IMMUNOLOGICAL RESPONSES IN HUMAN SALIVA
WITH ACUPUNCTURE STIMULATION

Kevin KW Ng
BDS (Adelaide) LDS (Victoria) DPHDent (Sydney)

A Treatise submitted in partial requirement
for the degree of
MASTER OF DENTAL SURGERY

Department of Preventive Dentistry
Faculty of Dentistry
University of Sydney
1988
SUMMARY:

The use of acupuncture for the prevention of pain during surgery is a modern adaptation of ancient technique and although there have been attempts to explain the phenomenon in terms of psychological conditioning or physiological adaptation, these hypotheses, as yet require further development.

The improvement in relationships between the People's Republic of China and the Western world has resulted in the introduction of acupuncture into the therapeutic regimen of medicine and dentistry in the West.

A number of research papers have been published in recent years, both in China and in other countries, to investigate the healing effects of acupuncture in terms of immunological responses after acupuncture stimulations.

This information has been reviewed by the writer together with the literature on the defense mechanisms of the oral cavity. The writer has attempted to establish a "correlation" between the immunological responses of the oral cavity and acupuncture stimulations using Secretory Immunoglobulin (IgA) in human saliva as a "linker".

An experiment was designed using 150 human saliva samples (25 test subjects, 25 control subjects, each providing 3 samples) collected
before, immediately after, and 24 hours after electro-acupuncture stimulations on a selected aural acupuncture point for 20 minutes (the internal secretory regulatory point).

The subjects were all Chinese aged over 8 and under 60 years, in good health with no gingivitis (CPITN scores of 0), and had no previous acupuncture experience.

The concentrations of Secretory Immunoglobulin A in the saliva samples were determined by the "Radial Immuno Diffusion" technique.

The results of the laboratory analysis revealed that there are obvious deviations in Secretory IgA levels in the experimental subjects before and after such stimulations.

On the other hand, there are no obvious changes in the concentration of IgA levels in the control subjects.

This positive result from this pilot study, although limited by its sample size, is most encouraging and further investigations in the field are indicated.
ACKNOWLEDGEMENTS

The writer would like to thank Associate Professor PD Barnard for his supervision and guidance, and Dr Jones and Miss Chu of the Department of Clinical Immunology, Queen Mary Hospital, Hong Kong for their laboratory support.
TABLE OF CONTENTS

Summary .......................................................... i
Acknowledgements .................................................. iii
Table of Contents .................................................. iv
List of Tables ...................................................... viii
List of Figures ...................................................... ix

1 INTRODUCTION

1.1 Acupuncture - The Chinese Healing Art .................. 1

1.2 Possible Role of Acupuncture in Human Immunological Response ....................................... 2

1.3 The Role of Body Defense Mechanisms in Dental Caries and Periodontal Disease .......... 5

1.4 Experiment in Human Volunteer Subjects on Immunological Response in Saliva to Acupuncture Stimulation ................................................................. 8

1.5 Aims of the Treatise ....................................... 10

2 THE TRADITIONAL CONCEPT OF ACUPUNCTURE

2.1 History and Background of the Ancient Chinese Healing Art .......................................... 11

2.2 The Traditional Phenomenon of Acupuncture

2.2.1 Yin and Yan ............................................. 14

2.2.2 Chi - The flow of internal body energy ........ 16

2.2.3 Bio-rhythm ............................................... 18

2.3 Auricular Acupuncture

2.3.1 Physiological foundations of auricular acupuncture ....................................................... 22

2.3.2 Points of auricular acupuncture ....................... 23

2.4 Techniques in Acupuncture Stimulations ............ 26
Table of Contents (Continued)

3  THE MODERN MEDICAL CONCEPT OF ACUPUNCTURE
   3.1 Acupuncture in Treatment of General Medical Complaints ..... 28
   3.2 Acupuncture in Dentistry ..... 34
   3.3 The Proposed Mechanisms of How Acupuncture can Stop Pain
      3.3.1 The gate theory ..... 38
      3.3.2 The endorphins concept ..... 39
      3.3.3 Psychological/hypnotic aspect ..... 41
   3.4 The Proposed Mechanism of How Acupuncture can Heal Disease
      3.4.1 The effect on immunological response ..... 43
      3.4.2 The effect on internal secretion ..... 49
   3.5 The Practice of Acupuncture in Western Countries ..... 54

4  IMMUNOLOGY OF ORAL DISEASE
   4.1 Immune Response to Dental Bacterial Plaque
      4.1.1 The nature and development of dental plaque ..... 58
      4.1.2 The components of dental plaque ..... 60
      4.1.3 Dental plaque and the immune response ..... 61
   4.2 The Oral Immune System
      4.2.1 Mucous membrane ..... 65
      4.2.2 Oral lymphoid tissue ..... 66
      4.2.3 Saliva
         4.2.3.1 Physiology of saliva ..... 68
         4.2.3.2 Composition of saliva ..... 69
         4.2.3.3 Immunoglobulin A in saliva ..... 71
Table of Contents (Continued)

4.3 Immunology of Dental Caries

4.3.1 Caries immunology ..... ..... ..... 75
4.3.2 Immunoglobulin A function in relation to dental caries ..... ..... ..... 77
4.3.3 Caries immunization ... ..... ..... ..... 80

4.4 Immunology of Periodontal Disease

4.4.1 The infectious nature of periodontal disease 83
4.4.2 Immune responses to periodontal disease ..... 86
4.4.3 Immunoglobulin A function in relation to periodontal disease ... ..... ..... ..... 91

5 DESIGN OF ACUPUNCTURE EXPERIMENT WITH HUMAN SUBJECTS

5.1 Subjects ..... ..... ..... ..... 97
5.2 Equipment ..... ..... ..... ..... 99
5.3 Technique ..... ..... ..... ..... 100
5.4 Procedures ..... ..... ..... ..... 101
5.5 Precautions ..... ..... ..... ..... 103
5.6 Controls ..... ..... ..... ..... 104

6 LABORATORY ANALYSIS

6.1 Quantitation of Human Secretory IgA (By Radial Immuno Diffusion)

6.1.1 Selection of the quantitation method ..... 105
6.1.2 Principles of the radial immuno diffusion technique ..... ..... ..... 106
Table of Contents (Continued)

6.2 Reagents  

6.3 Laboratory Procedures  

6.4 Photographic Illustrations of Experimental and Laboratory Procedures  

6.5 Data Measurement  

7 DATA ANALYSIS

7.1 Subjects in Study  

7.2 Experimental Results  

7.3 Statistical Analysis

7.3.1 Changes in control and test groups  

7.3.2 Changes after stimulation in all subjects  

7.3.3 Control versus test groups  

7.3.4 Control versus test groups after stimulation  

7.3.5 Replication of procedures  

8 DISCUSSION  

9 CONCLUSIONS  

10 REFERENCES  

vii
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acupuncture in Treatment of General Disease</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Acupuncture Results from the Soviet Union</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>Acupuncture Results from Paris</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>Acupuncture Results from China</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>Acupuncture Results from Shanghai</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td>Subjects Sex, Age and Occupation</td>
<td>120</td>
</tr>
<tr>
<td>7</td>
<td>S-IgA Concentration Results for Subjects</td>
<td>124</td>
</tr>
<tr>
<td>8</td>
<td>Changes in S-IgA after Stimulation for Control Groups</td>
<td>132</td>
</tr>
<tr>
<td>9</td>
<td>Changes in S-IgA after Stimulation for Test Groups</td>
<td>133</td>
</tr>
<tr>
<td>10</td>
<td>Changes in S-IgA after Stimulation in All Subjects</td>
<td>134</td>
</tr>
<tr>
<td>11</td>
<td>S-IgA in Control and Test Subjects</td>
<td>135</td>
</tr>
<tr>
<td>12</td>
<td>Control vs Test S-IgA after Stimulation</td>
<td>136</td>
</tr>
<tr>
<td>13</td>
<td>Replication of Procedures after 12 months for 1 Subject</td>
<td>137</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The Meridian Cycle</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Ear Acupuncture Points</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Selye's Theory on General Adaptation</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>Photographs of Laboratory Procedures</td>
<td>114</td>
</tr>
<tr>
<td>5</td>
<td>Standard Curve for S-IgA Concentration</td>
<td>122</td>
</tr>
<tr>
<td>6</td>
<td>Requirements for Dental Caries</td>
<td>141</td>
</tr>
<tr>
<td>7</td>
<td>Defense Mechanism of Oral Cavity</td>
<td>142</td>
</tr>
</tbody>
</table>
1.1 ACUPUNCTURE - THE CHINESE HEALING ART

Acupuncture is a medical science which dates back more than 2500 years to the first Chinese Dynasties. It has been constantly evolving since that time, particularly during the last 300 years, and more especially since 1950, when acupuncture science came to be widely developed both in theory and practice (WHO 1984).

The fact that acupuncture can effect the human body has generally been accepted, particularly its effects on killing pain. There is still not a single explanation for its action, but many theories have been put forward (Gross & Morse 1976).

Another area of interest to research scientists is the mechanism of how acupuncture can actually heal diseases.

For centuries, people have believed that it is impossible to propose a phenomenon that can explain the healing effect of acupuncture (Dimond 1971). Many still think of it as magic or supernatural.

The traditional Chinese physician has constructed the philosophy of 'Chi' - the flow of internal energy within the human body. Loss of balance of this internal energy flow could lead to the onset of different diseases within the human body (Chapman 1974). Acupuncture is believed to possess this specific power of regulating the energy flow, and can thus affect the healing rate of the affected individual (Mann 1978). Yet this phenomenon is generally unscientific and lacks physical or medical evidence and support.
Various technologies have been tried in recent years to investigate this healing effect and some workers have started to concentrate on the concept of human immunological response (Omura 1976).

1.2 POSSIBLE ROLE OF ACUPUNCTURE IN HUMAN IMMUNOLOGICAL RESPONSE

In Western medicine terms, there are three major factors that can affect the human disease process.

i. The actual number of micro-organisms that are present and affecting the body. These organisms can be of bacterial or viral origin.

ii. The virulence of the organisms that are physically present. These organisms can be bacterial, viral or fungal origin.

iii. The host resistance factor, the human immune system that can defend the host against infection by the organism.

These factors can be expressed by a dependence relationship formula:

\[
\text{Disease} = \frac{\text{Number of bacteria} \times \text{Virulence of the Bacteria}}{\text{Body Host Resistance (defense mechanism)}}
\]

An acupuncture needle surely cannot kill bacteria by itself.

An unsterilised acupuncture needle, in fact, can introduce bacteria to the body.

Unlike antibiotics, acupuncture treatment cannot decrease the number of bacteria present nor could it lower the virulence of the invading bacterial strains.
So the only factor that remains to be investigated is the body host resistance component.

A rise in levels of antibodies after acupuncture stimulation has been studied by Ma, Zhang, Lu and Lin (1979) and their results open a new insight into the traditional healing art of acupuncture.

Change of blood pressure after acupuncture has also been reported (Chen 1979). The drop in blood pressure may be caused by the effect of vaso-dilation following acupuncture stimulation (Omura 1976).

Vaso-dilation of peripheral blood vessels after acupuncture has also been observed by Selden (1978) and an increase in membrane permeability has resulted. This may lead the observer to suspect there is an increased outflow of white cells, plasma cells, T cells and antibodies to the surrounding tissue (Bi, Xu & Gao 1979).

Sabolovic and Michon (1978) conducted pilot studies on peripheral T & B lymphocytes responses to acupuncture. They have observed an altered composition of T & B cells in patients' blood before and after acupuncture, and the normal values returning together with improvement of B cells and humoral antibody production in subjects.

Ding, Roath and Lewith (1983) measured total and differential white cell counts, E rosetting and lymphocyte transformation with and without phytohemagglutin stimulation in nine healthy volunteer subjects before and after acupuncture. They found that lymphocyte transformation in the unstimulated cultures showed a clear increase post-acupuncture when compared to the pre-acupuncture levels in eight out of the nine subjects.
Sin et al (1983), of St Bartholomew Hospital, London, tested experimentally induced acute inflammation in the subcutaneous air pouch of rats by carrageenan injection. The test animals were subjected to electric acupuncture stimulation. The results show that there was a significant decrease in the number of exudate leukocytes in the inflammatory cavity after acupuncture stimulation. There were also increases in the peripheral white blood cell count. They have concluded that acupuncture stimulation can reduce leucocyte adherence to vascular endothelial cells.

All the above findings provide valuable information to the acupuncture researchers and they also give theoretical support to the design of this present experiment.
1.3 THE ROLE OF BODY DEFENSE MECHANISMS IN DENTAL CARIES AND PERIODONTAL DISEASE

Since the two major oral diseases, dental caries and periodontal disease are caused by bacteria and plaque, the accumulation of these agents or "antigens" is the key starting factor of the chain auto-immune reaction (Gibbons & Houte 1973, 1975).

The immunological response of the body defense mechanism plays a major role in combating this invasion (Lehner & Cimansoni 1980).

Evidence for local production of the major portion of secretory IgA has been presented from a number of laboratories. By utilising fluorescent antibody or in vitro culture technique, local synthesis of IgA has been demonstrated in human major and minor salivary glands (Hauptman & Tomasi 1975).

The primary site of synthesis of IgA is in the submucosal plasma cells. Immunoglobulins in secretion may reach the saliva either by transudation from serum or by local synthesis in the lamina propria (Tomasi & Bienstock 1965).

If the concentration of the secreted antibodies can lower the invasiveness and activity of the oral microbials, it would affect the disease process within the oral cavity.

In the case of periodontal disease, workers such as Bear and Morris (1977) and Lehner (1983) have demonstrated that the destructive effects to the periodontium are caused by the chain reactions triggered by the auto-immune mechanism of the affected host.
Page and Schroeder (1982) reported that B-cell activation and local immunoglobulin production by plasma cells in diseased gingival tissue as well as in culture of mononuclear cells from patients, are either exclusively polyclonal or a mixture of monoclonal and polyclonal activation.

Periodontal diseased tissue contains very large amounts of immunoglobulin derived from two sources: Some is produced locally and some enters the tissue from the blood as exudate.

Several investigators have searched for antibodies specific to plaque or to pocket bacterial determinates in the immunoglobulin present in diseased gingival tissue. All have found small amounts. (Berglund 1971, Kagan 1980 & Schenck 1985).

Mouton et al (1981) and Kagan (1980) have reported the presence of plasma cells in diseased human gingiva which specifically bind Bacteroides gingivalis, indicating local production of specific antibody. High antibody titres in patients with juvenile periodontitis decrease significantly after successful treatment.

