate consequences. This would also imply considerable risk for the operator. In order to reduce these risks, all but emergency dental treatment is now delayed until the infection has been controlled or eliminated. Even a periodontal examination, or probing to remove plaque, may sometimes be followed by systemic disturbance. Experience has shown that resolution rapidly follows institution of appropriate antibiotics.

Since E.gingivalis is about the same size as the blood cells, on which it feeds, (see next two chapters) instrumentation around a site infected with protozoa might produce a parasitæmia. It is not surprising, then, to find that Snyderman and McCarty, (1981) report similar pathology in rheumatoid arthritis and destructive periodontal disease. This similarity in pathologic processes might indicate a common ætiology. Over fifty years ago Kofoid (1929) reported finding entamœba in the bone marrow of some arthritic subjects. This might help explain the historical relationship between arthritis and periodontal diseases and the treatment of arthritis with antiprotozoal drugs. Following the elimination of E.gingivalis, not only is reversal of periodontal destruction observed, but some patients with arthritis report a dramatic reduction in signs and symptoms of the disease. This improvement is usually maintained unless the patient becomes reinfected. The converse is reported by Freeman (1980). Antiarthritic drugs were undergoing promising clinical trials for the control of periodontal disease (University of Toronto, Dental School). The writer has found that salicylate antiarthritic drugs do seem to suppress E.gingivalis to the point where the organism is hard or impossible to find in plaque. However, if therapy is stopped for a short time E.gingivalis will re-appear in plaque and the arthritic symptoms return. It may be too simplistic to think of periodontal disease as a single disease, or even just a local disease. For some this may be the case, while for others it may be the local manifestation of a systemic disease. In other cases it may be an oral disease with systemic implications and manifestations.

Of the drugs effective in the treatment of periodontal disease, it should be noted that Metronidazole, as well as a wide range of antibiotics are effective against E.gingivalis and a varying bacterial spectrum. However they have no effect on fungi. The antiamœbic, Emetine Hydrochloride, is effective against protozoa and yeasts but it does not interfere with bacterial metabolism. The common denominator appears to be that all of these drugs are effective against oral protozoa.

CONCLUSION

Stated briefly, the weight of evidence points to the strong likelihood of E.gingivalis being the primary periodontal pathogen, with systemic manifestations, in destructive periodontal disease. References in the literature, together with clinical experience reported in this text, clearly indicate that these organisms deserve consideration as systemic as well as oral pathogens.

Hilaire Belloc put matters succinctly:

"The microbe is so very small,
We cannot make him out at all."

He finished by saying:

"But scientists who ought to know
Assure us that it must be so.
Oh! Let us never, never doubt
What nobody is sure about."

CHAPTER III

ABOUT ENTAMŒBA GINGIVALIS
FURTHER EVIDENCE OF PATHOGENICITY

MICROBIOLOGY PREAMBLE - Entamœba Gingivalis Unmasked.

E.gingivalis feeds on red and white blood cells by "sucking" out the cell contents of the living cell. This was discovered on January 24, (Lyons, 1984) between 5:30 & 6:30 pm. Rather than being a harmless scavenger of cell debris, this amœba can now be seen as a uniquely adapted aggressive, predatory parasite which destroys living tissue.

In house observations on E.gingivalis support the published reports of others who have previously postulated on the pathogenicity of E.gingivalis. Rysky's (1977) observations on the ultra structure were preceded by Wantland et al (1958) who found that "both E.gingivalis and T.tenax are capable of cytolysis of epithelial cells, erythrocytes and leucocytes." Chandler (1958) also added that E.gingivalis possessed "a peculiar adhesive quality" and commented that Howitt (1926) had observed that red corpuscles, lying near E.gingivalis, "faded from view in a few minutes, indicating cytolytic action." Nolte (1977) states further that "the pathogenicity of a micro-organism is related to the sequence of its ability to:

1.) adhere,

2.) penetrate and grow in and on epithelial cells and

3.) bring about pathologic changes that result in disease."

Published reports regarding oral protozoa support a pathogenic role. The writer has personally observed that E.gingivalis possesses a very sticky cell membrane. Interesting plaque samples have frequently been observed until the slide dried out. The capillary attraction holding the coverslip down would be broken due to the loss of saliva by evaporation from the edges of the preparation, air would get under the edge of the coverslip and the cover slip would lift suddenly. This resulted in rapid

fluid movement between islands of bacterial plaque which can be likened to a river, during the spring run off, carrying off anything that got in the way.

The VCR recorded a column of amœbæ pushing their way through such a plaque sample. The cover slip lifted slightly and there was a sudden stream of bacteria, debris, leucocytes and erythrocytes gushing along the middle of the field of view. The lead amœba faltered slightly, then pushed forward across this stream creating a dam around which the fluid and cells had to flow. The amœba quite obviously controlled a limpet like tenacity, since it continued forward while the fluid pressure tore the adjacent jumbled mass of bacterial plaque apart. This adhesive characteristic would make it extremely difficult to dislodge by simple hygiene methods alone unless the stickiness of its cell membrane could be disrupted.

Since mixtures containing salt or baking soda or both, together with their inclusion in mouth rinses has traditionally been suggested to be effective, the author investigated the effect of saline solutions on E.gingivalis in some simple in vitro tests.

In Vitro Observations on the Effect on E.gingivalis of Various Liquids Including a Saturated Solution of Modified Torren's Powder

Plaque samples were prepared and examined as previously described. A drop of saturated solution of Modified Torren's Powder (Lyons 1980) was then placed at the edge of the cover slip while an area containing amœbæ was kept under observation. When the solution reached the amœbæ they immediately and rapidly reduced in size and became more opaque. Their internal structure could no longer be differentiated. Some amœbæ floated away, suggesting that they also lost their stickiness. They showed no sign of vitality, but observation for a further twenty to thirty minutes demonstrated that the amœbæ slowly expanded from these unrecognizable opaque masses to become, once again, clearly recognizable vital amœbæ.

The trial was repeated employing a skin cleanser containing Aloe Vera. This fluid not only disrupted the ability of the amœbæ to adhere, but cell membrane lysis occurred in a matter of seconds. With complete disruption of the cell, the contents dispersed.

Observations and Data Suggesting a Cyst Form of E.gingivalis

Wantland et al (1961) reported that E.gingivalis forms true cysts. However, most authors do not report them and believe that the biological necessity seems to have been eliminated. Chandler (1958) noted the ease with which the organism is passed directly from mouth to mouth, or as droplets from sneezing or coughing, or indirectly from contaminated articles. However, after ten years of observation the writer believes that there is either a cyst stage, or a resistant form, characterised by a slightly tougher and less sticky cell membrane. This conclusion is drawn because amœbæ have been observed apparently floating in plaque, largely rounded out and somewhat denser and more opaque than usual. The associated pocket was usually shallow (less than 2mm) and found in patients exhibiting no abnormality of oral or general health. Initially such observations were thought to be insignificant until it was subsequently discovered that such patients soon developed a flu like illness. This almost invariably was associated with a decline in periodontal health. The writer now regards finding "non-sticky" amœbæ as evidence of a very recent infection, usually within the last twenty four hours. Some patients seem to be aware of when they contracted the infection because they can relate the onset of symptoms to a particular event.

In Vitro Observations of E.gingivalis in Plaque Following In Vivo Application of Modified Torren's Powder

After clinical examination, plaque was taken as previously described. On first examination, the amœbæ were observed to be very active. The patient then patted Modified Torren's Powder onto the gingival margins (see Chapter X) and the plaque was re-examined about fifteen minutes later. An amœba was found in close proximity to a white granular mass which was presumed to be the salt/soda mixture. This amœba was kept under observation and photographed at regular intervals over the course of the next forty five minutes. A series of vacuoles were observed within the body of the amœba, each apparently filling with fluid before rupturing to discharge their contents. With the excretion of fluid the amœba became successively smaller and the cell membrane shrivelled. Finally, apparently unable to control its internal osmotic pressure in the presence of the powder, the cell membrane of the amœba ruptured, leaving the carcass resembling a burst sausage.

In Vitro Observations on the Effect of 17% Alcohol Applied In Vivo to E.gingivalis in Plaque

The effect of 17% alcohol was also observed. On examination of the plaque, motile amœbæ were observed. The patient agreed to savour a glass of sherry prior to a second plaque examination. When this second plaque sample was examined, about twenty minutes later, the amœbæ were now observed to be dormant, somewhat enlarged and rounded.

In Vitro Observations on the Feeding Mechanism of E.gingivalis

These observations were made at the end of the day. This might seem like an irrelevant comment, but the most frequent time when the writer has observed E.gingivalis doing anything other than lying relatively dormant in the plaque, has been at the end of the day. In this context, the end of the day means the end of the patient's day (i.e. when they are more likely to be a little fatigued from a full day's activity). This might suggest that E.gingivalis has a circadian rhythm associated with its life cycle.

The organism was observed in a saliva mounted plaque sample. The slide had been set aside after initial diagnosis on the top of the warm TV monitor for about forty five minutes. The temperature on the monitor was about 29°C. A number of E.gingivalis were observed to repeatedly "attack" living leucocytes. Cells with large single nuclei (lymphocytes) as well as comparably sized cells with lobed nuclei (polymorphs) were attacked. The amœba were seen to insert a finger like projection of cytoplasm through the cell wall of the leucocyte, locate the nucleus, penetrate the nuclear membrane, liquefy the nuclei and "suck" the liquefied nucleoprotein, together with the involuted nuclear membrane, down into the body of the amœba. The whole mass instantly became "the partly digested remains of a leucocyte nucleus in a food vacuole". The amœba then withdrew the "proboscis" leaving a denucleated cell. The cell membrane was apparently sealed at the termination of this predatory attack since no leakage was seen to occur. Individual amœbæ were repeatedly seen feeding on as many as four blood cells simultaneously. The time taken to denucleate a white cell was approximately two minutes.

E.gingivalis was also observed to attack other leucocytes and consume cytoplasm by "sucking" it from the cell. A broad pseudopod would be flattened against the cell membrane of a leucocyte and the central area would depress giving the appearance of an upper and lower jaw taking a

bite out of the leucocyte. The cytoplasm drawn into the amœba would become engulfed by the amœba cytoplasm. The amœba was seen to "swallow" globules of leucocyte cytoplasm, one after the other, with each globule passing into the amœba as if in a peristaltic wave. These globules seemed to be rapidly digested since they quickly shrank in size. Again, the cessation of the feeding cycle on the blood cell was accompanied by closure of the cell wall and no leakage was evident when the amœba detached itself. A paper by Horace Child (1926) had drawings of E.gingivalis ingesting leucocyte nuclei which match these observations.

In addition, E.gingivalis was observed "sucking" out all or part of the contents from erythrocytes. Often all that remained was the erythrocyte membrane. As an erythrocyte floated near to an amœba, the parasite put out a pseudopod to which the erythrocyte became stuck. With the two cells lying adjacent and seen to be just touching, the hæmoglobin could be observed flowing into the parasite. There was a thin black line running from the junction of cell membranes down into the body of the amœba. The tip of this line, once well within the parasite, expanded into a small dense black spot about the size of a single streptococcus. The distal end of the line faded as the erythrocyte emptied. Finally, all that remained was a small dense black dot that resembled an ingested bacterium. Again, apparent closure of the "wound" was observed when the feeding cycle was complete. This process, from the first indication that a stream of hæmoglobin was entering the amœba until the erythrocyte faded to the extent that all that could be seen remaining was the faint outline of the cell membrane, took only 18 seconds.

Observations on Protozoal Behaviour Patterns in E.gingivalis

Almost invariably, E.gingivalis from plaque, when examined in saliva by phase contrast microscopy, all seem to be in a similar phase of their lifecycle at any one time. For example, if one amœba is feeding, most of the colony are feeding. If one amœba is moving purposefully across the field of view, many will be travelling. Often they will be travelling in the same direction and when in groups they will often be in line astern, reminiscent of a wagon train in the Old West. If one amœba is apparently dormant, presumably digesting its food, the majority will be observed in the same state. When one finds one dead amœba, the nonvital remains of many of its compatriots will frequently be found littering the field.

