

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

A Manual of Microbiological Diagnosis and Nonsurgical Treatment

By

Trevor Lyons, BDS (U. Lond.), LDS RCS (Eng.), RM (CCM)  
In association with Eleanor Standfield, B.A.

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----- FORWARD -----

Forward to the first edition by Dr Murray Vimy  
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"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 it's 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 circumscision. 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 than 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 anaerobic species which

utilize amino acids and produce ammonia and urea as metabolic by-products. This in turn results in haemostasis, 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 *Entamoeba 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 anaerobic 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,  
Department of Medicine,  
Faculty of Medicine,  
University of Calgary,  
#615 401 9th Ave S.W.,  
Calgary, Alberta,  
Canada, T2P 3C5.

March 1989

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A Forward by Dr Brian McLean  
>C=N J=Y

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 gastroenterology. 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

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A Forward By Dr. Richard Christie

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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.

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A Forward by Dr John F. Coombs

>C=N J=Y

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 gingivae 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 *Entamoeba 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 amoebae harboured elsewhere in the body. My preliminary impression from very closely monitored cases of intestinal infection with so-called 'non-pathogenic' amoebae, 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 amoebae.

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

>C=Y J=N

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A Forward by Dr. I.M. Warrack.

>C=N J=Y

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 variuous 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.

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INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER I -----

## INTRODUCTION TO PARASITOLOGY - THE CINDERELLA OF THE HEALTH SCIENCES

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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-Russia 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 gastrointestinal 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 Amoebiasis.

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 non pathogenic 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 *Entamoeba 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 amoebae (e.g. of the genus *Naglaeria*) 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 shortlived.

#### 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 it's life cycle, then the parasite becomes extinct. Although there is archaeological 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 it's 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 *Ascaris lumbricoides*, do not have an intermediary host, neither can they complete their cycle if accidentally ingested by a non human 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 larvae lodge in the capillaries and break out into the alveoli where they grow and molt for about ten days. Migrating along the bronchi they enter the oesophagus 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 *Taenia*, 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 larvae (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

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

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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 larvae. 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

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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

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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

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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 it's life cycle without interference.

## CO-INFECTION, INTERDEPENDANCE and SYNERGISM

In addition to these main four 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 *Entamoeba 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 *Entamoeba gingivalis* and *Trichomonas tenax*.

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INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER II -----

## THE CASE FOR THE PATHOGENICITY OF THE ORAL PROTOZOA

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### PREAMBLE

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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 aetiology 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 "Legionnaires' Disease" was soon linked to "Legionella pneumophila". 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

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Oral Amoebiasis is an infection of the oral cavity with *Entamoeba 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); Lapiere and Rousset (1973); Markel and Voge (1976); Sutcliffe, 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.hystolitica*. 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 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 non-pathogenic parasite. Only the degree of pathogenicity might be questioned. Confusion over pathogenic potential is well illustrated by *Entamoeba 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.

Gros (1849) published the first descriptions of "*Endameba 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 aetiology 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

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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 non diseased 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 amoebae in plaque.

Since amoebae, 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 amoebae 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 Muenster, West Germany, has shown that, in destructive periodontal lesions, organisms recovered from the base of the pocket are anaerobic. 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 anaerobic is found in the research of Clayton and Ball, (1954). *E.gingivalis* was unaffected by anaerobic conditions, even in the presence of bacteriostatic concentrations of Penicillin.

#### MATERIALS and METHODS

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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 amoebae 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 haematoxylin smears. SAF is well suited to the preservation of oral specimens since it is relatively non toxic. 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.

After the initial case of Oral Amoebiasis 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 Cambron (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 amoebicides, 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

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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.

and if the infection had been recently contracted.

12) Protracted or repetitive influenza like symptoms were frequent

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.

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 ulcerative membraneous 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 ulcerative membraneous gingivitis which he stated would be consistent with a protozoal aetiology. 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 anti-protozoal 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 Amoebiasis.

#### 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 amoeba. In this state it often resembles a multinucleated giant cell (personal observations.)

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

"since this amoeba ingests both red corpuscles and leucocytes, and can dissolve tissues, the burden of proof falls on those who believe in it's 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 amoeba 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.

*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 Parodontosis 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.

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 Muenster showed that no matter what antibiotic was used to eliminate the bacteria, antibiotics inevitably resulted in the death of the amoeba 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.

Even if the oral protozoa, particularly *E.gingivalis*, prove to be nonpathogenic, their role in transmission of other micro-organisms, such as virus and viroid particles, requires careful evaluation. Elsdon-Dew (1976) found subcellular organisms in *Entamoeba histolytica*. Schuster and Dunnebacke (1974) reported virus like particles in a free living amoeba of the genus *Naegleria*. Armstrong and Pereira (1967) demonstrated that the infamous "Ryans Virus" variant of the Poliomyelitis virus was an amoeba of the genus *Hartmanella*. The virus particle so completely filled the body of the amoeba 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 amoebae (*Acanthamoeba* and *Naglaeria*) were "infected" with and might vector *Legionella* bacteria. Once inside the amoeba, the bacteria now protected from the environment, could be transported in water droplets. If inhaled, the cytoplasm packet (the amoeba) could rupture releasing a high concentration of bacteria into a single site. A single amoeba can vector more than enough bacteria to cause an infection. Thus, one amoeba, 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 amoeba can initiate infection, the time the patient spends in the "risk area" would not appear to be a significant factor in contracting Legionnaires' 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.

*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 non pathogen.

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 aerosol 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 parasitaemia. 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 aetiology. Over fifty years ago Kofoid (1929) reported finding entamoeba 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 anti protozoal 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). Anti-arthritic 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 anti amoebic, 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

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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:

>LM=15

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

He finished by saying:

>LM=25

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

ABOUT ENTAMOEBA GINGIVALIS - FURTHER EVIDENCE OF PATHOGENICITY  
-----MICROBIOLOGY PREAMBLE - Entamoeba gingivalis unmasked.  
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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 amoeba 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 it's 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 amoebae 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 amoeba faltered slightly, then pushed forward across this stream creating a dam around which the fluid and cells had to flow. The amoeba 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 it's 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

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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 amoebae was kept under observation. When the solution reached the amoebae they immediately and rapidly reduced in size and became more opaque. Their internal structure could no longer be differentiated. Some amoebae 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 amoebae slowly expanded from these unrecognizable opaque masses to become, once again, clearly recognizable vital amoebae.

The trial was repeated employing a skin cleanser containing Aloe Vera. This fluid not only disrupted the ability of the amoebae 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

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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 amoebae 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" amoebae 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

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After clinical examination, plaque was taken as previously described. On first examination, the amoebae 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 amoeba was found in close proximity to a white granular mass which was presumed to be the salt/soda mixture. This amoeba 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 amoeba, each apparently filling with fluid before rupturing to discharge their contents. With the excretion of fluid the amoeba became successively smaller and the cell membrane shrivelled. Finally, apparently unable to control it's internal osmotic pressure in the presence of the powder, the cell membrane of the amoeba 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

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The effect of 17% alcohol was also observed. On examination of the plaque, motile amoebae 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 amoebae were now observed to be dormant, somewhat enlarged and rounded.

#### IN VITRO OBSERVATIONS ON THE FEEDING MECHANISM OF E.GINGIVALIS

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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 it's 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 amoeba 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 amoeba. The whole mass instantly became "the partly digested remains of a leucocyte nucleus in a food vacuole". The amoeba 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 amoebae 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 amoeba would become engulfed by the amoeba cytoplasm. The amoeba was seen to "swallow" globules of leucocyte cytoplasm, one after the other, with each globule passing into the amoeba 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 amoeba 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 amoeba, 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 haemoglobin 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 amoeba. 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 haemoglobin was entering the amoeba 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*

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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 amoeba is feeding, most of the colony are feeding. If one amoeba 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 amoeba is apparently dormant, presumably digesting it's food, the majority will be observed in the same state. When one finds one dead amoeba, the nonvital remains of many of it's 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 spirochaetes attached by one end to a bacterial filament. The whole palisade of spirochaetes exhibit a uniform wave motion. In addition, many large motile bacilli (possibly "fusiformis"), some free swimming spirochaetes, non motile 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 amoebae, usually in clusters or nests. If this micro colony is close to the ACs, the amoebae 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 amoebae 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 amoebae will move toward the ACs from all directions. On arrival, the amoebae 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 amoebae seem to be in process of digesting their food.

#### COMPARISON WITH ENTAMOEBA 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", *Entamoeba histolytica*. When an amoebic 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 amoebae 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 amoebae 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 amoebae are at the base of an epithelial flap. The tip free gingival margin would correspond to the tip of the overhanging epithelial flap of the amoebic 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 amoebae 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 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, (amoebic tonsillitis, liver abscess.) This makes an interesting comparison when one considers that the amoebae 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 cytolysed 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

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The organisms in an anaerobic 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 Anaerobes, 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 anaerobic 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 non-pathogenic symbiant in a mixed anaerobic infection, it's 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 amoebae 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 amoebae might be a useful indicator that pathogenic bacteria had been eliminated. This could be a useful test for those ascribing a bacterial aetiology for periodontal disease. If one accepts that the mutual dependence and synergism exist between the organisms in a mixed anaerobic 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 amoebae 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 amoebae, clustered together in a nest, a greater destructive potential than the same number of amoebae 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 amoebae, 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, amoeboid behaviour in the same sample did seem to be in phase. This phase behaviour has also been found when two samples are taken from non contiguous sites in the same mouth and compared. On the basis of clinical observations the following scenario is proposed:

#### PHASE I

Initially the amoeba 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 amoeba 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 it's "fix" of haemoglobin, the amoeba then

secretes toxins into it's environment. The irritation and tissue destruction attracts leucocytes which migrate into the area. They are then sacrificed as further nutrition for the amoeba.

#### PHASE II

During this phase of the cycle the amoeba digests it's 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 amoebic nucleus is difficult to find.

#### PHASE III

After feeding and migration there is cell division, with small daughter cells (amebulae) 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 amoeba 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 "amebulae" cannot be easily seen with phase contrast microscopy.

The amebulae then feed and grow and the cycle repeats, with succeeding waves of amoebae invading, multiplying and dying. Greater risk to the patient occurs if invading amoebae do not all die, but continue to invade into the tissues. Even more risk occurs if amoebae 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 dessication 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 it's adaptability to changes in it's environment and it's ability to recover from adverse environmental conditions.

It's hardiness may be demonstrated by taking a sample at the end of the day (say 5pm): confirm the presence of active amoebae; 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 amoebae. 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 haemoglobin, which it previously ingested from erythrocytes as a countermeasure. When leucocytes are encountered, this haemoglobin, 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 amoeba, or a symbiont, 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).

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

#### EPIC THEORY, AUTO IMMUNITY AND THE PERFECT PARASITE

An alternate theory for the mechanism of destruction in oral amoebiasis 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 amoebae 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 amoebae about one hundred fold. Either the denudation or the "stinging" (see later this chapter) of leucocytes by amoebae 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 auto immune disease (Genco and Mergenhausen, 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 auto immune 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, *Entamoeba 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 amoebae than white cells. Since virus would then be free to multiply within amoebae, 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 amoebae, would be unassailable by the host as long as viable amoebae remained. The end result could be quite debilitating for the host.

#### COMPARISON OF INFECTIONS

It is observed with amoebiasis 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 non inflammatory 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 paradontal (amoebic) abscesses.

Since *E.gingivalis* thrives in an anaerobic 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 aerobe, even though it thrives in an anaerobic milieu and relies on the presence of anaerobic bacteria for survival. If this were the case, attacking red cells and consuming their haemoglobin would provide the amoebae 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 non pathogens 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 it's appearance in life. With a green filter on a phase contrast microscope, the whole field is green, especially the ectoplasm of the amoeba. 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 haemoglobin 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 amoeba 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 amoeba will be seen to approach a leucocyte and flatten itself against the leucocyte as the amoeba 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 amoeba 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 amoebae attack and feed on leucocytes. Feeding then, seems to be a two stage process. First the cell is "stung" and degenerates. Second, the amoeba comes back to feed on it's prey. The amoeba which stings a leucocyte moves on and stings others, often trailing a clump of host cells, stuck to the "tail" of the amoeba. The amoeba remains viable. The leucocytes degenerate. Other non attacked 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 amoebiasis, 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, it's 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 it's 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 amoeba 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 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 nausatium*, a free living protozoan which is a predator of *Paramecium* species.

*E. gingivalis* excretes undigestable 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 amoeba 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 amoebae only has amoebae 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.

>TM=1 BM=62 LM=5 RM=75

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER IV -----

## MICROSCOPY FOR THE DENTIST

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

## THE MICROSCOPE

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A microscope is composed of the sum of it's 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 diopetre 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, 40, 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 wearout or require servicing, so it may be best to purchase a microscope from a company that provides a local repair service for it's products.

#### PHASE CONTRAST CONDENSOR

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 CONDENSOR

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 amoebae 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:

>IM=15

Traverse rapidly about one field of vision.

Stop.

Adjust focus and examine the area.

Repeat the three previous steps.

>LM=5

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, amoebae 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 amoeba 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 discernable. 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 spirochaetes 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 haemoglobin that has been sucked from erythrocytes. Vacuoles which are mainly clear, but which have a few granules in them, contain cytoplasm from leucocytes.

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 it's way around immovable objects that stand in it's 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

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 a faint outline of the body of the cell, with typical but faint inclusions (remnants of leucocyte nuclei and granules of haemoglobin) 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 amoeba is it's 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 amoebae 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 flagellae 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 flagellae 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 flagellae. 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 flagellae. 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, hyphae, pseudohyphae and chlamydo spores. 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 hyphae have about the same diameter. Hyphae are long tubes with few cross walls. Hyphae 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. Pseudohyphae are medium length oval cells joined end to end. They are, in fact, elongated buds that have not quite separated on division. Chlamydo spores, the resistant disseminating form of the mould, are produced as a result of the union of a positive hypha with a negative hypha. Chlamydo spores 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 non branching, 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 gingivae. 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 non motile 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 cocco-bacilliary forms (CBs). These appear to be associated with decay and gingival irritation. *Actinomyces actinomycetum comitans* have the same appearance but cannot be positively identified other than by culturing.

#### BACILLI

Non moving 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.

#### SPIROCHAETES

Spirochaetes 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, spirochaetes will be observed still wriggling within the vacuole of an amoeba. Sometimes the spirochaete burrows out of the amoeba. I have seen spirochaetes bore their way into an amoeba, wriggle around and then leave. Subsequent to this, I observed the amoeba start to degenerate. One may speculate that rather than being a pathogen, spirochaetes may be commensal scavengers of cell debris, or perhaps even a natural enemy of the amoeba. However, some researchers believe that spirochaetes are not commensal but may locally suppress the immune response (Conversation with R.Mueller, Dip Bact. Univ. Muenster).

#### 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 non motile cocci. Within about five hours of thorough tooth surface debridement, 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 non motile 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 spirochaetes. Motile bacilli may not be present, in spite of the presence of spirochaetes, if the environment favours that development. As the plaque matures some of the cocci may colonize the branching filaments, (ACs). The presence of spirochaetes generally signals an anaerobic 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 examination 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 aetiological 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.

A SUMMARY OF SOME TYPICAL CASE HISTORIES  
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This chapter is divided into two sections. 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. *Entamoeba 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 (Sutcliffe et al, 1951) and tonsillar suppuration (Craig and Faust, 1970). Oral Amoebiasis is defined as an infection of the oral cavity with this protozoan parasite. Cases reported here include one case of amoebic tonsillar suppuration associated with oral disease and one case of amoebic 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, spirochaetes, amoebae, trichomonads, yeasts, ACs (branching filaments, probably a species of *Actinomyces* (A),

with cocci (c) attached), CBs (cocco-bacilliary 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. Amoebae were present in moderate (+) numbers. A scraping around the periphery of the base of the ulcer revealed many amoebae (++/+++), which became more apparent as the white cells lost vitality over the ensuing two hours. He made a rapid recovery with anti-amoebic 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 papillae of the mandibular incisors. Halitosis, bleeding and pain were pathognomic of acute ulceromembraneous gingivitis (synonyms: Vincents, ANUG). Entamoeba gingivalis was recovered from the site. Rapid improvement was obtained with anti-amoebic 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. Entamoeba 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-amoebic 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. Maxilliary Amoebic 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-paradontal 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 Entamoeba gingivalis.

Case Five: Age 18. Male. Recurring Amoebic 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 *Entamoeba 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 *Entamoeba gingivalis*. He was treated with systemic and contact amoebicides 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  
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Necessity and Chance, led to a series of events, the results of which challenge the widely held concept that *Entamoeba 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 *Entamoeba gingivalis*, little seems to have found it's 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 *Entamoeba 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 it's 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 anaesthesia, 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 non invasive 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 anaesthesia 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 amoebae 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.

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 non scientific 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+	+	*	+++
BACCILLI :	<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 liason 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.

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 non scientific 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+	+	++	+++
BACCILLI :	<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 = ***** ***** ***** ***** **

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Notes to the referring DDS or MD: Thank you for the referral. If you have any questions, please phone. Eleanor will be your primary liason 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 non scientific 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+	+	++	+++				
BACCILLI :	<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 = *****  *****  ****     **      *

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Notes to the referring DDS or MD: Thank you for the referral. If you have any questions, please phone. Eleanor will be your primary liason 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 it's 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 non scientific 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+            | +   | ++    | +++ |        |     |       |      |
| BACCILLI :               | <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 liason 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 amoebae, trichomonads or yeasts in her  
 plaque, which was minimal. A folow 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 non scientific 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+ + ++ +++  
 BACCILLI : \* 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. Eleanor will be your primary liason 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!  
The patient now seems to be free of infection  
Please proceed with routine treatment. I shall only see the patient again on request of the referring doctor or the patient if it appears that my services are needed again.

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

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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

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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

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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 amoeba, on occasion, but diagnosis and retreatment has always been promptly instituted.

Series Two: Case Six - Periodontal Disease, Epilepsy and Asthma  
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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 amoebae. *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  
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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 gingivae 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 gingivae was sent to the public health laboratory. *Actinomyces israelii* (a pathogenic anaerobic 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

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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

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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

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

>IM=15

Think amoeba.

>IM=25

Finally, just think amoeba first... BUT  
>LM=35

Don't think only amoeba.  
56



The Fungal Song: (anon)

...."Oh! What a beautiful morning!

....."Oh! What a beautiful day!

....."I've got a beautiful feeling.....

....."Everything's going to Decay!"

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, Hoeplich 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", analgaesics, 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, (Hoeplich, 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. Everpresent, 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

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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 Legionnaires 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 couriered, 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 haematological 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 anti candidal agents do not penetrate all organs, prevention is still the best remedy. Although candidaemia 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 gingivae, 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, energy 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 hyphae. 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 hyphae or tube cells) to the reproductive yeast stage (individual oval cells that "bud" daughter cells) at 37 degrees Celsius. 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 gingivae. The intermittent release of toxins into the tissue could alter the environment to permit the switching between buds and hyphae as the tissue temperature cycled above and below 37 C. (In vitro, 37 degrees is critical in governing the change between buds and hyphae. Above 37 C rapidly metabolising buds are formed, below this temperature, growth slows and buds elongate to form hyphae.) The invasive hyphae penetrate to warmer tissue and the cycle repeats. The critical temperature in vitro is not necessarily followed in vivo, but no contradicting data is available.

In vivo it was observed that the bud stage only lasts two to three days and is followed by buds elongating to become short cells. These are joined end to end, the strands referred to as pseudohyphae. The pseudohyphae are found mixed with buds. Two or three days more sees the mix changed to pseudohyphae, plus long branching septate filaments, the true hyphae. Another two or three days sees the mix as pure hyphae, with fat blunt ends. This is followed by the appearance of hyphae which are very slender, tapering to a sharp point. These sharp hyphae may sometimes be seen penetrating between individual epithelial cells. These may have dislodged in a clump when the plaque was taken. This stage is then followed by the appearance of many budding yeast cells. The entire cycle takes about ten days in an average individual.

## SPORES

If the environment becomes unfavourable for *Candida* it produces chlamydospores. These are thick walled cells which look like a swelling at the end of a pseudohypha, or a swelling between two of the segments of the pseudohypha. Chlamydospores should not be mistaken for buds since they do not represent increased metabolism, but are a resistant stage. When fungi experience a hostile environment, they produce resistant forms, or "spores" in order to spread more easily to a distant (i.e. more fertile) habitat. Often spores are produced sexually. Recently it has been shown that yeasts do have a sexually reproductive stage. Chlamydospores are now held to be the result of the union of a positive with a negative filament (a sort of sexual union) to produce a new genetic variant more capable of survival. Chlamydospores should therefore be regarded as a disseminating form, i.e. an infective stage.