Robertson et al (1978,80) studied 23 patients with primary immune deficiency. Included were those with selective IgA deficiency, agammaglo bulinaemia, cellular defects, and severe combined immunodeficiencies. The result showed significantly less gingival inflammation in the immunodeficient group.

Crawford et al (1978) provided valuable evidence indicating that secretory IgA antibody may regulate the colonization of the teeth and oral tissue by bacteria.
Page and Schroeder (1982) pointed out that previously immunized animals do not develop periodontal disease more readily, nor is the disease more severe in previously immunized than in non-immunized monoinfected animals. They have deduced that specific serum antibodies may provide a measure of protection and cell-mediated immunity may prevent or delay the onset of disease. Studies in humans lead to similar conclusions.

Dental caries is a disease caused by bacteria utilizing sugar in the diet for the production of acid. Streptococcus mutans is by far the most efficient cariogenic organism in germ-free rats and it is correlated with the presence of caries in man. Hence most of our immunological knowledge about caries concerns S. mutans.

In man, serum IgA, IgM and IgG antibodies, as well as cell mediated immunity to Strep mutans can be correlated with DMF index of caries. Salivary IgA can also be found with the same co-relationship. (Roitt & Lehner 1983)

The effect of body defense mechanism on dental caries has been studied (Evans & Genco 1973, Evans, Emmings & Genco 1975) and pilot experiments using monkeys have been performed to determine whether a vaccine can be developed that would trigger off the defensive mechanism of the body in prevention of dental caries (Newman 1980).
1.4 EXPERIMENT IN HUMAN VOLUNTEER SUBJECTS ON IMMUNOLOGICAL RESPONSE IN SALIVA TO ACUPUNCTURE STIMULATION

The discussion in sections 1.3 and 1.4 has a common element which is the body resistance through the production of antibodies. Both in the case of dental caries and periodontal disease, evidence for the involvement of sIgA antibody is increasing. Since acupuncture has been reported to affect the body resistance level, it would be of great interest to determine in human subjects the levels of salivary antibodies after stimulation with acupuncture. It has been shown that human saliva contains a certain amount of IgA which is believed to be one of the major components in the human defense mechanism against bacterial invasion (Baer & Morris 1977).

In the case of advanced periodontal disease the IgA level is raised. The antibody level is affected by the amount of antigen (or plaque) present (Newman 1980).

This treatise reports on a study planned and carried out by the writer to collect the saliva from experimental subjects before and after electro-acupuncture stimulation on chosen ear acupuncture points with the level of antibody being measured by Radial Immuno Diffusion Technique. The exact set up of the experiment will be discussed in detail in the later chapters.

A null hypothesis has been proposed in this treatise that no relationship exists between the secretion activity of human salivary sIgA antibody level and acupuncture stimulation.
If the results of the experiment shows that a scientifically significant relationship exists, one can reject the null hypothesis and deduce that the relationship does exist. If it can be proved there is a link between sIgA levels and acupuncture, this would open a new insight to this ancient healing art of acupuncture and explain some of its magic "healing" power scientifically.
1.5 AIMS OF THE TREATISE

The aims of this treatise can be summarised as follows:

(a) To review the recent relevant literature and textbooks on acupuncture and immunology.

(b) To design and carry out a pilot study to determine saliva antibodies secretions of sIgA following electro-acupuncture stimulation.

(c) To analyse the results obtained from the study with particular respect to any correlation between acupuncture and immunology.

(d) To stimulate and encourage further studies by both medical and dental professional workers towards this ancient Chinese healing act of acupuncture.
2.1 HISTORY AND BACKGROUND OF THE ANCIENT CHINESE HEALING ART

Acupuncture (L.acus=needle, punctura=puncture) is an ancient art of healing dating back to China in the sixth century BC at a time when the practice of medicine was already firmly established.

Acupuncture was first described in the oldest known Chinese book on medicine, "The Yellow Emperor's Classic of Internal Medicine" (Huang Ti Nei Ching). This is the oldest complete compilation dealing with all aspects of the normal and abnormal functioning of the human body, with diagnosis, prognosis, therapy and regimen (Chapman 1974).

The text is cast in the form of a dialogue between the legendary Emperor Hung Ti (The Yellow Emperor) 2697-2597 BC and his medical advisors. It is a product of the second century BC, although containing materials much older, exactly how much older depends on the school of thought. This text was revised in an English translation by Veith (1966). (Robinson 1985)

Acupuncture being an embodiment of a Chinese philosophy known as Taoism, suffered constraint in various historical periods under different rulers. This is clearly evident during the Thang period (653 AD) when Buddhist and Taoist monks were forbidden to practise medicine. (Dembek 1972)

Medical knowledge was traditionally confined to the social elite and was systematised by the establishment of the Imperial Medical
College (620-630 AD) which together with medical colleges in all chief provincial cities, awarded publicly accepted medical degrees from then onward (Dembek 1972).

Western civilisation knew nothing of Chinese medicine until the 17th century when Louis XIV of France sent the first missionaries, the Jesuits of the Scientific Mission to Peking. They studied the institution of Chinese civilisations and were amazed by what the Chinese physicians revealed to them with supporting evidence. They coined the word 'Acupuncture'. The first European treatise on the subject was published by Reverend Father Harvieu in 1671 (Baldry 1986).

A few years later, a Latin book on acupuncture was written by Rev Father Cleyer. Since that time, more than two hundred authors have written books on the subject (Mann 1978).

The one man principally responsible for acupuncture being taken seriously in the West was a Frenchman, GS de Morant. At the age of twenty, he was sent to China by a bank and in time became the French Consul in Shanghai. In Yunnanfu, during a cholera epidemic, he was surprised to find that in the hospital "treatment of patients by means of needles had better results than the medicine at the time". He studied this form of therapy to the degree that in 1908, the Viceroy of Yunnan conferred on him the title of "Master Physician" (Dembek 1972).

Morant's first public demonstration of acupuncture took place in Saint Antonine Hospital, Paris, when he cured a woman whose arm had...
been paralysed for years by hemiplegia (Dembek 1972).

Morant's *Précis de la Varal Acupuncture Chinoise* (Synopsis of the True Chinese Acupuncture) appeared in 1934, and the first two volumes of *L'Acupuncture Chinoise* (Chinese Acupuncture) in 1939. For a quarter of a century, all French books on acupuncture were inspired solely by his work.

In the final years of his life, he was offered a position as Professor of Acupuncture at an American University, and in 1950 was the French candidate for the Nobel Prize in Physiology (Baldry 1986).
2.2 THE TRADITIONAL PHENOMENON OF ACUPUNCTURE

2.2.1 Yin and Yang

To comprehend the methods of acupuncture therapy, it is essential to have an understanding of the ancient Oriental philosophical basis for medical practice. The concepts are founded on the assumption that man is a micro cosmic image of the universe and subject to the same tension disruptions as nature itself (Chapman 1974).

The immutable course of nature, Tao, was thought to act through two component forces which were constantly struggling with one another, moving toward a state of unity and balance (Mann 1978).

These two opposing forces of nature are Yin and Yang in which Yin is negative and Yang is positive. But they can mean so much more. Any object, person, taste, feeling can be defined as being Yin or Yang.

For example, cold, passive, dark, feminine are Yin. Hot, active, bright and masculine are Yang.

The Yin and Yang must be in equilibrium. In order to maintain a status of homoeostasis, there should be no excessive exhaustion or over supply of certain vital body elements. For the state of health to be maintained properly, the proper proportion of energy input and output must exist. (Baldry 1986)

Disease is nothing else than a disequilibrium between the Yin and Yang. What can be named Yang for a certain object, can be named Yin if we compared it to another object. For instance, the abdomen is Yang compared with the lower body, but it is Yin if we compare it with the upper extremities (Mann 1978).
Some examples of Yin and Yang (Deschepper 1985).

(a) The Ying and Yang in time:

The night is the darkness, the cold period, and the state of sleep, - hence related to Yin.

The day is the light, the warmth, the state of alertness, - hence the Yang.

If we put these motions on a circle, at the tip, which is Yang as opposed to the bottom, Yin. We find the maximum Yang in midday and maximum Yin in midnight. Going from bottom to top, there is an increase of Yang and at the same time Yin is decreasing.

(b) The Ying and Yang in seasons:

Summer - fullness of Yang and minimum of Yin. 
Winter - fullness of Yin and minimum of Yang.
Spring - increasing of Yang, decreasing of Yin.
Fall - increasing of Yin, decreasing of Yang.

(c) The Yin and Yang in space:

The Yin is below, at the interior and at rest. The Yang is above, at the exterior and movement.

We can see instantly some very practical deduction; heaven is Yang, earth is Yin. The human being is in contact with both. The head is the Yang, the lower extremities is the Yin. All these are important notions and are the basis of reasoning frequently used in acupuncture.

(d) The Ying and Yang in daily use:

Yang goes to the exterior which is expansion,
Yin goes to the interior which is condensation.

Some examples are listed: (Deschepper 1985)

<table>
<thead>
<tr>
<th>Yang</th>
<th>Yin</th>
</tr>
</thead>
<tbody>
<tr>
<td>the back</td>
<td>the front of body</td>
</tr>
<tr>
<td>the limbs</td>
<td>the trunk</td>
</tr>
<tr>
<td>skin</td>
<td>mucosae</td>
</tr>
<tr>
<td>head</td>
<td>feet</td>
</tr>
<tr>
<td>male</td>
<td>female</td>
</tr>
</tbody>
</table>
2.2.2 Chi - The Flow of Internal Body Energy

As there are twelve months in the year, the human body was thought to consist of twelve physiologic systems. (Man & Chen 1973)

Associated with each of these systems was a major visceral organ and a pathway of energy flow in the body termed Ching-lo (ie a meridian) (Chapman 1974).

The twelve meridian systems that associate with the twelve organs included the Bladder (B), Pericardium (P), Gall Bladder (G), Heart (H), Kidneys (K), Large Intestine (LI), Liver (Liv), Lung (L), Spleen (Sp), Stomach (S), Small Intestine (SI) and Triple Energiser (TE) (serves for warmth and nervous energy) (WHO 1984).

The meridian were made up of six Yin and six Yang, plus two trunk meridians in the midline of the front and the back of the body. They terminated at the fingertips or toes (Gross & Morse 1976).

A vital life force, the 'Chi', was thought to circulate through these pathways according to a circadian rhythm (Mann 1978).

Disturbance in the flow of this vital life force would occur as a result of disharmonies in the nature form or elements with the individual (Gross & Morse 1976).

Such disturbance if not corrected, would eventually lead to a disease state affecting either the organ associated with the pathway in which the blockage of the Chi occurred or an area at the same point along that pathway (Chapman 1974).

Acupuncture therapy was intended by ancient Chinese physicians to
correct blockages or excesses in the flow of the vital life force and to correct disharmonies or imbalance in the vital forces and elements can effect normal physiological functions of the body.

Each meridian was thought to run deeply within the body, although it surfaced occasionally (Chapman 1974).

When an individual had pain, this was considered to be a disease. Acute pain was related to excess Chi in some body areas and was treated by draining off the excess Chi. Chronic pain was thought to result from a deficiency of Chi in some part of the twelve physiologic system. It was treated by increasing the flow of Chi in that area (Gross & Morse 1976).

By acupuncture treatment, one could drain or increase the flow of Chi from one part of a system to another (Deschepper 1985).
2.2.3 Bio-rhythm

The interaction of Chi and transformation of air, food and water into Chi, blood and other substance form a cycle of changes. This is the transformation at work in the rhythms of growth and decay, in the changes from a flower to fruit or a child to an old man.

According to Mann (1978), the lungs are principally concerned with the Chi of the whole body. The spleen is concerned with the middle Chi, via its coupled organ the stomach which obtains the Chi from food. The kidney Chi determines the hereditary constitution, as the production of semen and ova is largely determined by the kidney.

The meridian cycle begins with that of the lungs as chi enters there. Also the Chi from the digestion of food and water as the stomach goes via the spleen to the lungs. Thus from the lungs, Chi of both sources is distributed around the body via the meridians in a certain order. Starting with the first point (or point of entry of the lung meridian), thence to the last point, (or point of exit) of the lung meridian, to the first point, or point of entry of the larger intestine meridian.

Figure 1 is a summary from Mann (1978) of the Meridian Cycle.
There are two major kinds of Chi (Mann 1978):

1. Nourishing Chi - Chi is not stable in the body; it circulates much as the blood. This circulation is of two main types, that of nourishing Chi through the meridians and blood vessels, and that of protecting Chi, between the skin and the flesh in the subcutaneous tissues.

2. Protecting Chi - the protecting Chi complements the nourishing Chi and, like it is formed by the digestion of food and water in the stomach (and spleen) and distributed hence to the rest of the body.

While the nourishing Chi is distilled from the purest elements, the protecting Chi emerges from the coarser products of digestion, and because of this crude origin has rougher and more aggressive properties. It therefore cannot penetrate the delicate meridians and vessels but instead circulates in the subcutaneous tissues. The protecting Chi warms the subcutaneous tissues, moistens the skin, controls the opening and closing of the pores and nourishes the space between the skin and the flesh. But its most important function is the protection of the body from 'outside invading evils'. If, for example, wind and cold invade the body, it meets the invasion by producing the desire for warmth and the manifestations of fever. Sweat is emitted, the fever subsides and the invading forces are dispersed. If, on the other hand, the invasion is successful, the patient will fall a victim to the disease. When the protecting Chi is too weak to permeate the subcutaneous tissue, the meridians will become empty and hollow, the flow of blood sluggish and uneven, the skin and flesh inadequately nourished. The patient may then become a sufferer from rheumatism; or, if the wind is cold and damp remaining in the body affect the meridians, vessels and joints, form an attack of arthritis.
The nourishing Chi comes into the same category as the Yin since it is composed of a rarefied substance and circulates with the blood in the interior of the body. The protecting Chi could be classified under Yang, since it is composed of coarser elements, circulates in the surface of the body and is associated with the blood stream but with Chi.

It is said that the protecting Chi every 24 hours completes 50 cycles in the body, 25 cycles parallel with the Yang during the day and 25 parallel with the Yin during the night. When it circulates through the Yang meridians in the daytime, it passes through those of the large intestine, the stomach, the triple warmer, the gall bladder, the small intestine and the bladder, in that order. Similarly at night, when it circulates through the Yin meridians, it passes from the kidney to the heart and hence to the lungs, the liver and finally the spleen. (Mann 1978)
2.3 AURICULAR ACUPUNCTURE

2.3.1 Physiological Foundations of Auricular Acupuncture

Physiologically speaking, there exists an intrinsic relationship between the auricle and the human body; and pathologically speaking, there also exists a fixed pattern of pathological responses on the auricle. (Lu 1975)

When diseases attack the body, sensitive points will show up in the corresponding regions on the auricle. Needling at such specially marked sensitive points to treat diseases is called "auricular acupuncture".