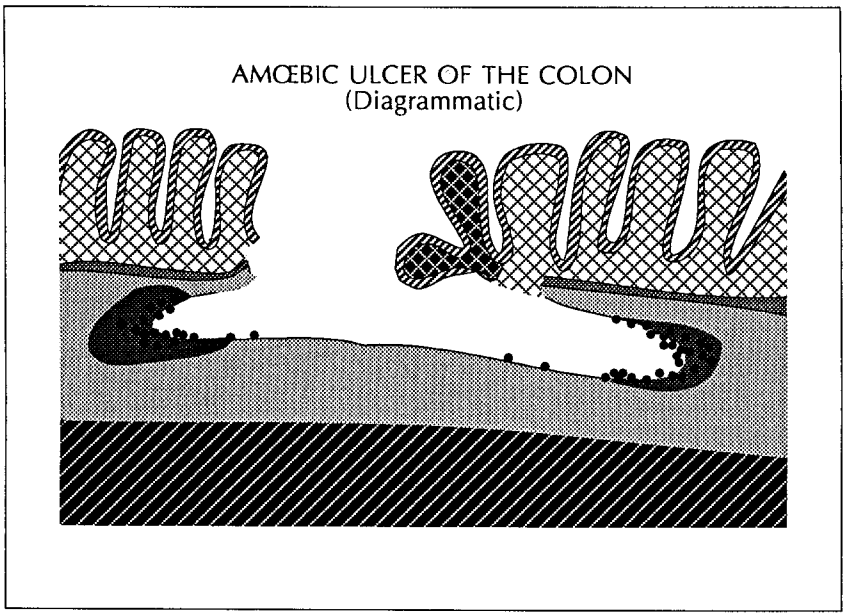
It is usual to find E.gingivalis (in severe destructive lesions) associated with a mixture of bacteria that include organized spirochætes attached by one end to a bacterial filament. The whole palisade of spirochætes

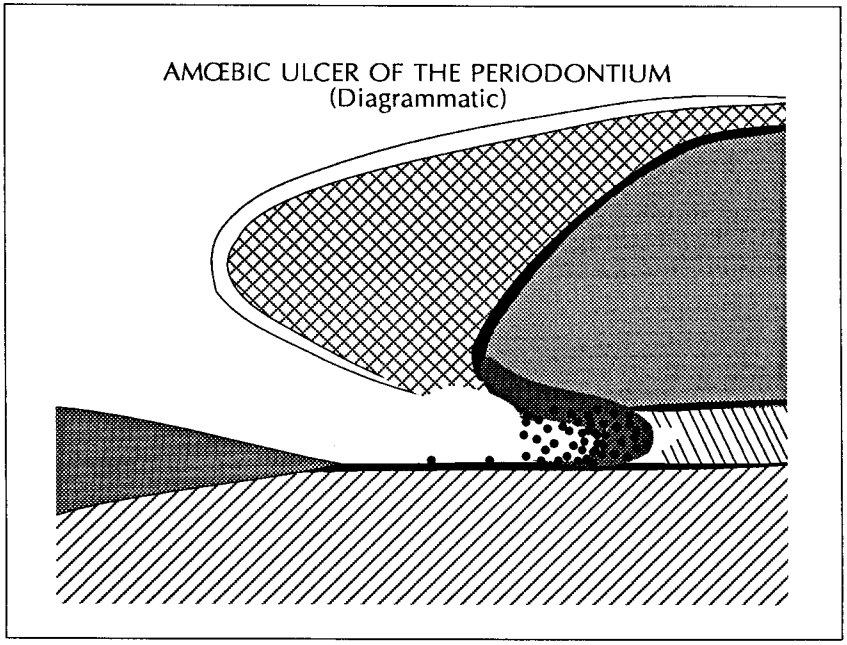
exhibit a uniform wave motion. In addition, many large motile bacilli (possibly "Fusiformis"), some free swimming spirochætes, nonmotile rods, cocci and filaments can be found. Intermingled in with all of the latter will be found branching filaments (a species of Actinomyces) to which small round bacteria (Cocci) are attached. This symbiotic colony is referred to by the first letter of each constituent genus: ACs. The appearance is of a piece of spaghetti which has been dipped in honey and then dipped in peas so that the peas are stuck to the spaghetti. In this bacterial mass will be found amœbæ, usually in clusters or nests. If this microcolony is close to the ACs, the amœbæ are often observed to be largely dormant, or slowly crawling over each other, like a litter of puppies. If they are away from the ACs, then the amœbæ are often seen to be moving purposefully, pushing and squeezing their way, as if following invisible tracks in the plaque. One behind the other, their march forward seems to be relentless. At times, apparently marching to instructions, the amœbæ will move toward the ACs from all directions. On arrival, the amœbæ crawl around each other, pushing and jostling until the whole colony settles down in the branches of the ACs. Engorged with the remains of blood cells in their food vacuoles, these dormant amœbæ seem to be in process of digesting their food.

COMPARISON WITH ENTAMŒBA HISTOLYTICA

A parallel must be drawn between the nesting behaviour of E.gingivalis as seen in dental plaque and the nesting behaviour exhibited by its close "cousin", Entamœba histolytica. When an amœbic ulcer of the colon is sectioned, nests of E.histolytica will be found under the overhanging margin of the ulcer, not on the floor. The ulcer spreads as the amœbæ migrate further under the intact mucosa, undermining it and disrupting the vascular supply. This ultimately becomes severed and the overhanging epithelium necrotizes and collapses. The amœbæ continue to invade laterally and the process repeats.

Now consider the parallel with the periodontal attachment of the tooth. The point of comparison is that the amœbæ are at the base of an epithelial flap. The tip of the free gingival margin would correspond to the tip of the overhanging epithelial flap of the amœbic ulcer in the colon. The surface of the tooth would correspond with the base of the ulcer. The gingival margin would correspond to the flap of the ulcer. In both cases the lesion spreads "laterally" as the amœbæ migrate parallel to the floor of the "ulcer". (Pocket deepens or ulcer widens). In both cases there is destruction of the epithelial flap (gingival necrosis; epithelial


AMŒBIC ULCER OF THE COLON
(Diagrammatic)


AMŒBIC ULCER OF THE PERIODONTIUM
(Diagrammatic)

necrosis). In both cases the base increases in size (gingival recession and loss of periodontal attachment; expansion of ulcer). In both cases the outcome could be chronic localized disease, acute localized disease, with either of the latter resulting in life threatening emergency, (periodontal abscess and cellulitis, perforation of the ulcer) or extension of infection to adjacent structures, (amœbic tonsillitis, liver abscess.) This makes an interesting comparison when one considers that the amœbæ are found at the junction between the epithelium and the "base" in both instances. E.histolytica has been described as moving less purposefully than E.gingivalis (Westphal, 1941). Both possess the ability to cytolyse red cells and epithelial cells but E.gingivalis also cytolyses leucocytes. Reports from the literature indicated neither are capable of initiating infection without the concomitant presence of bacteria. (Levine, 1973, p.147 re E.gingivalis. Grollman and Grollman, 1970, p.649 re E.histolytica). E.histolytica has long been recognized as a pathogen while E.gingivalis has been at the centre of continuing controversy.

DISCUSSION

The organisms in an anærobic infection have a mutual dependence on each other. The symbiotic relationships within such a complex flora may well result in a degree of synergism which considerably enhances pathogenicity. (56th Conjoint Meeting on Infectious Diseases, BIOP Symposium, Pathogenicity of Anærobes, Calgary, 1988.) Taken individually, the organisms may be relatively harmless or incapable of survival. Thus, it may well be that E.gingivalis belongs as a symbiant with the anærobic bacteria and is not, of itself pathogenic. However, this conclusion does not fully account for the ability of E.gingivalis to lyse epithelial cells, erythrocytes and leucocytes. Even if one accepts the argument that E.gingivalis is a nonpathogenic symbiant in a mixed anærobic infection, its presence could still be significant. Clayton and Ball (1954) described their experiments with E.gingivalis in bacterial plaque taken from the mouths of volunteers. Using Penicillin to achieve bacteriostasis, the amœbæ failed to multiply and died out. This finding could be applied in a clinical setting. The use of an antibiotic until such time that there were no further amœbæ might be a useful indicator that pathogenic bacteria had been eliminated. This could be a useful test for those ascribing a bacterial ætiology for periodontal disease. If one accepts that the mutual dependence and synergism exist between the organisms in a mixed anærobic infection, then the elimination of a target organism within this group may have the effect of collapsing the house of cards which comprises their ecosystem. It is interesting that after nearly thirty years of research into

the bacteriology of periodontal disease, "recent data indicate that the flora of actively progressing lesions is not of significantly different (bacterial) composition from that of matched sites that are not detectably active in the same person." (BIOP Calgary. WEC Moore. 1988).

Bacteria effect a pathogenic role by the liberation of toxins. These simple organisms with a simple life cycle, by sheer weight of numbers and rapidity of multiplication produce sufficient toxins for pathogenesis. The amount of toxin produced will be dependent upon their numbers and their metabolic rate. By comparison, protozoa are complex organisms with a life cycle that varies according to species and having a duration of several days. The amœbæ produce and store toxins within their bodies and meter it out in order to control their environment. If these parasitic organisms had some way of co-ordinating their life cycle, that co-ordination would give relatively few amœbæ, clustered together in a nest, a greater destructive potential than the same number of amœbæ scattered randomly and behaving independently from each other. It would be compatible with states of remission and exacerbation found in periodontal destruction and help explain why apparently few protozoa could have a more devastating effect than their sheer numbers alone might suggest. It could also explain why the numbers of amœbæ, present on microscopic examination, fluctuate from one day to the next if the same patient is repeatedly examined over several consecutive days.

As previously discussed, amœboid behaviour in the same sample did seem to be in phase. This phase behaviour has also been found when two samples are taken from noncontiguous sites in the same mouth and compared. On the basis of clinical observations the following scenario is proposed:

PHASE I

Initially the amœba arrives in a semi-resistant form. If there has been tissue injury, mechanical or bacterial, even transient irritation from bacteria metabolising sugar to acid, the amœba can survive because the environmental conditions are favourable. First the parasite feeds on erythrocytes which are already present in the pocket due to pre-existent injury. Having obtained its "fix" of hæmoglobin, the amœba then secretes toxins into its environment. The irritation and tissue destruction attracts leucocytes which migrate into the area. They are then sacrificed as further nutrition for the amœba.

PHASE II

During this phase of the cycle the amœba digests its prey. It remains rounded up and sluggish, putting out pseudopodia randomly. When the digestion phase is complete, it becomes active and moves apically, feeding as it goes. During the feeding stage the differentiation between ectoplasm and endoplasm largely disappears and the amœbic nucleus is difficult to find.

PHASE III

After feeding and migration there is cell division, with small daughter cells (amebulæ) budding off from the parent; the latter retains most of the food vacuoles. The budding of daughter cells occurs more than once before the parent cell, which is more apical than the daughter cells, dies. Death of the amœba releases toxins into the tissue which results in further destruction and bleeding. This provides a source of food for the succeeding wave of invading daughter cells. The latter are tiny, about half the size of an erythrocyte, i.e. about 4 microns. The nuclei of these tiny "amebulæ" cannot be easily seen with phase contrast microscopy.

The amebulæ then feed and grow and the cycle repeats, with succeeding waves of amœbæ invading, multiplying and dying. Greater risk to the patient occurs if invading amœbæ do not all die, but continue to invade into the tissues. Even more risk occurs if amœbæ gain access to the lymphatic or venous drainage systems. This might allow contiguous spread through tissues or transport to distant sites. Either of these effects could be exacerbated by any instrumentation at an infected site. Even a simple periodontal examination could carry an inherent risk.

VIABILITY

The successful parasite must develop ways to avoid being eliminated by the host response. Those that have not evolved such a mechanism simply have become extinct. While some parasites are "opportunistic" in that they can live either freely in the environment or within a host, E.gingivalis is an obligate parasite since it needs a host for survival. However, Cecil B. Hoare (1949) reported that E.gingivalis is surprisingly resistant to desiccation and a wide range of temperature and pH variation. It can survive at the freezing point for 18 hours, at 45°C for 20 minutes and for up to two days at room temperature provided it is not completely dried out. In vitro experiments with saline solution confirm its adaptabil-

ity to changes in its environment and its ability to recover from adverse environmental conditions.