## SYSTEMIC CORRELATION

Clinically significant features that have been observed:

>LM=15 RM=65

When patients are most fatigued, morose, unenergetic or depressed, Candida in the plaque is observed to be in the bud form.

When patients are at their most energetic, Candida is observed to be in the hypha form, with or without chlamydospores.

>LM=5 RM=75

Knowing the cyclical nature of Candida, it is then possible to observe it in plaque and relate the stage of the fungal cycle to the cyclical variation in mood, fatigue or energy of an individual. This is a valuable diagnostic test to determine if Candida has established to the point that the infection has started to seriously disturb the health and/or result in moderate to severe complications in treatment that might require the involvement of a physician.

It has been found that Candida is the most frequent organism which overgrows, following successful elimination of oral protozoa with antibiotic therapy. If this overgrowth is a biological embarrassment to the patient, the condition is called a superinfection. Typically, the aggressive, destructive phase of periodontal disease is arrested by the elimination of the protozoa but, if Candida remains, the tissue fails to heal properly. In the absence of Candida, appropriate therapy results in a good tissue response. These observations prompted the design a treatment regime for the elimination of Candida from the periodontal pocket.

## TREATMENT

Treatment for the elimination of the oral protozoa was modelled on that for the elimination of any lumen dwelling, potentially tissue invasive, parasite. Namely, contact and systemic antiparasitic agents used concurrently until the target organism have been eliminated (Cuttings' 1979). What worked against an animal parasite could also work against a fungal parasite, providing that the appropriate antimicrobials were chosen. The concurrent use of systemic and topically applied Nystatin eventually resulted in elimination of the fungus which was accompanied by further healing. In clinical practice, discontinuance of medication after elimination of Candida was not followed by relapse provided that treatment was continued until three months after all signs, symptoms and tests were negative.

It was never found that only topical (or only systemic) anti-Candidal therapy was successful. When such single phase treatment was employed, although the fungus might be significantly suppressed, discontinuance of medication resulted in rapid relapse.

## ORAL SIGNIFICANCE

Candida in the gingival crevice (or periodontal pocket) was often accompanied by a dull to bright red granular inflammation. Patients sometimes complained of prickling or burning sensation in the gums. The necks of the teeth were often hypersensitive. The gums were often tender

and would bleed with little provocation, but the pockets were seldom deep, frequently only 1mm. Deeper pockets associated with Candida were frequently those which had been previously infected with the protozoa and which had failed to heal completely, leaving a "residual" pocket 1-2 mm shallower than previously measured. Sometimes the tissue adjacent to an area at the gingival margin, infected with Candida, would be slightly white, or have faint white patches. Candida was not found to be associated with "Black Hairy Tongue".

#### CANDIDA, pH and CARIES

Candida survives between pH 3 and 8, but thrives in an environment that is acid (pH 5 to 5.5) and has up to 35% fermentable sugars available. Although the ideal temperature for fungal growth is between 20 C and 40 C, our experience showed it survived refrigeration without adverse affect on viability when the colonies were returned to the ideal range. It survives well in the oral environment, especially if there is active caries. Decay is classically accepted as caused by bacteria producing acid and driving the pH below 5.4. It was repeatedly observed that when Candida was present the rate of decay was exceedingly high. The type of rampant caries, associated with Candida, started as small lesions in the dentine just at the gingival margin. These lesions tended to rapidly spread circumferentially at and below the gingival margin. This decay could encircle a tooth in as little as three months and cause so much destruction that the tooth would be all but unsaveable in six months. The progress of the lesion was typically not associated with pain until the cavity was quite large. Recovering Candida from the surface of carious lesions, as well as from the base of pockets, would indicate that the organism grows in either aerobic or anaerobic conditions. However the metabolites under differing conditions would be expected to differ, a common finding with fermenting yeasts. Simply stated, under anaerobic conditions (the base of the pocket) Candida produces toxic substances, including acetaldehyde, which inhibits cell membrane permeability. Aerobically the yeast ferments sugars to acid, adding to that already produced by bacterial metabolism.

#### ANOMALIES AND OTHER MATTERS

Subsequent to the nuclear attack at Hiroshima a strain of Candida capable of actually metabolising fermentable substances to produce ethyl alcohol has been reported. The alcohol can then enter the blood stream of the unsuspecting patient and produce unwanted intoxication, (Iwata, U of Tokyo; Zwerling, 1984; Baker, 1982). Aerobically, the yeast may simply ferment sugars directly to acid, adding to that already produced by bacteria thus further depressing the pH and increasing the rate of decay. In West Germany, in the early eighties, there was an outbreak of rampant caries in the primary teeth of some children. The condition progressed to pulpal necrosis and multiple jaw abscesses from which only Candida could be recovered.

The healthy human body is covered by an unbroken layer of "skin". This protects the inner parts of the body from invasion by micro organisms. The skin changes from keratinized epithelium to mucous membrane as it enters body cavities. The covering remains unbroken, except in the mouth. Here the teeth are rooted in the jawbones and must pass from this inner part of the body to the surface.

#### FOCAL SEPSIS and INFECTIVE ENDOCARDITIS

The only part of the body where the epithelial covering is discontinuous is where the teeth are rooted and pass through the "gums". In health the gingivae tightly attach to the tooth to prevent the ingress of micro organisms. If *Candida* or the Protozoa are present at this site, and if the epithelium in the pocket has been destroyed by infection, then these parasites have direct access to the bone as well as to the soft tissues and the bloodstream. Instrumentation in such a site may drive these organisms deeply into the tissue or even the bloodstream. Moore-Gillon et al (1983) report thirty two episodes of infective endocarditis (between 1965 and 1982) in 30 patients with prosthetic heart valves. Of those that died from the infections, all were infected with organisms frequently found in the mouth. Two of these cases were candidal endocarditis (*C.albicans*). No cases of candidal endocarditis survived. Penetration of *Candida* to deeper structures, or dislodgement into the bloodstream, normally only occurs if the epithelial covering at the site of infection is not intact (ulceration), and/or if instrumentation is conducted at such a site, (Hoeprich and Rinaldi, 1983). If dislodged into the blood stream from such a site, *Candida* may not only settle on a prosthetic or a damaged heart valve, but also on a healthy heart valve in a non immuno compromised host. The prognosis for such an endocarditis is grim and further complicated by the risk of embolism. The frequency and size of emboli are greater in Candidal endocarditis than in bacterial endocarditis. Pockets infected with *Candida* frequently bleed upon the slightest provocation, indicating a break in the epithelium (micro-ulceration). Caution should be uppermost in the mind of the dentist before any instrumentation in such a site is contemplated.

Even the act of chewing can send showers of bacteria into the bloodstream. Normally our immune system copes with bacteria and viruses, but the parasite, *E.gingivalis*, feeds on the white cells which should destroy it. *C.albicans* and other fungi produce mannan, a soluble carbohydrate component that has been demonstrated to be immunosuppressive. Infections with such organisms, rather than being symptomatic of suppression of the immune system, more probably precede and contribute to suppression of cellular immunity. (Witkin, 1985). Thus animal parasites and parasitic fungi present a more insidious threat than mere bacteria.

For these reasons parasites should be treated with respect. Appropriate local and systemic agents should be prescribed to be used concurrently. Medication should be maintained until testing has shown that the infections have been eliminated, or controlled enough, before proceeding with routine dental care. The latter is always a necessary adjunct in treating these infections. Timing is all important to maximizing therapeutic response while minimizing complications.

No benefit accrues to the patient if the teeth are sitting in a bed of pus. Neither does the patient benefit if the end result of instrumentation in an infected site is the further spread of infection. Finally, for the physician attempting to treat a patient to eliminate *Candida*, treatment can be frustrating if a reservoir of *Candida* in the mouth receives no attention. Patients deserve a comprehensive team approach.

FOOTNOTE



Living with a parasite, animal or fungal, may be likened to living with a ticking time bomb. It is the role of the clinician to defuse this time bomb without exploding it. Carefully selected doses of appropriate anti-microbials, used to slowly kill off the parasite, reduce the Herxheimer's effect. (Showering of the host with antigenic material and virus resulting from the death of the parasite, animal or fungal.) Once the infection is controlled or eliminated, debridement and other physical treatment can be expected to be more successful, less painful and be accompanied by fewer complications.

The bottom line in the treatment of these types of infection is that patients must expect to get worse before they get better. Clinicians must strive to minimise the adverse effects of successful treatment.

>TM=1 BM=62 LM=5 RM=75

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER VII -----

DIAGNOSIS - THE FOUNDATION OF SUCCESSFUL TREATMENT

PREAMPLE

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For the purposes of this book, diagnosis of periodontal infections is divided into three broad categories:

>LM=10

- 1.) Destructive periodontal lesions, with or without inflammation.
- 2.) Inflammatory lesions of the gingivae, unaccompanied by destruction.
- 3.) Other lesions secondary to disturbances of systemic health.

>LM=5

The process of ecologic succession is well recognized in the colonization of the digestive tract by microorganisms. The micro ecosystem stabilizes after a series of changes in the microflora has occurred. Each dominant species maintains ascendancy as the environment is changed, each alteration to the environment permits ascendancy of a different species, or group of species, each, in turn, better adapted to the altered environment (Wilson et al, 1988). Once stability of the oral microflora has been established, little further change with age occurs unless the ecosystem is disturbed by external factors (Marsh, 1988). Neither *Candida* spp (Marsh, 1988) nor the oral protozoa (Barrett 1914; Bass and Johns 1914; Chiavaro, 1914; Chandler, 1955; Lyons et al, 1982;) can be considered normal residents of the oral microflora, although any of these organisms are frequently present when there is pathology in process.

In order to obtain all the information relative to a patient's periodontal condition, it is necessary to have as much information as possible. Prior to a periodontal examination, patients are instructed to follow a few simple rules to stabilise the ecosystem of their plaque in order that target organisms, if present, may be found. This also allows stabilisation of tissue response. The typical state of disease will then be observed, rather than an artificially enhanced state produced by superlative oral hygiene. The pretreatment instructions (see Chapter VIII and Chapter X for further details), allows for plaque stability and maturation while inhibiting the associated disease from running out of control.

To obtain an accurate diagnosis based on the microbiology of the plaque it is necessary to ensure that target organisms may be recovered for identification. Ideally, this would include viruses, bacteria, fungi and protozoa. There are technical problems associated with identification of all bacteria, since there may be more than four hundred species present. However, only a few species are considered potentially pathogenic. Viral identification also presents technical problems since most laboratories do not, at present, have facilities for viral cultures. Almost without exception, dental offices are not equipped for bacterial nor viral

culturing. Even if such steps were considered practical, the biosafety aspect should not be ignored. There is an increased element of risk to the environment, as well as to the office personnel, when dealing with concentrations of potentially pathogenic micro organisms. From the safety standpoint, therefore, such an approach to the diagnosis of periodontal disease might be ill advised.

For quick, safe and easy diagnosis, the phase contrast microscopic examination of plaque currently offers the most promise. Microscopy can be relied on to give consistently reliable results, especially with severe infections. Where there is light or recent infection, patients must observe a few simple rules to facilitate accuracy. It is best if the patient, as far as possible, adhere to the following instructions:

PATIENT INSTRUCTIONS FOR STABILISING PLAQUE IN VIVO  
-----

For five days prior to plaque examination:

- 1.) Brush once daily in the evening using the Bass Brush Technique
  - 1b) Use a Bass type of brush
  - 1c) Do not use commercial toothpaste.
  - 1d) Use floss, toothpicks, etc., only to remove food caught between the teeth, but do NOT floss or "pick" subgingivally.
- 2.) Do not use mouth washes or water irrigation devices.
- 3.) Use non sweetened liquids to rinse away all food debris after eating.
- 4.) If possible, take no medication for the five days,
  - 4b) Particularly "cold remedies" or
  - 4c) Acetylsalicylic acid and it's derivatives.
- 5.) DO NOT stop prescription medication unless authorised, by the prescribing physician, to discontinue.
- 6.) Brush the night before the appointment.
- 7.) Do not brush on the day of the appointment.
- 8.) Do rinse away food debris using plain water
  - 8b) Or any unsweetened beverage.
- 9.) Do not drink tart fruit juices before the appointment.
- 10) On the day of the appointment avoid eating food or snacks such as salted nuts,  
Sunflower seeds,

Fresh pineapple,  
Citrus fruits, or  
Other fresh fruit.

11) Do not use "breath mints" or other medicated lozenges.

12) Avoid fresh fruit juices and all carbonated beverages.

\*\*) Do follow the oral hygiene instructions given by the office

(See Chapter X - Patient Treatment Instructions

for the Pretreatment Programme which includes

Specific Oral Hygiene Instructions.)

If a negative plaque examination results when a positive is anticipated, carefully re-evaluate the diagnostic technique as well as the patient's food, medication and other habits; (chewing tobacco, brushing, use of breath fresheners, etc.)

#### MODIFIED PERIODONTAL EXAMINATION

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Once the patient is positioned in the dental chair, a brief clinical examination should be performed. To avoid injuring fragile tissue, or promoting bleeding, lightness of touch is important in the probing technique used for measuring pocket depths. Bleeding will make microscopy that much more difficult. NO scaling, prophylaxis, use of antiseptics, cavitron, prophyjet, etc., should precede the plaque exam.

All parameters are graded into five levels, for example, Good (G), Fair to Good (F-G), Fair (F), Fair to Poor (F-P) and Poor (P). Quantities are recorded from minimum (<o+) to maximum (+++) with the grades listed as: <o+, o+, +, ++, +++. The minimum clinical parameters that are recorded on the "K5" chart are:

>LM=10

Oral Hygiene, (OH) recorded from good (G) through poor (P).

Gingival Condition, (GC), recorded as above.

Plaque (P) (quantity), recorded from minimal <o+, normal o+, to maximum +++

Halitosis; from none -ve, then just detectable o+ through ++

Pockets (Poc - depths 3mm or more);

>LM=5

readings are taken mesial, buccal and distal for both the lingual and buccal surfaces of incisors, canines and premolars. For molars the readings are taken mesial, mesiobuccal, furcation, distobuccal and distal, as appropriate, on both the lingual and buccal arches.

>IM=10

Bleeding, the location is marked with a red B.

Lingual and buccal bleeding points are charted separately.

Mobility, from minimum <o+, the limit of normal being o+, to maximum +++

Inflammation, recorded as a solid red line on the chart.

>IM=5

If the inflammation is sporadic in a quadrant, it is shown as a dotted red line. Additionally, the inflammation is described as:

"None"

"Minimum-sporadic"

"Only in Stagnation Areas"

i.e. the areas the patient finds hardest to clean, labial to the lower anteriors, lingual to the lower molars, buccal to the upper molars and palatal to the upper anteriors.

"Four Quadrants (4Q) or GMD"

GMD is an acronym for Generalized Mauve Deterioration of the gingivae, i.e. If venous stasis is present.

"Detachment" or "Loss of Interdental Papillae"

are in the "most severe" category.

Sub-mandibular lymphadenopathy (SMG)

relative to both palpability and tenderness. Is recorded as "No" for not detected, L or R, meaning that the right or the left side is detectable but not tender, the next grade is L+R, meaning that both are detectable. In addition, tenderness is recorded using the same format.

Microbiological Parameters are recorded from <o+ through +++, except for filaments (Leptothrices) and cocci which are either recorded as normal (++) or abnormal, with the abnormality noted. For example, if streptococci or filaments in adherent ropes or bundles are observed, this is recorded as an abnormality. Cocco-Bacillary forms (CBs) are only recorded as "present" when observed. They are not graded.

The K5 chart has sufficient space to record this information in shorthand form. There is a simplified chart on which can be marked actual mobility, pocket depths and location of inflammation and bleeding. After patient identification, there is a prompt line and six charts so that a series of evaluations may be recorded on one sheet for ease of progress assessment:

>C=Y J=N

ILLUSTRATION SHOWS LAYOUT OF K5 CHART:

-----

>C=N LM=10

NAME:

FOLIO NUMBER:

Mob o+, +, ++, +++; Pockets 3 or 3>; Inflammation:red line

| DATE   | OH          | GC  | P   | Hal   | Poc | Mob   | I   | SMG | ACs             |
|--------|-------------|-----|-----|-------|-----|-------|-----|-----|-----------------|
| Lepto  | Cocci       | Mot | Bac | Spiro | Am  | Trich | Can | CBS |                 |
| Buc    |             |     |     | I     |     |       |     |     |                 |
| 8      | 7           | 6   | 5   | 4     | 3   | 2     | 1   | I   | 1 2 3 4 5 6 7 8 |
| Pal    |             |     |     | I     |     |       |     |     | MAX             |
| >LM=1  |             |     |     |       |     |       |     |     |                 |
| Pdx=   | -----I----- |     |     |       |     |       |     |     | Left            |
| >LM=10 |             |     |     |       |     |       |     |     |                 |
| Ling   |             |     |     | I     |     |       |     |     | MAND            |
| 8      | 7           | 6   | 5   | 4     | 3   | 2     | 1   | I   | 1 2 3 4 5 6 7 8 |
| Buc    |             |     |     | I     |     |       |     |     |                 |

| DATE   | OH          | GC  | P   | Hal   | Poc | Mob   | I   | SMG | ACs             |
|--------|-------------|-----|-----|-------|-----|-------|-----|-----|-----------------|
| Lepto  | Cocci       | Mot | Bac | Spiro | Am  | Trich | Can | CBS |                 |
| Buc    |             |     |     | I     |     |       |     |     |                 |
| 8      | 7           | 6   | 5   | 4     | 3   | 2     | 1   | I   | 1 2 3 4 5 6 7 8 |
| Pal    |             |     |     | I     |     |       |     |     | MAX             |
| >LM=1  |             |     |     |       |     |       |     |     |                 |
| Pdx=   | -----I----- |     |     |       |     |       |     |     | Left            |
| >LM=10 |             |     |     |       |     |       |     |     |                 |
| Ling   |             |     |     | I     |     |       |     |     | MAND            |
| 8      | 7           | 6   | 5   | 4     | 3   | 2     | 1   | I   | 1 2 3 4 5 6 7 8 |
| Buc    |             |     |     | I     |     |       |     |     |                 |

>LM=5 J=Y  
 PLAQUE EXAMINATION: DIRECT  
 -----

In order to achieve accurate and consistent results, it is important that the quality of the plaque is not altered by the sampling technique. The principles involved are:

- >LM=10
- A.) The plaque should be mature.
- B.) The quality of the plaque should be undisturbed by medication.
- C.) The quality should be undisturbed by local factors, such as acidic or salty foods, astringents or antiseptics, brushing just before an appointment, other over exuberant oral hygiene or recent dental work.
- D.) The sampling technique should be consistent and not introduce variables which were not present in the patient's mouth.

>LM=5

Assuming that the first three parameters have been met, the following technique has been shown to be reliable:

Having completed the clinical exam and identified the first pocket from which the plaque is to be taken, a drop of the patients own saliva is removed from the sublingual area and deposited in the middle of a clean microscope slide. Plaque from the suspect area is removed with a thin (and clean) instrument such as an explorer or fine perio probe. DO NOT use a wire loop, a curette or similar instrument. The probe must be taken to the base of the pocket without causing haemorrhage. The sample is then lifted clear and deposited in the saliva on the slide. Do not agitate or mix, but tease the plaque off gently, using a second instrument if necessary.

Once the plaque is in the saliva on the slide, drop a cover slip in place and squeegee the cover slip to produce a thin film of plaque. (Half a pipe cleaner, doubled over, makes a good squeegee. It is unlikely to break the cover slip and yet is capable of exerting sufficient pressure to produce a thin even film). The saliva should reach the edge of the cover slip over it's entire circumference and there should be no bubbles or grit under the cover slip. Supragingival plaque and detritus have little diagnostic value while reading the slide will be complicated by air bubbles, especially if they are numerous.

It is difficult to recover plaque from teeth which have been restored by crowning (particularly if the crowns are metal) since the plaque tends to stick to the crown margin instead of the dental instrument. Thus the plaque sample is very often lost onto the surface of the crown, to which the plaque adheres tenaciously.