Auricular acupuncture has been developed by experience accumulated by the Chinese throughout years of prolonged struggle with diseases.

There are numerous advantages of auricular acupuncture. For one thing, auricular acupuncture responds to a wide range of symptoms and effects instant cure; secondly, it has few side effects, is easy to administer, and also very economical in operation; thirdly, the essentials of auricular acupuncture are fairly easy to understand and thus not difficult to popularise (Lu & Needham 1978).
2.3.2 Points in Auricular Acupuncture

Auricular points - When the internal organs of the human body are diseased, or the body itself is diseased, they will show up as responses in the fixed regions of the auricle. Such responses often take the form of painful symptoms, or lowering of the electric resistance at the ear point, and occasionally accompanied by change in shape and colour. Needling is to be administered at such points, which are called auricular points.

Patterns of auricular points distribution - there are fixed patterns in the distribution of auricular points. Generally speaking, the auricle may be compared to an upsidedown fetus in the uterus with the head at the bottom and the extremities on the top, which may be described as follows:

The lobe corresponds to the face,
the antitragus corresponds to the head,
the cavity of concha corresponds to internal organs of the thorax,
cyma concha auriculae corresponds to internal organs in the abdomen,
the limb of helix corresponds to the diaphragm,
the anthelix corresponds to the trunk,
the concha edge of the anthelix corresponds to the spinal column,
the upper limb of the anthelix corresponds to the lower extremities,
the lower limb of the anthelix corresponds to the buttock,
the cumba concha corresponds to the upper extremities,
the triangular fossa of auricle corresponds to the sex organs,
the intertragic notch corresponds to the internal secretion,
the posterior surface of the auricle corresponds to the back (Lu & Needham 1978).

The ear point of internal secretion is selected for this stimulation experiment because it reflects the secretory glands including the major and minor salivary glands of the oral cavity.

The location of the internal secretion point is shown in Figure 2.

It is at the inferior vertex of the concha and reflects the region
of the head and neck parts of the body. There are no other specific acupuncture points located at the nearby areas except the lung points which are about 3-5mm above the internal secretion point.

Xu and Hou (1979) of the Nanjing Medical College reported that the auricular points of the internal organs are distributed mainly in agreement with the concha of the auricular branch of the vagus nerve. In compliance with individual variation of the distribution of the vagus nerve to the auricule, the location of the acupuncture points have also their relation specifications (Chapman 1974).

Experimental evidence has also supported that the use of clasps in the conduction of electric current could be used to detect certain points due to their lower resistance nature (Pomeranz, Cheng & Law 1977). Therapeutic effects are produced by ear needling at these low resistance points (Chapman, Gehrig & Wilson 1975).

The therapeutic effects introduced by electro-auricular acupuncture are closely related to regulatory function of the vagus and sensory nerves of the auriculae. The impulse by needling sensation or by electric signal is first conducted to the nucleus tractus pinalis trigenium then to reticular formation (Kaada, Jorum, Sagvolden & Ansethwoen 1979). The latter, being a higher sensory regulating centre affected by ear needling, exerts greater influence on the regulating function and sensitivity threshold of the internal organs (Xu & Hou 1979).
Figure 2 Ear Acupuncture Points
(Chapman 1974)
2.4 TECHNIQUE IN ACUPUNCTURE STIMULATIONS

There are five major techniques in modern day acupuncture practice.

1 Use of stainless steel acupuncture needles with or without manual or electric stimulations.

2 Use of finger pressure on selected acupuncture points. This is also called acupressure.

3 Injection of normal saline or vitamin B12 at the ear points to act as a stimulation to the point (Chapman 1974).

4 Moxabustion - this is to use the burning mugwort (a plant named artemisia vulgaris) rolled in tubes and ignited before applying to warm the selected skin points at a distance about two centimeters above the skin surface (Dembek 1972).

5 Use of electric stimulation on the skin surface above the selected acupuncture point by means of a conducting electrode. This technique is the most popular in modern use since there is no needle employed and the patient feels less discomfort during treatment (Dembek 1972). Another name for this method is transcutaneous nerve stimulation (Han & Terenius 1982).

All of these methods require an induction time of at least 15-30 minutes (Dubner 1976). The use of surface electrodes for acupuncture stimulation (i.e. the transcutaneous nerve stimulation) is employed by the writer in this saliva stimulation experiment and the induction time would be 20 minutes for all patients.

The term acupuncture is used for a number of related, but in terms of mode of action not necessarily identical, techniques.

It is therefore essential to define the stimulus procedure, as being mechanical (manipulation of needles), electrical via stimulation of needles (electro-acupuncture EA) or via surface electrodes (transcutaneous nerve stimulation, TNS) (Sjolund & Eriksson 1979).
The stimulus parameters may vary considerably according to the intensity, duration and frequency (Han & Terenius 1982).

The frequency can be low (less than 10 Hz) or high (100 Hz or more) and the intensity can be weak enough just to activate Ap fibres or high enough to activate Ag or even C-fibres. (Anderson, Ericsson, Holmgren & Lindqvist 1973).

Differences in stimulation characteristics lead to differences in the induced effects, and probably also in terms of mechanism of action. (Mayer, Price & Rafii 1977, Anderson 1979).

It is commonly believed that low frequency stimulation, which causes muscle contraction, mimics acupuncture in giving generalised analgesia with a prolonged induction latency period and prolonged after effect (Sjolund & Eriksson 1979).

High frequency stimulation of lower intensity (so called TNS) causes local or segmental analgesia and may activate other afferent pathways and the central nervous system (Wall & Sweet 1967).

It is for this reason that high frequency stimulation of about 100 Hz was employed in the present acupuncture study since the secretory function of salivary glands is affected by the central nervous system.
3.1 ACUPUNCTURE IN TREATMENT OF GENERAL MEDICAL COMPLAINTS

It would be going too far to say that there were no limits to acupuncture used in traditional Chinese medicine, but there can be no question that it was employed in many illnesses caused by the invasion of pathogenic organisms, that are well known today, as well as for those arising from malfunctions of various parts of the body. We may have a better picture of the situation if we attempt a classification of disease made by Lu and Needham (1978) as shown on Table 1.

It can be seen that the large number of disease entities fall under the heading of malfunction, whether in origin endogenous or exogenous. Such diseases as typhus, typhoid, cerebro-spinal meningitis, the various forms of encephalitis, or malaria or schistosomiasis, were certainly treated by acupuncture in pre-modern times, but it must always be remembered that the medieval physicians of China had a remarkable armamentarium of drugs with active principles at their disposal, and acupuncture was rarely used alone. Anciently, acupuncture was probably applied in cataract treatment, for neoplastic growths, and for the intractable pain of malignancy, situations where it is hardly used at all today (Lu & Needham 1978).

It may be considered that acupuncture works best where severe pain is involved in the syndrome, or the chronic affections respond better than acute ones (Melzack, Stillwell & Fox 1977).
<table>
<thead>
<tr>
<th>Pathogenic organisms</th>
<th>Allergies</th>
<th>Malfunctions</th>
<th>Geriatric conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacillary dysentery</td>
<td>asthma</td>
<td>gastric and duodenal ulcer</td>
<td>rheumatism arthritis hemiplegia prostatitis cerebral haemorrhage</td>
</tr>
<tr>
<td>cholera</td>
<td>hay fever</td>
<td>ulcer</td>
<td>arthritis hemiplegia prostatitis cerebral haemorrhage</td>
</tr>
<tr>
<td>ethmoidal sinusitis</td>
<td>other allergies</td>
<td>nephritis hepatitis lumbago</td>
<td>prostatitis cerebral haemorrhage</td>
</tr>
<tr>
<td>chronic colitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hepatitis</td>
<td>appendicitis</td>
<td>fibrositis</td>
<td>sciatica</td>
</tr>
<tr>
<td>post-herpetic neuralgia</td>
<td></td>
<td>migrane</td>
<td>sciatica</td>
</tr>
<tr>
<td>post-polio myelitic paralysis</td>
<td>trigeminal</td>
<td>sciatica</td>
<td></td>
</tr>
<tr>
<td>psoriasis</td>
<td></td>
<td>neuralgia</td>
<td>sciatica</td>
</tr>
<tr>
<td>tuberculous glands</td>
<td></td>
<td>haemorrhoids</td>
<td>sciatica</td>
</tr>
<tr>
<td>deaf-mutism (due to degeneration of auditory nerve)</td>
<td>varicose veins</td>
<td>sciatica</td>
<td></td>
</tr>
<tr>
<td>other auditory disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tonsilitis</td>
<td></td>
<td>haemorrhoids</td>
<td>sciatica</td>
</tr>
<tr>
<td>bronchitis</td>
<td></td>
<td>sciatica</td>
<td>sciatica</td>
</tr>
<tr>
<td>conjunctivitis</td>
<td></td>
<td>glaucoma</td>
<td>sciatica</td>
</tr>
<tr>
<td>laryngitis</td>
<td></td>
<td>dysmenorrhoea</td>
<td>sciatica</td>
</tr>
<tr>
<td>acne</td>
<td></td>
<td>cervical spondylosis</td>
<td>sciatica</td>
</tr>
<tr>
<td>eczema</td>
<td></td>
<td>&quot;slipped disc&quot;</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Parkinson's disease</td>
<td>sciatica</td>
</tr>
<tr>
<td>Dietary or Toxic beri-beri</td>
<td>Bell's palsy</td>
<td>goitre</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>epistaxis</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>haematemesis</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>haematuria</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>melaena</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>insomnia</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tachycardia</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bradycardia</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>renal colic</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>jaundice</td>
<td>sciatica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paget's disease (osteitis deformans)</td>
<td>sciatica</td>
</tr>
</tbody>
</table>
For illnesses that are primarily psychiatric, acupuncture is certainly still used in China today, though on the whole as an adjuvant to group therapy and the social approach for psychotherapy. On the borderline here, remarkable results are being reported for the cure of drug addiction.

The universal reproach directed against therapeutic acupuncture by modern scientific medicine is the lack of statistical evidence which would remove it from the sphere of folk belief and suggestion. The absence of adequate clinical control experiments, the existence of the 'placebo effect', and the relative paucity of quantitative remission and follow-up data even in contemporary China, is indeed a hindrance to many in taking therapeutic acupuncture seriously.

It is true that the lack of sophisticated statistical analysis hinders many scientific researchers and attempts to estimate the real value of therapeutic acupuncture. Yet, some valuable statistical data has become available during the past ten years from a number of modern medical researchers. For example, a group of acupuncture practitioners in London reported on one thousand cases treated by acupuncture, the age of the patients ranging from 3 weeks to 92 years (Mann 1973). There was cure or great improvement in 439 cases, and moderate improvement or considerable alleviation on a further 290 cases, so that one could say that 73 percent showed marked responses to the treatment.
A more extensive set of figures for treatment given in the Soviet Union during the five years before 1962 covered 10,719 patients. The results, as reported by Lu and Needham (1978) are shown in Table 2.

The two first grades of cure or marked relief was obtained in 70 percent of cases which was quite close to the figure obtained by the group in London. In this Soviet Union series the conditions were mainly malfunctions such as gastric ulcer, hypertension, stenocardia and incipient glaucoma, or allergic affections such as bronchial asthma.

About the same time Canas, describing 122 cases in private practice in Paris, reported 86 percent for the sum of the two first grades, and 68 percent for the first grade alone. His cases included lumbago, sciatica, torticollis, hydrarthrosis, sinusitis, acute laryngitis, expistaxis and ulcerating varicose veins (Lu & Needham 1978) (Table 3).

A very different type of morbidity was tackled by a group of Chinese physicians who reported on 63 cases of bacillary dysentery (Lu & Needham 1978). Somewhat to their surprise, acupuncture and moxibus- tion proved to be more effective than either sulpha-guanidine, phage or the traditional Chinese drugs (Table 4). All the patients were cured, there were no relapses during the following two years, and the treatment was adopted as standard.

Another statistical study which is worth examining is the experience of hospitals in Shanghai, Canton and Kirin with patients with appendicitis as reported by Lu & Needham (1978) (Table 5).
Table 2  Acupuncture Results from the Soviet Union  
(Researcher Vogralik cited by Lu & Needham 1978)

<table>
<thead>
<tr>
<th>Outcome of Acupuncture Treatment</th>
<th>No. of Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Cure very significant relief with long remission</td>
<td>3503</td>
<td>32.7</td>
</tr>
<tr>
<td>II Marked relief, with shorter remission</td>
<td>3986</td>
<td>37.1</td>
</tr>
<tr>
<td>III Milder relief</td>
<td>2045</td>
<td>19.1</td>
</tr>
<tr>
<td>IV No effect</td>
<td>1185</td>
<td>11.1</td>
</tr>
<tr>
<td><strong>Total Cases</strong></td>
<td><strong>10719</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Table 3  Acupuncture Results from Paris  
(Canas cited by Lu & Needham 1978 p200)

<table>
<thead>
<tr>
<th>Country Ref.</th>
<th>No. of Cases</th>
<th>% Effect by Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Cure/Marked Relief</td>
<td>Slight Effect</td>
</tr>
<tr>
<td>UK Mann, Whitaker et al.</td>
<td>1000</td>
<td>43.9</td>
</tr>
<tr>
<td>USSR Vogralik</td>
<td>10719</td>
<td>32.9</td>
</tr>
<tr>
<td>France Canas</td>
<td>120</td>
<td>68.0</td>
</tr>
<tr>
<td>USA Anon.</td>
<td>660</td>
<td>55.7</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>50.7</strong></td>
<td><strong>25.05</strong></td>
</tr>
<tr>
<td><strong>Average omitting French</strong></td>
<td><strong>44.1</strong></td>
<td><strong>27.5</strong></td>
</tr>
</tbody>
</table>
### Table 4  Acupuncture Results from China  
(Cited by Lu & Needham 1978 p200)

<table>
<thead>
<tr>
<th>Bacillary Dysentery</th>
<th>Acupuncture and moxa</th>
<th>Sulpha-guanidine</th>
<th>Phage</th>
<th>Chinese drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>63 cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to subsidence of symptoms</td>
<td>3.2</td>
<td>3.6</td>
<td>4.6</td>
<td>9.5</td>
</tr>
<tr>
<td>Days before return of faeces to normality</td>
<td>4.6</td>
<td>6.2</td>
<td>6.0</td>
<td>9.3</td>
</tr>
</tbody>
</table>

### Table 5  Acupuncture Results from Shanghai  
(Cited by Lu & Needham 1978 p201)

Appendicitis treated with acupuncture Chungshan Hospital, Shanghai.