Its hardiness may be demonstrated by taking a sample at the end of the day (say 5 pm): confirm the presence of active amœbæ; prevent the preparation from drying out by painting around the edge of the coverslip with immersion oil; leave the slide on the stage overnight, with or without heat from the illumination system; observe the slide the following morning. Providing the oil immersion lens has not been used, it is usually possible to find still viable, easily recognizable amœbæ. If the oil immersion lens is used, the dragging and pumping action transferred to the cover slip by stage movements and focus changes may cause rapid devolution of the preparation.

SUPEROXIDE THEORY

The feeding mechanism of E.gingivalis might be explained in the light of parasite avoidance mechanisms and the leucocyte response to pathogens:

Leucocytes liberate superoxide, a highly active form of oxygen, in the presence of foreign antigen. One may speculate that E.gingivalis employs the hæmoglobin, which it previously ingested from erythrocytes as a countermeasure. When leucocytes are encountered, this hæmoglobin, with its affinity for oxygen, might be used to absorb the superoxide thus leaving the leucocyte powerless and vulnerable to attack.

To encourage more leucocytes (food) into the area the amœba, or a symbiant, releases antigen which causes irritation, cell necrosis and venous stasis with leakage of whole blood into the tissue. Foreign antigen also significantly increases the concentration of pus cells (leucocytes).

Kofoid (1929) remarked that E.gingivalis exerted a chemotactic attraction for leucocytes. This surface antigen of the amœba, or some similar chemotactic substance, encourages the accumulation of leucocytes at the site of infection. Armed with hæmoglobin, to protect it from the leucocyte, the amœba is now provided with a plentiful and unending supply of nutrient cells which constantly migrate into the trap.

EPIC THEORY, AUTOIMMUNITY AND THE PERFECT PARASITE

An alternate theory for the mechanism of destruction in oral amœbiasis is provided by recent understanding of some of the enzyme pathways involved in destructive periodontal lesions. R. Mueller (1988) reports that polymorphs produce an enzyme ("elastase") which is proteolytic. This is normally bound to a circulating liver enzyme, ("proteinase inhibitor") to form "elastase proteinase inhibitor complex" (EPIC). If the formation of this complex is overpowered, rapid destruction results.

It has been stated (Hoare, 1949) that the numbers of amœbæ seen in relation to periodontal lesions could not be great enough to account for the degree of destruction observed. If subgingival plaque from an active rapidly destructive periodontal site is microscopically examined, it is found that the leucocytes outnumber the amœbæ about one hundred fold. Either the denucleation or the "stinging" (see later this chapter) of leucocytes by amœbæ might leave leucocytes in an uncontrolled state of maximum production and release of elastase. This would then locally overpower the EPIC resulting in rapid uncontrolled lytic activity.

Destructive periodontal disease has been considered as an autoimmune disease (Genco and Mergenhagen, 1982). The uncontrolled release of elastase resulting from disruption of leucocyte metabolism caused by E.gingivalis would be compatible with classification of destructive periodontal disease as an autoimmune disease. It would also emphasize the exquisite adaptation of E.gingivalis as an obligate parasite, completely dominating the immune response of the host. It might be argued that E.gingivalis is the epitome of parasites since the parasite commands the host to destroy its own tissues in order to promote a flow of blood cells for the sole purpose of feeding the invading organism. The supreme irony of this state of parasitism is that the very cells which should protect the host in fact destroy the host and are then, in turn, consumed by this predatory parasite, Entamœba gingivalis.

VIRAL VECTORING BY PROTOZOA

Contact between blood cells and E.gingivalis could result in the latter becoming "infected" and therefore become a vector for virus. The latter could easily be inserted into or removed from blood cells during the feeding process. Since E.gingivalis is not genetically similar to human cells, it is also possible that a virus would have less pathogenic affect on

amœbæ than white cells. Since virus would then be free to multiply within amœbæ, virus would also be beyond the reach of the human host's immune response. This could result in constant showering of the host with virus. Initially the host might be able to deal with viral showers. Ultimately, the viral source, protected by encasement within amœbæ, would be unassailable by the host as long as viable amœbæ remained. The end result could be quite debilitating for the host.

COMPARISON OF INFECTIONS

It is observed with amœbiasis that, at the site of infection, there is sluggish circulation. Venous stasis results in circulatory congestion and lowering of the tissue temperature. This could allow the parasite to overpower the local host defences and a rapid noninflammatory destructive disease might ensue. Should (bacterial) inflammation occur, the temperature would rise and tissue destruction would proceed more slowly. This could explain some of the clinical differences usually observed between periapical (bacterial) and parodontal (amœbic) abscesses.

Since E.gingivalis thrives in an anærobic milieu, the use of hydrogen peroxide, which releases nascent oxygen, would have obvious beneficial effects. Modified Torren's powder and hot saline rinses, if they exert similar action in vivo as they do in vitro, would also be of significant value in promoting resolution and healing in oral infections. Hydrogen peroxide is also toxic to yeast cells and is trichomonacidal, as are alcohol and some fruit juices. In clinical practice, it has been found that alcohol and fruit juices and some fresh fruits, such as pineapple, have a dramatic effect on the numbers and morphology of protozoans found on phase contrast examination.

E.GINGIVALIS AND BLOOD CELLS

An alternate explanation for the feeding habits of E.gingivalis might be that it is an ærobe, even though it thrives in an anærobic milieu and relies on the presence of anærobic bacteria for survival. If this were the case, attacking red cells and consuming their hæmoglobin would provide the amœbæ with oxygen.

Whatever the end result of research may show, the fact that erythrocytes and leucocytes are attacked and destroyed is not without significance. The ability to phagocytose erythrocytes has been held to be a significant factor in differentiating between pathogens and non-

pathogens since nonpathogens do not possess this ability. (Jaskoski, Transactions of the American Microscopical Society, Vol LXXXII, 1963).

Reports that E.gingivalis consumes "salivary corpuscles", (Child, 1926) evidence gained by examining stained slides, cannot be upheld by modern technology, using phase contrast microscopy. In wet mounts of dental plaque there are whole leucocytes. Seldom will nuclear remnants of dead leucocytes be observed. However in stained slides prepared from SAF fixed plaque, all that remains of the leucocytes, after the vigours of slide preparation, are the nuclear remnants. Child (1926) stated that the literature contains much that is inaccurate, with sweeping conclusions drawn from inconclusive results. The evidence presented has therefore been carefully amassed over the last eleven years in order to be sure that the conclusions drawn have a firm foundation.

GENERAL MORPHOLOGY OF E.GINGIVALIS

Having spent so many hours looking at E.gingivalis, it is relatively easy to overlook some pretty basic concepts. The descriptions of E.gingivalis from stained slides do not match its appearance in life. With a green filter on a phase contrast microscope, the whole field is green, especially the ectoplasm of the amœba. There is usually a clear deliniation between the ectoplasm and the endoplasm, the latter being a darker green. Inside the endoplasm can be seen a series of mainly round dark green to black objects. Some are in the range between pinpricks and the size of cocci; most are between a half to a quarter the diameter of a red cell. The former are hæmoglobin granules, while the latter are remnants of leucocyte nuclei. Some relatively clear vacuoles will also be seen, sometimes with a few black granules within. These vacuoles contain leucocyte cytoplasm complete with the leucocyte granules. The cytoplasm of the amœba is hyaline, not as stated in the literature, granulated. It looks like a blob of light green and dark green jelly which is translucent and of even texture.

"STINGING"

The cell membrane of E.gingivalis, as mentioned, is sticky. Often an amœba will be seen to approach a leucocyte and flatten itself against the leucocyte as the amœba slides past. Three phenomena may be observed. First, there is sometimes a sudden movement within the cytoplasm of the leucocyte, as if something has been injected. Second, starting at the cell membrane where the amœba touches the leucocyte, the granules in the leucocyte are seen to slow down, clump against the cell membrane and

finally stop moving. Third, this loss of movement of the granules spreads through the cell, the granules clump and clear open spaces replace the dense shimmering within the cell as it degenerates. The leucocyte never quite seems to die. It is in this state that amœbæ attack and feed on leucocytes. Feeding then, seems to be a two stage process. First the cell is "stung" and degenerates. Second, the amœba comes back to feed on its prey. The amœba which stings a leucocyte moves on and stings others, often trailing a clump of host cells, stuck to the "tail" of the amœba. The amœba remains viable. The leucocytes degenerate. Other unattacked leucocytes remain viable.

BINARY FISSION

E.gingivalis reproduces by binary fission. The writer has been unable to observe typical mitotic activity. There appears to be two reasons for this. Firstly, the mitotic division is probably atypical (Stabler, J. Morph. Vol 66 No 2) and secondly, E.gingivalis does not often display binary fission during normal working hours.

ENVIRONMENTAL RESPONSE AND RHYTHM

Both Child (1926) and Kofoid (1929) postulate that E.gingivalis becomes dormant, or shocked, if suddenly disturbed, as occurs when a plaque sample is taken. It is observed that, if additional time beyond that which is necessary for diagnosis of oral amœbiasis, is taken, E.gingivalis frequently becomes more active as the slide warms from heat generated by the microscope. Further, since protozoa are more complex than bacteria, its life cycle cannot be measured in minutes. From careful observations the writer believes there is a diurnal rhythm in which this parasite is more active when its host is tired or sleeping. This would certainly explain why marked activity has normally been observed (feeding, moving or multiplying) late in the day of the patient. For shift workers, the amœba is active when they finish their shift. Increased parasite activity when the host is tired might also explain why patients with E.gingivalis often report night sweats which cease after the infection has been eliminated.

Night sweats are commonly known to be symptomatic of parasite infections. Diurnal rhythms of disease are well known with malaria. The symptomology coincides with stages of the cycle of Plasmodium, (the

malarial parasite). Different species have different cyclical rhythm. This matches the differences in the rhythms of types of malaria. For some variants of the disease, blood tests must be done in the middle of the night, or results will be negative, since organisms can only to be found in a blood smear during a relatively short period of the night.

INGESTION AND EXCRETION

E.gingivalis does not put pseudopodia out on either side of an object to be engulfed. It inserts a finger like projection into white cells; it puts a flat pseudopod against other objects, including the occasional bacterium, and sucks. This is similar to the feeding mechanism of Didinium nausatum, a free living protozoan which is a predator of Paramecium species.

E.gingivalis excretes undigestible portions of leucocyte nuclei by bringing a vacuole to the cell membrane and disgorging the contents. These, the excreted nuclear husks from leucocytes, probably represent the bulk of the leucocyte nuclei which may occasionally be seen in plaque.

FASTIDIOUS FEEDER

Apart from the rather obvious comment that E.gingivalis is no scavenger of cell debris, the writer has never seen it consume a leucocyte nucleus that just happened to be lying around. Should there be any present, the amœba simply skips right past and finds a nice juicy whole live leucocyte on which to feed.

It does not, for the most part, seem to like a diet of bacteria. The writer has seen E.gingivalis spit them out right after engulfing them. Further, E.gingivalis seldom seems to contain bacteria. Occasionally a patient infected with amœbæ only has amœbæ which have ingested bacteria. These patients seem to have little untoward with their dental or general health. This strain of E.gingivalis seems to be infrequent and while it may be of apparent low pathogenic potential at the time of examination, reduction of host immunity, antigenic variation or mutation of the parasite may give rise to an aggressive pathogen at an indeterminate later date. (The ticking time bomb.)

CONCLUSION

The concluding comments for this chapter are simple. Although there is a possibility that E.gingivalis is not pathogenic, the overwhelming weight of evidence points to it being an aggressive pathogen. Furthermore, the writer believes that it is the primary pathogen in most destructive periodontal disease. It may also play a key role in serious, debilitating and incapacitating disturbances of the general health. The full extent of its adverse impact on the human race will remain unappreciated unless it is fully researched, not just as a specific agent of oral disease, but as a general agent of systemic disease.

CHAPTER IV

MICROSCOPY FOR THE DENTIST

The purpose of this chapter is to outline what the dentist should look for in a microscope, explain its basic construction and functions and describe the typical appearance of frequently observed micro-organisms observed by phase contrast microscopy.

THE MICROSCOPE

A microscope is composed of the sum of its parts so that a custom microscope may be built to a customer's specifications by selecting the appropriate components. The basic piece is a flat base which is wide, long and heavy enough to provide stability. It often contains a transformer, switches, rheostat and circuitry for the built in light source. The latter will normally have a lens and an iris diaphragm.