For phase contrast microscopic examination of living amoebae, the use of liquids, other than the patient's own saliva, as a mounting medium, causes temporary distortion of the amoebae, which almost invariably makes them unrecognizable during the time that a slide would normally be examined.

The spotting and recognition of protozoan and fungal parasites is not within the scope of this chapter. It is assumed that the reader will seek additional assistance in the form of tutoring and training if needed. (See Chapter X for a guide to spotting and recognition of protozoa and other organisms commonly seen by phase contrast microscopy.) Once the slide has been read and a tentative diagnosis reached, it would be prudent for the neophyte to obtain confirmation of the diagnosis by submitting material to a reference laboratory.

PLAQUE EXAMINATION: INDIRECT  
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Confirmation of micro-organisms identified, for all practical purposes, may be divided into two classes:

PROTOZOA for which fixed plaque in sufficient quantity must be submitted to a parasitology laboratory.

FUNGI for which a swab may be taken in order to grow the material in culture. A dried slide from a suspect area may also be submitted to a mycology laboratory.

## PARASITOLOGY : COLLECTING PLAQUE FOR THE LABORATORY

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### Introduction

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*Entamoeba gingivalis* was discovered in 1849 by Gros, but interest in this protozoan parasite has fluctuated as opinion about its pathogenicity has varied. The question of the degree of pathogenicity of this lumen dwelling parasite remains partially unanswered. The difficulty surrounding laboratory procedures for producing permanently stained slides, and for culturing this protozoan have compounded the enigma. Although wet mounts of plaque mounted in saliva may easily be used to demonstrate amoebae in the deepest (most apical) portion of subgingival plaque, taken from an infected site, certain practical problems arise for the dental practitioner and researcher alike.

When a patient, suspected of harbouring an amoebic infection, presents, several factors may interfere with proper diagnosis, for example:

- 1.) the microscope may not be readily available,
- 2.) the microscopist may not be available,
- 3.) the first pockets searched may be negative,
- 4.) there may not be time to conduct the microscope search at all,
- 5.) there may not be time to conduct the microscope search immediately,
- 6.) confirmation of the diagnosis may be required, or
- 7.) the search may be interrupted by other more pressing matters and
- 8.) the slide may dry out before the search is completed.

The routine laboratory procedures employed for the examination of faecal smears for protozoa were modified in order to overcome the preceding barriers. The new method, (Palmer, 1981, see Chapter XI) produces permanently stained slides of *Entamoeba gingivalis* in dental plaque which clearly demonstrates the nuclear structure.

Although the plaque samples are very tiny, difficulty in obtaining sufficient material for high quality slides will not be experienced, providing that all steps are meticulously followed. Sloppy technique in the dental office, clinic or laboratory only serves to jeopardise the results. In order to achieve consistent results, the steps described in this chapter for plaque collection should be carefully followed.

### BULK FIXATION OF PLAQUE IN SAF

For each patient tested, plaque from a number of pockets can all be placed in one container of SAF fixative which was developed by Yang and Scholten (see Chapter XI). SAF is available from medical supply houses in bulk or in individual kits originally designed for stool sampling. The kits are used by many medical laboratories, including some Provincial Health Laboratories. In the United States kits may be obtained from



Meridian Diagnostics, 3471, River Hills Drive, Cincinnati, Ohio, 45244. Phone 800.543.1980 or 513.271.3700. In Canada Meridian products are distributed by BioMega Diagnostic, 10900, Rue Hamon, Montreal, PQ, H3M 3A2. Phone 800.361.9615 or 514.331.7520.

The plastic bottle containing the SAF fixative fluid must be marked with the name of the patient and the name of the doctor submitting the sample. Only about 15cc of the fixative fluid should be in the container, therefore pour out any excess before starting to collect the plaque. Plaque from each pocket is taken (as for direct examination) and immediately deposited in the SAF fixative by gently agitating the instrument in the fluid to dislodge the probed material. The instrument is wiped dry before returning to the mouth for the next sample from the next pocket and the process repeated until sufficient material has been collected. The data sheet must also be completed and should be clearly marked DENTAL PLAQUE. Do not forget the name of the patient, the name and return address of the doctor. Inclusion of your own microbiological readings as they apply to Motility, Baccilli, Spirochaetes, Yeasts, Amoeba and Trichomonas may be of value since they may help the laboratory personnel.

Since plaque in SAF stores well (provided that the plastic container also has a plastic lid), there is no need to rush the material straight to the lab. However, do not stockpile specimens for more than a week or more than about two dozen kits. This will help the laboratory have an orderly flow of incoming work.

Unfortunately there is only one commercial laboratory in North America, at the time of writing, who have expertise in handling plaque preserved in SAF. The laboratory, Penpar Laboratory in Mississauga, Ontario, may be contacted at 3043a, Hurontario Street, Mississauga, Ontario. Phone 416.361.3387. The protocol was developed by Palmer and Scholten, to whom enquiries should be directed: Ontario Ministry of Health, Central Laboratory, Parasitology, 81 Resources Road, Weston, Ontario, M9P 3T1. Phone 416.235.5722. Plaque samples are no longer processed at the public health laboratory. The protocol, which is in Chapter XI, was released at the 11th Annual Education Conference of the International Academy of Preventive Medicine, 1981.

Trichomonas tenax is difficult to find by both direct and indirect examination. As yet, we do not have a reliable method for confirming T.tenax. A positive finding in either office or laboratory should be considered positive. The typical movement of Trichomonads make them unmistakable in a live wet mount, even if the numbers are sparse. In a stained slide they are very difficult to find unless the slide is "loaded".

#### SUMMARISED INSTRUCTIONS FOR COLLECTING PLAQUE IN SAF

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The instructions for collection of stool samples do not, of course, apply to the collection of dental plaque. Here are the relevant directions for the collection of dental plaque:

- 1.) Collect plaque at the beginning of the appointment.
- 2.) The plastic bottle should contain 15 cc fluid.

- 3.) Pour excess fixative into a spare container.
- 4.) Locate affected areas: pockets 3mm or more and/or inflamed areas.
- 5.) Collect sub gingival plaque ONLY with thin explorer.
- 6.) Agitate instrument in fixative to deposit plaque.
- 7.) Wipe instrument dry before returning to mouth.
- 8.) Collect plaque from 6 to 10 pockets.
- 9.) Patients must not brush on the day plaque is to be collected.
- 10) Patients should not floss for at least five days prior to the appointment.

On the day of the appointment:

- 11) Patients should avoid the use of tooth picks,
- 12) water irrigation devices,
- 13) strong antiseptics and some
- 14) drugs, even aspirin, may depress the number of parasites in the plaque to the point where they cannot be easily found.

Disregard of any or all of the above instructions may result in a false negative result.

Follow the directions for plaque collection implicitly, starting with instructions to the patient. Please also remember that other organisms, such as Candida species, may also cause problems and should be tested for separately. C.albicans may be cultured by using BiGGY Agar (also called Nickerson Medium.)

#### MYCOLOGY : COLLECTING PLAQUE FOR THE LABORATORY

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#### Introduction

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The most reliable test for Candida albicans is the use of Nickerson's Test Kit for the Selective Culture of C.albicans:

----BACTO BiGGY AGAR----made by----DIFCO LABORATORIES----

These kits are made by Difco in Detroit (Mich). They are available in Canada through British Drug House, (B.D.H.) in Toronto, on special order. (Nickerson Medium, Difco product # 0635-42-3 (20 tubes) 0635-80-6, 100 tubes.) They should also be available from a medical supply house or a pharmacy. In Canada B.D.H. may be contacted at 1.800.268.2129 or Toronto area (416) 255.8521 and Montreal area (514) 335.1621. The number

for Difco in Detroit is (313) 961.0800. or 1.800.521.0851 from anywhere in the USA except Michigan where the number is 1.800.344.8526. PML Microbiological (USA) also supply Nickerson's medium, their number is 1.800.547.0659.

Kits show positive cultures in as little as two days, as long as two weeks.

Discard, only after sterilisation,  
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after four of weeks incubation to rule out false negatives. If colonies are observed (they are usually chocolate brown, pale milk chocolate or even dark chocolate to black) the the entire kit, unopened, may be sent to a reference laboratory for confirmation. Mycology kits containing live cultures should be handled with appropriate precautions, preferably in a fume hood. Positive cultures should be confirmed as yeast because between 2% and 5% are false positives. Some positive cultures are not fungal at all, but are a species of oral bacteria observed as free floating cocci displaying Brownian movement. The species is yet to be identified.

Other kits that can be used for Mycology swabs are either Mycology kits, complete with the proper data sheet, or Bacteriology kits. The data sheet must be either amended to read MYCOLOGY or the proper data sheet substituted. Be sure to fill out the clerical details including the name of the patient and ANY MEDICATION the patient is taking, the name and return address of the doctor, the name of the doctor and the patient must BOTH be shown on the specimen bottle.

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Be sure to fill out data sheets and identify specimens properly. Send them to the appropriate reference laboratory, observing the proper protocol for transport of biologically hazardous material. NOTE that unmarked specimens arriving at the laboratory for either mycology or parasitology must be destroyed.

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Once the paperwork is complete, including putting identification on the ground glass section of the slide, if applicable, take a sterile swab from the sealed packet. Try to make sure that the packet is opened at the "handle" end of the package (i.e. don't grab hold of the cotton swab and thus contaminate it). The cotton end of the swab is then run around the gingival margins of all of the maxillary and mandibular teeth on both the buccal and lingual surfaces as one continuous sweeping movement. (Or rubbed over a specific area to be swabbed.)

If using a Mycology kit (an empty sterile container):  
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The wooden part of the swab just above the cotton end is then grasped with a pair of orthodontic or similar pliers and the wooden "handle" broken off. The shortened swab is then placed in the sterile mycology tube and the top fastened.

If using a Bacteriology kit (a transport medium in a small bottle):  
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The swab should be placed in the container of transport medium, taking care not to sink the swab into the medium as this can make the material difficult to recover at the lab.

If submitting a dried slide:

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A slide with a ground glass section, on which the names of both the doctor and the patient can be written, should be used. Probed material is spread out as a thin film on the slide with the instrument with which it was taken. Make sure the material is put on the same side of the slide that has the names on! Allow the slide to dry thoroughly before putting it in the cardboard protective sleeve. Secure it with the elastic band and place the slide, kit and form in the mailing tube.

Before submitting to the Lab:

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For all specimens other than cultures already growing in Nickerson's medium, put the prepared specimen, (in it's mailing tube) in the refrigerator for five to eight days. Then send the kit to the reference laboratory.

If using Nickerson's test kits:

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After swabbing the suspect area, gently wipe the swab over the surface of the agar and dispose of the swab. Replace the lid on the test tube. Be careful to avoid breaking the surface of the medium when wiping it with the swab. Do not leave the swab in the tube. Allow the tube to incubate at room temperature. Keep tube under observation daily. If colonies grow, do not open but submit to a reference laboratory. If you are well versed in handling pathogenic material a slide may be prepared for examination. To confirm the presence of yeast cells use tap water as a mounting medium for immediated phase contrast examination. Sub culturing may be done to identify the species of yeast using appropriate media. Remember that this tube contains a concentration of potentially pathogenic organisms. Handle with care!

At the end of a month, if nothing has grown, release the cap, keep tube supported in a tray so that the medium will not run out when it is heated, place in the autoclave and sterilise before discarding. (e.g. Two thirty minute cycles at 15 psi in a steam autoclave.)

Whenever possible use the services of the Parasitology Laboratory and the Mycology Laboratory to confirm your chairside findings. Initially this will help you to establish your accuracy of diagnosis: proficiency testing is a common practice with public health laboratories and 70% correlation should be the minimum expected. The backup of an accredited microbiology laboratory in the form of a written report can also have it's own intrinsic value.

Once diagnosis is established treatment may be commenced. For treatment to be successful it has been found it necessary to impart a good understanding of the nature of the disease. Patients who experience problems associated with release of antigenic material from dying organisms (Herxheimer's Reaction) should phone the office for advice.

Appropriate changes in medication can then be implemented before the situation gets out of hand. These concepts are dealt with in the next chapter and the rationale is explained in ensuing chapters.

>TM=1 BM=62 LM=5 RM=75

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER VIII -----

## CASE MANAGEMENT

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## PREAMBLE

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The rationale for the antibiotics used has already been discussed. The recommended dosages, together with the special formulations will be found in Chapter IX. Outlines for treatment of lumen dwelling protozoa may be found in any good text on Parasitology (e.g. Beaver, Jung and Cupp), Pharmacology (e.g. Grollman and Grollman) or Infectious Diseases (e.g. Hoeprich). The principles of therapy used for the routine treatment of lumen dwelling parasites, such as those found in the alimentary canal (of which the mouth forms a part) and the reproductive tract have been modified to better suit the oral environment.

## TREATMENT PRINCIPLES

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The basic principle is the elimination of a lumen dwelling parasite using systemic and topical antiprotozoal drugs including antibiotics. This is co-ordinated with appropriate dental care (usually deep scaling) which is timed to coincide with the appropriate response to chemotherapy. Patients must, at all times, maintain adequate levels of oral hygiene. The exact treatment regime for any particular patient must be tailored to their individual need. Therefore there is no specific protocol. Each case must be judged on its merits and the treatment protocol adapted to meet the patient response rather than simply repeat a course of treatment which is not working. Emphasis is placed on biological interference with the metabolism of plaque by antiseptic and other antimicrobial agents rather than by intense mechanical interference.

Complications are often seen and should be expected. They fall into three main categories:

### Group 1. Herxheimer's Reaction

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The host (patient) reacts adversely to the toxins, antigens, virus and viroid particle which are released into the body of the host on the death and disintegration of the parasite. While the parasite lives, it controls the rate of release of toxic substances in order to maintain its environment. Disruption of the metabolism of the parasite results in the uncontrolled release of the cell contents of the parasite. The degree of the reaction will be dependent on numbers of parasites, nature and quantity of the released material and the tolerance of the host to these foreign substance.

### Group 2. Superinfection

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With the elimination of the target organism, other co-infecting organisms that are not eliminated, expand into the ecological niche vacated by the offending parasite. This overgrowth of nonsusceptible organisms may require treatment (e.g. Candida). In some cases the overgrowth is self limiting and no additional treatment may be necessary (e.g. Black Hairy Tongue). The latter seems to be due to the overgrowth of pigment producing bacteria and may be exacerbated by ingested pigment or tobacco smoke.

### Group 3. Suppression of Normal Flora

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Suppression of normal bacteria in the digestive tract may result in gastro intestinal disturbance. Since bacteria originally entered the digestive system with food, the imbalance is usually self limiting and only temporarily maintained while the patient is taking antibiotics. When antibiotic therapy ceases, the imbalance frequently redresses naturally.

### MANAGEMENT OF COMPLICATIONS

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With the treatment of a parasite infection, there will inevitably be a release of toxins resulting from the disintegration of the parasite. This is frequently greater than the release of toxin that normally occurs during that stage of the natural life cycle of the parasite. These toxins must be eliminated from the system because they may make the patient feel ill. Consequently, the patient should drink extra water or unsweetened tea or coffee to help "flush" the toxins out of their system. Alcoholic beverages, milk, soft drinks and fruit juices are not helpful. Treatment should be tailored to minimize the rate of toxin release. This would include selection of the appropriate antibiotic and timing of therapy. In order to minimize the Herxheimer reaction, a less effective antibiotic regime should be used with more severe infections. This may necessitate increasing the duration of antimicrobial therapy. Such a regime will stress the patient less by minimizing peaks in the flood of antigenic material, to which the patient is exposed as a consequence of treatment. In general, a younger, more robust individual could tolerate a more severe stress from more massive or rapid release of toxic substance than could an older or more debilitated individual.

Reducing the antibiotic dosage to minimize the unwanted but unavoidable Herxheimer's Reaction is inadvisable, since the minimum inhibitory dosage may not be achieved and the end result may be the selective breeding of an antibiotic resistant organism.

The overall perceived severity of the infection, it's duration, the age and general health of the patient should all be taken into account when choosing appropriate therapy. Moreover, the clinician must also be sensitive to the patient's social history and the likely impact that a moderate to severe reaction might have on the individual. Careful assessment of the risk factors and discussions with the patient about the likely complications and their impact are essential to effective case management. Should the patient exceed their individual tolerance and suffer undue malaise as an unwanted side effect of treatment, the therapy should be stopped for a short period (3-5 days) in order to eliminate toxins. Therapy should then be re-instituted at the previous level. Under most circumstances, the first reaction (Herxheimer's reaction) on

death of the parasite, has been found to be more severe than subsequent reactions. This might indicate that initial therapy eliminates most of the organisms. Upon restarting therapy, the reservoir of infection is not great enough for there to be a release toxins that will exceed the threshold tolerance of the individual.

#### FREQUENT COMPLICATIONS

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Complications often encountered in Group One are headache, nausea and malaise. With maxillary infections, the treatment may result in feelings of irritability and unreasonableness. Transiently increased arthritic symptoms are sometimes noted and there may also be transient increase in the mobility of those teeth affected by the infection. Loss of appetite and altered sense of taste may also fall into this category. One of the more frightening side effects is a sensation of the heart pounding, particularly at night. Underlying health disorders may temporarily exacerbate during an antigenic flood. Side effects in Group One usually disappear before the completion of anti-amoebic therapy.

Complications in Group Two frequently include Black Hairy Tongue. This usually disappears during medication or shortly thereafter. Superinfections may also occur. These require diagnosis and appropriate attention. Alteration of therapy may be required. Superinfections with Candida species, especially *C.albicans*, fall into this category. *C.albicans* is a fungus which has both a yeast and a mould phase. If it is present in subgingival plaque, it may grow unmolested by the host immune response. *C.albicans* may pose a real threat due to the proximity of the gingival vascular bed, and the ability of the organism to invade through intact mucosa. Candida has been shown to produce a polysaccharide (MPPS) which stimulates suppressor cells of the immune system. Once thought of as an opportunistic organism that only infected persons with suppressed immunity, recent studies suggest that the converse is true and that the infection with *C.albicans* actually contributes to immunodepression (Piccolella, 1981; Rivas, 1983).

Group Three complications are usually related to disturbances of the gastro-intestinal (GI) tract and tend to be self limiting. Not all disturbances of the GI tract fall into this category; some disturbances may be due to superinfection with Candida species or intestinal parasites. The latter two categories require diagnosis and treatment.

#### MEDICAL CONSIDERATIONS

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For obvious reasons a good medical history is prerequisite to treatment. The information should contain the name of the patient's physician, who should be contacted as appropriate. This is of particular importance in the case of female patients who may develop vaginitis due to candidal overgrowth during antibiotic therapy. This would require treatment by the physician as well as temporary cessation of antibiotics. Female patients who are pregnant or nursing should not be on systemic medication unless prescribed by their attending physician. It is also wise to note that the elimination of infection, particularly with the use of antibiotics, has been known to enhance fertility. Patients should be so advised.



Clinical experience indicates that treatment is tolerated better and compliance improved if the patient is not subject to extremes of malaise resulting from parasite death and subsequent resultant shower of toxins, virus or viroid particle or other antigenic material. Placing patients who have not previously undergone therapy, or who have long standing infections, onto a "holding" programme for at least one month prior to starting systemic therapy greatly reduces adverse reactions.

#### STABILISING THE PLAQUE ECOSYSTEM

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In order to stabilise the plaque and inhibit proliferation of protozoa, many patients are initially put onto a pretreatment programme. This involves the use of simple antiseptics and an oral hygiene routine that avoids the use of commercial products, most of which contain sweeteners. Some of the ingredients in commercial toothpastes and mouthwashes, while seeming to temporarily suppress protozoa in plaque do not seem to be effective in arresting pathologic change. The suppression of the protozoa may make them almost impossible to find and result in false negatives. If the use of these products is discontinued for a few days, the protozoa rapidly reappear in plaque. This suggests that the protozoa might be retreating into the tissue and would explain why attempts to eliminate lumen dwelling protozoa solely with contact amoebacides frequently fails. While the luminal phase may be suppressed by contact amebacides, the tissue phase is not. As soon as therapy stops, there follows a prompt relapse as protozoa once again recolonize the lumen.

The key to tolerable therapy is initial suppression of the protozoa. Once initial control has been established by following the pretreatment instructions, patients usually experience some improvement in oral health. This reassuring experience will bolster confidence for the next stage of treatment, where side effects of antimicrobial therapy are to be expected.