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Cured (%)</th>
<th>Improved (%)</th>
<th>No. Effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple uncomplicated</td>
<td>500</td>
<td>323 (64.6%)</td>
<td>139 (27.8%)</td>
</tr>
<tr>
<td>With local peritonitis</td>
<td>78</td>
<td>28 (35.9%)</td>
<td>17 (21.8%)</td>
</tr>
<tr>
<td>With appendicular abscess</td>
<td>12</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Follow-up within 1.5 years

<table>
<thead>
<tr>
<th>Number of Cases</th>
<th>No Recurrence (%)</th>
<th>Chronic Symptoms (%)</th>
<th>Recurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>391</td>
<td>118 (30.2%)</td>
<td>108 (27.6%)</td>
<td>165 (42.2%)</td>
</tr>
<tr>
<td>40</td>
<td>17 (42.5%)</td>
<td>9 (28.5%)</td>
<td>14 (35.0%)</td>
</tr>
<tr>
<td>11</td>
<td>5 (42.5%)</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Follow-up within 4 years (1530 cases)

<table>
<thead>
<tr>
<th>No Recurrence (%)</th>
<th>Chronic Symptoms (%)</th>
<th>Recurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.3%</td>
<td>38.4%</td>
<td>41.3%</td>
</tr>
</tbody>
</table>
3.2 ACUPUNCTURE IN DENTISTRY

Another area in which acupuncture has potential applicability in contemporary dentistry is in the treatment of chronic pain (Council of Dental Research 1974).

In some instances chronic pain appears concomitant to demonstrable organic tissue damage, (eg: toothaches), but in other patients it appears when tissue damage is minimal or lacking (eg: temporomandibular joint pain).

Some chronic pains such as TMJ pain are often highly resistant to drug and even surgical therapy, and many patients who have undergone multiple operations or extensive drug therapy suffer from iatrogenic problems (Chapman 1974).

The most dramatic aspect of contemporary Chinese practice is the prevention of painful sensation normally with surgical and dental procedure by means of acupuncture stimulation (Beecher 1974). Over four hundred thousand operations have been performed under acupuncture with a success rate of about 80 percent to reduce pain during operations from more than 100 types of surgery (Chapman 1974).

Dentists at the Fourth Hospital at Hsi'an Chu studied the effect of auricular acupuncture in the extraction of 1250 teeth. No pain was evident in 65.6 percent of extraction and in 31.5 percent only slight pain was reported. Some 2.9 percent were classified as a failure (Chapman 1974)
Conventional nerve block by local anaesthetic was studied in 110 extractions by the same dentist who found 81.8 percent showed no pain and 15.5 percent showed slight pain, only 3 percent were reported a failure (Chapman 1974).

When normal saline is injected into the acupuncture point in the ear tooth extraction point in Figure 2, heat paresthesia followed the injection, and it is believed that the presence of the heat paresthesia insures the success of the anaesthetic. Studying an additional 530 patients, Hsi'an dentists found that 81.3 percent of the 290 reporting the heat sensation after injection were totally pain free during extraction of teeth and another 18 percent of these same patients had only slight pain. This indicates the patients with heat paresthesia yielded a success rate comparable to the obtained with nerve block with local anaesthetic agent (Chapman 1974).

In the 240 patients who did not report heat sensation, 64.6 percent were pain free and another 32.5 percent had slight pain during extraction of teeth.

These observations suggested that acupuncture is effective in the prevention of dental pain and that heat sensation following injection of saline into ear acupuncture point greatly increases the probability of successful analgesia (Chapman 1974).
At the University of Washington, Chapman (1974) and an oral surgeon (JD Gehrig) have initiated pilot research on acupuncture anaesthetic for dentistry. Several procedures have been carried out following needling of the body point or injection of saline to the ear. These include general drilling, placement of gold crowns, pin amalgam build up and extraction of teeth. All of the procedures have been free of pain or nearly so. (Chapman 1974)

Following injection of vitamin B into several sites on the pinna of the ear, Dr Gehrig was able to extract a maxillary third molar from a 35 year old British anaesthesiologist. The extraction resulted in an opening into the floor of maxillary sinus which required suturing. The procedure was largely painless.

The patient returned two weeks later for extraction of the contra-lateral third molar, and volunteered to reject an anaesthetic to provide a control for the acupuncture extraction done 2 weeks previously. The sensation during attempted extraction proved to be painful and required injection of 2ml of local anaesthetic solution before the molar could be removed painlessly.

However, Chapman concluded that acupuncture is time consuming and the nerve block procedures are fast and highly efficient for pain prevention and therefore outweigh the application of acupuncture as a routine dental anaesthetic method at present. Yet acupuncture is a harmless and inexpensive form of therapy which may prove useful with certain patients when other procedures have failed or are contra-indicated (Chapman, Murphy & Butler 1973).
Dubner in 1976 also reported eleven cases of painless tooth extraction seen in China under acupuncture anaesthetic and concluded that acupuncture anaesthetic is particularly useful for patients with heart disease and those instances where the possibility exists of a dry socket after extraction. Local anaesthetic used in dentistry usually contains epinephrine which may contribute to a reduction of blood supply to the extraction site and subsequent blood clot breakdown.
3.3 THE PROPOSED MECHANISMS OF HOW ACUPUNCTURE CAN STOP PAIN

3.3.1 The Gate Theory

The Gate theory was developed by Melzack and Wall (1965) and it has been used to interpret acupuncture. In this theory, peripheral impulses are believed to travel centrally along two pathways via small, myelinated delta fibres, which carry the pain sensation and unmyelinated C fibres, which transmit the sensations of pressure and touch (Gross & Morse 1976).

There is a 'gate' in the substantia of the spinal cord that can close out the central interpretation of painful stimuli if there is an increased activity of the large C fibres. Transmission by the small fibres which carry pain may be modified by increasing the activity of the large fibres which carry sensation of touch and pressure. An increase in the activity of the large fibres can act upon the gate in such a way that the information carried by small fibres is gated out (Chapman 1974).

Acupuncture manipulation or stimulation is supposed to generate a barrage of pressure activity that can close the spinal cord 'gate' and decrease the central perception of pain (Melzack & Wall 1965).

Man and Chen (1973) proposed that in addition to the gate in the substantia gelatinosa of the spinal cord, there is a second gate at the thalamus. The closing of the second gate prevents pain perception from areas innervated by cranial nerves. Manipulating acupuncture needles to a peripheral branch of the cranial nerve effectively closes the thalamic gate.
3.3.2 The Endorphins Concept

Early studies revealed that the analgesic effect of acupuncture was blocked by procaine infiltration into acupuncture points and that it was not possible to induce analgesia in paraplegic or hemiplegic patients, pointing to the importance of afferent transmission. (Han & Terenius 1982)

Involvement of certain transmission mediators in the central nervous system was suggested from experiments when the cerebro-spinal fluid of donor rabbits given acupuncture was infused into the cerebral ventricles of recipient rabbits, increasing their pain threshold. (Han & Terenius 1982)

Anderson et al (1973) verified the effect of acupuncture on thresholds to pain that was experimentally induced by tooth pulp stimulation in healthy volunteers.

By applying signal detection therapy, Chapman et al (1973) proved the existence of a biological component for acupuncture as well as a central biasing effect when he compared the effect of dental analgesia acupuncture with 33 percent nitrous oxide relative analgesia.

However, the most exciting finding was the report by Mayer, Price and Rafii (1977) that the analgesic effect of acupuncture on electrically induced tooth pulp in man could be partly reversed by naloxone, a specific opiate substance, indicating the participation of endogenous opioids endorphins in acupuncture analgesia.

The results of Mayer and associates were confirmed in studies by Sjolund and Eriksson (1979) on volunteer patients with chronic pain
and by Pomeranz and Chiu (1976) in laboratory animals. The isolation of opiate-like peptides from the central nervous system, coupled with the discovery of specific receptors for these endogenous substances, has provided the basis for a new theory of modulation of pain (Hughes et al 1975).

There are two essential approaches which have been taken to establish the role of endorphins in acupuncture analgesia. The principle approach uses naloxone, under the assumption that it is a specific antagonist for opioid receptors mediating analgesia. The second approach is the direct measurement in the brain, or cerebral fluid (Han & Terenius 1982).

Reports that acupuncture analgesia can be temporarily and at least partly blocked by naloxone are abundant. Pain relieving action of acupuncture was reversed by intravenous doses of 0.4 - 0.8mg of naloxone (Han & Terenius 1982).

Cheng and Pomeranz (1979, 1980) reported that the analgesic effect of electro-acupuncture in mice would be completely reversed by naloxone or other opiate receptor antagonists including naltrexone, cyclozocine and piprenorphine.

Pomeranz, Cheng and Law (1977) studied the effect of acupuncture on electro-physiological and behavioural responses to noxious stimuli in mice. The observation that the analgesic effect of acupuncture was abolished after hypo-physectomy led Pomeranz to the conclusion that a morphine like pituitary peptide mediates acupuncture analgesia.
3.3.3 Psychological/Hypnotic Aspect

According to some western scientists acupuncture is merely a form of hypnosis or is related to the placebo effect (Alexander 1973).

This placebo effect can be demonstrated by the experiment carried out by Chapman in 1974 in which he had shown that in almost 60% of the cases of acupuncture dental analgesia for relief of post operative pain, a placebo effect was shown to be as effective as morphine administration for the relief of post operative dental pain. He had suspected that acupuncture may be somewhat similar to that of the 18th century mesmerism which was the forerunner of hypnosis. (Chapman 1974)

The Chinese people are alleged to be stoical, are ready to accept indoctrination, and are faithful believers in their leaders and other people of authority. It is well known that hypnosis is more effective when a person believes in and accepts the authoritarian role of the hypnotist (Gross & Morse 1976).

In many cases it is reported that in addition to acupuncture, the patient receives narcotic analgesic and or sedative before the acupuncture. It may be that the calming effect of these drugs plus suggestion are sufficient to account for the analgesic effect of acupuncture.

Barber and Mayer (1977) studied the neural mechanism of hypnotic analgesic procedures in experimental and clinical dental pain and suggested that acupuncture may act as a placebo or by suggestion. The results concerning naloxone reversal of acupuncture analgesia
should be considered in this regard. However, hypnotic analgesia is generally not reversed by naloxone, and placebo analgesia can only be reversed by very high doses of naloxone (7.5 - 10mg) but not by doses effective in narcotic overdosing (0.4 - 2mg) (Levin, Gorden & Field 1979).

This suggests a difference in underlying mechanisms between acupuncture analgesic on the one hand, and placebo and hypnotic analgesia on the other (Mihie & Binkert 1978).
3.4 THE PROPOSED MECHANISM OF HOW ACUPUNCTURE CAN HEAL DISEASE

3.4.1 The Effect on Immunological Response

Lu and Needham (1978) proposed that acupuncture, apart from its actions in terms of central and autonomic nervous conduction, effects neuro humoral responses.

They believe the latter effect may play an important role in the therapeutic effects of the cells of the reticulo-endothelial system, largely in the spleen, which can generate clones of cells producing antibodies against almost any foreign protein molecule.

Thoughtful acupuncture physicians in the early fifties already saw fairly clearly that these must be the way in which the "natural healing power of the body" could be intensified. (Lu & Needham 1978)

At the present time, a good deal of research on these subjects is going on; largely in China and Japan (Lu & Needham 1978). Quite a lot has been done on changes in the total leucocyte count after acupuncture, and marked increases found both in China, Japan and the West. This increase is of the order of 40-60 percent and is relatively transient, occurring whether or not the stimulation sites were classical acupuncture points, and increase is more marked after electro-acupuncture than manual stimulation (Zhou & Cao 1979).

This phenomenon of acupuncture affecting the human body defense mechanism has agreed with Lu and Needham's theory of antibody production and enhancement of phagocytosis by white blood cell after acupuncture stimulations. (Lu & Needham 1978).
Bi and Gao (1979) and their collaborators have found the increase to be greatest in the mono-nuclear leucocytes which are especially connected with the reticulo-endothelial system.

In China, the work of Sian Neurological Hospital Unit established a four fold increase in typhoid and paratyphoid antibodies in rabbits after acupuncture and a six fold increase after electro-acupuncture. (Lu & Needham 1978)

Acupuncture also causes prolonged increases in the antibody titre of the blood, both in human patients and experimental animals. Serum complement was also reported to be markedly raised when compared with controls (Lu & Needham 1978).

The work of Omura (1976) has provided a basis for understanding the immunological effects induced by acupuncture. He found that in most subjects there are three consecutive changing phases in the vaso constriction present after acupuncture.

The first phase is vaso constriction, next a phase of quasicontrol and lastly, a vasdilation. With the use of infrared thermography, Omura showed increases in skin temperature from 19 to 25 degrees celous. Changes in blood chemistry and in complete blood cell count often begin appearing approximately four hours after acupuncture and can become significant after 24 hours in some patients.

The changes in complete blood cell counts are characterised by a segmented neutrophilia, lymphoctopenia and an eosinopenia.
Song, Zhang, Sun and Yu (1979) in China studied surgical specimens including four lower and two upper amputated human limbs from diabetic patients.

Before amputation, acupuncture was given to each patient. After the needling reaction was well obtained, one percent solution of methylene blue was injected into each point as an indicator. After amputation, specimens of 0.5 x 0.5 x 0.7 cm were taken from the amputated levels with the blue spot as its centre. Specimens were fixed and sectioned to 5 μm thickness and stained according to Riley’s method (Lu & Needham 1978).

Under 400x microscopic observation they were able to conclude that mast cells in the acupuncture point were significantly more than in the control sites. This finding has provided further support of the immunological basis of acupuncture.

Other important findings in China during recent years on the immunological effect of acupuncture are numerous. Some of the most interesting findings have been summarised as follows.

Zhao and Wang (1979) studied the effect of electro-acupuncture on cell mediated immunological response in rabbits in which they found there is enhancement in function of the cell mediated immunity of the rabbit which suggests the host resistance to pathogenic agents is elevated after acupuncture.

The Research Group of Acupuncture of Department of Histology and Biology in Hanan Medical College reported in 1979 that acupuncture shows some regulating effect on cell mediated immune response and
the enhancement effect usually persists at least for 24 hours.