Rising vertically from the base is the stand, to which will be attached the moveable stage and the observation tube, monocular, binocular or trinocular. The latter will allow the mounting of a 35mm or a TV camera. With a trinocular tube there are two eyepieces as well as the phototube. The focusing mechanism will also be built into the stand. This comprises a large wheel, for racking the stage up and down, together with a smaller concentric wheel for fine adjustments of the stage height, i.e. for fine focussing of the image seen through the eyepieces.

PARFOCAL

If a camera, particularly a TV camera, is to be mounted, it is beneficial if the eyepieces and the camera are parfocal. That is, when the observer looking in the eyepieces sees the image sharply in focus, the image is also crisply in focus for the camera and a clear image will be displayed on the TV screen of the monitor. Some systems require the light to be cut off from the eye pieces when the camera is in use, and vice versa. Such systems are very inconvenient in a dental setting because the dental personnel and the patient may wish to simultaneously view the plaque.

Other systems use a "light bar" which intersperses a prism between the objective lens and the trinocular head. The greater versatility of this system, which splits the light between camera and eyepieces, allows simultaneous viewing. Alternative settings direct the light only to the binocular eyepieces, or only to the camera tube, to maximize the light for crispness of the perceived image.

EYES

The two eyepieces should be adjustable for width so that the interpupillary distance of the operator can be matched. The eyepieces should also be capable of individual adjustment for focus to allow for differences in the dioptre requirements of each eye. Some eyepieces can be used while wearing spectacles, which can save constantly removing and replacing them. These features allow the microscopist to work without eyestrain.

MAGNIFICATION

Under the observation tube, attached to a horizontal projection from the stand, is a revolving turret on which a series of objective lenses can be mounted. Common magnifications available are 10x, 20x, 40x, and an oil immersion lens which gives 100x. When viewed through 10x eyepieces the total magnification will be the product of the two magnifications of lenses employed. Current optical systems allow for good resolution up to 1,000x total magnification. Although lens systems can magnify beyond that, some clarity of detail may be lost ("empty magnification"). Such extra magnification may not necessarily prove useful.

MECHANICAL CONSIDERATIONS

The lens system is used to observe a specimen on a slide which is held on the stage by the specimen holder. The specimen holder grasps the slide at either end by spring loaded jaws. The holder can be adjusted left and right as well as forward and backwards by two vertical knobs which protrude below the stage. These knobs connect to the holder via a gear and toothed bar mechanism. The latter, as well as the gears for the focussing mechanism, can wear out or require servicing, so it may be best to purchase a microscope from a company that provides a local repair service for its products.

PHASE CONTRAST CONDENSER

Beneath the stage is an optical device called the phase contrast condenser. This device will, when matched to the appropriate phase contrast lens, provide light which will permit observation of unstained living objects. The latter absorb so little light that the human eye cannot clearly differentiate them if a normal light source is used. By "phasing" the light source the contrast is improved so that moving objects down to .25 micron may be observed in much greater detail and clarity than would be possible with a plain light system.

CENTERING

For maximum clarity of detail, it is necessary to carefully adjust the microscope according to the manufacturer's directions. The light entering the microscope system must be centred, the light must be focussed on the object and the phase contrast condenser must be centred, or the image in the eyepiece will be distorted, even if it is properly in focus!

ADJUSTING the CONDENSER

The phase contrast condenser is carried on a condenser mount which can be racked vertically via an adjustment knob on the stage of the microscope. This movement allows the light source to be focussed on the object (i.e. the slide). Two adjuster screws on either side of the condenser allow the light beam to be centred in relation to the field of vision in the eyepieces.

USE of the TELESCOPE

Two further adjuster screws allow the actual phase condenser to be adjusted. In order to do this it is necessary to use an accessory lens in one eye piece tube. This accessory lens is called the telescope and it can be focussed. With it, one can see inside the phase condenser. When looking through the telescope, which must be focussed, the images of two rings may be observed. These must be adjusted to be concentric so that the light will be properly phased. Each objective lens has a matching light annulus in the phase condenser. After selecting an objective lens, and focussing the image in the eyepiece, the telescope should be used to adjust the matching light annulus. This step is repeated for each pair of lens and annulus.

ALIGNMENT and MISALIGNMENT

The directions of the manufacturer of your microscope should be observed when setting it up. Become familiar with the procedure for it will be required routinely. Most microscopes need to be adjusted from time to time since alignment can be altered by moving or bumping the equipment, or by uninformed persons (patients, office staff, cleaners, etc.) fiddling with the knobs! Even the microscopist can misalign it by inadvertently racking the condenser instead of the stage when examining a specimen.

PHASE CONTRAST DARK FIELD

With only one exception, always use matching phase contrast annulus and objective lens. They will be coded by colour, number, symbol, etc. The exception to this rule is that, provided the two objective lenses to be used are related by a factor of 10, the phase contrast annulus for the higher power objective may be used with the low power objective. The result is phase contrast dark field illumination with the low power objective and phase contrast bright field illumination with the high power objective. The advantage is that, when changing directly from the low to high power it is not necessary to change the phase contrast annulus. I find that the dark field low power setting allows me to scan and spot amœbæ with greater ease before going to higher magnification to confirm the identification.

FUZZY IMAGE?

In all other cases a mismatch between the light annulus in the condenser and the objective lens provides a fuzzy distorted image. The same is true if the light source is off centre or not focussed on the slide, if the glass of the slide is not matched to the objective lens requirements, particularly if the slide is not of the correct thickness. The same holds true for the cover slip, which must be on top of the slide! Dirt or oil can also affect the clarity of the image. The high power oil immersion lens must be used with the correct oil. With other lenses oil causes distortion. Old oil on the high power lens may reduce the clarity of the image. Sometimes, when starting a fresh container of oil there will be distortion of the image if any of the old oil remains on the lens. Old and new oil, it seems, do not mix.

PRECAUTIONS

When removing or placing a slide on the stage, always rotate the lens turret to an empty space so that there is ample room above the stage to remove or place a slide without fear of damaging lenses or breaking slides. Always rotate the lens turret by grasping the outer edge of the turret and not by using the lenses as little handles. Although the latter action may seem natural, it has a tendency to unscrew the lenses. The latter can make focussing difficult as well as risk having the lens drop off the microscope.

To maximize the life of the illumination lamp, always turn the lamp rheostat down to zero before turning the power off, make sure it is at zero before turning the power on and don't leave the lamp burning when not in use.

To protect the camera, do not allow the light source to burn the camera out by allowing excess light through the system, for example, when adjusting it. Again, the life of the camera can be prolonged by selecting no light to the camera and turning it off when not in use.

SAFETY CONSIDERATIONS

It may seem to be restating the obvious, but do not bump or abuse the microscope since this may put it out of adjustment. The risk of accidental damage can be reduced by the site selected for the microscope. Placing a dust cover over it is a wise move since it also makes it more visible and therefore less likely to be damaged. Within the latter context, it is important, in the dental setting, to install the microscope in an area away from dust or fume production. Atomised particles from cavitron, air rotor or prophyjet, together with dust from grinding and polishing, splatter from various sources or caustic fumes released in laboratory procedures could all be harmful. Within the same context of care to be taken, recommended procedures for the disposal of biologically hazardous waste should be observed. If one admits that the material on the slide is pathogenic then it is wise to dispose of slides and cover slips and not re-use them!

In order to emphasize techniques developed by the author, such as phase contrast pseudo dark field scanning, or findings which are at variance with the literature, the first person has been used as appropriate.

SCANNING

Having examined the patient, taken plaque and prepared a slide, we are now (finally) ready to actually look at it. Assuming the microscope to be have been adjusted according to the manufacturer's instruction manual, place the slide on the stage, switch on the power, increase the brilliance of the lamp so that the light shows as a bright spot on the slide. Using the control knobs adjust the stage so that the light falls in the middle of the specimen, check that the phase annulus selected matches the 100x objective. I then rotate the turret to bring the 10x lens into position. Look down the eye pieces. If a green filter is used on the light system it will impart a green hue to the field of vision. All "open space" will appear black. All "solid" areas, i.e. plaque, will appear as an almost fluorescent green. Traverse the field using the stage control knobs with one hand. The other hand is used to constantly adjust the fine focus, as required. The pattern of movement is:

Traverse rapidly about one field of vision.

Stop.

Adjust focus and examine the area.

Repeat the three previous steps.

This procedure is continued until an area requiring close inspection is found or the whole slide has been examined. I prefer to start at the edge and work around the circumference of the plaque sample before examining the central section, scanning from one side to the other, row by row, from top to bottom.

SPOTTING

Protozoa are often to be found in areas of intense bacterial activity or areas with many leucocytes. They may also be spotted at this magnification. Using the phase contrast pseudo dark field technique, amœbæ appear as "black holes" which have a bright circumferential green line delineating them from the adjacent area. In the centre of the "black hole" will be a bright green mass. Having spotted a suspicious object, centre it in the field of view. Swing the 10x lens out of the way, place a drop of oil on the central (lit) portion of the slide and rotate the turret again. The 100x lens is brought into position. Look through the eyepieces again, focus, and if the field was properly centred, the suspicious object should be in view. The cell can then be differentiated.

THE TROPHOZOITE

The moving, living amœba is called a trophozoite. This nonencysted stage is also referred to as a vegetative stage. The typical appearance of the trophozoite of E.gingivalis under high power is of a cell about the density of a red blood cell and without any visible dancing granules. The cell membrane, ectoplasm and much darker endoplasm should be plainly discernible. Within the endoplasm will be found a series of circular dark structures a little smaller than red cells. Sometimes one can make out that these are contained within vacuoles. Within the endoplasm will also be seen small intense dark spots with no apparent vacuolation. Occasionally ingested bacteria will be seen within vacuoles. Sometimes one may observe live spirochætes wriggling around in the vacuoles. The large round dark masses are the partly digested remains of the nuclei of leucocytes. The tiny dark granules are granules of hæmoglobin that has been sucked from erythrocytes. Vacuoles which are mainly clear, but which have a few granules in them, contain cytoplasm from leucocytes.

Locomotion

Movement of the trophozoite falls into two categories. When dormant (digesting food?), the trophozoite lazily puts out pseudopodia randomly, it shows no purpose of motion. At other times the trophozoite will be seen purposefully pushing through the plaque, squeezing its way around immovable objects that stand in its way. The ingested food, the size or behaviour of the trophozoite is not indicative of a species differentiation, rather an indication of the stage reached in the life cycle

Motionless?

Dead trophozoites may be recognized by loss of the hyaline appearance of the cytoplasm, loss of the differentiation into ectoplasm and endoplasm and loss of movement! The cytoplasm additionally appears as if made of "Swiss cheese" because of the large number of vacuoles, particularly small empty ones. The nucleus will also be more pronounced. Sometimes all that can be seen of the dead trophozoite is is a faint outline of the body of the cell, with typical but faint inclusions (remnants of leucocyte nuclei and granules of hæmoglobin) with a distinct nucleus outside the ruptured cell membrane. Occasionally, all that remains of the trophozoite is the nucleus, apparently within a capsule.

Under adverse environmental conditions, E.gingivalis assumes the appearance of a series of adjacent "grease blots" joined to each other by a thin strand of cytoplasm that remains intact. Only one of these "blots" contains the nucleus and food vacuoles. Occasionally a "blot" will separate from the nucleated cell, but the "blot" continues to move aimlessly. Whether E.gingivalis is observed as one of these aberration or as a normal trophozoite, with time the cytoplasm shrinks and becomes more dense.

THE NUCLEUS

The most important feature of the amœba is its nucleus. This will be about the size of a (food) vacuole but appears as a circle (size about 4 microns) with an offset dot or tiny disk in the middle. The space between the outer (chromatin) ring and the inner karyosome is mostly clear but one may sometimes see a fine web, consisting of a few strands which join the chromatin ring to the inner karyosome. The whole resembling a bicycle wheel, with rim, a few spokes and an offset hub. One may also sometimes observe thickenings on the chromatin ring which may represent the chromosomes. Stabler (Journal of Morphology Vol.66, No.2 pp 357-367) notes: "The chromosome number appears to be five, with one element slightly smaller than the others." However, both Wantland et al (1961) and Child (1926) claim there are six chromosomes. The numbers of chromosomes are more significant to the researcher than the clinician for it is on the identification of the nucleus that the cell is confirmed as E.gingivalis. Without a high quality microscope, that has been accurately set up, diagnosis is difficult.