Effective treatment is dependant upon good liaison between the patient and the office. Therefore, at all stages of care, especially when side effects are experienced, or questions arise regarding their care, patients should be encouraged to phone the office for advice and clarification. Positive reinforcement and reassurance by telephone contact can be an effective way of ensuring compliance. One member of the dental office staff, an empathetic listener who can accurately relay messages between patient and doctor, should be trained for liaison.

Informative patient instruction sheets, given to each patient at the time that a prescription is made, or a change in home care is recommended, also help patients to understand the aims and scope of treatment at home and in the office. Prior to treatment by the author, patients are advised to alter home care in order to stabilize the plaque for microbiological diagnosis. Ideally the pretreatment programme will be followed for one month prior to the plaque microbiology appointment. Within a few days of starting the new oral hygiene routine most patients experience an improvement in oral comfort. This pleasant experience bolsters confidence against future Herxheimer's Reaction. The pretreatment instruction sheet which patients receive in the author's practice is reproduced below:

#### PRETREATMENT PROGRAMME

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"Periodontal disease, gingivitis and "Pyorrhea", are three of the names commonly used to describe infections of the gum in the area where the tooth is rooted. A soft white film called plaque is found in this area. It is more abundant if there is infection. Plaque causes gum disease. There may be over 400 different types of "germ" in the plaque, but only a few actually cause disease. The purpose of a plaque examination is to identify target organisms: specifically two types of one celled animals (protozoa) as well as certain kinds of fungi (yeasts and moulds). You will see living germs, including bacteria, on the TV monitor attached to the microscope. Once a diagnosis is made, antibiotics and antiseptics can be prescribed to eliminate the target organisms so that natural healing can occur. To speed this healing, your own dentist will have to do some dental treatment (scaling, stain removal, etc.,) at the appropriate time. Initial control or elimination of the infection usually minimizes dental treatment and also makes it less painful and more successful.

In order to get an accurate diagnosis some changes in your oral hygiene will be required to avoid false negatives and also to control the progress of any infection until your appointment for a plaque examination. These changes in outline are:-

Throw out your old tooth brush because it is infected.

Your toothpaste, which probably contains the sweetener mannitol should also be thrown out. Mannitol is used to culture the bacteria that cause decay.

Brush once daily, using 1% peroxide on the brush instead of toothpaste. Peroxide is an antiseptic which kills some mouth germs.

Use Modified Torrens Powder once daily. It also kills some germs but it's main purpose is to stimulate the gums to better health.

Only use floss to remove food particles, such as meat or fibre, that you know is stuck between your teeth.

Do not use commercial mouthwashes because many of them contain chemicals which make plaque examinations more difficult and time consuming.

In the five days prior to your appointment for plaque examination it is ideal to have the plaque stable. That is, do not use anything for cleaning other than a toothbrush and water.

Try to avoid floss and any medication in the five days before your appointment. (HOWEVER YOU MUST CHECK WITH YOUR PHYSICIAN BEFORE ALTERING OR STOPPING PRESCRIPTION DRUGS.) You should not have been on any antibiotic for at least two and preferably six weeks prior to your appointment.

On the day of the appointment do not brush, floss, use any mouthwash, drink fruit juices, eat citrus or other fruit or fresh pineapple, salted nuts, sunflower seeds, suck "breath mints" or chew gum.

On the day of the appointment do rinse your mouth thoroughly with water, or any unsweetened beverage, after any meal or snack, so that there are no food particles in your mouth.

At the time of the appointment we want 12 to 18 hour old stable plaque, uncontaminated by residual food particles from your last meal. Some medications, particularly cold remedies and aspirin, etc, make our target organisms more difficult to find.

Recent dental treatment, especially within two, but upto six weeks, can also disturb the plaque and result in false negatives.

If difficulty is encountered in finding target organisms additional tests, or appointments may be necessary.

It will only take a few more moments to read the the next page which details your new oral hygiene routine. You will probably find it simpler and less time consuming than your present technique. You should find that it works as well, or better, than anything else you have tried. MODIFIED TORRENS POWDER (MTP). (For Tissue Conditioning).

The formula is one part salt plus six parts baking soda, (mix for 5 minutes in a blender to make a fine powder.) Put about a teaspoonful of the powder into an egg cup, or similar. Pat the powder onto all the gum margins using a saliva wetted finger. Spit out all the excess. Try not to eat, drink or rinse for the next hour. For those on a low sodium diet use the preventive paste made with Epsom Salts instead of MTP. Use MTP in the morning.

For a sore or painful mouth or gums, a useful mouthwash is 3 teaspoons full of powder in 4 to 6 ounces of hot water. Rinse gently and keep it in your mouth while it is hot. When it cools, spit out and take another hot mouthful, etc. Do this as often as brings relief. An alternative is unsweetened tea or coffee. All act as a hot poultice, but the MTP rinse works best. Second best is hot, strong, clear tea.

PREVENTIVE MOUTH RINSE: (Anti-plaque Anti-septic)

1% Hydrogen Peroxide is made by diluting 3% Peroxide: 1 part peroxide with 2 parts water makes a 1% solution when fresh. Use about three teaspoonsful to rinse for three minutes. \*NOTE\* Hydrogen peroxide "goes off" slowly after the bottle has been opened. Buy small bottles. Keep the main supply refrigerated. Keep a smaller bottle of 1% in the bathroom. The shelf life, once opened, is so short that when the bottle is half gone it may not need to be diluted as much as at first. It may even be down to 1% by the end of the month. Judge the strength by the fizz! Use at night after brushing.

PREVENTIVE PASTE: a taste alternative to MTP.

Mix a few drops of 3% hydrogen peroxide with about a teaspoonful of MTP to make a stiff paste. Apply the paste to the gum margins for tissue conditioning. Use your toothbrush, but don't "brush". Spit out the excess.

BRUSHING: Teeth and Gums.... I prefer the Bass Brush Technique.

Dip the toothbrush into 1% peroxide and brush the area covered by the brush, redip the brush and brush the next section and so on. After brushing, rinse thoroughly with water, then rinse with the peroxide.

Brush last thing at night; Modified Torrens Powder, alone or with hydrogen peroxide, should be used in the morning.

GENERAL NOTES including a quick summary of oral hygiene:

After the use of the peroxide or the powder, you should try not to eat, drink or rinse for the next hour. During the day rinse with water after all meals and snacks to remove food debris; use floss if necessary to remove food, but be careful not to hurt the gum. Don't saw with the floss. Remember that food debris encourages growth of bacteria which cause tooth decay and inflammation of the gums.

Change your toothbrush every week because it becomes infected with the germs from your plaque within two weeks. Continue this until the target germs have been eliminated by treatment. Use a Bass type of brush, for example, the Butler SUB-G (Dr Bass Right Kind.) It is soft because the brush should wear out so that you don't! Do not use the rubber tip.

Use MTP in the morning and spit out the excess but do not rinse the residue away. However, after food or beverages always rinse (and swallow) with water to remove food residue. Brush once daily, with peroxide, before you go to bed. After brushing rinse out the foam with water then rinse with 1% peroxide. Spit out the excess but do not rinse the residue away.

By following the preceding regime you will not only start yourself on the road to recovery but also minimize the time needed for your first appointment. Finally, if you have any questions, please phone."

<-----End-of-Patient-Instructions----->

After diagnosis, those patients who should be on a holding programme are instructed relative to the appropriate medications and home care. The Holding Programme comprises the use of topical antiprotozoal agents together with the continued use of Modified Torren's Powder, plus brushing once daily with 1% hydrogen peroxide. The Torren's Powder soaks up exudate from the pocket. This fluid must then be replaced. The fluid re-entering the pocket will be drawn from the surrounding gingival tissue and contain a greater concentration of antibodies with a lower concentration of the byproducts of microbial life. Provided that the patient has not just brushed, the use of the powder also helps to reduce gingival oedema. Since brushing frequently causes microscopic scratches and abrasions on the tissue, brushing the tissues with the powder or the powder/peroxide mixture is inadvisable. Likewise the use of the powder right after brushing is discouraged since salt/soda getting into the wound can cause irritation and oedema.

In over ten years of clinical experience, the modification of the powder originally described by Torren in the British Dental Journal nearly half a century ago remains an excellent simple remedy, providing it is used as described. Brushing with this or similar mixtures can cause a lot of irritation and discredit an effective technique through sloppy mismanagement of Torren's original brilliant concept.

THE HOLDING PROGRAMME

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(See Chapter IX for a full description of all pharmaceutical preparations and regimes referred to in this section)

In conjunction with these simple changes in oral hygiene and antiseptics, the patient uses MA paste, four to eight times daily, for up to two months or the tetracycline rinse, also four to eight times daily, for one month. The rinse is sometimes preferable when the pockets are deep, since a fluid should penetrate more effectively than a paste. A "water pik" device could also be used for delivery of the rinse to the affected area, provided that the pressure is not excessive and that the rinse is further diluted 9:1. Both the rinse and the paste are very bitter. An advantage of the paste is that it is applied with a toothbrush and helps prevent the toothbrush from acting as a vector for infection. A disadvantage of the rinse is that staining of the teeth occurs, black hairy tongue is frequent and the use of 40% ethanol gives rise to a burning sensation of the oral soft tissues. However, unlike the paste, which is most concentrated on the gingivae, the rinse will affect almost all tissue in the mouth.

#### ACTIVE TREATMENT

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After successful completion of the holding programme, a re-evaluation of the patient allows the selection of the next phase of treatment. If the evidence now suggests a light or well controlled infection, a highly effective antiamebic could be selected, provided that the patient is aware of the increased likelihood of more severe malaise experienced with these drugs. Minocin (which is expensive) or Metronidazole in Regime #1, #3 or #4 might be selected depending on such factors as the age and health of the patient, sociological considerations regarding alcohol consumption and the experience of the prescribing doctor.

A more severe periodontal infection generally requires more protracted treatment. Tetracycline, a weakly antiamebic antibiotic might be selected, particularly if the patient has a history of any arthritic change, other systemic disturbance or metabolic disorder. These indicate that treatment is likely to be protracted due to potential drug interactions or antagonisms.

An alternative antibiotic of intermediate effectiveness between the last two groups is Penicillin V. However clinical experience has shown that it must be used for a minimum of 30 days to eliminate amoebae. A course of therapy lasting 35 days is not unusual.

NOTE: The use of the Tetracycline mouth rinse, in the Holding Programme, for four weeks prior to starting systemic therapy reduces side effects and shortens treatment time. The rinse can also be used in conjunction with systemic tetracycline in recalcitrant cases.

Do not just prescribe:

- .....First diagnose;
- .....Second monitor;
- .....Third adjust dosage and duration;
- .....Fourth monitor and etc.
- .....Finally.....Expect complications and reinfections.

#### RESISTANT CASES

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Do not prolong treatment with Metronidazole, (see your pharmacopeia) but rotate with a series of antibiotics, including Penicillin V, Tetracycline, Minocin and the Erythromycins or even anti-protozoals such as Atabrine. Metronidazole can be used in conjunction with some antibiotics in order to potentiate the therapeutic effect but side effects will be maximized. Increased dosage, above that usually recommended for deep seated infections is inadvisable. DON'T even contemplate Emetine hydrochloride.

Cases that do not respond satisfactorily may be due to misapplication of the programme by the patient, selection of an inappropriate antibiotic or the selection of an insufficient dosage. Other factors which may impede response include underlying medical disorders, multiple infections, focii of oral irritation or stagnation which may require remedial action such as the removal of calculus deposits or the elimination of overhanging margins. Ineffective therapeutic response may also be due to drug interaction or antagonism, for example, tetracycline may become bound to heavy metals in the digestive tract and thus not absorbed in therapeutic quantities. Constant re-infection from exogeneous sources must be considered as must reinfection from close personal contact or even infection from close proximity to an infected person in a small enclosed space (e.g. an automobile). Poor air circulation which occurs with some "sealed" buildings may result in the air in the building becoming stale and an increasing load of environmental pollutants as well as pathogenic micro-organisms may produce an environment which is not conducive to successful therapy. Undue resistance in therapy may be due to multiple infections, in the mouth or elsewhere, particularly infections with both E.gingivalis and T.tenax or either parasite and C.albicans, or all three together. Refractory cases may also be due to antagonism with other medications, inadequate absorption or concurrent non oral infections with Candida, intestinal parasites or other systemic condition which may have to be treated first. The aware clinician may often find it prudent to confer with medical colleagues.

#### SPECIAL CONSIDERATIONS FOR ROUTINE DENTAL CARE

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Although the concept of Oral Amoebiasis is an alternative approach to the aetiology of some periodontal infections, the treatment of Oral Amoebiasis must be supplemented by traditional dental care. Oral Amoebiasis and "usual and customary" dental care are not alternatives to each other. It has been found that the best results are obtained if the clinician and the patient pay careful attention to detail and maintain open lines of communication for effective team work. Frequently one finds that most "in-office" treatment can be delayed until the infection is controlled or eliminated. The case can then be re-assessed and treated accordingly.

In some cases it may be found that deep pockets (greater than 5mm) do not respond adequately because of inadequate penetration of the topical antiamoebic paste. Daily subgingival application of the treatment paste into such areas should be considered. Some patients can be taught to gently fill easily accessible pockets with paste, using a 10cc syringe with a blunted one and a half inch 18 gauge needle. If the patient is unable to perform this task, daily visits to the office may be required. In conjunction with systemic antibiotic therapy such an effort may be rewarded by such rapid progress that the needle can no longer be inserted

in as little as three weeks. This technique should also be considered for unresponsive bifurcation and trifurcation involvements.

For those patients with dentures or orthodontic appliances, care must be taken to prevent the appliance from vectoring the disease. Once daily the appliance should be "sanitized". Betadene has been found to be an effective surface disinfectant for smooth surfaces, (Best et al, 1988) with the additional advantage that it is generally non irritant to mucous membrane (CPS, 1988) and does not stain. Soaking in betadene solution at full strength for one hour should be followed by rinsing the solution from the appliance and use of a commercial denture cleaner. Before reinserting the appliance in the mouth a little of the prescription paste should be applied to the fit surface, thus utilizing the appliance to prolong the contact time of the paste with the tissues. The appliance should also be removed from the mouth and both should be cleaned of all debris after meals, then a little paste should be applied to the fit surface before reinserting it.

Patients should be made aware of possible sources of reinfection, that is, any object, cutlery, crockery or food that may have become infected from the mouth of another person. Shared food or beverages, chip dips and other communal sources of food into which licked food or fingers might have been placed and unhygienic practices engaged in by food servers or preparers all present potential for reinfection. Droplet infection from coughs and sneezes should not be dismissed as unrealistic, particularly if the potential "victim" has just eaten something sweet. The irritation from the byproducts of bacterial metabolism or the microscopic scratches and abrasions from recent brushing may create a suitable environment for infection by protozoa should an amoeba be introduced to such an environment. Auto reinfection from toothbrushes, bathroom cups, cosmetics, musical instruments, pens and anything else that goes in the mouth must also be considered as possible vectors. Household pets which may carry the infection, particularly older dogs (*E.gingivalis*) or cats (*T.tenax*) would also be suspect and may have to be treated by a veterinarian.

Patients often report that when the infection has been eliminated and the tissues have healed, periodontal procedures are less painful and rarely require anaesthesia. The most appropriate time to initiate periodontal therapy is when no further clinical improvement is observed after elimination of infection. Now the remaining calculus hinders progress and should be removed. Clinical observation suggests that once the infection has been eliminated and the tissue response stabilised, there seems to be less subgingival calculus, which is easier to remove than first anticipated. By eliminating the infection prior to scaling, both patient and operator are at lower risk to the spread of infection.

During active phases of treatment, the patient should use the appropriate paste, (MA, MK or MC). Peroxide and MTP remain as the cornerstones of the oral hygiene programme. Use of the paste, the powder and the peroxide should continue until three to six months after treatment is complete, in order to facilitate healing during the convalescent phase. After completion of treatment, the patient will change to the preventive programme, which basically means the continued use of effective non prescription oral antiseptics. At the time of writing, the mainstay of the preventive programme is Modified Torren's Powder and 1% hydrogen peroxide. An alternative to the latter is a perborate based dentifrice, the formulation for which has been modernized from an old dental

compendium of pharmaceuticals (Dilling and Hallam, 1954). From an initial pilot survey, brushing with "Viadent" paste followed by a water rinse and then the "Viadent" rinse, twice a day, looks promising.

Some patients will need to use a proxabrush. The best results are obtained with the #612 head, which is like a tiny bottle brush, rather than a Christmas tree. The patient who needs a proxabrush should be instructed to use it to loosen interdental plaque, so that it may be rinsed away, then to use the proxabrush to apply treatment paste to the interdental areas which have just been cleaned. Floss is not to be taken subgingivally rather it is used for the removal of impacted food particles, or for loosening plaque under a bridge pontic. Rinsing will then remove the dislodged debris. Treatment paste should be applied to all accessible areas of the mouth after plaque removal. The more intense use of treatment paste (MA or MK) by the patient, immediately before and after appointments where there may be some tissue injury, such as scaling, also helps tissue response by reducing the chances of postoperative infection and soreness. Likewise, hand scalers may be dipped in the paste frequently and the areas from which calculus has been removed may be additionally dressed with a small quantity of the paste at the end of the scaling, curettage or rootplaning session.

The main principle is to interfere with the ecology and maturation of the plaque CHEMICALLY not mechanically. Each individual patient will ultimately develop their own successful variation on the theme under the guidance of each particular practitioner.

.....There are no hard and fast rules.....

.....just the application of general principles.

Following successful therapy to eliminate the protozoa, patients often report a series of improvements in both their oral and general health. These reports vary widely and seem to be related to the pretreatment status of the patient. Feeling of a cleaner mouth, loss of halitosis, absence of bad taste, especially on rising, absence of gingival bleeding, feeling that the teeth are firmer or stronger, absence of hot, cold and touch sensitivity, ability to eat comfortably and having a moister mouth are some of the oral improvements reported. General health improvements often reported are fewer headaches and reduced malaise and fatigue once the protozoa have been eliminated. Although there have been some reports of improved arthritic symptoms, the association is not clear at this time. However, the general feeling of "wellness" (which most patients report) is often first noted as a "wide awake" feeling immediately after elimination of *E.gingivalis*. Sometimes this feeling is so intense that patients have difficulty sleeping for the first night after elimination of the infection. Thereafter, normal sleep patterns return and patients report that they feel more energetic. Some patients find that they require less rest following the elimination of a long standing chronic infection. For patients with underlying metabolic disorders, such as diabetes, the disorder is often found to stabilise following elimination of the oral infection enabling them to enjoy a more active lifestyle.

As a footnote to this chapter it should be emphasised that the use of antimicrobial agents must be supplemented by effective oral hygiene as well as thorough dental care. Failure of the patient to comply with the antibiotic or antiseptic regimes, failures in oral hygiene routine, failure to remove all sub and supra gingival calculus at the appropriate



time, failure to treat adequately, or at all, open carious lesions, ignoring faulty restoration or crown margins, the presence of ill fitting or unhygienic dentures and the failure to correct dietary factors, or any other factors which promote rapid proliferation of plaque, will all lead to a less than satisfactory result.

The purpose of this book is not to provide a universal panacea which makes other forms of dental care redundant, rather it is to provide information on organisms whose presence can jeopardise the success of regular dental care. Once infection has been eliminated, primary non surgical periodontal care completed and sufficient time has elapsed to permit healing, each case can be re-evaluated. In the absence of aggressive osteolytic periodontal disease, the patient with a healthy mouth has more treatment options available. Such a patient may choose orthodontic repositioning of previously loose teeth, or may elect crown and bridge as a more viable alternative to extractions and full dentures. The clinician with a "maturing" practice will find that dental practice is no longer an uphill battle against ever widening odds.

>TM=1 BM=62 LM=5 RM=75

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER IX -----

>C=Y J=N

PHARMACEUTICALS & ANTI PROTOZOAL REGIMES

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>C=N J=Y

Principles of Therapy

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The therapeutic principal employed, is the concurrent use of appropriate topical and systemic antimicrobials. For maximum therapeutic effect, this must be supplemented by the use of Modified Torren's Powder and 1% hydrogen peroxide in the oral hygiene programme. The timing of all phases of dental care is determined by clinical and microbiological assessments. Once the infection has been controlled, dental treatment can proceed. The aims of such an approach are the best possible therapeutic response, coupled with less pain for the patient, greater ease for the dentist and less risk of infection for both the patient and the dental team. The purpose of identifying and eliminating target organisms with antimicrobial therapy is to produce an ideal operative environment and increase the chances of operative success for those procedures which are hindered by the presence of blood.