(Bi, Xu, Gao 1979)

Bi, Xu and Gao (1979) studied the anti-inflammatory effect of acupuncture in Zhonggngo Medical College and found there is an increase in local circulation and lymphatic drainage at and around the stimulation area. Ma and Chang (1979) also found that acupuncture can also promote transformation of normal T lymphocytes into lymphoblasts.

In a preliminary study on the effect of moxibustion on phagocytosis activities, the Biochemistry Laboratory, Shanghai Medical College found there are increases in phagocytic function of polymorphonuclear cells after acupuncture stimulation.

Zhang et al (1979) of the Department of Microbiology, Shanghai Medical College concluded that there is enhancement of antibodies forming and particularly immunoglobin M after moxibustion on acupuncture points.

Sin (1983) demonstrated in mice that acupuncture stimulation can cause an increase in the phagocytic activity, leading to an increase in the rate of clearance of foreign substances injected into the blood of mice.

In 1983 Sin et al were able to show that there was a significant decrease in the number of exudate leucocytes in the inflammatory cavity of subcutaneous air pouch of rats after acupuncture stimulation. An increase in the peripheral white blood cell count was also observed. They have concluded that acupuncture treatment can reduce leucocyte adherence to vascular endothelial cells.
The report by Ding et al (1983) also provides valuable information on the immunological effect of acupuncture. Total and differential white cell counts, E rosetting and lymphocyte transformation were measured in nine healthy human subjects before and immediately after acupuncture. Lymphocyte transformation in the unstimulated culture showed a clear increase 3 hours post-acupuncture as compared to the pre-acupuncture levels in eight out of nine subjects.

Hatai et al (1977) studied the morphological changes of the lymph node and the influence on the immune mechanism in animals caused by acupuncture stimulation. Results showed that the antibody value in the experimental group of animals was much higher than in the control group. Other changes were also observed. These included enlargement of lymph sinus, increase in mast cells and a picture of degranulation. A rapid increase of plasma cells was also observed after 48 hours.

Rogers and Bossy (1981) reported stimulation by acupuncture caused increased leukocyte phagocytosis and increase in specific and non-specific antibodies, including complements and the appearance of a bactericidal compound in plasma. They proposed the mechanism involved in the body's response to acupuncture may include the autonomic nervous system and defence control centres in the hypothalamus.

Li (1979) of the Fifth Hospital of Ningxia, China, observed there was significant increased gammaglobulin in patients after electric needling of nerve trunks. This increase was most significant 3 hours after needling and become normal the next day.
The research by the Festering Moxibustion Research Group in Cili Country of Hospital of Henan, China, (1979) reported serum IgE levels were elevated in asthma patients after acupuncture treatment.

Chen et al (1979) also found marked increased IgG levels in rabbits after acupuncture stimulation.
3.4.2 The Effect on Internal Secretion

The neuro humoral effect induced by acupuncture stimulation could lead to the arousal to greater activity of the cells of the suprarenal cortex which produces cortisone and related steroids.

This effect would be brought about either directly by autonomic neural stimuli reaching the suprarenal cortical and gonadic interstitial cells, or indirectly by neural activation of hypothalamus and pituitary, followed by hormonal stimuli (Lu & Needham 1978).

Pomeranz, Cheng and Law (1977) studied the acupuncture effect on reducing electro physiological and behavioural responses to noxious stimuli and concluded that they have evidence to believe that pituitary hormones could mediate the acupuncture analgesia.

Another important finding in internal secretion after acupuncture is reported by Omura (1976) in which he found that the changes in blood chemistry usually occurred with an increase in serum glucose, significant decrease in serum glyceride and serum phospholipids, a slight decrease in serum cholesterol, an increase in alphaglobulin and in betaglobulin, and a delayed increase in gammaglobulin. Analysis of these findings also lead Omura to suspect there is probably an increase secretion of adenocorticotropic hormones (Selden 1978).

Han and Terenius (1982) reported that there is an increase of cerebral content of hydroxytryptamine and its metabolic product 5 hydroxy-indoleacetic acid in experimental rabbits and rats under electro-acupuncture for 15-60 minutes. This most prominent and
consistent change took place in the lower brain stem especially in the raphe area and in the spinal cord. The physiological and pharmacological implications of 5HT in pain and analgesia, especially in relation to morphine action, was summarised by Messing and Lytle (1977) and will not be discussed in detail here.

Other evidence obtained from China to support the increase in existence of 5HT in midbrain from experimental animals is provided by Zhu, Jiang and Wen (1979) and Ye, Feng, Zhao and Zhang (1979).

During the National (China) Symposium of Acupuncture in 1979, there were numerous reports regarding experimental evidence both on human subjects and animals in supporting the theory that internal secretion can be affected by acupuncture stimulation.

These can be summarised as follows.

1. Increase secretion in acetylcholine esterase (ACHE) (Wang, Yu & Liu)
   - in different brain areas of rat (Xiong et al)
   - in human whole blood (Wan, Zhang, Wang, Gu)
   - in locus coeruleus of rat (Ai, Ru & Luo)
   - in thalamus of rat (Ai et al)

2. Increased secretion in certain monamine and amino acids in various regions of rabbit brain stem. (Zhu et al)

3. Increased secretion of atropine in rats. (Ren et al)

4. Increased secretion of eserine, acetylcholine and hemicholinium - 3 from electro-acupuncture analgesia in rats (Guan et al).

5. Increased turnover rate of CNS norpinephrine in rats. (Han, Guan & Xu)

6. Increased activity of dopamine - hydroxylase and cholinesterase in blood (Cai & Lu).

7. Increase in histamine level in blood (Lu & Cheng, Xu & Hou).
8. Increase release of cyclic 3'5' adenosine monophosphate level.

9. Increased concentration of calcium and phosphorous in blood. (Wan et al)

10. Increase in plasma prostaglandin level in human subjects. (Zhou & Chen)

11. Activation of endocrine glands (pituitary and adrenal glands) secretion. (Bi & Gao)

12. Increased the turnover rate in CNS norephiphrine in rats. (Han, Guan & Xu)

It is evident that the whole subject needs to be brought into relation with the interesting work of the school of Hans Selye at Montreal on 'stress' and what he has called since 1936 the 'general adaptation syndrome'. Selye and his collaborators established that in any stress state, such as the entry of a pathogen, or the malfunction of a psycho-physiology system, induces first an 'alarm reaction' followed by a stage of increased resistance, and finally by a stage of exhaustion (Lu & Needham 1978) (Figure 3).

The alarm reaction to the stress on 'bodily changes in pain, fear and rage', floods the circulation with adrenaline from the supramedulla, raises the blood pressure, constricts the peripheral circulation, speeds the heart, dilates the bronchi, mobilises liver glycogen and activates the sympathetic (adenergic) nervous system.

Then follows the process of general adaptation. The stressor excites the hypothalamus to produce the substance that stimulates the pituitary gland to liberate the adrenocorticotrophic hormone (ACTH) and this in turn causes a liberation of steroids such as cortisone and cortisol from the supra-adrenal glands cortex.
Figure 3 Selye's Theory on General Adaption
(Lu & Needham 1978)

normal resistance level

hyper-heterostasis

heterostasis

homoeostasis

emergency discharge of adrenalin

resistance stage

coming into play of the neural-hypothalamus pituitary ACTH-adrenocortical-corticosteroid

--- the possible effects of acupuncture
The upshot of these effects is the stage of increased resistance, a "re-setting of the thermostat" at a higher level (heterostasis), but as the adaptation energy is finite, a stage of exhaustion eventually ensures.

The relevance of all this to acupuncture is that by intensifying the neural signals to the hypothalamus it could potentiate the pituitary/ACTH/adrenocorticoid axis, either raising the resistance to an even higher heterostatic level or prolonging the stage of resistance, or both.

Perhaps it could also act more directly upon the suprarenal cortex by way of the splanchnic nerves through the coeliac and renal autonomic plexuses.

Acupuncture could also work by increasing the output of gonad stimulating hormones (GSH) from pituitary glands, or perhaps directly through the pelvic splanchnic nerve supply of the testis and ovary via their appropriate autonomic plexuses.

Heterostasis is a matter of strengthening the body's natural non specific defence, the body's own autopharmacological effects, to combat the stress factor in disease. This might almost be taken as an epigram for what therapeutic acupuncture may always have been doing (Lu & Needham 1978).
3.5 THE PRACTICE OF ACUPUNCTURE IN WESTERN COUNTRIES

Dembek (1972) has presented a comprehensive coverage of acupuncture in a number of countries.

Acupuncture in Japan and other Eastern Countries (Dembek 1972)
Acupuncture is used in other Asian countries, but not to the extent seen in China. Japan, Korea, Vietnam, India, and Tibet all contain practitioners in one form or another, derived from their contact with China. Japan's three national associations of acupuncturists collectively enroll over 22,000 members. Acupuncture was introduced in Japan in the year 562 AD as a system of therapeutics. Of separate academic interest is the sophistication of Tibetan medical work. This country, for all its isolation, borrowed widely from Sanskrit, Pali, and Chinese works for its medical sources.

Acupuncture in the USSR (Dembek 1972)
The Soviet Union does not have the numbers of acupuncturists that China has, but Russian research and usage of acupuncture is among the most advanced in the world. Russian emphasis on the practical application of acupuncture has led to its usage in anaesthesia and treatment of liver, kidney, stomach, and facial disorders. The usage of needles has been slowly discontinued in favour of electrical stimulation and there is currently experimentation with laser beams. The most recent breakthrough in USSR acupuncture studies is the invention of the tobioscope, which is supposed to photo-electrically record skin resistance over acupuncture points and meridians. Its inventor, Dr Victor Admenko, seems to have been a jump ahead of Dr William A Tiller of Stanford University, who was
also experimenting with similar devices (Dembek 1972). More recently, they have shown successful results using laser beam to stimulate acupuncture points.

Acupuncture in France (Dembek 1972)

France is the leading western country in its integration of traditional Chinese medicine with modern western medical practice. There are presently about one thousand MD's practicing acupuncture in France. The school teaching acupuncture first requires an MD degree of the prospective practitioner and then a programme of instruction lasting four to five years.

Acupuncture in England (Dembek 1972)

England is the home of many practising acupuncturists. Its most noted practitioners are Dr Joshua S Horn, Dr JR Worsley and Dr Felix Mann. Dr Mann, President of the Medical Acupuncture Society in London, combines western medical knowledge, applied study in China, and over twenty years of acupuncture treatment of patients. His books are the most extensive to be found today in the English language. Untold hundreds of English speaking people have been introduced to acupuncture through Dr Mann's publications. His books written specifically for the physician and student of Chinese medicine, will play an important role in any current attempts to integrate acupuncture with present medical concepts in the United States. In July 1972 he conducted a demonstration of acupuncture techniques at Stanford University's Memorial Auditorium before 1500 US physicians (Mann 1978).
Acupuncture in Austria (Dembek 1972)

Pioneer work in acupuncture anaesthesia is being performed in the Vienna Polyclinic, Vienna General Hospital in Austria under the direction of Dr Johannes Bishko and Dr Edward H Majer. Dr Bishko has spent fourteen years learning the art in Hong Kong. The greatest hindrance in further study, according to Dr Majer, Head of the Ear, Nose and Throat Clinic is the unavailability of western translations of much of the classic and recent Chinese work. The obstacle will hopefully be overcome in the future through the growth of national and international acupuncture organisations.

Acupuncture in the USA (Dembek 1972)

In the United States there has been a flood of acupuncture articles in the popular press. This flurry of articles represents a re-introduction of acupuncture due in part to the visits to China by Drs E Grey Dimond, Edger Snow, James Reston, Arthur Galston, E Signner and President Nixon. In 1972 there were only a handful of licensed acupuncturists in the United States. The greatest problem acupuncture integration faces is the efforts of the more conservative members of the society to condemn its usage.

The potential value of acupuncture practice in the USA will hopefully be remedied through current studies, increased relations with mainland China, and the establishment of institutions devoted to the study of acupuncture. The integration of eastern and western medical knowledge can almost surely lead to significant improvement in the general practice of medicine (Dembek 1972).
Acupuncture in Australia

Acupuncture treatment for general medical complaints such as tennis elbow, headaches, arthritis and neurological disorder have been increasing in popularity in Australia. There are medical acupuncture associations in each state of the country and the number of members is increasing each year.

The use of acupuncture to assist pain control during childbirth delivery is also available in many Australian hospitals. (Gibb 1981)

A number of private health insurance companies have also included the rebate for treatment fees from qualified acupuncturists throughout the country. This indicates there is an increase in demand for such service in Australia. (Gibb 1981)
4.1 IMMUNE RESPONSE TO DENTAL BACTERIAL PLAQUE

4.1.1 The Nature and Development of Dental Plaque

Dental plaque is an aggregation of a large number and variety of microorganisms on the tooth surface. The ecology of plaque is extremely complex and plays an important part in the nature and development of plaque. (Krasse 1977)

Initially aerobic organisms attach to enamel surface pellicle. These are Gram positive cocci and rods and Streptococcus Sanguis is perhaps the prevalent organism. These bacteria form micro colonies and often develop in columns perpendicular to the tooth surface. (Gibbons 1980)

There is then a shift to anaerobic organisms such as veillonellae and fusobacteria. This development of anaerobic conditions with increased bacterial accumulation, nutritional interaction between plaque bacteria and the formation of extracellular polyaccharides mediating interbacterial adhesion may determine the properties of plaque. (Gibbons 1980)

Formation of lactic acid by Streptococcus mutans can be utilized in the metabolism by V. alcalescens which increase in number. Dextran formed from sucrose may mediate interbacterial aggregation between Streptococcus mutans or S. sanguis and Actinomyces viscosus. Salivary agglutinins may also play a part in aggregating certain bacteria. (Roitt & Lehner 1983)
Fully formed plaque contains about $2.5 \times 10^7$ aerobic and $4.6 \times 10^7$ anaerobic organisms per mg of plaque.