SIZE RANGE

On average, E.gingivalis ranges from about the size of a leucocyte to up to between two to three times the diameter of a leucocyte, (i.e. 10-25 microns). The very smallest amœbæ which I have seen are a little smaller than an erythrocyte (which is about 7 microns in diameter) while the very largest have been up to five or six times the diameter of a white cell, or almost half the diameter of the field of view with the oil immersion 100x objective lens, (i.e. in the region of 60 microns).

LEUCOCYTES

The white cells seen most often in plaque are polymorphs, they are about double the diameter of a red cell. Polymorphs have up to four lobes on their nuclei and have granules shimmering in the cytoplasm. It is thought that the number of lobes of the nucleus of polymorphs increase with the age of the cell.

PLATELETS

Aggregates of small dark cells considerable smaller than red cells which are slightly ovoid or oblong with rounded edges are platelets with an average size of 2x3 microns. They are significantly larger than cocci, but smaller than Candida buds.

TRICHOMONAS TENAX

Another organism to be found in plaque is Trichomonas tenax. This flagellate is unmistakable because of its vigorous activity. It is difficult to make out at low power. Intermediate or high power may be required to spot and identify this creature. When rounded up, it is about the size of a red cell or a little larger. Normally it will be elongated, like a sausage with tiny whips thrashing about at one end. In length it may be about the diameter of a leucocyte and at its widest about the diameter of an erythrocyte. It is almost colourless, particularly at lower magnifications. The four flagella may be observed to act as a propulsion device when it releases its hold on the environment. It appears to have at least two tiny hooks near the base of its body with which it anchors itself to surrounding debris. It then uses its flagella to scoop food (cell debris) into an undulating membrane and thence into a "mouth" about one third along the body from the base of the flagella. A very fine oval, consisting of an outer membrane and a clear central portion, may sometimes be seen within the body close to the base of the flagella. This is the nucleus. Trichomonas which are about twice this size may sometimes be seen. The two variants may be different species.

CANDIDA

Candida appears in plaque as buds, hyphæ, pseudohyphæ and chlamydospores. Occasionally, but with difficulty, dense shrivelled buds may be found in necrotic tissue that dislodges with the plaque. Candida is difficult to spot at low power and must usually be sought at intermediate power, such as x400, and confirmed at high power. A clue that it may be present is the observation at low power that there is no motility and the plaque looks more granular than normal. The difference in appearance may be likened to the difference between salt and sugar.

ACs and CANDIDA

In appearance, Candida has about the same diameter as ACs, but a totally different structure. ACs have an inner filament with cocci palisading along it, the diameter of the cocci and the filament appear equal. Sometimes the colonization of the filament has only occurred at the tip. This appearance is similar to a bullrush. By contrast, Candida has a smooth outer shell, lined with cytoplasm. An inner vacuole sometimes has a granule dancing in it. Sometimes the cell is filled with cytoplasm. Candida buds and hyphæ have about the same diameter. Hyphæ are long tubes with few cross walls. Hyphæ may be seen to branch. One end may come to a fine point. This is the actively growing end of the hypha which is thought capable of insertion between intact layers of epithelial cells. Pseudohyphæ are medium length oval cells joined end to end. They are, in fact, elongated buds that have not quite separated on division. Chlamydospores, the resistant disseminating form of the mould, are produced as a result of the union of a positive hypha with a negative hypha. Chlamydospores are about twice the diameter of buds.

LEPTOTHRICES and ACTINOMYCES

Recognition of bacterial types in plaque using a phase contrast microscope is relatively simple. Long strands, thin and nonbranching, are filamentous complex bacteria which belong to the genus Leptothrices. Similar appearing filaments which branch belong to the genus Actinomyces. Both have about the same diameter as cocci. Slightly thicker and denser filaments with irregular thickness are sometimes found, in association with sensitive or inflamed gingivæ. They may be pathogens, may be soil saprophytes with pathogenic potential or may be

harmless. The current complexities of identification of bacteria from plaque leaves their classification and significance an enigma.

COCCI, CBs and AAC

Small round nonmotile bacteria found in clusters are cocci. If small and arranged as if in a tight string of pearls they may be pathogenic streptococci. Short straight chains of fatter cocci are a frequent finding and are apparently normal. Streams of floating round to oval cocci that exhibit Brownian movement I refer to as coccobacillary forms (CBs). These appear to be associated with decay and gingival irritation. Actinobacillus actinomycetemcomitans (AAC) have the same appearance but cannot be positively identified other than by culturing.

BACILLI

Nonmoving rods are included with cocci in my plaque assessments but motile rods are differentiated into small and large bacilli. From clinical experience the former are associated with decay, the latter with gingival inflammation.

SPIROCHÆTES

Spirochætes are unmistakable: tiny spiral organisms that wriggle around. Sometimes they can be seen swarming on debris, like piranha attacking prey. Sometimes in association with severe destructive lesions they are palisaded down actinomyces filaments, all "pumping" together. One gets the impression of a wave in a football crowd. Although they produce an unpleasant odour when grown in culture, I have never been able to make any correlation between their presence and any changes in oral health or disease. On occasion, spirochætes will be observed still wriggling within the vacuole of an amœba. Sometimes the spirochæte burrows out of the amœba. I have seen spirochætes bore their way into an amœba, wriggle around and then leave. Subsequent to this, I observed the amœba start to degenerate. One may speculate that rather than being a pathogen, spirochætes may be commensal scavengers of cell debris, or perhaps even a natural enemy of the amœba. However, some researchers believe that spirochætes are not commensal but may locally suppress the immune response (Conversation with R. Müller, Dip Bact. Univ. Münster).

CHRONOLOGY of COLONISATION

There is a chronological order for the colonisation of the tooth surface by the micro-organisms that comprise dental plaque. The first to colonize are nonmotile cocci. Within about five hours of thorough tooth surface débridement, this colonization will be well developed. Long filaments will be next, which mix with the cocci. By the twelfth hour, there is a well formed matt of nonmotile rods, cocci and filaments. Within this ecosystem, if environmental conditions permit, motile bacilli and other organisms capable of sugar fermentation will make an appearance and will be joined later by spirochætes. Motile bacilli may not be present, in spite of the presence of spirochætes, if the environment favours that development. As the plaque matures some of the cocci may colonize the branching filaments, (ACs). The presence of spirochætes generally signals an anærobic environment, a state which may be reached in about five days. ACs, on the other hand seem to be late arrivals. It is in such a mixed habitat that the protozoa may find a congenial home. In other words, they can only establish in dental plaque if there has been a pre-existent bacterial infection. However pre-existent trauma could also prepare the habitat for colonization by either protozoa or fungi. Generally, Candida will not colonize unless there has been pre-existent infection, sometimes by bacteria only, but usually by the protozoa. Left untreated, the protozoa may ultimately be suppressed by the presence of fungus. It has been discovered recently that some fungal species produce antiviral compounds. (Aspergillus Niger: paper presented at 56th Conjoint Meeting on Infectious Diseases, Calgary, 1988). The zone of inhibition which may be observed around some fungal colonies is indicative of the general ability of fungi to inhibit other life forms: many antibiotics, some of which are antiprotozoal, are produced by fungi.

RATIONALE for STABILISING PLAQUE by the PRETREATMENT PLAN

The pretreatment plan was developed in order to stabilise the plaque prior to examination. It was felt that it would be best if plaque could be examined in whatever stage of development it might have attained in the patient's mouth. The purpose of the plan was also to prevent disease from going out of control while not suppressing target organisms to the point where they could not be found. In practice it worked extremely well, with fewer reasons to believe that a negative reading might be false. Patients who were on the plan for about a month prior to plaque exami-

nation often reported improvements in oral health before plaque examination and the examiner noted a reduction in halitosis and bleeding.

TOXIC SHOCK

One other symbiotic colony to be regarded as of pathogenic significance is Candida colonized by cocci. The appearance is similar to ACs, except that the central element is a fungal hypha, although yeast cells are sometimes colonized. The association of Candida and Staphylococcus aureus has been identified as one of the ætiological agents in toxic shock syndrome (Truss, 1984); Nolte, 1977).

MICROSCOPIST'S SHOCK

I seldom find any other organisms in plaque, but they can occur. One day a patient arrived whose home was being renovated. They had been tearing out walls in their home. There had been a lot of dust in the air. She had developed some mouth and throat irritation. During examination of the plaque at low power, something quite large moved. Careful inspection revealed the squashed but still twitching body of a microscopic (dust) mite.

CHAPTER V

A SUMMARY OF SOME TYPICAL CASE HISTORIES

This chapter is divided into two sections. The illustrations for some of the cases are in the Illustrations section at the end of the book. The first section contains five case histories selected from the first group of patients treated. Each case illustrates a different facet of infection or treatment. These cases have lack of complication in treatment as a common denominator. The second section contains a series of case histories which present unusual features, or where the patients had underlying medical disorders which complicated treatment, or where a longer history exists. One case is presented by courtesy of a colleague, Dr. Brian Maclean. The second section includes a case report illustrated by the "Periodex Evaluation". This is a computer generated professional opinion report on periodontal status which the author developed in order to assist in assessing the progress made by patients during treatment. It also helps patients understand the nature of their infection and associated disease processes, thereby improving motivation and "compliance". "The Periodex" reports included (relative to the patient, Series Two, Case Two) are in exactly the same format as originally presented to the patient. The system has been extensively modified during the last five years. A more detailed explanation of "The Periodex" will be found in Chapter XII.

Although the microbiology of plaque has been extensively researched, little attention has been paid to organisms other than bacteria. While bacteria may predominate and be easy to culture, it must not be forgotten that viruses, fungi and protozoa are also to be found in dental plaque, particularly plaque associated with periodontal infections. Entamœba gingivalis is a frequent inhabitant of plaque associated with oral disease. This chapter focuses on clinical aspects of oral disease associated with this parasite. Although E.gingivalis, a lumen dweller, is frequently found in the mouth, it is sometimes found elsewhere, for

example in pulmonary suppuration (Sutliffe et al, 1951) and tonsillar suppuration (Craig and Faust, 1970). Oral Amœbiasis is defined as an infection of the oral cavity with this protozoan parasite. Cases reported here include one case of amœbic tonsillar suppuration associated with oral disease and one case of amœbic granuloma.

At the outset no sterile broth nor normal saline was available, so the expedient of using each patient's saliva as a mounting medium for the plaque was employed. This protocol is still used because it was found that other liquids caused distortion of protozoa which made them unrecognizable. Since 1978 plaque for diagnosis has always been taken from a site suspected to be diseased, rather than from an arbitrary "standard" site.

The following clinical parameters were recorded for each patient, together with any other pertinent findings such as saliva production or specific pathology: oral hygiene; gingival condition; plaque quantity; halitosis; pockets; inflammation and submandibular lymphadenitis. In addition, microbiological data was recorded at low power as leptothrices, cocci and motility. At high power, the presence (or absence) of the following were recorded: bacilli, spirochætes, amœbæ, trichomonads, yeasts, ACs (branching filaments, probably a species of Actinomyces (A), with cocci (c) attached), CBs (coccobacillary forms), epithelial cells, erythrocytes, leucocytes and any other pertinent findings were noted. Explanatory note: quantitative assessments from minimum to maximum, which are used in this text are: <o+, o+, +, ++, +++.

Case One: Age 51. Male. Palatal Ulceration

At his regular re-examination appointment, the patient complained of recent headache and general flu like symptoms which included sore and itchy eyes and mouth. He had a small grey ulcer, with little surrounding inflammation, in the palate. Although his oral hygiene was good, the gingival condition was fair with sporadic areas of chronic inflammation. Plaque was within normal limits (o+), with minimal motility (<o+), which was due to a low number (o+) of small bacilli. Amœbæ were present in moderate (+) numbers. A scraping around the periphery of the base of the ulcer revealed many amœbæ (++/+++), which became more apparent as the white cells lost vitality over the ensuing two hours. He made a rapid recovery with anti-amœbic therapy.