Periodic testing ensures that antibiotic therapy may be precisely tailored to the presence of target micro-organisms. Once these have been eliminated antimicrobial therapy may be immediately discontinued. This avoids under or over utilization of antibiotics. Superinfection with nonsusceptible micro organisms can also be detected, allowing prompt changes in antibiotic regimes before major complications ensue. After successful completion of therapy, routine retesting can identify reinfection before significant tissue changes occur.

The following antibiotics and anti protozoal medications have been of value in treating Oral Amoebiasis. The formulations for the special pharmaceutical pastes were developed in conjunction with local pharmacists to ensure appropriate strength, proper consistency, safety of active and adjuvant agents and quantity to be dispensed.

Pharmaceutical reference sources were consulted regarding possible drug interactions, antagonisms and synergisms. Specific brand names are sometimes mentioned, if clinical results were consistently good and/or if unwanted side effects were minimal. When side effects were consistent with a specific brand, the most likely explanation is a reaction to one of the adjuvants, fillers or flavours.

Within the context of obtaining a medical history for each patient, and updating it at the time that any prescription is made, it must be emphasized that certain antibiotics, including tetracyclines, metronidazole and ketoconazole, are inadvisable for pregnant or nursing women. It is also suggested that the MK paste not be used by pregnant or nursing mothers. Women of child bearing age should therefore be careful to use adequate contraception whilst taking these medications.

My collaboration with researchers at the University of Muenster, West Germany, who have been investigating the oral protozoa, suggests that the minimum period of antibiotic therapy needed to eliminate *E.gingivalis* in mixed infections is 35 days. At Muenster, in vitro cultures of *E.gingivalis* were started from periodontally diseased sites. In order to obtain a pure culture, various antibiotics were used to suppress the bacteria. No matter which antibiotic was used, elimination of bacteria resulted in death of the protozoa in culture. Since the potentially pathogenic bacteria cultured with the amoebae were eliminated before all the amoebae died, the presence of amoebae remains a useful guideline relative to the destructive potential of periodontal lesions and indicates whether an environment conducive to pathosis still persists.

Clayton et al (1954) found that the minimum concentration (MIC) of Penicillin necessary to achieve bacteriostasis in mixed cultures of *E.gingivalis* and bacteria from dental plaque also prevented the amoebae from multiplying. At lower concentrations the amoebae flourished as did the controls which contained no penicillin. It took seven and a half times the MIC of penicillin in vitro to actually cause death of the amoebae. These observations suggest that penicillin, or any antibiotic, should be effective in the treatment of Oral Amoebiasis providing that the dosage and duration of therapy are appropriate to maintain less than ideal in vivo conditions.

This theory has been borne out in daily clinical practice. Repeated microscopical examinations of plaque has shown that infection with *E.gingivalis* is eliminated after 25 to 35 days of combined therapy with Penicillin V and topical amoebicides. This observation was also valid for other antibiotics, including some which are not generally considered effective in periodontal infections. Elimination of protozoa coincided with resolution of the disease. However, not all cases responded uniformly. Some cases resolved more rapidly, while others were more resistant and responded slowly. In those cases where the infection with amoebae persisted, motile bacteria remained in the plaque in association with the amoebae. Clayton (1954) also observed this phenomenon with *E.gingivalis* in vitro. He noted that in vitro conditions required by *E.gingivalis* were like those required by the pathogen *E.histolytica*; namely, the amoebae required the presence of other living cells in order to survive.

The combination of in vitro experiments and clinical experience provides a rational explanation for the observations that:

As the amoeba go,

.....So goes periodontal destruction.

The application of these principles helps explain why the current usage of antibiotics, such as Tetracycline, as an adjunct to conventional periodontal therapy, is so effective. The use of the microscope simply helps determine the choice and duration of antibiotic therapy for each patient. Microscopic examination of plaque helps prevent over or underuse of antibiotics, helps prevent persevering with an inappropriate antibiotic and allows more advantageous timing of standard dental treatment. Elimination of infection prior to surgical periodontal procedures ensures success and also minimizes patient discomfort. The latter is a factor which should not be underestimated as being of practical importance.

SYSTEMIC AMOEBACIDAL ANTIBIOTICS  
-----

TETRACYCLINE: Weakly amoebacidal antibiotic.

Peak side effects normally between 8th to 15th days

Sample Prescription

Rx.Tetracin 250mg Caps

Mitte qs 35/7

Sig ii qid

Rpt x1 prn

General Comments

The usual dose employed until the protozoa have been eliminated is (Pfizer "Tetracin") 250 mg caps. Two caps twice daily. This is used in conjunction with MA Paste, Modified Torrens Powder and Hydrogen Peroxide.

Warnings

Usual warnings to the patient include increased skin sensitivity to direct sunlight and avoidance of "heavy metals" at the same time as the tetracycline is taken. In particular, polyvalent cations, such as aluminium, calcium, magnesium and iron, bind with tetracyclines in equal molecular ratio, thereby preventing absorption of the antibiotic. Tetracyclines should not be taken at the same time as products or foods which are likely to hinder absorption. Included in this list would be milk and dairy products, antacids and some vitamin/mineral preparations. Therefore, tetracyclines should be taken on an empty stomach, i.e. one hour before or two hours after food. As with all antibiotics used to eliminate an infection, there is a probability of increased fertility or decreased effectiveness of contraceptive measures. Prolonged antibiotic therapy increases the chance of superinfection, particularly with the yeast, *Candida albicans*. Female patients are especially at risk. Frequent side effects encountered with amoebacidal therapy include headache, nausea, irritability, altered sense of taste, (metallic taste), gastro intestinal (GI) disturbance, malaise, exacerbation of arthritic symptoms and "general aches and pains", loss of spatial orientation including vertigo and difficulty with depth perception.

Follow up

After the infection has been eliminated, if the tissue is not yet fully recovered, particularly with reference to bone regeneration, continue the prescription at half dosage (i.e. one cap twice daily) until healing is complete. Frequently it is necessary for the duration of this second prescription to be double the duration of the first. The continued antibiotic coverage helps prevent reinfection during the healing (or convalescent) phase of therapy. This is modelled on one form of treatment for *E.histolytica*, the cause of amoebic dysentery. The patient should be periodically re-examined both clinically and microbiologically in order to determine the next phase of treatment.

MINOCIN: Potent amoebacidal antibiotic.

Side effects normally start about the 2nd day and peak by the 4th or 5th day. After the tenth day the side effects may diminish slightly but then stay more or less constant for the duration of therapy.

#### Sample Prescription

Rx.Minocin 200 mg Tabs

Sig. ii Stat

followed by i bid

Mitte qs 14/7

No Repeats

#### General Comments

This antibiotic in clinical practice is as effective, or more effective than Metronidazole. A good drug to keep in reserve when all else fails. Side effects encountered with Minocin may include nausea, vomiting and extreme malaise unless the infection has been well controlled prior to therapy with other less potent antibiotics.

Loading dose 100mg tabs x2, followed by one tablet twice daily for two weeks.

#### Warnings

No dairy products should be taken with the tablets which are preferably taken on an empty stomach, but can be taken with food. The side effects with this antibiotic (tetracycline family) are comparable to Metronidazole used at full dosage. It seems to be very effective against the protozoa, but is very expensive and makes patients feel really ill.

#### Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.

ERYTHROMYCIN: Amoebacidal antibiotic.

#### Sample Prescription

Rx. PCE

333 mg tabs

Sig i tid

Mitte qs 10/7

Repeat, number of times: x2 prn

#### General Comments

PCE is claimed to reduce the severity of GI disturbance, but with most erythromycins expect moderate to severe GI upset. Take with or without food.

Erythromycin tablets or capsules: for all erythromycins the daily dosage is 1000 mg, sometimes taken as one dose, but usually taken as three or four divided doses over at least ten days, or until infection has been eliminated.

Erythromycin base should be taken one hour before food, unless gastro intestinal upset occurs, then take with food.

Both stearate and estolate are hepatotoxic. The stearate is used primarily in dermatology. Neither in common usage.

Erythromycin (estolate): 250 (or even 500) mg qid 10/7. Expect moderate to severe GI upset. Take with or without food.

Erythromycin Ethyl Succinate (EES) 600 mg tid. Well tolerated. Take with food. Also hepatotoxic.

ERYC: encapsulated, enteric coated pellets of erythromycin. 250mg take one hour before meals, qid. A version of the base. (Made by Parke Davis).

PCE 333mg tabs. Polymer coated erythromycin base particles. Very expensive. (Abbott's reply to ERYC.)

Because of the severity of the GI disturbances encountered with this antibiotic, little experience has been gained. Some clinicians favour the drug, but patients do not appreciate it, because of the severity of the GI disturbances.

Erythromycins appear to be highly effective against the oral protozoa, particularly the soluble variant if used for thirty or more days. This form of the antibiotic is available in Germany, it comes as individual packets of powder, each containing 1000 mg. The contents are dissolved in water and swished around the mouth before swallowing. It is used twice daily. North American availability is unknown.

#### Warnings

The warnings for all antibiotics are basically the same since side effects seem to be more related to the nature of the infection than the nature of the treatment.

#### Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.

ROVAMYCIN 500mg: Amoebacidal antibiotic.

#### Sample Prescription

Rx. 500 mg caps

Sig ii qid

Mitte qs 5/7

#### General Comments

Can cause very severe diarrhoea. Two caps four times daily for three to five days or until two days after symptoms cease. This antibiotic is expensive and seems to be no more effective than tetracyclines. To eliminate protozoa it must be used for a comparable period. In spite of limited experience, it seems to hold promise as a useful short term drug when rotating from one antibiotic to another, in stubborn cases.

#### Warnings

The same general warning as applicable to all amoebacidal antibiotics.

#### Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.

PENICILLIN V: Amoebacidal antibiotic.

Peak side effects normally 10th - 20th days.

#### Sample Prescription

Rx. Pen V 300 mg tabs

Mitte qs 35/7

Sig ii qid

#### General Comments

300 mg = 500,000 IU. Two tabs four times daily for thirty five days. For patients who are not allergic to penicillin this antibiotic is safe, inexpensive, effective and consumption of food and beverages is not critical at the dosage and duration employed. For Oral Amoebiasis, increase duration up to one month, or longer, if necessary.

#### Special Note

Low doses of Penicillin, for example, half the minimum inhibitory concentration required for *Streptococcus mutans*, can have unwanted effects. At this dosage the ability of *S. mutans* to bind to saliva coated hydroxy apatite is enhanced, although the ability of the organism to bind to other tissue may be unimpaired or even reduced. (Crawford and Russell, 1988)

#### Warnings

As for other amoebacidal antibiotics, but the severity of side effects may be less since it would not appear to be a potent amoebacide.

#### Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.

METRONIDAZOLE 250 mg tabs. Amoebacidal and effective in anaerobic infections. Not a true antibiotic but rather an antimetabolite.

Peak side effects normally 3rd - 8th days.

Sample Prescription

Rx. Metronidazole 250 mg tabs

Mitte 30 tabs

Sig i bid

Do not repeat

Warnings

WARNING: do not consume alcohol while taking this medication, nor for 24 hours before nor 48 hours after.

General Comments ..... Regime #1.

Peak side effects normally 3rd to 5th days.

The original dosage was 30 tabs over 10 days with the first day's dosage split over two days: 1st day one tablet. 2nd day one tablet twice. 3rd and each subsequent day: one tablet three times a day. Should be taken with food but can be taken on an empty stomach.

General Comments ..... Regime #3.

Peak side effects normally 5th - 10th days.

30 tabs: one twice daily. Since this dosage does not encompass 30-35 days it should be followed by a second course of anti protozoals as deemed appropriate, unless testing demonstrates that this minimum dosage has been successful. Regime #3 is sometimes all that is needed for very light and recent infections in young adults.

General Comments ..... Regime #4:

Two consecutive Regime #3. Side effects only occur once, timing as above.

General Comments --- Regime #2

This was a gynaecological regime. It did not translate into an effective regime for oral infections. The dosage, one gram at bedtime for one, two or three successive nights, duration dependent on severity of infection, was not found to be effective in most cases.

Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.



## ANTIFUNGAL AGENTS

-----

NYSTATIN Tablets: relatively insoluble antifungal.

Peak side effects in first five days or after six weeks, depending on sites of infection.

### Sample Prescription

Rx. Nilstat tabs

Mitte qs 3/12

Sig ii bid

Repeat once

### General Comments

Nystatin is almost insoluble, only about 2% being absorbed systemically. For this reason it has been advocated for treatment of candidal infections of the gastro-intestinal tract since it is NOT well absorbed. Nilstat brand. Each pink tablet contains 500,000 IU active nystatin. Dosage is two tabs twice daily. For patients unable to tolerate this, a graded dosage based on the powder usage (see later section, this chapter) is as follows:

First month, one tablet daily. If this is well tolerated,

Second month, one tablet twice daily.

Third month, one tablet thrice daily.

Fourth month, one tablet four times daily.

Fifth month, and all succeeding months, two tablets twice daily.

For each month that the tablets are taken, the patient must have had no difficulty tolerating the treatment before the dosage is increased. When dosage is well tolerated dosage is increased by one tablet for the next month, until full dosage has been reached. Re-examination is then scheduled after the end of the month in which the dosage is changed to two tabs twice daily.

### Warnings

Side effects are not normally encountered until about six weeks into medication at full dosage or at the 180 tablet level from onset of medication with the graded dosage.

Because of the complexities of signs and symptoms for patients with mycotic (fungal) infections, it may be frequently necessary to consult with the patient's physician in order to co-ordinate treatment and ensure that medical care and dental care are provided as appropriate.

Nystatin may cause gastro-intestinal disturbance if there is significant gastro-intestinal infection. Since Nystatin causes increased permeability of the fungal cell wall, the leakage of cellular contents from *Candida* into the gastro-intestinal tract may cause irritation.

For other tissue use Ketoconazole, since it is well absorbed, but may be hepatotoxic. Blood testing is advisable if it is used for a protracted period, or in conjunction with Metronidazole, other hepatotoxic drug or if liver function is impaired. These parameters suggest consultation with the patient's physician.

The two most frequently encountered side effects in relation to the elimination of *C. albicans* with nystatin are thought to be due to the release of cellular contents. If *Candida* is in the gastro-intestinal tract, low abdominal pain may be encountered within an hour or two of taking the tablets. This indicates release of irritants comparable to the tissue response seen on first acute infections with this organism (Thrush). The pain may be accompanied by loose bowel movements which may be so severe as to cause urgent diarrhoea or even fresh blood in the stool. The best response is immediately stop the pills, allow the condition to settle, then restart therapy with the graded dosage. It may be advisable to consult a physician familiar with the latest concepts in the treatment of candidal infections. The patient should always be advised of possible complications at the outset.

If marked GI disturbance occurs, it may be accompanied by fatigue and/or depression. The latter may be severe and quite frightening for the patient. If gastro-intestinal disturbance is not an initial complication then fatigue, lethargy and depression may appear at about the sixth week or 180 tablets into medication with the graded regime. This latter complication may be due to the release of cellular contents from dying *Candida* colonies affecting brain cell metabolism. The mechanism could be an alteration in the balance between phenylalanine and serotonin. No matter what the explanation, in practice, the following typical history has been observed. Waves of fatigue and/or depression sweep over the individual which seem unrelated to social or factors other than the prescription for Nystatin. The first wave is the worst and may last about a day, followed by a return to normal then a second wave of slightly less severity a day or two later. These waves become less severe and further apart as treatment progresses.

There have been reports of patients with severe infections with *Candida* becoming so depressed that suicide is attempted. Experience indicates that this could be a real risk. Patients should be warned that infection with *Candida* has been associated with suicidal tendencies. For many patients it comes as a relief to know that a fungal infection can have such devastating effects. A significant number of people with *Candida*, when apprised of this information, will admit to having contemplated suicide. Knowing there may be a reason for feeling rotten will give many persons the strength to persevere. Over the last ten years two patients did attempt suicide. Both had oral infections with *C. albicans* that could only be diagnosed by culture. In neither case was the organism found on direct examination, although one patient had been diagnosed by direct examination about a year prior, the infection had "spontaneously resolved" and culturing was done to confirm the absence of infection. In both cases the attempt was made while awaiting the lab result. One of the two attempts was successful.

Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.

NYSTATIN Lozenges: antifungal.

#### General Comments

Nystatin powder in polyethylene glycol base with bitter orange flavour. 200,000 IU per lozenge. 24 per package. Each lozenge scored for half dose application. Sig: half a lozenge upto 8x daily to supplement MC paste. The pharmacist prepares the lozenges in trays. Clotrimazole or Miconazole can be substituted for Nystatin with slight cost increase.

Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.

KETOCONAZOLE: highly soluble and well absorbed anti fungal.

#### Sample Prescription

Rx. Ketoconazole 200 mg tabs

Mitte qs 1/12

Sig. i daily with evening meal.

#### General Comments

Dosage varies from one daily to two four times daily, although the latter dose would hardly be applicable in dental infections. The drug is hepatotoxic. If therapy is contemplated for more than a month, or if a repeat course of treatment is envisaged, the patient should be monitored serologically as well as microbiologically. The dentist should consider working with the physician in such cases. The main use for this drug is in the final stages of treatment to clear the last vestiges of Candida in the final weeks of treatment. Preferred dosage is minimum and short duration (check a current pharmacopoeia for more detail) unless the patient is being closely monitored by a physician. Nystatin tablets remain the treatment of choice as adjuncts to the MK paste.

#### Warnings

The most common side effect noted with the use of Ketoconazole is fluid retention. In addition, many patients experience a sense of woolly headedness, as if they had had a little too much to drink. (Ethyl alcohol, it must be remembered is produced by yeast fermentation and is a yeast toxin. Other fungi also produce hallucogenic compounds, viz "magic mushrooms".) This latter side effect may also be noted with other antifungal drugs. With severe oral infections even the use of the paste may have unexpectedly severe repercussions. Treatment should always be tailored to the tolerance of the patient.

Because it is so effective, use Ketoconazole with discretion once the full extent of the infection has been determined. Extreme reactions to

fungal toxins, from the indiscriminate use of this drug, could be life threatening.

Follow up

Re-examine patient clinically and microbiologically after completion of therapy to determine next stage of therapy.

NYSTATIN POWDER made by Cyanamid: (1/8 tspn = 500,000 units):

General Comments

The dosage recommended by physicians who use this form of nystatin are:

a pinhead four times daily (qid) for one month (1/12),

then 1/8 tspn qid 1/12;

then 1/4 tspn qid 1/12;

then 3/8 tspn qid 1/12;

then 1/2 tspn qid 1/12;

then decrease at same rate.

tspn = teaspoon. Medical colleagues, who employ this form of therapy, prescribe the powder in bulk, to be measured out by the patient, suspended in water and drunk. Alternatively a pharmacist may make up capsules of predetermined strength for the patient to swallow if tablets are contra indicated. Patients who need this form of therapy will usually already be under the care of a physician. Treatment is often a medical dental team approach.

SUMMARY AND GENERAL COMMENTS

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Ultimately, with the elimination of the infection, there should be a slow return to normal oral health. Most, if not all, patients treated to eliminate oral Candida also report improvements in other areas of health which is one of the more pleasant side effects encountered.

The listing of the various remedies, together with the therapeutic results that have been reported herein, the warnings and etc. should not be construed as being an encyclopaedic dissertation on the drugs mentioned. This chapter is not intended to be a pharmacoepia nor a substitute for one. Side effects listed in the pharmacoepia for any antibiotic that is effective against *E.gingivalis* may occur with any of the antibiotics used against this organism. For example, side effects listed as common with metronidazole may occur with penicillin, even though one would not expect the particular response in question.

A small group including the author, who reacted badly to the first course of Metronidazole taken to eliminate *E.gingivalis*, became curious. Were the side effects due to the drug or a Herxheimer Reaction? About three months after completing treatment and having retested negative, we again took the medication, each in varying doses. There were no reactions. Thus it could be concluded that we had either developed a tolerance to

Metronidazole or the original side effects were a Herxheimer's Reaction. About three months after this experiment, it became necessary to be retreated. Side effects on this second course of Metronidazole to eliminate E.gingivalis were similar to the first course. From these observations it might be inferred that most of the side effects encountered were a true Herxheimer's Reaction rather than a drug reaction.

#### TOPICAL ANTIMICROBIALS -----

TETRACYCLINE MOUTH RINSE: Amoebacidal antibiotic. For topical use only.