Change in diet texture and vast increases in consumption of fermentable carbohydrate are features of civilization. Examination of teeth from isolated or primitive human communities show low incidence of caries and severity of chronic inflammatory periodontal disease. (Newman 1980)

The narrow embrasures of teeth and relative lack of movement between contiguous approximal surfaces, and the lack of occlusal wear, permit the impaction of bacteria and food producing abundant, stagnant plaque. The outline of the approximal caries lesion seems to follow plaque. Chronic periodontitis originates in relation to the most stagnant portion of approximal plaque. (Newman 1980)
4.1.2 The Components of Dental Plaque

Dental bacterial plaque may be considered to have three functional components:

1. Cariogenic organisms -- the most important are:
   (a) Streptococcus Mutans, S. Sangius
   (b) Lactobacillus acidophilus
   (c) Actinomyces viscosus

   (Tanner et al 1986)

2. Periodontal diseases inducing organisms -- the most significant organisms are:
   (a) Bacteroides asaccharolyticus (gingivalis)
   (b) Actinobacillus actinomycetemcomitans
   (c) Actinomyces viscosus
   (d) Bacteroides melaninogenicus
   (e) Veillonella alalectens
   (f) Fusabacterium nucleatum
   (g) Spirochaetes
   (h) Wolinella recta
   (i) Bacteroides inter medius
   (j) Bacteroids gingivalis
   (k) Peptocococcus micros
   (l) Eikenella corrodens

   (Page & Schroeder 1982)

3. Adjuvant and suppressive agents -- the most potent of which are:
   (a) Lipo-polysaccharides (LPS)
   (b) Dextrans
   (c) Levans
   (d) Lipoteichoic acids (LTA)

   (Monefeldt et al 1986)
4.1.3 Dental Plaque and the Immune Response

In order that dental plaque should be able to induce an immune response or cause direct toxic effects, it must penetrate through the epithelial barrier of the gingival crevice. (Monefeldt et al 1986)

The junctional epithelium is a highly permeable tissue which allows substances, probably up to a molecular weight of 700,000, to pass from gingival sulcus to the connective tissue and vice versa.

The substances pass through the intercellular spaces of the junctional epithelium and among the large variety of substances tested, tritium labelled collagenase, albumin and endotoxin are particularly significant. (Roitt & Lehner 1983)

Dental plaque broken up by ultra-sonication can induce increased DNA synthesis of lymphocytes which have been previously sensitized to some of the plaque antigen. (Ivanyi & Lehner 1971)

Both T and B lymphocytes respond to plaque antigens. T lymphocytes respond to protein fraction whereas B lymphocytes respond to the lipoprotein of Veillonella. (Schroeder 1977)

There is a significant correlation between the proliferative response of lymphocytes stimulated by dental plaque as compared with a single organism, such as Veillonella alcalescens, or Actinomyces viscosus. (Roitt & Lehner 1983)
Sensitized lymphocytes respond to dental plaque by the release of soluble mediators or lymphokines. These are released by both T and B cells, though some lymphokines, such as mitogenic and chemotactic factors, are released predominantly by T cells and others such as osteoclast activating factor (OAF) by B cells. (Ebersole et al 1982).

A release of macrophage migration inhibiting factor might localize macrophages to the site of lymphocytes activation. (Genco & Slots 1984)

Another mediator, lymphotoxin, is cytotoxic for human gingival fibroblasts which are concerned in laying down collagen in periodontal membrane. OAF is also released by activated lymphocytes and causes bone resorption, and may cause destruction of supporting alveolar bone. (Cogan et al 1986)

The release of mitogenic factor may recruit unsensitized lymphocytes to proliferate, so that the cellular reaction is boosted. (Ivanyi & Lehner 1971).

The effects of plaque accumulation in vivo on the immune response have been tested in dental students. They were asked to abstain from oral hygiene for 28 days and the plaque accumulated, gingival inflammation and cellular and humoral immune responses were tested.

Accumulation of dental bacterial plaque and the associated gingival inflammation were corelated with an increase in lymphocyte transformation and release of macrophage migration inhibiting factor (MIF). Lymphocytes were activated by sonicates of an autologous bacterial
plaque, streptococci, veillonella, and actinomyces. The cellular response was of limited duration and had returned to base-line values 28 days after plaque was removed. (Roitt & Lehner 1983). These results clearly show that accumulation of dental bacterial plaque adjacent to the gingiva can stimulate the cell-mediated immune response of the host.

Memory cells to some plaque antigen may also be stimulated, as has been observed by repeating the plaque accumulation experiment in the same subjects. Significant lymphocyte transformation was found. It was greater in magnitude and lasted longer in the second as compared with the first plaque accumulation experiment.

A variety of bacteria and their products are capable of non-specific stimulation of B-lymphocytes to produce immunoglobulin. (Ebersole et al 1985)

These substances are termed polyclonal B-cell mitogens. (Tolo & Schenk 1985)

Among the most important are LPS from Gram negative bacteria, dextrans, levans and antigen from Actinomyces viscosus. (Ebersole et al 1987)

Polyclonal B-cell activation might account for the increase in the concentration of immunoglobulins in experimental gingivitis and periodontitis but its biological role in the development of periodontal disease is unknown. It is possible that non-specific activation of B-cells to produce antibodies and lymphokins might be involved in the development of periodontal disease. (Ebersole et al 1982).
IgA, IgG and IgM antibodies to most oral micro-organism have been found in control subjects.

This evidence suggests that dental plaque organisms elicit a serum antibody response as part of the host response to any microbial antigen. (Tolo & Schenk 1985)
4.2 THE ORAL IMMUNE SYSTEM

4.2.1 Mucous Membrane

The health of the mouth is dependent on the integrity of the mucosa which does not normally allow microorganisms to penetrate.

The mucosa is in continuity with a number of anatomical structures and these are particularly vulnerable if the oral defences are breached. It is in direct continuity with the skin of the lip at the mucocutaneous junction and with the pharynx and larynx via the oropharynx. (Squier, Johnson, Hopps 1976)

The major and minor salivary glands open through their ducts into the mouth.

The mouth is colonized by a variety of micro-organisms from the time of birth and though most of them are commensals, they may become pathogenic when the host responses are altered. The factors which are responsible for maintaining oral health are the integrity of the mucosa. (Roitt & Lehner 1983)

The basement membrane of the epithelium is another barrier to penetration of microbial and other agents. In the lamina propria adjacent to the basement membrane are a few lymphoid cells which might deal with an agent which succeeds in penetrating the overlying barriers.

Antibodies in saliva can decrease the penetration of mucosa probably by forming an immune complex with the corresponding antigens. (Taubman 1982)
4.2.2 Oral Lymphoid Tissue

The extraoral lymph nodes comprise a fine network of lymph capillaries commencing superficially in the mucosa of the tongue, floor of the mouth, palate, cheeks and lips, as well as from the gingiva and the pulp of the teeth. These capillaries join to form larger lymph vessels which are joined by other lymph vessels originating from a deep network in the muscle of the tongue and other structures. (Squier et al 1976)

Any microbial antigen which has gained entry through the intact epithelium into the lamina propria may enter the lymphatics directly or may be carried there by phagocytes. The antigen will then be transported to the anatomically related lymph nodes where it may induce an appropriate immune response. (Lehner 1982)

Unlike the gut associated lymphoid tissue and bronchus associated lymphoid tissue, the intra oral lymphoid tissue has no well-defined oral associated lymphoid tissue. Nevertheless, there are four types of lymphoid aggregations in the mouth, each of which may play some part in the immunological surveillance of the oral tissue. These four aggregations include: a pair of tonsils, lingual tonsils and pharyngeal tonsils.

Tonsillar cells respond in vitro to T cell and B cell mitogens and antigen and give primary and secondary antibody response. They resemble gut associated lymph tissue (GALT) only in their generating a significant number of IgA forming cells and in their sub-epithelial location. (Cebra et al 1977)
However, most IgA containing cells produce monomers without J chains and secreting component cannot be detected in tonsilar epithelium.

Antibodies and sensitized cells can pass through the epithelium and therefore may have a local protective function in guarding the entry to the digestive and respiratory tracts.

(Husband, Monie & Gowans 1977)

Lymphocytes and plasma cells are found in the major salivary glands (parotid, submandibular and sublingual), as well as in the minor salivary glands scattered under the oral mucosa. The lymphoid cells are localized in small clusters adjacent to ducts or they are scattered between acini.

Most of the plasma cells secrete IgA, IgM or IgG. It appears that most IgA secreted in saliva is synthesized locally by the gland associated plasma cells. The IgA synthesized locally is dimeric, unlike the serum IgA which is monomeric. (Tomasi & Hauptman 1975)

The connective tissue of the gingiva may be diffusely infiltrated by plasma cells. IgG producing cells predominate with a ratio of IgG/IgA varying between 4/1 and 7/1. The preponderance of IgG producing cells over IgA producing cells follows the pattern of a classical secondary immune response. (Roitt & Lehner 1983)

Gingival aggregation of plasma cells, lymphocytes, macrophage and polymorphonuclear leucocytes is probably the most important lymph collection in the immunological response to dental plaque.
4.2.3 Saliva

4.2.3.1 Physiology of Saliva

A definition of saliva is the secretion from the parotid, submandibular, and sublingual glands and the large number of minor salivary glands spread over the mucosa of the palate, cheeks and lips. The secretions from the submandibular/sublingual glands, which enter the mouth through a common duct, will dominate the liquid phase in the bottom of the mouth and on the lingual surfaces of the lower jaw. The liquid layer covering the hard palate and the mucosal surface of the lips will be dominated by the secretion from the minor glands. By moving the tongue, lips and the mimic muscles of the face, the different secretions will spread over larger areas and mix. (Bratthall & Gibbons 1980).

Saliva plays a central role in maintaining normal physiological condition of oral tissue. It contains several important antibacterial systems along with Ca-binding proteins and electrolytes with buffer properties. When the efficiency of the system is lost by impaired salivary gland functions, the risk of caries increases. In extreme cases if most function of salivary glands is lost, severe conditions with the dry mouth syndrome xerostomia may occur. It is characterised by rampant caries and is a painful condition. (Brandtzaeg 1983)

Mechanical cleansing by the muscular actions of the tongue, cheeks and lips plays an important part in maintaining hygiene of accessible sites in the mouth. This is greatly aided by saliva
which in addition to lubricating the movements during speaking, chewing and swallowing makes it possible to swallow bacteria, leucocytes, tissue and food debris into the stomach where bacteria or noxious substances are inactivated. (Roitt & Lehner 1983)

There is a continuous flow of saliva, the resting flow rate (no added stimulation) is about 19 ml per hour. This rate will increase with psychic stimuli, such as the thought of food, and the presence of food in the mouth.

4.2.3.2 Composition of Saliva

The major components of saliva can be listed as follows:-

LYSOZYME (or muramidase) - This enzyme shows bactericidal activity by splitting the bond between N-acetyl glucosamine and N-acetyl muramic acid in the mucoprotein components of the bacterial cell wall.

Lysozyme may effect the development and keep down the total load of commensal organisms in the mouth, possibly by interacting with other salivary components such as IgA. Yet, there is little evidence to suggest that salivary lysozyme plays an essential role in control of caries. (Baer & Morris 1977)

PEROXIDASE - This is a heat-labile enzyme found in saliva which in the presence of thiocyanate ions and hydrogen peroxide kills lactobacillus acidophilus by inhibiting the uptake of lysine and may inactivate some streptococci by inhibiting their glycolytic enzymes. Whole saliva may contain low molecular weight inhibitors of peroxidase and one of these may be hydrogen peroxide produced by some
Unlike systemic immune response which has a well-established immunological memory, there is little convincing evidence for this in the local synthesis of sIgA. There is little secondary response upon repeated administration of an antigen by mouth. This is one of the disadvantages of oral immunization.

Secretory IgA has at least two functional advantages:

(a) It is preferentially transferred from the gland to the mucosal surface, possibly by the so which may have special receptors on duct epithelial cells.

(b) sIgA is more resistant to proteolytic degradation by bacterial and digestive hydrolases than other immunoglobulins, so that it is particularly suited to function on mucous membranes.

The function of sIgA has been described as an antiseptic point for mucosal surfaces. This may prevent absorption of the vast array of food and bacterial antigens from the gut and thereby prevent overloading the immune system and development of undesirable allergic responses.

sIgA in saliva interferes with the adhesion of microorganisms to the mucosal membrane. (Carlsson & Krasse 1968)

This adherence-inhibition potential of sIgA may play a role in the prevention of oral microorganisms from adhering to the teeth and forming dental plaque. (Baer & Morris 1977)

The protein attached to the sIgA dimer give some protection to the action of the salivary proteolytic enzymes.

This mechanism would overcome the difficulty that IgA probably does not fix complement and therefore cannot induce bacteriolysis.
sIgA contains high molecular weight glycoprotein which can agglutinate microorganisms in a selective way. (Bratthall & Gibbons 1975) Several salivary components can agglutinate microorganisms under proper conditions. sIgA lysozyme and B - microglobulin are examples.

sIgA is of great importance in viral infections, by preventing viral particles from penetrating host cells (viral neutralization). This function of sIgA is evident in response to virus vaccines such as the live, attenuated polio virus vaccine when administered orally. (William & Gibbons 1972)
4.3 IMMUNOLOGY OF DENTAL CARIES

4.3.1 Caries Immunology

The development of dental caries requires:-

(a) The presence of cariogenic bacteria that are capable of rapidly producing acid below the critical pH required for dissolving enamel.

(b) A sugar in the diet that favours colonization of these bacteria and that can be metabolized by the bacteria to form acid.

This process can be interfered with by the presence of an effective immune response. (Hamada & Slade 1980)

In principle, dental caries is no different from other diseases caused by microorganisms, in being dependent on the microbial attack on the one hand and the resistance of the host on the other.

Strep Mutans appears to be the most efficient cariogenic organism as it induces caries rapidly in germ-free rodents, though other cariogenic organisms may also contribute to the disease. (Roitt & Lehner 1983)

Antibodies to this organism are increased in patients with caries. (Ivanyi & Lehner 1978)

To elucidate the protective effect that the immune system may have on dental caries, several attempts have been made to relate caries experience to levels of antibody reactive with S. Mutans, either in serum, saliva or dental plaque. (Evans, Emming & Genco 1975)
Strep Mutans can induce human lymphocytes both to proliferate and to release macrophage migration inhibition factor. These functions are good evidence for the presence of cell-mediated immunity to the most important cariogenic organism in man. (Roitt & Lehner 1983)

Although the response of lymphocytes is rather modest, this can be boosted by the immuno potentiating effect of plaque accumulation. Under these conditions, a negative correlation was established between caries index and stimulation index of lymphocytes and this is consistent with the findings between serum antibody and the caries index. Hence, the lower the caries index, the higher are the lymphoproliferative responses and antibody titres. (Roitt & Lehner 1983)

As the stimulated cells are T lymphocytes, it is probable that they represent the T cell helper population which is involved in helping B-cells to produce antibodies. (Michalek & McGhee 1977)

There are two principal immune mechanisms of protection against dental caries. The salivary secretory IgA antibodies affect the salivary domain, whereas the gingival crevicular fluid, containing IgA antibodies, complement and polymorphonuclear leukocytes among other of the blood components, affect the gingival domain.