Case Two: Age 19. Female. Acute Necrotizing Ulcerative Gingivitis

Patient attending for routine care was observed to have ulceration occurring on the tips of the interdental papillæ of the mandibular incisors. Halitosis, bleeding and pain were pathognomonic of acute ulceromembranous gingivitis (synonyms: Vincents, ANUG). Entamœba gingivalis was recovered from the site. Rapid improvement was obtained with anti-amœbic therapy.

Case Three: Age 31. Male. "Failed" Root Canal Therapy

Patient attended complaining of pain and swelling around upper front teeth. On examination there was an inflamed swelling buccal to the upper left lateral incisor. Pus was discharging down the periodontal membrane on the distal where the pocket depth was 8mm. Entamœba gingivalis was recovered from this site. Radiography revealed a previous root canal filling which the patient said had been completed about one year prior. Rapid resolution occurred with anti-amœbic therapy and the existing root canal filling was then judged to be successful. Pocket depth returned to less than 2mm and the condition remained stable for six and a half years, at which point the patient was posted out of the country. (1979-1985)

Case Four: Age 52. Male. Maxillary Amœbic Granuloma

Patient had a long history of periodontal problems. A cantilever bridge had been constructed at an indeterminate earlier time. It replaced the first premolar on the upper right, using the second premolar as the abutment. Root canal therapy on this tooth had apparently failed. Following a recent acute episode, there had been a history of surgical intervention, with loss of a portion of the buccal plate. Radiography revealed a periapical translucency with a periapical-parodontal defect. The patient had been treated previously for oral parasites but they had recurred at this site. After removal of the tooth the periapical lesion was curetted and submitted for parasitological laboratory examination. The tissue was positive for Entamœba gingivalis.

Case Five: Age 18. Male. Recurring Amœbic Tonsillar Suppuration

The patient, attending for routine treatment, appeared to be unwell. It was observed that his lips were dry, his tonsils were inflamed and discharging pus. He had a submandibular lymphadenitis on the left side. For approximately two-and-a-half years prior to this he had suffered recurrent tonsillitis with associated symptomology. Although his oral

hygiene was good, his gingival condition was only fair. There were some areas of chronic inflammation (i.e. venous stasis at the gingival margins), pockets were in the 2 to 3mm range with one at 5mm. Direct phase contrast examination of plaque was positive for Entamœba gingivalis. Plaque was also submitted for laboratory examination in SAF fixative. His physician also submitted tonsillar pus in a separate SAF fixative kit. Both samples were positive for Entamœba gingivalis. He was treated with systemic and contact amœbacides and retested after completion of his prescription. He was then negative for oral parasites and both his dental and general health improved. He remained stable for over four years until suffering facial injuries in an auto accident. As a complicating factor to his injuries he became re-infected and was retreated. He did not experience tonsillar infections with these later episodes, neither did he experience a periodontal breakdown. (1979-1989)

THE BEGINNING: Summarised

Necessity and Chance, led to a series of events, the results of which challenge the widely held concept that Entamœba gingivalis has no effect upon oral and systemic health. This situation might never have arisen had sterile broth or normal saline been available on the day that a phase contrast microscope was first tried in my dental practice. Although much has been previously published about Entamœba gingivalis, little seems to have found its way into dental texts.

But for one patient's dislike of harbouring a parasite in her body - "parasites aren't supposed to be good for you, are they?" - no action might have been taken. Subsequent to that comment, the expertise of dentists, parasitologists, physicians, pharmacists, historians, librarians, translators and many others was pooled. The resultant information will hopefully give impetus for further scientific research to investigate and re-evaluate the pathologic role of Entamœba gingivalis. Some additional case histories are now presented to further illustrate the pathogenic potential of the oral protozoa.

The remainder of this chapter is summary of material presented at meetings of the Canadian Dental Association, August 25 1986, Halifax, Nova Scotia and The British Society for Oral Medicine, September 21 1987, London, England. These cases have been selected because they had been followed over a longer period than had been done with the first series, or because the cases were more complex. In clinical practice it was found that antibiotics, used to treat the oral protozoa, seemed to be less effective than anticipated, if the patient was taking anti-inflammatory

drugs, particularly corticosteroids, concomitantly. It was also observed that infections with the oral protozoa seemed to be less responsive to therapy if the patient had multiple infections, underlying disorders of the general health, was taking antihistamines, aspirin or its derivatives, tranquilizers, narcotics or other mood altering drugs.

An investigation of household pets, known to be infected with oral protozoa, revealed that after the veterinarian had induced full general anæsthesia, no protozoa could be demonstrated in plaque. This indicated that the narcotic agent might have a similar pharmacological effect on both the host and the parasites.

Series Two: Case One (Aug 1977)
Severe Periodontal Disease 11 year report

On presentation, the patient had a severe periodontal problem. He had been told that his remaining teeth would have to be removed in order to preserve the bony ridge for full dentures. He still had most of his natural dentition and was anxious to retain them, if at all possible. Traditional nonradical periodontal care was instituted with some limited success. However, some of his teeth remained mobile and had to be splinted. His rate of deterioration was slowed but not satisfactorily arrested.

In 1978 he was recalled for the examination of his plaque from a diseased site. This was found to be infected with E.gingivalis. Elimination of the protozoa, using the same treatment regime as previously prescribed, proved successful. One course of medication, augmented when necessary by traditional noninvasive periodontal care, resulted in partial filling of the previous vertical bony defect. The result was, however, a little short of expectations. Most of the splints could be removed after the course of treatment because the teeth were no longer mobile. The lateral incisor did not tighten completely and was left splinted. This site was later found to be infected with the yeast, Candida albicans, which may have been responsible for the less than perfect healing. The patient improved further with treatment to eliminate the yeast (1988).

Series Two: Case Two (August 26 1983)
Aggressive Destructive Osteolytic Periodontal Disease

The patient presented with loose teeth and painful bleeding gums which made it difficult for her to maintain normal oral hygiene. She required anæsthesia for any professional cleaning and had suffered a number of abscesses recently. Her condition had deteriorated over the

past year and the tissue was an unhealthy purple/mauve colour typical of the venous stasis seen in advancing, inflammatory, destructive periodontal disease. There were many 3-5 mm pockets, marked mobility, halitosis and excessive plaque. Many amœbæ and trichomonads were observed in the plaque from diseased sites. A radiograph of the left central incisor, taken by her dentist, revealed a vertical bony defect on the mesial, with about 25% support. The initial "PERIODEX EVALUATION" scored one hundred.

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Dr.Trevor Lyons, 45, Rosebery Avenue, Ottawa, Ont, tel:236-2233
""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""
Patient  Name:-   Series Two Case Two          DATE:    Aug 26.83
Report sent to Referring Doctor?  Yes          (First Report)
************************* PERIODEX EVALUATION ****************
This is an open ended scale.  The lower your score, the better.
Scoring:- Read * for your score in each category.  Score totals
automatically in this nonscientific scale.  Use it to compare
your progress. Your Periodex score reflects my clinical opinion
==================Hi-normal Lo-normal= Fair === Poor === Bad ===
ORAL HYGIENE:        *       F to G    Fair      F to P    Poor
GINGIVAL CONDITION Good      F to G    Fair        *       Bleeding
PLAQUE :           <o+       o+          *       ++        +++
HALITOSIS :          *       o+        +         ++        +++
POCKETS :          <1        1-3       3+          *       6+
INFLAMMATION :     None      Min/Spor  Stagn       *        Detached
MOBILITY:          <o+       o+        +         *         +++
SUB MANDIBULARS:   No        L or R      *        1 Tender 2 Tender
Leptothrices:        *       Any variation
Cocci :              *       Any variation
5 Stages Infection-First---Second---Third----Fourth---Fifth----
MOTILITY :         <o+       o+        +           *       +++
BACILLI :          <o+       o+          *       ++        +++
SPIROCHAETES :     <o+       o+        +           *       +++
ENT. GINGIVALIS :  <o+       o+          *       ++        +++
TRICHOMONAS TENAX:<o+        o+          *       ++        +++
CANDIDA :          <o+       o+        +         ++        +++
CB FORMS :         PRESENT
A/C :              <o+       o+          *       ++        +++
============================================================
PERIODEX SCORE =         100
PERIODEX GRAPH     ********* ******** ******  *****     **
A PERFECT SCORE =          9
A PERFECT GRAPH = ***       *
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Notes to the referring DDS or MD:   Thank you for the referral.
If you have any questions, please phone.  Eleanor will be your
primary liaison person.  If she cannot answer, she will ask me.
Please delay all but emergency treatment until the infection is
controlled or eliminated.  I will advise you accordingly.
I have prescribed medication & issued appropriate instructions.
I will continue to monitor the patient on a regular basis.
```

On October 7 1983, about six weeks after treatment to eliminate the protozoa had been initiated, the plaque was sparse. There were no protozoa, but C.albicans was found in the plaque from diseased sites. There had been a dramatic tissue response with improvement in colour, texture and tone. The hard and soft tissues were much less sensitive. Pockets were reduced in depth to 1-3 mm with less than 1mm gingival recession. Mobility was half the August reading. The "PERIODEX" score was reduced to 47 from the initial score of 100.

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Dr.Trevor Lyons, 45, Rosebery Avenue, Ottawa, Ont, tel:236-2233
""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""
Patient  Name:-   Series Two Case Two          DATE:    Oct 07 83
Report sent to Referring Doctor?  Yes          (Second Report)
************************** PERIODEX EVALUATION ****************
This is an open ended scale.  The lower your score, the better.
Scoring:- Read * for your score in each category.  Score totals
automatically in this nonscientific scale.  Use it to compare
your progress. Your Periodex score reflects my clinical opinion
=================Hi-normal Lo-normal= Fair === Poor === Bad ===
ORAL HYGIENE:        *      F to G   Fair     F to P   Poor
GINGIVAL CONDITION Good       *      Fair     F to P   Bleeding
PLAQUE :             *      o+       +        ++       +++
HALITOSIS :          *      o+       +        ++       +++
POCKETS :          <1          *      3+       4-5      6+
INFLAMMATION :     None      Min/Spor   *      4Q or GMD Detached
MOBILITY:          <o+       o+         *      ++       +++
SUB MANDIBULARS:   No          *      Both/firm 1 Tender 2 Tender
Leptothrices:        *      Any variation
Cocci :              *      Any variation
5 Stages Infection-First---Second---Third----Fourth---Fifth----
MOTILITY :         <o+       o+       +        ++       +++
BACILLI :          <o+       o+       +        ++       +++
SPIROCHAETES :     <o+       o+       +        ++       +++
ENT. GINGIVALIS : <o+        o+       +        ++       +++
TRICHOMONAS TENAX:<o+        o+       +        ++       +++
CANDIDA :          <o+       o+       +          *      +++
CB FORMS :         PRESENT
A/C :              <o+       o+       +        ++       +++
=============================================================
PERIODEX SCORE =          47
PERIODEX GRAPH     ******** ******** ****     **       *
YOUR LAST SCORE =         100
YOUR LAST GRAPH = ******** ******** ******** *****    **
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Notes to the referring DDS or MD:   Thank you for the referral.
If you have any questions, please phone.  Eleanor will be your
primary liaison person.  If she cannot answer, she will ask me.
Please delay all but emergency treatment until the infection is
controlled or eliminated.  I will advise you accordingly.
I have prescribed medication & issued appropriate instructions.
Please do the scaling & etc.  NOW!
I will continue to monitor the patient on a regular basis.
```