#### Sample Prescription

Rx.

Misce 250 mg caps Pfizer "Tetracin"; dissolve contents i cap per 5cc 40% Ethyl alcohol; filter out the filler before dispensing; FLAVOUR: use bitter orange or bitter almond flavouring 2-3%; spirit compound or unsweetened commercial (i.e. supermarket) variety is compatible with the bitterness of the medication, a syrup or any sweetened flavouring is unsuitable and should not be used. All ingredients should be suitable for internal use.

Mitte. Bottles of 150 cc. DO NOT SUPPLY MORE THAN 150 cc PER WEEK. SHOW EXPIRY DATE AS 7-10 DAYS FOLLOWING Rx PREPARATION.

Sig. Rinse mouth four or eight times daily: see details of patient instructions in Chapter IX.

Note: Tetracin is not available in all jurisdictions. A reasonable substitute brand of tetracycline is always available and should be selected for ease of preparation, minimising of side effects and patient tolerance re taste and texture.

The addition of nystatin powder to this rinse has not proved to be highly effective but may be of limited value:

#### General Comments

See Patient Instructions in Chapter IX for further details relative to patient instructions and complications.

#### Sample Prescription

Rx: Tetracin/Nystatin Mouth Rinse:

To the following Tetracin Mouth Rinse is added Nystatin Powder or finely crushed Nystatin Tablets. One tablet (or 500,000 IU) per 250 mg Tetracycline (and 5cc alcohol). Sig as per Tetracin Rinse but add: SHAKE WELL BEFORE USING.

#### General Comments

See Patient Instructions in Chapter IX for further details relative to patient instructions and complications.

The formulae for the various pastes are as follows:

Rx: MA TREATMENT PASTE Amoebacidal treatment paste that also suppresses Candida.

Misce: 30 grams 10% Metronidazole cream with three finely crushed Nystatin tablets (Nilstat or an equivalent quantity Nystatin powder), plus 10 drops of bitter orange or 2 ml oil of Anise for flavour. Each Nystatin tablet contains 500,000 i.u. Nystatin. The paste should not be runny but have the consistency of margarine. DO NOT USE A SWEETENED FLAVOUR NOR A SWEETENER.

Mitte: 30 gms plus a 10cc "PeeDee Dose" Syringe loaded with paste.

Sig: Use two to four times daily during treatment and thereafter once daily until the paste is all gone, normally about six months. Rinse away food debris after meals or snacks, after the evening meal rinse away food debris with water, then apply the paste. Last thing before going to bed: rinse with water, brush with peroxide, rinse with water then apply the paste. (A strip of paste from the nozzle of the syringe (1mm) across the width of the brush (6mm) is sufficient for the gum margins of either the upper or lower jaw. Wipe onto the gum margin from left to right on the cheek side of the teeth, then back on the tongue side. Repeat for the other jaw.) Continue to use the paste sparingly until further tests have remained negative for about three months.)

Rx: MK TREATMENT PASTE: Anti fungal treatment paste that suppresses protozoa.

Misce: 30 grams 10% Metronidazole cream with three finely crushed Ketoconazole tablets to produce, as nearly as possible a paste which is 10% metronidazole and 2% ketoconazole. Paste should be unflavoured but 10 drops of bitter orange could be added for flavour. The paste should not be runny but have the consistency of margarine. DO NOT USE A SWEETENED FLAVOUR NOR A SWEETENER.

Mitte: 30 gms plus a 10cc "PeeDee" Dose syringe loaded with paste.

Sig: Use two to four times a day until further notice: rinse away food debris after meals or snacks, after the evening meal rinse away food debris with water, then apply the paste. Last thing before going to bed: rinse with water, brush with peroxide, rinse with water then apply the paste. (A strip of paste from the nozzle of the syringe (1mm) across the width of the brush (6mm) is sufficient for the gum margins of either the upper or lower jaw. Wipe onto the gum margin from left to right on the cheek side of the teeth, then back on the tongue side. Repeat for the other jaw.) Continue to use the paste until further tests have remained negative for about three months.)

Rx: MC TREATMENT PASTE: Anti fungal treatment paste that suppresses protozoa.

Misce: 30 grams 10% Metronidazole cream with nine finely crushed Nystatin tablets (Nilstat or an equivalent quantity Nystatin powder), plus 10 drops bitter orange or 2 ml oil of Anise for flavour. Each Nystatin tablet contains 500,000 i.u. Nystatin. The paste should not be runny but have the consistency of margarine. DO NOT USE A SWEETENED FLAVOUR NOR A SWEETENER.

Mitte: 60 gms plus a 10cc "PeeDee" Dose syringe loaded with paste.

Sig: Use four to eight times daily as per MK paste (above). The MC paste is useful if the ketoconazole contained in the MK paste is contra indicated.

SPECIAL NOTE: None of the above taste good, and the fluoride tastes even worse.

Home Application of Fluoride in Custom Trays.  
=====

An adjunct in the treatment of Oral Candidosis if the conditions warrant. Also effective in controlling caries.

Fluoride: use once daily; Germiphene 0.5% Neutral.

Fluoride: use once weekly; Germiphene 1.23% acidulated Sodium Fluoride, lemon flavour only.

Supply patient with trays and suitable supply of both fluoride gels.

In mild cases use only .5%; in moderate cases use both and in severe cases use 1.23%. In all cases a daily application is required.

Suggested regime: apply fluoride in trays for 10 minutes, remove trays, spit out excess but do not eat, drink or rinse for one hour.

Continue use until all tests are negative or clinical condition resolved and stabilised, or both.

This may be useful in the treatment of Candida (in conjunction with other measures, of course, including Nystatin tablets.) However, only the "Germiphene 1.23% acidulated" seems to be effective and it tends to remove the glaze from porcelain.

Some general comments and notes gleaned from various sources  
-----

Emetine Hydrochloride, an alkaloid derived from ipecacuanha, is a powerful amoebacide, but hazardous in use since it irreversibly inhibits protein synthesis in mammalian and yeast cells. Used topically it exerts an irritant effect, if swallowed this irritation (emetic action) can cause salivation, nausea, vomiting, muscular weakness, depression, perspiration and tachycardia. It is considered as a dangerous drug which should only be administered in a hospital where the emergency cardiac support services are readily available. It is worth noting that it has no effect on bacterial metabolism and was at one time extensively used for the successful treatment of periodontal infections. Successful, that is, if the occasional fatality is discounted. It may cause a complete cardio vascular collapse with topical use.

To the discerning reader, it should be obvious by now that in some cases the complications likely to be encountered can be quite complex. From the text it may not be apparent that many of the complex cases which come to my office do so by referral from physicians or dentists. It is frequently necessary to work as part of a team to rehabilitate patients

to a better state of health. Findings are reported to the referring doctors. Consultations among health care professionals provide the patient with comprehensive co-ordinated care. The dentist should not take responsibility for treating medical conditions, but co-operate with the attending physician when there is a mixed medical-dental problem. Patients must be kept fully informed and aware that there may be an interplay between oral infection and systemic disease. If there is, then there will be a combination of care given by dental and medical practitioners, each according to their field of expertise.

#### A FEW INTERESTING FACTS

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MILK upsets body chemistry and is not a good source of calcium; Ca++ is better obtained from vegetable products.

Fungi:

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Nickerson's Tests: About 5% of cultures are false positives. Bacteria, seen as CBs have not yet been identified.

Fruit juices are often contaminated with *Torulopsis* (*glabrata*) and *Candida* (*tropicalis* and *albicans*).

90% vaginal infections are now *Candida* as against 25% in the pre antibiotic era.

*Candida* tends to concentrate at either end of the gastro-intestinal tract.

All species of Genus *Candida* are susceptible to Nystatin, a polyene antimicrobial.

Sweet cravings have been treated with Nystatin by some physicians. Does this mean that some sweet cravings are due to an upset in body chemistry caused by *Candida*?

There are many dermatologic symptoms of *Candidal* infections, some of them allergic in origin.

PROTOZOA

\*\*\*\*\*

*Didinium nausatum*. A free living protozoan, it is a predator on *paramecium*. *D.nausatum* is between 80 and 200 microns. Proboscis penetrates prey, sucks out contents, involutes membrane and then consumes remains.



>TM=1 BM=62 LM=5 RM=75

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER X -----

TREATMENT OF ORAL AMOEBIASIS

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Reproduced in this chapter are the actual information sheets which are given to the patients attending the author's practice. The general format has been developed over the years to the point where the material seems to be understood by the average patient. These instruction sheets are presented "as is" and without further editing.

Tetracycline Mouth Rinse used in the Holding Programme

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The patient is initially prescribed a bottle of 150 cc. which will remain stable for not more than ten days following the preparation of the Rx. It should be used within this time period at a rate of 15cc per day. Used four to eight times per day, it is then a matter of simple arithmetic to arrive at the individual amount to be used at each rinse.

Patient Instructions

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Sig. Rinse mouth four times daily for ten minutes or more frequently for shorter durations as outlined:

"If you have used MTP, eaten or drunk anything one hour prior to using the rinse: rinse mouth thoroughly with water before use. Take one half to one teaspoonful (3-5cc) and swish around teeth and gums for ten minutes, then spit. (This gives you 100 points). 60% of the benefit comes in the first 60 seconds of rinsing, 20% in the next three minutes and 20% in the last six, so, if the lesser volume is used, or if the duration is reduced, increase the frequency to achieve 400 points daily plus or minus 10%. Do not rinse away with water, drinks, food, etc, if possible, for one hour after use. If you do consume anything within an hour after using the rinse, simply do an extra rinse. The bottle is to be finished in not more than ten days. If the rinse makes you feel nauseated you probably took too big a mouthful.

If there are infected areas of "skin" in your mouth, they may turn yellow and eventually peel off. If the underlying skin is not pink and healthy, i.e. if it is raw, sore or bleeding, phone at once.

Because you have an infection, because the rinse contains 40% alcohol as well as an antibiotic, you may find it feels like your mouth is on fire, especially if the infection is severe.

Beware: extremely bitter. Expect Black Hairy Tongue. If treatment is working also expect yellow/brown stains on teeth which may feel "gritty".

To be used for at least two and preferably four weeks. Do not discontinue without checking with doctor. Maintain your personal oral hygiene regime; i.e. continue to use Modified Torrens Powder and Hydrogen Peroxide, etc., as instructed, but discontinue the bitter paste (MA or MC) for the time being."

ALTERNATE METHOD: dilute to 4% and use in a waterpik: put one teaspoonful (5cc) of the rinse in the waterpik tank and add nine teaspoonsful (45cc) water to dilute the rinse. Use all of the resultant mix at the lowest possible pressure setting (= 100 points). Repeat this four times daily or use any combination of rinse at full strength or use of diluted rinse in waterpik that you find convenient in order to obtain 400 points. Clean tank thoroughly after each use.

Repeat, on request(\*), two times.

-----Preamble-to-Systemic-Medication-----

The following instructions are given to patients, in conjunction with other material on the general nature of parasite diseases, the specific nature of their infection, and the details of their individual prescription. The information package is rounded off with a copy of their "PERIODEX" which also contains a summary of instructions relative to medication, antisepsis, oral hygiene and the timing of dental treatment.

Patient Treatment Instructions for Oral Amoebiasis  
-----

#### PRE TREATMENT RINSE FOR SORENESS IN THE MOUTH

A saturated solution of Modified Torrens Powder is made by dissolving 3 teaspoons of the powder in four ounces of hot water. Rinse and hold in mouth until the solution cools then spit out. Repeat. If the solution in the glass cools off, add two more ounces of hot water. (Don't burn yourself!) Continue until you have used all the rinse. Repeat as often as brings relief. An alternative rinse is hot, strong, unsweetened, clear tea; use as above but you can swallow instead of spitting. To be used only if absolutely necessary.

#### PREVENTIVE MOUTH RINSE: (Anti-plaque Anti-septic)

This is 1% hydrogen peroxide. Dilute the 3% Peroxide, which you buy: Add one part peroxide to two parts of water. Use this, instead of toothpaste, to brush your teeth. (Peroxide slowly goes flat with time. Judge the strength by the "fizz". Make sure it is reasonably fresh so that it will work properly.)

PREVENTIVE PASTE is a taste alternative to Modified Torrens Powder.

Mix a few drops of 3% hydrogen peroxide with about a teaspoonful of Modified Torrens Powder to make a stiff paste. Apply the paste to the gum margins, (in the morning) using your toothbrush, don't scrub! Spit out the excess but don't rinse. (This mixture may also be used occasionally to brush teeth, but not gums, to remove stain.) The use of this mixture during treatment is discouraged.

BRUSHING: Teeth and Gums.

Brush last thing at night: dip the toothbrush into the dilute peroxide; brush the area covered by the brush, rinse the brush, redip in the peroxide and brush the next area and so on. After brushing rinse

thoroughly with water, then apply MA paste. Do NOT brush with MTP - pat it on the gums in the morning to improve tissue healing.

#### HOLDING PROGRAMME:

Brush at night (wet your brush with 1% peroxide!); use Modified Torrens Powder (MTP), for tissue conditioning, in the morning; the pretreatment rinse, if needed, and MA Treatment Paste or Tetracycline Rinse (TR) as prescribed, at least four times daily.

#### STERILISATION: Preventing Re-infection.

Dishes may be "sterilised" (to kill amoebae and trichomonas) by filling the sink with only hot water, adding a cap full of bleach, double the usual amount of detergent, a kettle full of boiling water then leaving the dishes to soak until you can comfortably put your hands in the water: i.e. 30 to 45 minutes. Wash the dishes, rinse, then stack to dry. Alternatively, if you have a dishwasher, use it on the heating cycles: i.e. Heated Wash and Hot Air Dry.

#### MEDICATIONS: (complications and side effects.)

With prescriptions for Metronidazole, no alcoholic beverages should be consumed for 24 hours before starting the medication, for the duration of the prescription plus an additional 48 hours after finishing the pills.

With prescriptions for tetracycline, "Tetracyn" is normally prescribed because it has been found to work reliably with minimal effect on the digestive tract. The normal dosage is two capsules twice a day until the infection has been eliminated. This is often two to four weeks. Some patients require longer and the need (and dosage) is assessed according to the microbiology and also the patient response. Extended periods of antibiotic coverage to minimize re-infection while healing is occurring can be accomplished at half dosage (i tab x2 daily) until parameters warrant discontinuation of medication.

The absorption of tetracycline is hindered and in some cases prevented, if it is taken at the same time as "heavy metals", (for example, calcium contained in dairy products. "Heavy metals" are also contained in saccharin, Modified Torrens Powder, antacids, MSG, most vitamin preparations and mineral supplements.) Ideally you should take tetracycline one hour before or two hours after meals or "heavy metals".

Side effects must be expected in the treatment of any parasite infection. Most side effects (e.g. a flare up of arthritic symptoms,) are due to the release of toxins from the dying amoeba. This is called the Herxheimer's Reaction. Some side effects are due to the overgrowth of non susceptible organisms, (e.g. Black Hairy Tongue, an overgrowth of pigment producing bacteria.) Other side effects are due to temporary suppression of normal bacteria. Elimination of any infection may increase fertility and thus increase the chances of pregnancy.

Apparent allergy, especially rash and itch, two to three weeks after starting medication, may be due to overgrowth of other animal or fungal parasites. Such symptoms require investigation so that the cause may be eliminated. Gas and bloating may also fall into this category.

MA PASTE: Metronidazole paste for treating Amoeba.

Because the MA paste is concentrated, you only need a tiny amount. Squeeze out 6 mm of paste across the width of the toothbrush, (the brush is about 6 mm wide.) This is enough paste to treat the gums in only one jaw. Wipe the paste around the gum margin of the upper teeth: start on the cheek side at the back and wipe to the opposite side. Immediately place the brush on the palate side and wipe back to the starting tooth. Repeat this procedure for the lower jaw. The paste should be used so sparingly that there is no excess to spit out. If you eat or drink within the hour of using the paste, simply apply the paste again after your food or beverage. The paste is meant to be swallowed. It helps your treatment to swallow the residue. The paste should be used four to eight times daily during the holding programme and two to four times daily during treatment. One month after the infection is cured, the paste should still be used, sparingly, each evening, until it is all gone, (normally four to six months.)

#### A SUMMARY and SOME HINTS:

Rinse all food debris away thoroughly, after meals and snacks. After the evening meal, rinse and then apply the MA paste. Brush last thing at night with peroxide, rinse with water then apply MA paste. In the morning use Modified Torrens Powder. After the use of any of the special antiseptics (the paste, rinse or powder,) you should try not to eat, drink or rinse for the next hour. Remember that any food debris in your mouth will cause bacteria to grow and increase the chance that you will catch the infection. It is caught by direct and indirect mouth to mouth contact so be careful about what goes in your mouth.

Take the capsules or tablets as prescribed. For the first month, renew your tooth brush each week. For the second month, renew it every two weeks and thereafter start each month with a new tooth brush. A Bass type of brush is best; for example, Butler's "Dr Bass Sub G Right Kind". Many tooth brushes are too stiff and can abrade the teeth or gums.

The pills and paste are ordered for a minimum time so make sure you take them as prescribed, each day, until they are all gone. IF YOU RUN INTO PROBLEMS, PHONE AT ONCE. Continuity of treatment is important. The MA paste should be used at least twice a day while you are taking medication. The applications should be one to four hours apart, the second application near bedtime. Use the paste more often, but sparingly, rather than a large amount just once. Large portions of paste may make you feel ill, especially if used close to consuming alcohol. If you use too much paste, the treatment may not work. The syringe makes it easier to measure the paste accurately. When the syringe is empty, disassemble and clean it by squirting water through. Dry the barrel before reloading. If the paste has been supplied in a tube, squirt new paste into the syringe from the tube. If the paste was supplied in a jar, then use a wooden stir stick, a butter knife, or similar to quarter fill the barrel. Refit the plunger, expel the air, then refit the cap.

GLOSSARY: Entamoeba gingivalis, the amoeba that is the target of treatment, lives in the absence of oxygen, together with bacteria. Moving bacterial rods (bacilli) break sugars into acid which causes inflammation and decay. Tiny moving snake like bacteria, spirochaetes, cause odour and inhibit healing. ACs, bacterial filaments with cocci attached, cause inflammation.

-----Preamble-to-Preventing-Reinfection-----

Once patients are found to be free of infection and all prescription items have been discontinued, they will change to the preventive programme, which is designed to minimize the chances of reinfection. If patients do become reinfected despite application of the preventive programme, it has been found that the programme, which bears similarity to the pretreatment regime helps to control the infection until rediagnosis and retreatment can be instituted. Where patient instructions are identical to previous instructions, reference is made to procedures and techniques, but without elaboration.

Patient Instructions for the Preventive Programme  
-----

Continue to use MODIFIED TORRENS POWDER (MTP). (For Tissue Conditioning) once daily, as before.

Oral hygiene routine will also be as before with the use of:  
1% hydrogen peroxide to wet the brush as previously described, or MTP/peroxide paste (so called preventive paste) which is wiped onto the gingival margins with the toothbrush. This paste is not intended for brushing the gingivae, although it does make an effective dentifrice, or "Viadent" toothpaste and mouth rinse each used twice daily. Before brushing all loose debris should be rinsed from the mouth with water. The teeth are then brushed with "Viadent" which is then rinsed away with water. About 2cc of "Viadent" rinse is then used for about one minute.  
Or

The following home made toothpaste (TLT PASTE) can be used for brushing in place of peroxide. Because it contains salt, unless it is thoroughly rinsed away after use, it is inadvisable to use this just before going to bed since it may engender thirst in the night. At all other times, if used as directed by persons who have no restrictions about salt intake, this paste is an effective dentifrice. Monitoring has shown this inexpensive dentifrice exerts good control over dental plaque. Any other (commercial) toothpastes and/or mouthwashes are not a part of this programme.

TLT PASTE (PERBORATE DENTIFRICE: Anti-plaque, Antiseptic.)

This non abrasive stable toothpaste combines the benefits of hydrogen peroxide with the convenience of commercial toothpaste: Mix two parts of MTP with one part of "Amosan" in a blender to form a fine (dust like) powder. (Mix about 30 gms or 1 oz.) Add Mineral Oil USP to make a creamy paste. Store in a dry closed jar. Half fill a Peedee Dose syringe and squeeze a blob about the size of a match head onto the brush. Alternatively, use a dry spatula to put a blob onto the toothbrush. Wipe the paste onto the teeth and gums, then brush them. Spit out excess. Repeat for the upper jaw. Rinse with water. Then wipe some more paste onto the gum margins, spit out the excess but DO NOT RINSE. Use once daily instead of peroxide for brushing.