There is however, no overwhelming evidence in favour of one or the other mechanism playing a predominant role in protection against caries operating under the particular experimental conditions. (Roitt & Lehner 1983)
4.3.2 Immunoglobulin A Function in Relationship to Dental Caries

Numerous studies in animals have shown that increased antibody levels to S. mutans (either sIgA or IgG) can enhance the elimination of S. mutans from the oral cavity and interfere with its cariogenic activities. (Thylstrup & Fejerskov 1986)

There may be some differences in the salivary components between persons with high and low caries experiences. A consistent and meaningful pattern has not been found. However, the concentration of sIgA in whole saliva is significantly less in subjects with high caries as compared with those with low caries experience. (Arnold, Merteky & McShee 1976)

Specific sIgA antibodies to S. mutans have been detected in saliva by haemagglutination and agglutination assays. A significant increase in salivary sIgA to S. mutans was not found in subjects with low caries experience. It appears that the salivary IgA antibodies increase with the number of past carious lesions, so that they may reflect the cumulative caries experience. (Brandtzaeg 1983)

Salivary sIgA antibodies to S. mutans have been induced in man by swallowing daily capsules filled with $10^{10}$ organisms, but the duration of antibody titre was limited, even on secondary immunization.
The effect of sIgA responses in relation to protection of the tooth surface can be summarized as follows:-

(1) Inhibition of bacterial adherence by:
   (a) blocking of adherence determinants
   (b) reduction of hydrophobicity of bacteria
   (c) agglutination of bacteria
       (enhanced by sIgA, glycoprotein interactions)

(2) Inhibition of bacterial enzymes.

(3) Anti-inflammatory activity in mucosal tissue.

(Thylstrup & Fejerskov 1986)

As salivary IgA may function by binding to the antigenic determinant of oral bacteria and thus may block bacterial surface from adhering to the oral surfaces such as enamel, it is possible that this mechanism is highly efficient in most subjects, as the exposed smooth surfaces of teeth seldom develop caries. However, the development of caries at the susceptible sites (fissures, approximal and cervical sites) may not be accessible to the salivary component and a protective relationship has been found between the antibody titre and caries index. (Roitt & Lehner 1983).

It can be assumed that Strep Mutans in saliva is swallowed and although the organism does not colonize gut mucosa, it may nevertheless, induce an immune response in the gut associated lymphoid tissue (GALT). Sensitized cells may then home to salivary glands to produce the sIgA antibodies found in saliva.

(Hamada & Slade 1980)
It has been shown that the sIgA antibody titre in saliva increases with the caries index, so that salivary antibodies lack a protective relationship to caries but may be an indirect index of the frequency and possibly magnitude of colonization by Streptococcus mutans. (Roitt & Lehner 1983)

The ability of man to produce an effective immune response to Streptococcus mutans may depend on the Ia (immune associated) gene. This might develop only in the few individuals who are caries free. (Arnold et al 1976)

It appears that man has the potential to mount the cellular and humoral immune responses to Streptococcus mutans but that under conditions of natural immunization, these are usually inadequate.
4.3.3 Caries Immunization

The increasing interest in salivary immunoglobulin has inspired attempts to find means of immunization which elicit high levels of sIgA antibody to Streptococcus mutans in saliva.

The following procedures have been used:-

(a) Local immunization in, or in the vicinity of, salivary gland tissue (Challacombe & Lehner 1980)

(b) Perioral or intra-gastric immunization
(Michalek et al 1976)

(c) Passive transfer of sIga antibody with milk from donors.
(Thylstrup & Fejerskov 1986)

Passive transfer of IgA antibodies with colostrum of immunized rats has been shown to transfer caries immunity to their offspring. This observation has led to the interesting proposal that it might be possible to obtain protection in humans by the consumption of milk from cows immunized with S. mutans. (Thylstrup & Fejerskov 1986)

Salivary IgA antibodies have been invoked as the protective agents in multiple subcutaneous immunization of rats near the salivary glands. (Taubman & Smith 1974)

Furthermore, prolonged oral immunization of germ-free rats before and during dental implantation of Streptococcus mutans has led to a significant diminution in caries and this was attributed to an increase in salivary IgA antibodies. (Michalek et al 1976)

The role of secretory IgA in the mechanism of prevention of caries has been supported by transferring secretory antibodies in the milk of lactating germ-free rats to their litter, which had minimal serum
antibodies, and showing that there was a significant reduction in
caries (Michalek & McQhee 1977). Although these experiments have
been interpreted almost entirely in favour of secretory IgA
antibodies IgA antibodies in the serum, saliva or milk of the rats
might have also played a part in these investigations.

The gingival crevicular fluid contains most of immune components
present in blood and these have direct access to the smooth and
approximal surfaces of teeth and by mixing with saliva the resulting
"oral fluid" may reach the occlusal surfaces. Subcutaneous and oral
submucous immunization with S. mutans induces predominantly serum
IgG, IgM and IgA antibodies.

(Lehner et al 1975, 1976) (Challacombe & Lehner 1980)

Active and passive immunization in the Rhesus monkey has been
explored in order to investigate the mechanism of protection against
dental caries by immunization with Streptococcus mutans.
Significant levels of serum IgG, IgM and IgA antibodies to S. mutans
were elicited only in monkeys immunized subcutaneously. (Bowen et al
1975)

Oral immunization induced a modest increase in salivary IgA
antibodies to S. mutans, although a slight increase in IgA
antibodies were also found in the saliva of all other groups of
immunized and control monkeys. A small, though not significant,
reduction in dental caries was found in the monkeys immunized
orally, whereas subcutaneous immunization with S. mutans
consistently elicited a significant reduction in caries.

(Challacombe & Lehner 1980)
IgG induces significant protection. IgA and IgM may compete or interfere with the protective effect of IgG antibodies and the ratio of IgA and IgM antibodies might be an important factor in immunization against dental caries.

The results of active and passive immunization suggest that immunoregulation is governed by four sets of cells and their products: antigen-presenting cells, T helper cells, T suppressor cells and B cells. A high avidity IgG class of antibody to S. mutans appears to play an essential part in protection against dental caries.

(Roitt & Lehner 1983)

Unlike systemic immune responses which have a well-established immunological memory, there is little convincing evidence for this in the local synthesis of sIgA. Thus, repeated administration of an antigen by mouth may not induce a brisk secondary response, with prolonged and high titre of antibodies. This apparent lack of memory on the part of secretory B cells might be a disadvantage in oral immunization which relies on a brisk secondary immune response, when a micro-organism is encountered, some time after immunization.

(Roitt & Lehner 1983)
4.4 IMMUNOLOGY OF PERIODONTAL DISEASE

4.4.1 The Infectious Nature of Periodontal Disease

Periodontal disease is the outcome of host reactions to oral bacteria and their products. (Nisengard 1977) Evidence for bacterial specificity in periodontal disease is accumulating.

Gram-positive anaerobic bacteria and spirochetes are found in large numbers in periodontal pockets. (Palenstein-Helderman 1981)

The presence of Gram-negative bacteria in gingival connective tissue has been shown in both advanced adult periodontitis (Frank & Voegel 1978) and localized juvenile periodontitis (Saglie et al 1982) suggesting the possibility of direct antigenic stimulation in gingival tissue. (Smith et al 1985)

Recent findings suggest that anaerobic black pigmented and asaccharolytic rod Bacteroides gingivalis plays a role in the development of periodontal disease. (Schenck 1985)

Patients with periodontitis harbor pigmented Bacteriodes in higher proportions and more frequently than patients with clinically healthy gingiva. (Zambon, Reynolds & Slots 1981)

Non-fermentative black pigments strains show a predilection for pockets in patients with periodontitis (Spiegel et al 1979), and are found in high number proportion in severely inflamed pockets and in pockets showing periodontal breakdown (White & Mayrand 1981).
In several studies, elevated levels of serum antibodies against whole cells or crude tracts of B gingivalis were observed in periodontitis patients. (Taubman 1982) (Mouton et al 1981) (Tolo & Brandtzaeg 1982)

Studies have encompassed a number of antigens, some of which may be associated with periodontitis, and others that are unrelated to the disease. By using ELISA the binding of antibody to disease-associated antigens may be observed by the binding to unrelated antigen. It is therefore of interest to examine the level of antibodies to the isolated fraction of the bacterial antigens.

In 1985, Ebersole, Taubman, Smith and Haffajee reported that a local response to members of the micro flora colonizing different sites may be detectable by monitoring antibody levels in the crevicular fluid. Extension of their observations provided information that these local responses exhibit bacterial specificity and may be limited to certain components of the local flora. These local responses with bacterial colonization may identify those sites that represent a higher risk of disease development.

A multitude of immune components are present in the healthy and diseased gingival crevice including antibody, T lymphocytes, B lymphocytes, polymorphonuclear leukocytes and complements. The interaction of these components with bacteria has been demonstrated in gingival fluids from periodontally diseased subjects.

(Attstrom 1975)

Each immune component has protective and destructive potential.

(Wilton 1982)

The measurement of these immune responses in local fluid should be helpful in unravelling the relationships among the immune responses and the bacteria which initiates and perpetuates these responses.

(Smith et al 1985)
4.4.2 Immune Responses to Periodontal Disease

There is strong evidence suggesting that humoral and cellular immune responses may play both protective and destructive roles in periodontal disease. (Baer & Morris 1977)

The presence of plasma cells, small lymphocytes and neutrophils in the gingival connective tissue of man may be considered part of the normal surveillance system of the body.

The antibodies secreted by the plasma cells and the lymphokines released by the T cells can flow outward into the crevice. Lymphocytes and leukocytes can also migrate through the junctional epithelium into the crevice. (Craddock, Longmire & McMillan 1971)

Thus, there is a protective mechanism present to counteract the effects of noxious substances which might gain entrance through the junctional epithelium.

The microbial plaque is one of the primary etiological factors initiating periodontal disease. It is known that bacterial products not the organisms themselves, can penetrate the crevicular epithelium. (Gibbons & Von Houte 1973)

Several reports have shown that a number of bacterial components when applied to the periodontium will induce inflammatory responses such as immune complex reactions and chronic allergic reactions.

Antigens have been shown to induce inflammatory changes upon repeated application to the periodontium of several animal species. Patients with periodontal disease have circulating antibodies
directed against specific oral microorganisms and exhibit hypersensitivity reaction against several antigens of plaque microbes. (Baer & Morris 1977)

Strains of Actinomyces viscosus of human and animal origin have induced periodontal disease in experimental animal model systems. Periodontal destruction has been induced in gnotobiotic rats monoinfected with human isolates of Actinomyces naeslundii. Histopathological changes were similar for disease initiated by these plaque microorganisms, and the cell infiltrate was characteristic of an immune response.

Therefore, evidence suggests that there is interaction between the surveillance system and the bacterial products which penetrate the gingival lining.

This interaction may be initially protective because of antibody-mediated opsonization. (Nisengard & Beutner 1970)

Evidence also seems to suggest that microbial surface components may initiate a cellular response in the periodontium. This response may be protective to the host, but the local release of lymphokine may lead to the alteration of tissue remodelling with subsequent alveolar bone resorption.

Immune mechanisms may represent only one possible manner in which periodontal destruction can occur. However, the interaction between plaque components and the surveillance system can explain both the inflammatory process found in the gingiva and the destruction found in the periodontium.
Microbial components which have the ability to gain access to the surveillance system and which have the potential for initiating periodontal destruction include endotoxin, enzymes, chemotactic factors and cell surface antigens. (Gibbons & Houte 1973)

Endotoxin is a lipopolysaccharide of the cell walls of gram-negative bacteria. It is a potent inflammatory agent capable of penetrating an intact gingival sulcus and by itself can activate the complement system by the alternative pathway which release C and C that are 3 5 chemotactic for neutrophils.

The neutrophils ingest foreign substances and release lysosomal enzyme which cause tissue destruction.

Endotoxin can also react with serum component and mediate the release of alpha-globulins which release histamine and heparin from mast cells found in the gingival tissue. Histamine is a potent vasoactive amine and increases vascular permeability. This response can explain the edematous condition, erythematosus colour of gingiva and increased flow of crevicular fluid seen in gingivitis. Endotoxin has also been shown to stimulate bone resorption in vitro. (Baer & Morris 1977)

Plaque microorganisms produce enzymes which can penetrate the sulcular lining. Certain of these enzymes are capable of destroying the intercellular matrix of the sulcular epithelium, providing an easier access for larger microbial components, and carbohydrates, proteolytic enzymes and collagenases that can cause direct tissue damage. (Fullmer & Gibson 1966)
Chemotactic factors of low molecular weight (1,000) from plaque microorganisms can easily penetrate sulcular lining. They are capable of attracting poly-morphonuclear leukocytes that elicit inflammatory response. This interaction of the factors with the complement system also has the potential for the release of the chemotactic factors C3a and C5a.

Plaque micro-organisms contain characteristic cell surface antigens. Plasma cells containing immunoglobulins have been identified in gingival tissue. (Taubman & Smith 1974). There is also widespread occurrence of circulating antibodies specific for oral microorganisms. Immunoglobulins and complement components have been identified in the perivascular areas of inflammed human gingiva. These findings suggest that immune complexes may play a role in gingivitis. (Genco et al 1974)

The immune complexes activate the complement system with release of histamine and chemotactic factors. The complexes can also be phagocytosed and provide a mechanism for protection.

In chronic periodontal disease there is a large influx of small lymphocytes and monocytes into connective tissue which is characteristic of cell-mediated immune response. (Ivanyi & Lehner 1971)

The release of lymphokines by transformed lymphocytes can mediate the pathological changes seen in periodontal destruction. (Mergenhagen 1973)
Roitt & Lehner (1983) summarized four immunopathological stages which have systemic immune counterparts:

(a) The initial lesion is found in the normal state with localized inflammatory response of polymorphonuclear leukocytes. Complement activation and chemotaxis generated by plaque antigens and possibly immune complex may account for this stage.

(b) The early lesion shows a localized infiltration of predominantly T with a few B lymphocytes. In the circulation, lymphocytes are sensitized at this stage to plaque antigens, as shown by their ability to release lymphokines.

(c) The established lesion is characterized by a local plasma cell infiltration and peripheral blood lymphocytes can be stimulated to proliferate with plaque antigens. This stage can persist for years with early pocket formation.