November 16 1983. After just over 5 weeks of antifungal medication there was a further improvement, but the patient had some stain on her teeth. She was referred back to her dentist for routine scaling, curettage, etc. The "PERIODEX" score reflected the continued improvement, being reduced to a "still at risk" value of 34.

```
Dr.Trevor Lyons, 45, Rosebery Avenue, Ottawa, Ont, tel:236-2233
Patient  Name:-   Series Two Case Two          DATE:    Nov 16.83
Report sent to Referring Doctor?  Yes          (Third Report)
************************** PERIODEX EVALUATION ***************
This is an open ended scale.  The lower your score, the better.
Scoring:- Read * for your score in each category.  Score totals
automatically in this nonscientific scale.  Use it to compare
your progress. Your Periodex score reflects my clinical opinion
=================Hi-normal Lo-normal= Fair === Poor === Bad ===
ORAL HYGIENE:         *      F to G   Fair     F to P   Poor
GINGIVAL CONDITION Good        *      Fair     F to P   Bleeding
PLAQUE :              *      o+       +        ++       +++
HALITOSIS :           *      o+       +        ++       +++
POCKETS :          <1           *      3+       4-5      6+
INFLAMMATION :     None         *      Stagn    4Q or GMD Detached
MOBILITY:          <o+       o+           *     ++       +++
SUB MANDIBULARS:   No        L or R   Both/firm 1 Tender 2 Tender
Leptothrices:         *      Any variation
Cocci :               *      Any variation
5 Stages Infection-First---Second---Third----Fourth---Fifth----
MOTILITY :         <o+       o+       +        ++       +++
BACILLI :          <o+       o+       +        ++       +++
SPIROCHAETES :     <o+       o+       +        ++       +++
ENT. GINGIVALIS :  <o+       o+       +        ++       +++
TRICHOMONAS TENAX:<o+        o+       +        ++       +++
CANDIDA :          <o+       o+         *      ++       +++
CB FORMS :         PRESENT
A/C :              <o+       o+       +        ++       +++
=============================================================
PERIODEX SCORE =          34
PERIODEX GRAPH      ******** ******   ***       *
YOUR LAST SCORE =         47
YOUR LAST GRAPH =  ******** ******** ****      **       *
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Notes to the referring DDS or MD:   Thank you for the referral.
If you have any questions, please phone.  Eleanor will be your
primary liaison person.  If she cannot answer, she will ask me.
Please delay all but emergency treatment until the infection is
controlled or eliminated.  I will advise you accordingly.
I have prescribed medication & issued appropriate instructions.
The infection is controlled.
Please proceed with routine treatment, cautiously.
I will continue to monitor the patient on a regular basis.
```

January 12 1984. By now the routine noninvasive periodontal care was complete and the patient was receiving orthodontic treatment to restore the upper left central incisor to its place in the arch. The "PERIODEX" score was now 18 which is just outside normal, reflecting the continued, but reduced presence of the yeast. Mobility was further reduced and was now within the range of normal.

```
Dr.Trevor Lyons, 45, Rosebery Avenue, Ottawa, Ont, tel:236-2233
""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""
Patient  Name:-   Series Two Case Two          DATE:    Jan 12.84
Report sent to Referring Doctor?  Yes          (Fourth Report)
************************* PERIODEX EVALUATION ****************
This is an open ended scale.  The lower your score, the better.
Scoring:- Read * for your score in each category.  Score totals
automatically in this nonscientific scale.  Use it to compare
your progress. Your Periodex score reflects my clinical opinion
==================Hi-normal Lo-normal= Fair === Poor === Bad ===
ORAL HYGIENE:         *       F to G   Fair      F to P    Poor
GINGIVAL CONDITION    *       F to G   Fair      F to P    Bleeding
PLAQUE :              *       o+       +         ++        +++
HALITOSIS :         -ve       o+       +         ++        +++
POCKETS :           <1          *      3+        4-5       6+
INFLAMMATION :      None        *      Stagn     4Q or GMD Detached
MOBILITY:           <o+         *      +         ++        +++
SUB MANDIBULARS:    No          *      Both/firm 1 Tender  2 Tender
Leptothrices:         *       Any variation
Cocci :               *       Any variation
5 Stages Infection-First---Second---Third----Fourth---Fifth----
MOTILITY :          <o+       o+       +         ++        +++
BACILLI :           <o+       o+       +         ++        +++
SPIROCHAETES :      <o+       o+       +         ++        +++
ENT. GINGIVALIS :   <o+       o+       +         ++        +++
TRICHOMONAS TENAX:  <o+       o+       +         ++        +++
CANDIDA :             *       o+       +         ++        +++
CB FORMS :          PRESENT
A/C :               <o+       o+       +         ++        +++
=============================================================
PERIODEX SCORE =          18          (Ideal Range = 12 to 17)
PERIODEX GRAPH      *******  ***       *
YOUR LAST SCORE =         34
YOUR LAST GRAPH =  ******** ******   ***       *
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Notes to the referring DDS or MD:   Thank you for the referral.
If you have any questions, please phone.  Eleanor will be your
primary liaison person.  If she cannot answer, she will ask me.
Please delay all but emergency treatment until the infection is
controlled or eliminated.  I will advise you accordingly.
I have prescribed medication & issued appropriate instructions.
Please proceed with routine treatment.
I will continue to monitor the patient on a regular basis.
```

April 18 1984. Treatment was now complete, demonstrating a remarkable improvement in both the oral and general health of the patient. This was reflected in the "PERIODEX" score of 13 (the ideal score is 14, the ideal range is 12-17). There were no amœbæ, trichomonads or yeasts in her plaque, which was minimal. A follow up radiograph, taken by her dentist in May 1986, showed the defect (mesial to the upper left central incisor) to have filled in, giving about 50% bony support.

```
Dr.Trevor Lyons, 45, Rosebery Avenue, Ottawa, Ont, tel:236-2233
""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""""
Patient  Name:-   Series Two Case Two        DATE:   Apr 18.84
Report sent to Referring Doctor?  Yes        (Fifth Report)
************************** PERIODEX EVALUATION ****************
This is an open ended scale.  The lower your score, the better.
Scoring:- Read * for your score in each category.  Score totals
automatically in this nonscientific scale.  Use it to compare
your progress. Your Periodex score reflects my clinical opinion
=================Hi-normal Lo-normal= Fair === Poor === Bad ===
ORAL HYGIENE:          *       F to G  Fair     F to P   Poor
GINGIVAL CONDITION  *          F to G  Fair     F to P   Bleeding
PLAQUE :            <o+          *     +        ++       +++
HALITOSIS :         -ve        o+      +        ++       +++
POCKETS :           <1           *     3+       4-5      6+
INFLAMMATION :      None         *     Stagn    4Q or GMD Detached
MOBILITY:              *       o+      +        ++       +++
SUB MANDIBULARS:    No           *     Both/firm 1 Tender 2 Tender
Leptothrices:          *       Any variation
Cocci :                *       Any variation
5 Stages Infection-First---Second---Third----Fourth---Fifth----
MOTILITY :          <o+        o+      +        ++       +++
BACILLI :              *       o+      +        ++       +++
SPIROCHAETES :      <o+        o+      +        ++       +++
ENT. GINGIVALIS :   <o+        o+      +        ++       +++
TRICHOMONAS TENAX:<o+          o+      +        ++       +++
CANDIDA :           <o+        o+      +        ++       +++
CB FORMS :          PRESENT
A/C :               <o+        o+      +        ++       +++
===============================================================
PERIODEX SCORE =          13           (Ideal Range = 12 to 17)
PERIODEX GRAPH       *****     **       *
YOUR LAST SCORE =         18
YOUR LAST GRAPH =    *******   ***      *
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Notes to the referring DDS or MD: Thank you for the referral.If you
have any questions, please phone. The patient now seems to be free
of infection and I have issued appropriate instructions. Please do
the scaling and proceed with routine treatment. I shall see the
patient again only on request of the referring doctor or the
patient if it appears that my services are needed.
```

Series Two: Case Three
Severe Periodontal Disease, Case Report by a Colleague

This patient was treated by Dr. Brian Maclean, who reported that the patient had such a severe periodontal problem that many extractions were contemplated. Examination of the plaque revealed an infection with E.gingivalis. These were eliminated using systemic and topical antiprotozoals. Routine periodontal care (scaling and curettage) was initiated at the appropriate time. The results suggested elimination of destructive periodontal disease since the teeth tightened, vertical bony defects healed, the tissue appearance and pocket depths returned to normal. There was no further bleeding or discomfort. Instead of denture therapy the patient elected crown and bridge rehabilitation.

Series Two: Case Four
Periodontal Disease and Multiple Sclerosis

This patient had multiple sclerosis (MS). When first seen she had a periodontal problem and protozoal involvement. The infection was difficult to treat because of apparent antagonism between her various medications. There was the additional complication of Candida albicans, present in her plaque. The latter was also recovered by culturing a swab of a red granular lesion that covered most of the hard palate. When the MS was active the palatal lesion was bright red. After treatment to eliminate oral infection, the lesion abated, Candida could no longer be found on direct examination, nor cultured from her mouth, her periodontal condition and oral health improved and the MS went into remission. It was noted, on subsequent reinfection that the same pattern repeated itself.

Series Two: Case Five
Periodontal Disease and Diabetes

The patient, a diabetic, had to be most careful with his diet, insulin dosage, exercise and general life style. He had diabetic crises several times a year. Some of these required hospitalisation to readjust his insulin. E.gingivalis was discovered in his plaque. After elimination of the protozoa, he regenerated much of the lost bone. Given that other variables affecting insulin requirements appeared to remain unchanged, it is noteworthy that he also experienced better stability of his diabetes. In fact he became quite cavalier about the timing of his meals and insulin injections, being able to lead a less regimented lifestyle. He has become more active and has not had a relapse in his oral or general health since the initial elimination of the protozoa. In particular, there has been no

recurrence of diabetic crises. He has become reinfected with amœba, on occasion, but diagnosis and retreatment has always been promptly instituted.

Series Two: Case Six
Periodontal Disease, Epilepsy and Asthma

The patient suffered severely from painful bleeding gums. She had to take Dilantin for recently developed Grand Mal epilepsy and also used a Beclovent inhaler for asthma. Her plaque was extremely active with large numbers of amœbæ. Trichomonas tenax and C.albicans were also recovered from time to time. The first application of antiprotozoal treatment paste in the office was immediately followed by an aura, a possible warning of an imminent seizure. The paste was quickly removed by irrigation and no seizure occurred. This course of events was not a surprise since metronidazole (one of the ingredients in the paste) is contraindicated with active CNS disorders. It took twenty months to eliminate the infections. This was not unexpected in light of the complexity and seriousness of the disorders of her general health. Since she had to take so many different medications, the potential for antagonism or drug interaction existed.

Series Two: Case Seven
Creeping Reattachment and the Interdental Papilla

This patient actually showed rebuilding of the interdental papilla between the central and lateral incisors. This site had been previously infected with protozoa, with cratering of the papilla plus a 7mm pocket. Elimination of the protozoa and the continued use of antiseptics in conjunction with appropriate home and office care coincided with "creeping reattachment" and normal appearance of the tissue. Pocket depth was reduced to 1mm without apparent recession.

Those cases already described in which C.albicans seemed to be playing a part were significant because the condition did not fully resolve while the yeast was still present. The last two cases, case number eight and case number nine are included as a reminder that other factors which we may not be able to identify immediately, do play a role in some periodontal infections.

Series Two: Case Eight
Peripheral Actinomycosis

Prior to seeing this patient, he had received extensive periodontal therapy, including both surgery and courses of several (different) antibiotics. However, his gingivæ remained so tender that he was unable to eat normally spiced foods. Pocket depths were shallow, with good bony support and no abnormal mobility, but the tissues appeared abnormal. A swab from the affected gingivæ was sent to the public health laboratory. Actinomyces israelii (a pathogenic anærobic filamentous micro-organism regarded by some authorities as a fungus and by other authorities as a species of "higher" bacteria) was cultured. It was eliminated with long term antibiotic therapy. The result was a return to normal comfort and a better appearance of the tissue. The patient was then able to return to a normal diet, including spicy food, with no discomfort.

Series Two: Case Nine
Stomatitis of Unknown Aetiology

This parallels the previous case except that no specific organism could be consistently identified. Protozoa and yeasts were observed in his plaque from time to time and he improved with treatment to eliminate these target organisms. Heavy filaments in ropes were observed in the plaque, but culturing was unsuccessful in identifying them. After treatment, he tended to relapse into states of soreness and bleeding. He never developed deep pockets, mobility or evidence of bony destruction. Treatment was empirical, antibiotic and/or antiseptic mouth rinses were intermittently used as appropriate. His progress was monitored, clinically and microbiologically, in order to maintain his oral health and comfort. Heavy stain, which he developed subsequent to the use of anti microbial mouth rinses, was acceptable to him as a trade off for oral comfort. Following treatment he was able to eat without pain.