GENERAL NOTES:

If you get stains on your teeth you can also clean them by brushing with either "TLT" or "PREVENTIVE" pastes. Do not brush the gums while doing

this. Immediately after brushing, rinse out thoroughly with water. This technique is for cleaning teeth, not conditioning tissue. During the day rinse (swish and swallow) with any liquid to remove food particles after eating. Water is preferable, but any unsweetened beverage is OK. Any rinse is better than no rinse at all. Use floss, if necessary, to remove food. Be careful not to hurt the gum. Don't saw with the floss. Food debris encourages growth of bacteria, this can lead to tooth decay, inflamed gums, even reinfection with protozoa or yeasts. Start each month with a new tooth brush, preferably a Bass type of brush: The brush is supposed to wear out. A stiff brush is hard on the gums and may hasten recession. If instructed to, use a proxabrush with #612 refills. #1612 is the "Traveller".

#### TREATMENT OF ORAL CANDIDOSIS

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For patients with oral candidosis, the treatment and the information had to be modified to suit the condition, but the principles of treatment remain the same. Modified Torren's Powder, hydrogen peroxide and a treatment paste are prescribed in conjunction with appropriate antifungal tablets. The oral hygiene instructions remain the same as previously described in this chapter, the different nature of the infection results in variations in medication and the different instructions are outlined below:

#### Patient Treatment Instructions about Oral Candidosis

-----

Candida, (previously called "monilia") is a fungus which has two forms. The mould phase: long strands (hyphae) grow slowly and can penetrate skin. The yeast phase: single cells (yeast cells) grow rapidly, release poisons (mycotoxins) at a higher rate. It switches back and forth between the (invasive) mould and the (reproductive) yeast over about a ten day cycle. In plaque it lives on sugar from food, fermenting sugar directly to acid. I find Candida in gum line cavities when the rate of decay is extremely high: usually, the rate of decay slows after Candida is eliminated. I find Candida in deep pockets that have not healed after successful treatment for Oral Parasites. Candida may cause white areas in the mouth; ulcerations; painful cracks at the corners of the mouth and associated raw areas on the adjacent skin. My other observations, associated with Oral Candidosis, include "stinging" in the gums, and sensitive or painful teeth for which no other cause can be found. Usually, relief of symptoms coincides with elimination of Candida.

The Dallas Environmental Unit, other similar diagnostic centres and many physicians now report Candida as the only abnormal finding for some patients with environmental illness, undue fatigue and even some types of depression. Improvement is observed when Candida is eliminated. Recently, the latter has been shown to be due to Candida toxins interfering with normal brain cell chemistry. Up to March of 1984 there had been seventy nine Candida mycotoxins discovered. Nine out of fifty-eight species of Candida are associated with disease.

The following partial list of conditions, sometimes associated with Candida, is useful in helping to determine if your Candida might be

affecting you. However, Candida is NOT the sole cause of these, or any condition. Finding Candida should not exclude other tests or diagnoses:  
>LM=6 RM=75

allergy symptoms; some cases of infectious endocarditis. Disease of collagen which may include mitral valve prolapse and some types of joint or ligament disorder; digestive system disorders including gas, bloating and diarrhoea; blood poisoning; hair loss; infected nail beds; urinary and reproductive system infections; hormone imbalance, cystic acne; "thrush" (e.g. diaper rash.) Candida infections, zinc deficiency and loss of sense of smell are interrelated as are Candida and deficiencies in Iron, Vitamin A, B3, B6, folate and pyridoxine: this could lead to blood disorders, such as a reduction in the white cells that belong to the immune system. In fact, recent research has shown that some of the toxins produced by Candida reduce circulating T-cells from 35% to 5%. Candida can dramatically suppress the immune system, rather than being symptomatic of suppression of immunity. It has also been reported as one of the co-infection factors that, if left untreated in HIV infected individuals, may weaken their immune system to the point where they may develop AIDS. However, infections with Candida do not mean a person will, or even might develop AIDS. To get AIDS a person must be infected with the virus. Without the virus persons with Candida do NOT develop AIDS. Candida is treatable, thus the risk factor can be reduced. Other symptoms associated with Candida infections include loss of short term memory, headaches, hyperactivity in children, sweet cravings, abnormalities in blood sugar, multiple jaw abscesses, suicidal tendencies and the "Drunken Charlie Syndrome." Elimination of Candida often brings improvement in associated signs and symptoms of disease.

>LM=2 RM=79

Although this list is incomplete and may sound frightening, remember that you don't necessarily have to have any of the above. It is only if the infection has affected your general health that you might have a problem. Your particular problem would depend on the severity of the infection and it's location, your genetic susceptibility, the genetically determined pathogenesis (ability to cause disease) of the strain of Candida, etc.

Candida interferes primarily with cell function, rather than cell structure. Most Candida related disease, while apparently bizarre and unrelated, have disturbance of cell metabolism as a common denominator so they are usually largely reversible.

Although Candida is all around us, not everyone becomes infected. A positive finding of Candida must be related to signs or symptoms of disease. If the suppression or elimination of Candida brings an improvement in health it can reasonably be assumed that the Candida was, at least in part, responsible. A positive finding of Candida with no symptoms may indicate an incubation stage.

Taking an antibiotic will not give you Candida. But if you are taking an antibiotic and already have Candida, then the suppression of the other "germs" (which compete with Candida for space and nutrients in your ecosystem) upsets the balance and allows Candida to overgrow and cause a problem.

Taking everything into consideration, relative to the importance of this yeast, if it is associated with ill health I advise treatment to

eliminate the Candida. Systemic and topical anti-fungal agents are used until further testing is negative for Candida and the health has stabilized. Retesting is normally done about every three months. Treatment is continued for three months after all tests, signs and symptoms are negative.

NYSTATIN TABLETS: brand "Nilstat" (pink tablets) is preferred.

Take two tablets in the morning and two at night, unless your Rx instructions are different. Nystatin is only slightly absorbed from the digestive system. It must be taken for a long time in order to achieve concentration in tissue enough to kill Candida. When this happens (toxic) cell contents are released into the body. This is called the Herxheimer's Reaction and can cause side effects which may include an initial upset of the digestion, followed by fatigue and/or depression. These may occur after a few days or at about six weeks. This seems to depend on the location of Candida colonies. The latter two complications may recur on a cyclical basis until succeeding generations of the fungus have all been killed. When taking the tablets no longer causes any side effects (release of toxins) this indicates that the yeast is nearly eliminated.

MK PASTE: Metronidazole paste for treating Candida.

Apply the paste two to four times per day, in the same manner as MA paste. The same routine as outlined for MTP and brushing is also followed. The paste is more effective if use is concentrated over several hours to produce one peak concentration each day. (Applying the paste twice/day is minimum, four times/day is maximum.) Candida is a hardy bug that can be difficult to eliminate. Research has shown that Candida can grow on toothbrushes. Try to prevent this by rinsing it after cleaning your mouth, then massaging a little fresh MK paste into the bristles and let it dry. For the first month, use a new brush each week; use a new brush each two weeks for the second month and then start each month with a new tooth brush.

The pills and paste are ordered for six months, so when you run out of either go back to the pharmacy for your repeat prescription.

GLOSSARY: Candida albicans, the yeast which is the target of treatment, lives in the absence of oxygen, together with bacteria, in the pocket producing toxins which may enter the circulation. Above the gum line there is oxygen so the fermentation of sugars, by Candida, can proceed to acid formation. Moving bacterial rods (bacilli) also break sugars into acid. The acid can cause inflammation or decay. Tiny moving snake like bacteria, spirochaetes, cause odour and inhibit healing. ACs, bacterial filaments with cocci attached, also cause inflammation.

The preceding patient instruction bulletins are the 1988 versions. From time to time the instruction sheets are updated to reflect changing patterns of disease and treatment. If patients consistently misunderstand part of the instructions, these areas are edited to improve comprehension. Newer remedies and changes in protocol can also be immediately included since the files are stored on computer disk and printed out as needed. Variations in patient instructions can be included in the printout so that patients can receive a personal instruction sheet, not just a printed copy.



The instructions for the Bass Brush Technique, as taught in the author's practice, are appended.

"Rinse the brush with water and dip it in 1% hydrogen peroxide. Place (the side of) the brush against the sides of the teeth and gums. Twist, so that the bristles tuck between the teeth. Push, so that the handle of the brush begins to bend. Keeping the bristles locked in position, "shimmy" to dislodge the plaque. To avoid scratching, do not let the bristles move across the teeth or gums. Remove the brush, rinse it, redip in peroxide, replace the brush in the mouth, placing the bristles on the next area to be covered, but use a slightly overlapping stroke to avoid missing an area. Twist, push, shimmy. Continue this technique until all areas have been brushed. Rinse out loosened plaque, foam and debris with plain water, as often as needed. Don't forget to brush the biting surfaces. Brush forward and backwards to dislodge anything on the tooth.

In order to avoid missing hard to reach areas, turn your head left or right as needed. For example, as you approach the midline, turn your head away from the hand holding the brush. When you reach the midline, turn the brush (from left to right, or vice-versa) and turn your head at the same time to ensure even attention to all quadrants on both the cheek and tongue surfaces of the upper and the lower teeth. Work by feel and do not watch yourself in the mirror as this may hinder access.

After brushing, use the antiseptic advised. Substitute TLT paste for hydrogen peroxide if advised and modify technique accordingly."

>TM=1 BM=62 LM=5 RM=75

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- CHAPTER XI -----

LABORATORY PROCEDURES - PARASITOLOGY

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Introduction

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The steps outlined have been shown to be a reliable method to demonstrate the oral presence of *Entamoeba gingivalis*. When compared to the results of direct phase contrast examination of plaque there was a better than 95% correlation between the two methods. This study involved a total of 1074 samples in a six year period (\* previously unpublished data.)

Plaque samples were examined by direct phase contrast microscopy and plaque for bulk fixing in SAF was collected as previously described in Chapter VII. If confirmation of diagnosis was required, or if primary diagnosis was uncertain, plaque from all "suspect" pockets in the same mouth was taken and deposited in 15cc of SAF contained in a plastic bottle (bulk fixed). This was submitted to the reference laboratory for the preparation of a permanently stained slide which was examined for oral protozoa.

The result of direct and indirect methods of plaque examination was:

484 positive direct plaque examinations were confirmed positive by the laboratory. The laboratory reported a further 10 cases in which the direct examination had not been 100% conclusive (97.98% confirmed positive diagnoses). Where diagnosis was negative by direct examination but positive by laboratory examination, the number of amoebae was very low. Therefore they were difficult to find by direct microscopy or there were very few amoebae in the sample submitted to the laboratory.

541 negative direct plaque examinations were confirmed negative by the laboratory. A further 39 cases were found positive by direct examination but were negative by laboratory examination (93.28% confirmed negative). In all cases, the amount of plaque was sparse and/or the numbers of amoebae were very low (1 or 2 on a slide.)

There were 7 samples which were submitted relative to only *Trichomonas tenax*. Six were positive by direct microscopy and confirmed by the laboratory. One was positive by direct microscopy but insufficient plaque was submitted to the laboratory to permit preparation of a slide. Some of the samples which were positive for *E.gingivalis* were also positive for *T.tenax*. Because of the very low number of infections where *T.tenax* was the only protozoan present, statistical analysis relative to this organism could be misleading since there were insufficient cases for a reliable data base. These cases were all negative for *E.gingivalis* by direct and indirect examining methods, so they have been included in the number of confirmed negative *E.gingivalis* samples. The microbiology laboratory at Muenster achieved reliable and accurate results by culturing for *T.tenax*. This method would seem to hold the most hope for accurate laboratory testing for the organism.

Of the 1074 slides examined by the laboratory, there was agreement between the direct and indirect method with 484 positives and 541 negatives. The overall confirmation rate was 95.44%. Those cases where there was a discrepancy (4.56%) could be explained because there was very little plaque, or the amoebae were very small, or they were very few in number. In all cases they would be hard to find. This indicated that if either the direct or the indirect method showed positive, it was probable that the numbers of "free" protozoa in the plaque had been "captured" by only one of the methods. Therefore it appeared that either method is equally accurate. However, direct microscopy allows better monitoring of the patient since there is no delay. The clinician receives the microbiological data at the time of the patient's appointment and is better able to direct continuity in medication.

>LM=3 RM=77 J=N

Examination of Dental Plaque for Entamoeba gingivalis. Nov 1978-Feb 1984  
 =====

|                     |          |
|---------------------|----------|
| Laboratory Findings |          |
| *****               |          |
| E.g +ve Slide       | = 494.00 |
| E.g -ve Slide       | = 580.00 |
| T.t +ve Slide       | = 6.00   |
|                     | -----    |
| TOTAL               | 1074     |
|                     | =====    |

|                     |          |
|---------------------|----------|
| Chairside Findings  |          |
| *****               |          |
| E.g +ve confirmed   | = 484.00 |
| E.g +ve unconfirmed | = 39.00  |
| E.g -ve confirmed   | = 541.00 |
| E.g -ve unconfirmed | = 10.00  |
| T.t +ve confirmed   | = 6.00   |
| T.t -ve unconfirmed | = 1.00   |
|                     | -----    |
| TOTAL               | 1074     |
|                     | =====    |

|                                           |   |        |
|-------------------------------------------|---|--------|
| Total number of chairside E.g positives   | = | 523    |
| Percentage of unconfirmed chairside -ve   | = | 1.72%  |
| Percentage of unconfirmed chairside E.g   | = | 7.89%  |
| Discrepancy Rate (i.e. % not confirmed)   | = | 4.56%  |
| Correlation Rate (Percentage)- Both +ve   | = | 97.98% |
| Correlation Rate (Percentage)- Both -ve   | = | 93.28% |
| Percentage of chairside E.g confirmed     | = | 94.46% |
| Percentage of chairside -ve confirmed     | = | 93.28% |
| Correlation Rate (Percentage) +ve and -ve | = | 95.44% |

>LM=1 RM=79 C=Y J=N

A LABORATORY TECHNIQUE TO DEMONSTRATE ENTAMOEBA GINGIVALIS IN STAINED SMEARS

=====

Developed by J.C.Palmer, A.R.T. (1981)

>C=N  
Preparation of Reagents.  
-----

A. SAF Fixative:

|                              |         |
|------------------------------|---------|
| Sodium Acetate, anhydrous    | 0.9 gm  |
| or with three molecules      |         |
| water                        | 1.5 gm  |
| Glacial Acetic Acid          | 2.0 ml  |
| Formaldehyde, 40% Commercial | 4.0 ml  |
| Water                        | 92.5 ml |
| Final pH                     | 4.15    |

B. Physiological Saline.

Prepare a stock solution of 8.5 gm NaCl in 100 ml water. For use, dilute one part of the stock solution with nine parts of water.

C. Mayer's Albumin.

Thoroughly but gently mix the white of a fresh egg with an equal amount of glycerine. (To avoid trapping air bubbles, use a magnetic stirrer on slow speed.) Strain through a gauze; store in the refrigerator in a brown bottle with a crystal of thymol added to reduce fungal growth.

D. Picric Acid.

Prepare a saturated solution by dissolving picric acid in lukewarm water until some crystals remain undissolved in the bottom of the container. Cool to room temperature overnight.

Dilute by adding 25 ml of the supernatant with 25 ml of distilled water to produce 50 ml of half saturated working solution.

IMPORTANT NOTE: Solid picric acid should be stored under WATER and NOT dry because it is EXPLOSIVE when completely DRY.

E. Ethyl Alcohols.

To prepare an x% alcohol solution, dilute x parts of 95% ethyl alcohol with (95-x) parts of water.

F. Carbol-xylol Solution.

|                                |        |
|--------------------------------|--------|
| Phenol, (liquefied by warming) | 250 ml |
| Xylol                          | 750 ml |

Mix by adding the phenol to the xylol.

Store in a closed brown glass bottle.

Modified Iron Haematoxylin Stain (for SAF preserved plaque.)

---

Haematoxylin-mordant solution:

- a) Haematoxylin 10 gms  
Alcohol (absolute or 95%) 1000 ml

Allow to "ripen" in light for one week, (or longer.)

- b) Ferrous Ammonium Sulphate 10 gms  
Ferric Ammonium Sulphate 10 gms  
Concentrated Hydrochloric Acid 10 ml  
Distilled water 1000 ml

WORKING SOLUTION

- Solution a) 25 ml  
Solution b) 25 ml

Mix and place in a coplin jar

To Test Solution:

Add a few drops of the working solution to some alkaline tap water.

The drops of working solution should rapidly change to BLUE  
This indicates that the solution is still fresh and may be used.  
If the brown colour persists, the solution has deteriorated and should be  
discarded and fresh working solution made as above.

Method for preparing SAF fixed E. gingivalis for staining.

---

1. Resuspend SAF fixed specimen by shaking.
2. Pour into 2 conical centrifuge tubes.
3. Centrifuge at 2000 rpm for 5 minutes.
4. Pour supernatant back into original container.  
(to prevent any loss of specimen.)
5. Add 1 to 2 ml isotonic saline. (to both tubes.)
6. Resuspend the sediments.
7. Combine both specimens in one tube.
8. Fill the tube with saline.
9. Centrifuge at 2000 rpm for 5 minutes.
10. Decant supernatant very carefully.  
(Drain tube well.)
- 10a. Repeat steps 5 to 10. This second wash is to  
ensure the removal of excess SAF as it interferes:  
with adherence of specimen to the slide and  
with the quality of staining. After the second  
wash the tube is drained well and
11. An equal quantity of Mayers Albumin (usually one or  
two drops) is added to the sediment in the  
centrifuge tube.
12. Mix well in a vortex mixer.
13. Remove some of the suspension with a capillary  
pipette and place 1 or 2 drops on a microscope  
slide.

14. Place slides horizontally in a 37 degree incubator.
- 14a. Leave overnight.
15. Spread the material out slightly on the slide, (by squashing with an applicator stick,) to give areas of varying thickness.

Method for staining amoebae with modified iron haematoxylin

-----

- |     |                                                                            |              |
|-----|----------------------------------------------------------------------------|--------------|
| 16. | Place slide in 70% ethyl alcohol<br>(to coagulate the albumin.)            | 10 mins      |
| 17. | Place in alkaline tap water                                                | 10 mins      |
| 18. | Place in Haematoxylin-mordant<br>working solution                          | 10 mins      |
| 19. | Place in distilled water                                                   | 1 min        |
| 20. | Place in picric acid for<br>(as determined by experimentation)             | 5 to 10 mins |
| 21. | Place in running tap water                                                 | 20 mins      |
| 22. | Place in 50% alcohol (to which a few<br>drops of ammonia have been added*) | 10 mins      |
| 23. | Place in 70% alcohol (to which a few<br>drops of ammonia have been added*) | 10 mins      |
| 24. | Place in 85% alcohol                                                       | 10 mins      |
| 25. | Place in 95% alcohol                                                       | 10 mins      |
| 26. | Place in 100% alcohol or carbol-xylol                                      | 10 mins      |
| 27. | Place in xylol                                                             | 10 mins      |
| 28. | Place in fresh xylol                                                       | 10 mins      |
| 29. | Mount in a neutral mounting medium<br>& dry (preferably at 37 degrees)     | overnight    |

Notes:

- \* 3 to 4 drops of ammonia in 50 ml alcohol
1. Gomori's trichrome could be used instead of modified iron haematoxylin.
2. Do not agitate slides during processing or specimen may be dislodged and lost.
3. Stained slides should be scanned systematically at 10X & 40X to spot amoebae and identification confirmed with the 100X oil immersion lens.
4. Except for the staining and destaining, (steps 18 & 20 above,) the times given are not critical for modified iron haematoxylin, but should be followed as closely as possible. Staining time should be adjusted to produce good colour contrast (see below:)
5. The background debris should stain pale blue while the trophozoite (body of the amoeba) should stain medium blue. The nuclear chromatin should stain dark blue or even blue/black. By contrast, the cytoplasm of the leucocyte should be almost colourless but the nucleus should be dark blue.
6. The typical entamoeba gingivalis trophozoite has an entamoeba nucleus,

consisting of fine periphereal chromatin applied to the nuclear membrane. There is a slightly diffused central karyosome. The cytoplasm is divided into ectoplasm and endoplasm. Within the endoplasm are food vacuoles which usually contain the partly digested remains of leucocyte nuclei, leucocyte cytoplasm or even haemoglobin removed from erythrocytes. Bacteria are sometimes seen within the food vacuoles while some amoebae even have no ingested food. This does not hinder identification, however, because the nucleus is quite typical.