COMMENT

Those cases where no specific organism could be identified, fell into a very small minority. Taken with those cases where the periodontal problems seemed to be due to underlying systemic disorders, they accounted for less than 1% of the cases seen.

Longitudinal Study

During long term experience with the same group of patients in general practice, it became very apparent that there seemed to be a corre-

lation between deterioration of periodontal health and deterioration of general health. The presence of protozoa in plaque preceded both in most cases. My observations over the long term were that if periodontal disease could be stabilised by the elimination of target organisms, the patient enjoyed better general health. It is not clear whether patients were simply better able to deal with an underlying general disorder once the oral infections were eliminated, or whether the oral infections were, in fact, more intimately involved with the general health disturbances. Either way, elimination of oral infections brought about benefits to both oral and general health. Therefore one must conclude that some periodontal diseases may be oral diseases with systemic manifestations while others are systemic diseases with oral manifestations.

Although convinced that the oral protozoa, particularly E.gingivalis, are indeed pathogenic, the writer cautions, especially in relation to periodontal problems:

At first, when all else fails:-

Think amœba.

Finally, just think amœba first... BUT

Don't think only amœba.

CHAPTER VI

The Fungal Song:

"Oh! What a beautiful morning!
Oh! What a beautiful day!
I've got a beautiful feeling...
Everything's going to Decay!"

- Anonymous

ABOUT YEASTS and ORAL CANDIDOSIS

This chapter contains some original observations about the yeast, Candida albicans. Variation in literary style has been employed to focus the attention of the reader on points that might otherwise be overlooked. A partial list of the most significant signs and symptoms, both oral and general, associated with oral infections with C.albicans, will be found in the patient instructions in Chapter X. Infection with this fungus has recently been the subject of intense interest and there are now many books and articles available. References to C.albicans and other fungi cannot be covered in detail in this chapter, or even this book. The reader is referred to other works to provide detail for the skeletal information herein supplied. Particularly, readers should avail themselves of the works of Nolting, Eagleson and Kane, Hoeprich and especially Truss. Their works are listed in the bibliography. There are many medical and microbiological conferences held annually which cover mycology, including C.albicans, in great detail. To fully understand the importance of mycoses, it is imperative for readers to expand their knowledge by further reading and by attending symposia on medical mycology. Even with additional knowledge, if the dental patient is to receive the full benefit of recent discoveries, the dentist will still find it necessary to co-operate with physicians skilled in the management of mycotic disease.

INTRODUCTION

Educated Incapacity was defined by the noted philosopher, Herman Kahn, as meaning "the acquired or learned inability to understand or even see a problem, much less a solution." The health sciences have long known about disease associated with Candida albicans but this yeast/mould has only recently come under renewed scrutiny. Candida has been termed an opportunistic pathogen. In modern society, people do not rest when they are sick, instead they take "cold remedies", analgesics, antibiotics and other prescription drugs in order to "keep going". As a result, Candida is afforded plenty of opportunity to effect a pathogenic role, particularly if the antibiotic employed is tetracycline. Although infections with Candida were once rare, they have now become commonplace, (Hoeprich, 1983).

From an ecological standpoint, fungi (yeasts and moulds) are agents of putrefaction. The decomposed remains of animals and rotten vegetation are degraded to fertilize and enrich the soil. This permits luxuriant germination of the next generation of vegetation, which supports herbivores, which fall prey to carnivores. Ever-present, the saprophytic fungi await death in order to survive. Unfortunately, some saprophytic fungi also strike living organisms, living as parasites until the living organism dies, whereupon fungi resume the role of saprophytes.

Long after the last living plant and creature on this planet dies, fungi will survive to rot the remains. Taken in this context the yeast, Candida, a saprophyte turned parasite by opportunity is an organism to be avoided. Although it may now (1989) be frequently present, it should not be considered a normal part of the human flora. Animal parasites, by contrast, have a vested interest in a vital host, since the death of the host leaves the parasite without a habitat. Therefore the order of treatment should be fungi first, animal parasites second.

ORAL CANDIDOSIS

Following successful therapy to eliminate oral protozoa, clinical and microbiological assessment of patients revealed that resolution was not always complete. The most frequent inhabitant of pockets that failed to heal was the yeast Candida. Elimination of this fungus was accompanied by further healing.

The alternate term "candidosis" has been used in this text in order to emphasize a subtle difference from the conditions usually referred to as "candidiasis". Very often the presence of Candida in the sub- and supragingival areas will not be marked by gross pathology nor accompanied by localised signs and symptoms, (e.g. those associated with Thrush or Leukoplakia). It was felt that the subtle changes in health observed required a specific description. The term Oral Candidosis has therefore been used to designate an infection of the oral cavity with less marked signs and symptoms than would be the case with a frank infection. In the latter case the term Oral Candidiasis would, in the writer's opinion, remain more appropriate.

Compared to bacterial or viral invaders, protozoan and fungal parasites are complex organisms. Complexity of the organism also leads to complications in treatment. Protozoa have been shown to vector (carry) simpler organisms such as viruses (Schuster, 1974) or even bacteria (Wright, 1988). This can be significant in transporting "packets" of infecting germs into a host. With the disruption of this "packet" the host is showered with infecting microbes which may cause an infection. This may be the critical factor in Legionaires Disease (Rowbotham, 1980). Even if other germs are not carried by the parasite, death of a relatively large and complex organism, as a result of antiparasitic therapy, may release toxins into the host causing malaise (Compendium of Pharmaceuticals and Specialities, 1984). This phenomenon, the Herxheimer's Reaction, may be no more than a nuisance. It is frequently experienced with treatment for the oral protozoa, but in some cases, such as treatment of Toxoplasmosis, (a parasitic infection), side effects may be serious or even life threatening. Although the side effects encountered in the treatment of Oral Candidosis are seldom so severe, they can sometimes cause alarm in persons not anticipating a reaction. Patients should be encouraged to phone immediately if a reaction is experienced, since alteration or temporary discontinuance of therapy may be advisable.

SURVEY ON THE ORAL INCIDENCE OF CANDIDA

Because it was observed that the incidence of Candida in the mouth was significantly less than was generally reported, it was suggested to me that a random survey should be done. On December 1 1982, everyone who entered my dental office, whether a patient or not, was asked to volunteer for an oral swab to assess the incidence of Candida. A sterile swab was wiped around the buccal mucosa of each person and then sealed in a sterile container. This was refrigerated for five to eight days,

then sent to a Laboratory which only processes mycology specimens for analysis. The purpose of the refrigeration was to allow Candida to establish colonies in the transport tube. Refrigeration, it was concluded, should retard the growth of bacteria more than a fungus and allow fungi, if present, a chance to establish colonies.

This protocol had been developed during the two years prior to the study. Direct observations of Candida in plaque had not always been followed by laboratory confirmation. Reports on known Candida positives would sometimes come back reported as negative for fungi or "overgrown by bacteria".

After a period of trial and error, it was found that refrigeration of specimens in the office, prior to submitting them to the laboratory, improved the correlation rate to close to 100%. Samples were then sent via courier, once a week, to the Ontario Ministry of Health Mycology Laboratory. Because the dental office was 300 miles (500 km) distant from the reference laboratory, storage without degradation of specimens was a prerequisite for accuracy in the confirmation of office microscopy. The most accurate results were obtained by refrigeration of specimens for between 5 and 8 days prior to transmission. Some swabs were now reported as positive even though Candida had not been found on direct examination. Careful re-examination of these patients frequently resulted in direct confirmation of the lab test. The validity of either method of testing had now been established by demonstrating each to have comparable accuracy.

Initial results obtained with 211 patients showed that the incidence of Oral Candidosis was 22.27%. 36 patients were infected with C.albicans (17.06%). A further 11 patients (5.21%) were reported as "Genus Candida", but the species was not further identified. Some species, other than C.albicans, are also pathogens. Patients with C.albicans had oral and/or systemic problems which resolved when the oral infection was eliminated. A few of the positive swabs were from asymptomatic people. However, when C.albicans was left untreated, unexplained fatigue, or other disturbances of oral and/or general health frequently ensued.

The final results of the survey, which spanned seven months, involved 408 patients: 89 (21.81%) were infected with C.albicans; 29 (5.15%) were infected with Genus Candida; 4 with "a yeast, not a pathogen"; 1 with Trichosporon; 4 reported as overgrown by bacteria and 285 were negative.

CANDIDA AND AIDS

The necessity for treatment should always be governed by signs and symptoms of disease as well as positive microbiological findings. When positive direct or indirect finding are accompanied by an absence of clinical findings, the clinician must be careful not to dismiss the microbiological findings as insignificant. These patients may be at risk or in an incubation stage. Of the 28.92% of persons with Candida (21.81% C.Albicans), none went on to develop AIDS. Candida does suppress the immune system. It is of significance since it may predispose an individual to other infections. For patients with other illness, superinfection with Candida compounds the problem. The aware dentist is in the front line of the fight for better health, particularly when the office is equipped to make microbiological investigations.

As a result of recent technological changes, many more species of Genus Candida (Symposium on Fungal Diseases, CACMID, 1988) have recently been identified. Of more than 140 species identified by 1986 (Nolting), at least 9 are considered pathogenic. The most common and most virulent is C.albicans, variant albicans. The most frequent of the other species identified as pathogens include C.stellatoidea, C.krusei, C.tropicalis, C.pseudotropicalis and C.parapsilosis (Nolting, 1987). The oral incidence of Genus Candida, some of which might not be pathogenic, together with those instances of positives for C.albicans labelled as in the incubation stage (i.e. before the appearance of symptoms) may be an explanation for the common belief that Candida is a "commensal." From the evidence gathered, Candida would not appear to be a normal inhabitant of the mouth. Rather it is one which is associated with oral and/or systemic disease. If left untreated in an apparently symptomless host, infection with Candida is invariably followed by deterioration of the oral and general health of the patient.

The mouth is the portal of entry for many micro-organisms. From the gingival margin the organism is afforded the opportunity:

for lymphatic or hæmatological dissemination,
to gain access to deeper structures of the mouth,
for aspiration into the bronchi, or
to pass with food into the digestive tract.

No internal organ is immune from infection with Candida, but since all anticandidal agents do not penetrate all organs, prevention is still the

best remedy. Although candidæmia has rarely been reported, failure to demonstrate Candida in blood or tissue of patients suspected to have systemic or endocardial candidal infection, is frequently followed by confirmation of the clinical diagnosis at autopsy (Hoeprich, 1983.) Systemic illness may also be due hypersensitivity or to immune response. From the capillary bed in the gingivæ, cellular components, Candidal metabolites or mycotoxins, may be absorbed. Cellular components which have been identified include proteins, polysaccharides and lipids. For example, glucan, chitin, mannan, ergosterol and triglycerides have been all identified. Although antibodies are produced against antigenic components of Candida species, anergy may result if the infection overpowers the host response (Hoeprich, 1983). Microbiological findings in relation to oral and systemic signs and symptoms should be carefully evaluated in view of what is now known about mycoses in general, and C.albicans in particular. One may well question whether Candida can be considered as commensal or any part of the normal flora.

SWITCHING

Candida albicans and some of the other pathogenic species of this Genus have been shown to be dimorphic in both culture and tissue. Dimorphism in tissue has been considered as one of the criteria of pathogenicity (Hoeprich, 1983.) As investigations progressed, it was observed that patients who attended the office with a history of vague illness, for which there was no apparent cause, sometimes had Oral Candidosis. It also became apparent that there was a correlation between symptomology and the observed stage in the life cycle of Candida.

When Candida is present in dental plaque, it may be observed in one of three basic forms. Long filaments, called hyphæ. These have been described as capable of inserting themselves between intact layers of epithelial cells, (i.e. an invasive stage). Candida converts from this mould stage (the hyphæ or tube cells) to the reproductive yeast stage (individual oval cells that "bud" daughter cells) at 37°C. The available data indicates that in the yeast form there is not only more rapid metabolism but also more rapid release of Candidal toxins which might enter the patient's circulation from the capillary bed in the gingivæ. The intermittent release of toxins into the tissue could alter the environment to permit the switching between buds and hyphæ as the tissue temperature cycled above and below 37°C. (In vitro, 37°C is critical in governing the change between buds and hyphæ. Above 37°C rapidly metabolising buds are formed, below this temperature, growth slows and buds elongate to form