>TM=1 BM=62 LM=5 RM=75  
INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS  
----- CHAPTER XII -----

>C=Y J=N  
THE PERIODEX (c)  
-----

>C=N J=Y  
Following the integration of computer technology with the practice of dentistry, the author developed "The Periodex" to assist in evaluating the clinical and microbiological data for each patient. "The Periodex" is a computer generated report together with a numerical readout ("score") and a bar graph. The clinical and microbiological parameters, recorded on the patient chart during the examination, are entered in the computer and the patient receives a report at the end of the appointment. The "PERIODEX EVALUATION", a copy of which is kept by the patient, is useful for motivation and to help patients understand their progress. "The Periodex" can be tailored to reflect the clinician's judgement, with extra emphasis being placed on those criteria deemed most important. A copy of the report can also be sent to a referring dentist, or to the patient's physician. Only the "score" is recorded on the patient chart, since the information used to generate the report is already a part of the patient record. The simplicity of the final score has been found beneficial in assessing progress.

The computer software, which is the basis for "The Periodex" is simply a spreadsheet, such as "VisiCalc", (registered programme of Tandy/Radio Shack) or Lotus 1-2-3. Each block to be used as a "label" is filled in, as illustrated in the "Periodex Evaluation" sample file contained in this chapter. The areas to be used to generate the score are initially filled with prompt words (labels) which will ultimately be replaced by a numerical entry. The advantage of "Visicalc" is that the numerical entry is not shown on the screen (or printout) as a number but rather as an asterisk. The usual numerical entry for any block is one, which is shown as a single asterisk. The programme is instructed to multiply the numerical entry for any position by a preselected number. For example, entries in column two are multiplied by two in the first eight rows. The system can be weighted to give extra emphasis in the final score to those factors deemed most important by the clinician. For example, gingival condition might be rated as fair (enter 1 in the appropriate box) but bleeding may occur with gentle probing. A second entry could be made on this line to record this fact by entering 1 in the appropriate box. For clarity, the adjacent box to the right of this entry would be now be filled with the explanatory note "Bleeding". The programme adds the value of each entry made to make the final score. A bar graph is simultaneously generated. The starting score when the programme is loaded is always zero. The previous score is shown as ?. The "ideal" score is 14 with a five point spread being acceptable. Most patients experience no difficulty maintaining a score in the ideal range in the absence of infection. On subsequent appointments the score from the previous appointment is entered against "Previous Score".

At the beginning of the appointment, "The Periodex" master file is loaded from disk into the memory of the computer. Each clinical and microbiological parameter recorded for the patient is also entered on the



computer display. The computer programme "VisiCalc" changes numbers to asterisks, makes the calculations, produces the bar graph and displays the entries, score and graph as seen in the examples. At the end of the examination the reports are printed, including one for the patient. To the bottom of each report is added the specific instructions which are selected from the information contained in the master file. By using a simple spread sheet to record information, produce calculations and issue a summary of instructions, the clinician can have a flexible programme which can be quickly tailored to individual requirements without the necessity to understand computer programming or hire a computer consultant.

After initial diagnosis, appropriate therapy is instituted. The patient is then seen at appropriate intervals to assess progress, make any necessary alterations in therapy and co-ordinate the most advantageous timing of routine office care. Each subsequent appointment follows the pattern of the first with the clinical and microbiological assessments necessary to produce the "Periodex Evaluation". Integral to successful treatment are patient consultations in order that each person adequately understand their problems, their specific instructions relative to medication and home care. In addition to the summary of instructions printed on "The Periodex", details of the prescription, home care and other pertinent instructions are given to the patient on pre printed instructions at the end of the appointment. (For examples of these, see Chapter X.)

>C=Y J=N  
 SAMPLE OF "THE PERIODEX" MASTER FILE

>LM=2 RM=78 C=N

-\* "PERIODEX-EVALUATION" (c) 1989 Dr Trevor Lyons & Eleanor Stanfield.\*-  
 Documentation for this programme is contained in Chapter XII of the book "INTRODUCTION TO PROTOZOA & FUNGI IN PERIODONTAL INFECTIONS"(a Manual of Microbiological Diagnosis & Nonsurgical Treatment) by Trevor Lyons. Send for more information: 45 Rosebery Avenue, Ottawa, Ont., K1S 1W1, CANADA. Before using this non scientific programme, replace lines 1+2 with your own heading & delete rows three through ten. Customize the programme for your own requirements. The author & copywrite owners are not responsible for use or misuse of this programme. No warranty is implied or intended. -----<"PERIODEX-EVALUATION">---( developed 1983; (c) 1989.)-----

Patient Name:-  
 Report sent to Dr  
 -----<"PERIODEX-EVALUATION">---( developed 1983; (c) 1989.)-----

A computer generated professional opinion report on periodontal status. A (\*) for each category replaces the appropriate prompt word and has a value automatically assigned. The total is also shown as a bar graph and the totals per column show the pattern of clinical & microbial findings.

| On Examination:    | "A"  | "B" <+>  | "C"   | "D"      | "E" (A = Best) |
|--------------------|------|----------|-------|----------|----------------|
| ORAL HYGIENE:      | Good | F to G   | Fair  | F to P   | Poor           |
| GINGIVAL CONDITION | Good | F to G   | Fair  | F to P   | Bleeding       |
| PLAQUE :           | <o+  | o+       | +     | ++       | +++            |
| HALITOSIS :        | <o+  | o+       | +     | ++       | +++            |
| POCKETS :          | <1   | 1-3      | 3+    | 4-5      | 6+             |
| INFLAMMATION :     | None | Min/Spor | Stagn | 4Q/GMD   | Detached       |
| MOBILITY:          | <o+  | o+       | +     | ++       | +++            |
| SUB MANDIBULARS:   | None | One      | Two   | 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+     | +             | ++    | +++ |        |     |       |       |

=====  
Total per Column  
PERIODEX SCORE = 0 (Ideal Range = 12 to 17)

PERIODEX GRAPH  
YOUR LAST SCORE = ?  
YOUR LAST GRAPH =

.....

Notes to the referring dentist or physician. Thankyou for the referral.  
If you have questions, please phone the office for clarification.  
DDS only, please delay all but emergency treatment until the infection is under control or cured. I will advise you of your patient's progress. I have prescribed medication & issued appropriate patient instructions. The infection is controlled enough for you to do the scaling & etc. now. I advise polishing restorations to reduce irritation. Please arrange it. I advise adjusting the bite to reduce traumatism. Please proceed with it There is need to restore teeth; xrays may be necessary, especially: I recommend a new upper/lower denture/bridge to assist with the therapy. The patient now seems to be free of serious infection. Now is the time to proceed with all routine treatment, especially the scaling & etc. I will continue to monitor the patient on a regular basis & report. I shall only see the patient again if there is a perceived need Improved relative to gum condition, inflammation, swelling, etc Review instructions re timing, method & dosage. If you brush near a meal Brush before eating & rinse afterwards. Watch for hidden sugars! Prescription depends on result of swab for Candida: phone in 3 & 30 days Use a "Proxabrush" with #612 refills for cleaning & applying the paste. DENTURES: After food: wash clean. Daily soak in "Betadine" for one hour, then in a denture cleaner. Wipe paste onto fitting surfaces, 2-4x daily. See your dentist for construction of custom trays for home application of Fluoride Gel. Germiphene 1.23% in close fitting trays works best. Use once daily for ten minutes. Do not swallow. Too much may be nauseating. Change Rx to:

Holding programme:Tetracycline Rinse or MA Paste four to eight times/day pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night Metronidazole Regime #3: 30 tabs. Take one twice daily preferably with food. No alcohol for the duration of Rx plus one day before & two after. plus MA paste twice daily until further notice-approximately 6pm & 10pm. pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night Metronidazole Regime #4: 60 tabs. Take one twice daily preferably with food. No alcohol for the duration of Rx plus one day before & two after. plus MA paste twice daily until further notice-approximately 6pm & 10pm. pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night Continue with Rx

Tetracycline Capsules: two caps twice a day until the infection has gone This is usually for four weeks, followed by 1 cap x2 daily for 8 weeks. plus MA paste twice daily until further notice-approximately 6pm & 10pm. pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night

Minocin 100 mg tabs: Loading dose of two tablets, then 1x2 daily for two weeks. Can be taken with food, but not with milk or milk products.  
plus MA paste twice daily until further notice-approximately 6pm & 10pm.  
pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night  
Penicillin V (300 mg tabs) Two tabs four times daily for 30 or more days  
plus MA paste twice daily until further notice-approximately 6pm & 10pm.  
pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night  
Change Rx to:

Nystatin Tablets: 2x2 daily for at least 6 months with testing at three month intervals to check progress & for 3 months after negative tests.

plus MK paste, two to four times daily, ideally x3, until further notice  
pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night

Nystatin Tablets: one daily for a month, then 1x2 daily for a month then 1x3 daily for a month, then 1x4 daily for a month, then 2x2 daily til K5

plus MK paste, two to four times daily, ideally x3, until further notice  
pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night

Ketoconazole: one tablet x1 daily: Report any side effects immediately.

plus MK paste, two to four times daily, ideally x3, until further notice  
pat MTP on the gums in the am & brush with 1% hydrogen peroxide at night

Nystatin lozenges: dissolve slowly x4 daily, plus use MC paste x4 daily.

WARNING: Use of antibiotics to treat infection may enhance fertility.

WARNING: This medication may make your skin more sensitive to sunlight.

Change to Preventive Programme

Use MTP in the AM, brush with hydrogen peroxide or TLT PASTE in the PM.

Brush daily with "VIADENT", rinse with water then with "VIADENT" rinse.

(Use a teaspoonful to rinse then hold in mouth for at least one minute.)

>J=Y LM=5 RM=75

The file is available for uploading from some computer bulletin boards, such as Genie. The file was uploaded to this board in ASCII format and should be downloaded in the same manner. "The Periodex" should run, without major modification, using VisiCalc. With other spread sheets, such as Lotus 1-2-3, some modifications will be necessary in order for "The Periodex" to operate.

"THE PERIODEX" & VISICALC

-----

Using your communications software, download "The Periodex" and save to disk as PERIODEX with the extension /VC. After logging off from the bulletin board, exit the communication programme, load VisiCalc and then PERIODEX. Edit the first ten lines as indicated, save the edited file to disk and "The Periodex" is ready to run.

"THE PERIODEX" & LOTUS 1-2-3

-----

Proceed, as with VisiCalc, to download and save to disk as PERIODEX/VC. Exit the communications programme and load Lotus. Use the self prompting "translate" feature, to convert the file to be readable by Lotus and save it to disk with a .WKS extension. It may now need considerable editing to make "The Periodex" workable. The text portion should be self explanatory, but error messages indicate that formatting or formulae may need editing.

Global format is left aligned, 9 characters per column, all numbers are shown by the computer as \* except for the "Periodex Score" and the

"Previous Score" which show the actual value. Calculation is automatic and the order of recalculation is across the rows first. Columns I through M in the rows adjacent to the prompt words contain the multiplication formulae used to generate the score.

Example: Oral Hygine good (position C 11) is referenced by position I 11 which multiplies a numerical entry at C 11 by one. J 11 multiplies the entry at D 11 by two, K 11 multiplies E 11 by three & etc.

Each of the five positions in each row is scored one through five and the totals are calculated by adding the column totals, then adding each total with the formula in position C 32.

At position C 33 the total at position C 32 is multiplied by .4 and the answer shown as a single asterisk. Asterisks are used in VisiCalc for the generation of graphs.

At each of the positions C through H in row 33 the total score from position C 32 is multiplied by a successively smaller number; .4; .2; .1; .05; .025; and the answer is shown as asterisks thereby producing a bar graph.

The same formula is used in row 35 relative to position C34.

The formulae for all positions on "The Periodex" are appended for ease of trouble shooting. The printout starts with the LAST position in the file and works logically through, row by row. The first ten lines have been edited in accordance with the instructions at the top of the sample printout contained in this chapter.

#### PROGRAMMING NOTES

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At position A101 the spread sheet shows "Brush dai" the word is completed in box B101 and further text is added. The quotation marks at the start of each label block tell the computer programme that this is a "label", i.e. text, and neither a numerical entry nor a formula. The comment "Print -> H 102" does not appear on the worksheet at this position but is shown at the top left of the screen. It is the prompt to tell the computer operator to print from the first position (A 101) to the second position (H 102) if the instructions on these two lines are applicable for this patient.

Positions A 36 to H 36 create a line of colons across the worksheet. The slash (/) is a computer command meaning Special Instruction? The dash (-) means repeat the next symbol across the position. The symbol is a colon (:). The same command is given in the adjacent position and the result is a line across the worksheet appearing as ::::::::::::::::::::::::::::::

From position G 35 to I11 will be found the formulae which calculate the score and generate the bar graphs.

The last six entries are formatting instructions. /W1 means one window. /GOR means that the global order of calculation is across the rows. /GRA means that the global calculation is done automatically each time a value is entered on the worksheet. /GF\* means that all values displayed on the worksheet will be disguised as asterisks. /GC9 means that each column is nine characters wide. Words longer than nine characters must be

completed in the next position on the worksheet. The final entry is a code used within VisiCalc to identify the file.

&gt;TM=1 BM=62 LM=5 RM=75 LS=2

&gt;C=Y J=N LS=1

INTRODUCTION TO PROTOZOA AND FUNGI IN PERIODONTAL INFECTIONS

----- APPENDIX -----

Some final comments by the author.

-----

&gt;LS=2 C=N J=Y

An "appendix" may be thought of as a useless blind extension to the intestine. Derived from the same root, an "appendage", such as a thumb, forms a useful extension of a limb. The reader will have to assess into which category this addition to the book belongs.

&gt;LS=1

CONCEPTS REVISED

-----

&gt;LS=2

Two parallel concepts previously referred to have been left with no apparent connection. Chapter II detailed stages of infection with *Entamoeba gingivalis*, starting with an incubation stage. This was followed by a severe or repetitive 'flu' like illness during which a periodontal deterioration was observed to commence. Following the apparent recovery of the patient, it was frequently noted that rather than a return to normal health, the patient had seemingly acclimatised to a diminished state of health. This was characterized by fatigue and often by more frequent headaches. Patients experienced greater problems with maintaining oral health. Excessive plaque production (often an accompaniment to a cough, a cold or a 'flu' like illness), gingival bleeding and a bad taste on arising were often matched by the observation that this individual had unpleasant halitosis, vaguely reminiscent of garlic. It should be noted that many providers of dental care develop an unpleasant halitosis within a few years of commencing their training. This phenomenon does not go unnoticed by the general public and is regularly the subject of newspaper columns. (Viz: "Dear Abby, my dentist is a nice fellow and very good at his job, but he has overpoweringly bad breath. How can I tell him not to eat garlic?/Should I tell him he has halitosis?" etc.)

Chapter II details the stages of infection with *E.gingivalis* and also indicates that, despite good oral hygiene, aerosol spray created by dental instrumentation could put the dental team at risk of infection. For both patient and dental staff, the risk of pulmonary infection from such a source also exists. Although pulmonary amoebiasis is usually reported as due to *E.histolytica*, (Blyth and Pirie, 1978), *E.gingivalis* has been recovered in cases of pulmonary suppuration, (Sutcliffe, Green and Suter, 1951). Blyth and Pirie stated the reason for identifying the organism as *E.histolytica* rather than *E.gingivalis* was because of the presence of ingested red cells. They were almost certainly unaware that *E.gingivalis* ingests red cells, since this fact was not reported until 1984 (Lyons). Blyth and Pirie found no evidence of hepatic or intestinal amoebiasis, which they stated was unusual in cases of pulmonary amoebiasis, but they stated that bronchial embolism leading to pulmonary infection was associated with *E.gingivalis*. My colleagues in public

health laboratories have also reported the presence of *E.gingivalis* in sputum samples submitted by patients with chronic lung infection, but this fact has only infrequently been reported in the literature. The situation is further compounded because dentists are not normally exposed to medical or public health literature and so would remain largely unaware.

In Chapter VII it was stated that, for the purposes of this book, diagnosis would be divided into three broad categories. It now remains to tie these categories to those classes of periodontal diseases which have been traditionally described. Pyorrhea alveolaris, literally pus in the gums, was the early term used to describe destructive periodontal disease. Gingivitis, a non destructive inflammatory lesion of the gingivae became recognised as a separate entity. Even today it is recognized that gingivitis does not necessarily proceed to destructive lesions.

Destructive periodontal lesions came to be subdivided into three main classes.

Periodontitis Simplex was described as destructive lesion, where the bone loss was horizontal; it was accompanied by inflammation.

Periodontitis Complex was described as a destructive lesion with vertical bone loss, i.e. intra bony lesions. It too was accompanied by inflammation.

Periodontosis on the other hand was described as a non inflammatory destructive lesion.

Applying the concepts of Oral Amoebiasis, as outlined in this book, to these four main classes of periodontal diseases it has been observed that:

>LS=1  
BACTERIAL GINGIVITIS  
-----

>LS=2  
Gingivitis, with pocket depths ranging up to 3mm, is associated with motile bacilli or ACs, (*Actinomyces* filaments with cocci attached) or CBS (cocco bacilliary forms.) The latter may be *Actinomyces-actinomycetum comitans* (AAC), but other species have a similar appearance.

>LS=1  
CANDIDAL GINGIVITIS  
-----

>LS=2  
Gingivitis, characterized by a red granular appearance, shallow pockets, which are frequently only about 1mm deep and fragile gingivae which bleed with slight provocation, is frequently found in association with *Candida* spp or other fungi. This type of gingivitis is not associated with infection with protozoa. It should not be confused with a superinfection with *Candida* spp which is sometimes a sequel to infection with protozoa. Pockets which fail to heal and which are infected with *Candida* spp do not fall into this category of gingivitis.

>LS=1  
INFLAMMATORY DESTRUCTIVE PERIODONTAL LESIONS.

-----  
>LS=2

These lesions are typically infected with one or both species of the oral protozoa together with large numbers of motile bacilli, ACs and ropes of bacterial filaments. Spirochaetes are frequently also present in abundance.

>LS=1

NONINFLAMMATORY DESTRUCTIVE PERIODONTAL LESIONS.  
-----

>LS=2

These lesions are found to be infected with one or both species of the oral protozoa but without accompanying bacterial activity.

>LS=1

OTHER DESTRUCTIVE LESIONS.  
-----

>LS=2

Applying the diagnostic criteria outlined in this book, these lesions form a small minority of the cases of periodontal disease seen in a mixed general dental practice in Ottawa, Canada. Only about 2% of cases seen over the period in which data for this book was compiled fall into this category. Most of the patients had no specific target organism in their plaque. Rather there was an underlying disorder of the general health which had escaped medical diagnosis at the time of dental diagnosis. The two most frequent disorders associated with generalized and unexplained periodontal destruction were either diabetes mellitus or infection of the intestinal tract with protozoa.

Some apparently destructive lesions (perhaps .1%, that is one tenth of one percent of the total number of persons for whom a plaque examination was made) appeared to be of bacterial aetiology. This total includes one case of juvenile periodontitis seen in the practice of a colleague.

In conclusion, then, it appears that the vast majority of patients with destructive periodontal lesions are infected with oral protozoa. Elimination of protozoa is followed by arrest of the disease and resolution, including regeneration of alveolar bone.

Protozoal infection calls for long term systemic and topical treatment to eradicate the infection.

Bacterial infection calls for topical treatment alone. Systemic therapy is seldom required.

Mycotic infection calls for extended long term therapy with anti-fungal agents.

In all cases the aim is to eliminate the offending target organisms.

All cases of periodontal infection require correction of local factors by changes in home care and effective dental treatment. The timing of the latter should be dictated by the microbiological status of the patient.

In the absence of target organisms which might be implicated in periodontal disease, consider a systemic aetiology.



Always consider that there may be a systemic component to periodontal infection.

Simple Simon, simple soul,

Bought a book on plaque control.

Judging from his gum condition

He got the unrevised edition.

Trevor Lyons.

>LS=1

Reference quoted:

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>LS=2

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>TM=1 BM=62 LM=2 RM=78

HS>C=Y J=N

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Page -<P>##>-

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