(d) The advanced lesion marks the transition to a destructive immune pathological mechanism with ulceration of the pocket epithelium and localized destruction of collagen and bone. This is a progressively destructive process leading to loss of teeth.

The immunological processes are complex and may involve types IV, III, II, and I reactions with the protective destruction mechanism of lymphocytes and macrophage function antibodies and complement activation.
4.4.3 Immunoglobulin A and Periodontal Disease

The presence of immune components in fluids of the gingival crevice has been known to man for many years. These exudates have been shown to contain a variety of serum proteins, most notable IgA, IgG and IgM. (Holmberg & Killander 1971)

Based on the concentration of Immunoglobulins in these fluids, it was suggested that crevicular fluid is a dilution of serum and the volume of fluid reflects the degree of gingival inflammation. (Scherkein & Genoo 1977)

These suggestions imply that the concentration of antibody in crevicular fluid should be similar throughout the mouth and would simply be an indicator of systemic antibody levels. (Ebersole et al 1985)

Lally et al (1980), Lovelace et al (1982) and Ebersole et al (1985) suggested the existence of local antibody synthesis and/or accumulation of specific antibodies to periodontal disease associated bacteria in tissue and fluids of gingiva.

Ebersole et al (1985) suggested that a local host response to members of the microflora colonizing different sites that may be detectable by monitoring antibody level in crevicular fluid. These local responses exhibit bacteria specificity, and may be limited to certain components of the local flora. These local responses with bacterial colonization may identify those sites that represent a risk of periodontal disease development.
Smith et al (1985) reported that the levels of antibody consistent with local antibody synthesis or accumulation were detected from static crevicular fluids obtained from approximately 10% of sites sampled in patients with periodontal disease.

Evidence from laboratories suggested that specific local immune responses occur in gingiva to antigens of periodontopathic organisms. (Lally et al 1982)

In humans, low levels of circulating antibodies to oral microbes are known to exist and in different studies, immunoglobulins have been reported to increase in periodontitis. Evidence for the presence of IgA & IgG, in inflammed gingiva has been reported by Platt, Crossly and Dalbow (1970)

Smith et al (1985) reported that elevated sIgA antibody concentrations were observed in gingival homogenates from patients with periodontal disease. By means of ELISA technique, they were able to compare the antibody activity in static crevicular fluid and gingival homogenate. They have concluded that a significant portion of antibody found in the gingival homogenates and static crevicular fluids of periodontally diseased subjects may be locally derived.

Ebersole et al (1980) and Listgarten, Lai and Evian (1981) reported that elevated serum levels of IgA and IgG antibodies to A actinomycetemcomitans Y4 in juvenile periodontitis. Taubman (1982) demonstrated there are increased frequencies and high levels of sIgA antibodies to the bacterium in stimulated parotid saliva of juvenile periodontitis patients.
The stimulation of secretion will actually decrease the concentration of sIgA in parotid saliva, but the average secretion rate is increased about 2.5X, according to Brandtzaeg (1971).

Smith et al (1985) studied stimulated parotid saliva of young adults and found a significant association between high levels of sIgA antibodies to A. actinomycetemcomitans Y4 and the number of deep gingival pockets.

Quantitative human immunoglobulin data show sIgA to be present in saliva at higher levels in patients with periodontal disease. (Lindstrom & Folke 1973)

Unstimulated whole saliva from individuals with healthy periodontium contains about 19mg IgA per 100 ml. In periodontitis patients, the mean amount increased to about 37mg IgA per 100 ml of saliva. (Brandtzaeg 1971)

Most recently, Sandholm, Tolo and Olsen (1987) have conducted studies on salivary immunoglobulins activities to Actino bacillus actinomycetemcomitans in periodontal diseased patients using the ELISA technique. They have also confirmed the finding that the level of salivary sIgA was influenced by the periodontal condition of the patient though this alteration level is not as dramatic as IgG. They have found elevated levels of sIgA antibody to A. actinomycetemcomitans Y4 were found in the saliva of 3 out of 11 patients with untreated juvenile periodontitis and the mean level in the 3 was 3X times higher than in the healthy subjects. Eight out of 39 patients treated for juvenile periodontal diseases had elevated levels of sIgA antibody and the mean level of such antibody
was 10X higher than in the healthy subjects. The level of sIgA antibody was elevated in 22 out of 131 patients with periodontitis and the mean level of antibodies in these high responses was 12X times higher than in the healthy subjects. They have further concluded that the salivary IgA antibody levels were positively correlated to the collection time for the sample.

Comparison of IgA and IgG antibody activity in diseased gingival homogenates revealed that IgA concentrations were not as elevated as the IgG antibody responses. This pattern was seen with every microorganism tested. This difference may represent a preferential response in IgG isotype to antigens present in gingiva. (Smith et al 1985)

On the other hand, Mattioli and Tomasi (1973) have shown that IgA plasma cells from mice have life spans in the order of 4 to 5 days. Whereas, IgG plasma cells generally are much longer lived. Since experimental specimens were not necessarily collected during periods of specific antigenic stimulation, the IgA response could have decreased more rapidly than the IgG response. (Smith et al 1985)

Despite the fact that the level of salivary IgA was less influenced by the periodontal condition of the test subjects, elevated level of salivary IgA have been observed in severe adult periodontitis. This indicates that in periodontitis also, IgA is released into saliva, mainly by glandular secretion. (Sandholm, Tolo, Obsen 1987)

sIgA is the predominant component of all oral secretions and in saliva. IgA is also the chief immunoglobulin of dental plaque. The fact that local immunity has provided effective function in the
induction of sIgA and a related lowering of the caries experience in rats substantiates the existence of a protective local immune system. A similar protective local immunity phenomenon may occur in soft tissue surrounding the teeth. (Taubman & Smith 1974)
Since the collection of saliva for laboratory analysis for the presence of immunoglobulins after acupuncture stimulation has not been carried out by other workers, this study is a pilot one. It is the aim of the author to design a control acupuncture experiment on human subjects with the use of a relatively simple procedure and instrumentation in order to obtain maximum co-operation and minimum discomfort for the subjects.

Special precautions have been placed on patient selection, co-operation and safety for control and test subjects.

In order to test the null hypothesis proposed in section 1.4, the experiment was designed to test the relationship between acupuncture stimulation and the salivary sIgA secretion activity of the oral cavity.

The null hypothesis was assumed that no relationship exists between the two variables. In the design of this experiment, the clinician performing the acupuncture stimulation would not carry out the saliva analysis procedure and meant that the technician had no idea which saliva samples were control and which saliva samples were acupuncture stimulated. This was to avoid bias in the reading and measurement of the analytical results.

All of the saliva samples were collected in the writer's clinic and later sent to the pathology laboratory of the Queen Mary Hospital (Hong Kong) for analysis.
5.1 SUBJECTS

Subjects were chosen from a random selection from the writer's patients record section in his private practice in Hong Kong. They were telephoned by the writer and given an elementary explanation of the study and to obtain unofficial permission for the patient's participation in the study. This was followed up by a letter of confirmation enclosing a written consent form to be filled in and returned to the office for reference.

These patients received instructions at a personal interview one week before the stimulation experiment in the clinic and understood that the whole of the experiment was for scientific research purposes only and the possible complications minimal. The patient was assured that he was not at risk of any sort since acupuncture has been used for decades and the number of accidents recorded is almost negligible.

All patients participated on a voluntary basis and receive no reward or souvenir in return. They received a letter of appreciation after the experiment.

During their first interview, patients' previous medical history was obtained. Subjects suffering from any severe illness such as heart disease, diabetic, high blood pressure, immunological disorder, lung, kidney or liver problems were not accepted for the study. The subjects were chosen after the interview if they had satisfied the requirements of good physical health and were willing to cooperate.
Patients under 8 years and over 60 years of age were rejected due to co-operation problems. For patients aged between 8 to 18, parental consent was also obtained.

During the selection procedure, a total of 225 patients were approached and invited to participate in the experiment. Some 86 patients were medically unsuitable for the experiment either because of history of severe illness or health reasons, 56 had previous acupuncture experience and 33 were rejected because of non-cooperation or being unable to give up the time required.

A total of 50 patients from different age and sex groups was selected. They were all with good and healthy periodontal status. The Community Periodontal Index of Treatment Needs (CPITN) was used as a guideline for this selection (Cutress et al 1986). Patients with a CPITN score of 1 or above were not accepted. This was to ensure uniformity in gingival flow rates as patients with periodontal inflammation would tend to have higher gingival flow rates and could affect the concentration of immunoglobulins measured.

All of the chosen subjects were of Chinese origin and none of the patients had received any acupuncture treatment of any nature at any time in their previous medical history.
5.2 EQUIPMENT

The Electro-acupuncture Stimulator:
The stimulator consists of an oscillator which can send out electric signals at 1-10 cycles per second and is operated by a 9 volt dry battery. The electric current is conducted through two connecting wires to the copper ear electrodes.

The Conducting Wire:
On one end of the connecting wire is a pair of clamps which are able to be connected to the ear electrodes. At the other end, it is connected to the stimulator by means of a plug.

The Ear Electrodes:
The ear electrodes are designed in the form of an ear ring. The blind ends are condensed into a ball shape for maximum contact with the ear. The metal ring has an elastic nature, so that light pressure only is needed to distend it and place it onto the selected points without any pain or discomfort. There is absolutely no discomfort or damage to the skin surface during placement.
5.3 TECHNIQUE

Effective acupuncture stimulation relies on either proper manual stimulation or electric impulse. The principle used in this experiment is based on continued stimulation which is generated by electric impulses produced by an electro-stimulator.

Conduction of such electric signals can be done either by acupuncture needle or by electrode. In this experiment, copper ring electrodes, were used to conduct this impulse onto the selected point at the skin surface. No acupuncture needle was used and therefore there was no need to penetrate into the skin surface. This minimised discomfort or pain felt by the subjects.

Soft music or assorted video music was provided constantly during all of the acupuncture stimulation procedures to relax the patient during the 20 minute stimulation time. This applied to all test subjects and control subjects.
5.4 PROCEDURES

The subject was instructed to rinse vigorously a few times with water to get rid of food debris before the acupuncture stimulation proceeded.

The saliva collected times were standardized as follows, for both test subject and control subjects:

1st sample (11:00 am)
2nd sample (11:20 am)
3rd sample (11:20 am - 24 hrs later)

Saliva (5 ml) was collected one minute before the acupuncture stimulation.

The copper ring electrodes were placed at the selected points on the ears and electric wires connected to the electrode by use of a clamp.

The stimulator was in the off position when the other end of the connecting wire was plugged into the terminal.

When all connections were completed, the subjects were advised that they would feel a funny sensation on the ear.

At first, the patient might feel nothing, but as the intensity of the stimulation gradually increased the next sensation would be tingling or itchiness. This may be described as "ants" walking along the ear surface.

The patient was advised to notify the operator to stop increasing the level of stimulation immediately he felt the tingling sensation becoming too painful. This intensity is considered the required
intensity for adequate stimulation to produce effective acupuncture and was to be maintained and continued for 20 minutes. The subjects were advised not to move or to touch the connecting wires and the ear electrodes.

According to Hahn & Terenius (1982), 20 minutes is the average stimulation time for effective transcutaneous nerve stimulation to take place. Therefore, all of the test and control subjects in this experiment received 20 minutes electro-acupuncture stimulation on the same selected ear point.

At the end of this 20 minute stimulation, the power switch at the stimulator was turned off.

Connecting clamps were removed from the ear electrodes which were then removed from the ear.

Further saliva collection was carried out at this stage. Collection of saliva was achieved by asking the patient to spit into a sterilized text tube until it reached the marked level of approximately 5ml. The use of a conical funnel was helpful if the patient found it difficult to spit accurately into the narrow opening of the test tube.

Before being dismissed the patient was re-assured and advised to return to the clinic 24 hours later to enable collection of the third saliva sample. Patients were advised to return to the clinic at the exact time designated for the experiment for saliva collection. Those who were late for the appointment were reappointed at the same time on the next day.
5.5 PRECAUTIONS

Basically, there were no specific complications that could have arisen from this experiment.

There should be no chance for cross infections between patients since there was no need to use acupuncture needles and contamination of instruments could be avoided.

Collection of saliva was straightforward and all containers and paper cups were disposable. The clinician wore rubber gloves during collection of saliva.

To avoid contamination by saliva, rubber gloves were worn during laboratory procedures. Test tubes for storing the saliva and pipettes used were discarded after use.

The accurate medical history was essential for selection assessment. Patients with cardiac pacemakers did not participate in the test because the electric impulse and current may have been upset by such stimulation.

Patients with other medical problems such as epilepsy or physically mentally handicapped persons were rejected for co-operation reasons.
The saliva sample collected from the volunteer subjects before the experiment was used as the baseline "control" for the study.

The subjects were advised verbally not to take any drugs, medication or therapeutic treatment of any nature to the body for one week prior to the experiment time to avoid any delayed body reaction which may have arisen by previous exposure to other stimulating factors. The same reason applied to any history of previous acupuncture treatment. None of the subjects should have received any acupuncture treatment in any form at any time previously.

All of the acupuncture stimulation appointments were made at eleven o'clock in the morning in order to obtain uniformity. Patients who were late were re-scheduled to another visit.

Patients were also instructed to have breakfast at about 7.30am that morning and to brush their teeth thoroughly after breakfast to avoid accumulation of food debris in the saliva sample.

After collection the saliva samples were immediately delivered to the saliva concentrator for the concentration process. Out of the 50 chosen subject, only 25 subjects received acupuncture stimulation. The other 25 subjects received no electric acupuncture stimulation but came to the office at the same time of the day and observed the same rules and timing for breakfast and brushing. All they were required to do was to provide three saliva samples at 11am, 11.30am and 24 hours later in order to act as controls.
6.1 QUANTITATION OF HUMAN SECRETORY IgA (BY RADIAL IMMUNO DIFFUSION)

6.1.1 Selection of the Quantitation Method

Clinical laboratories presently include total serum protein determinations and serum electrophoresis as routine screening procedures for patient's sera.

It is the general view that, whenever the immunoglobulin diffusion region is increased or decreased on the electrophoretic analysis, further examinations by quantitation of immunoglobulin GA and M (IgG, IgA and IgM) is indicated (Davies & Monto 1976).

Many methods for quantitative assessment of the immunoglobulin have been described. Three of these currently considered to be of greatest value are:

i. Radial immuno diffusion
   (with time diffusion or with limited diffusion).

ii. Automated immune precipitation.

iii. Electro immunossay
   (Davies & Monto 1976)

Radial immuno diffusion technique has been employed in this study for quantitation of secretory IgA in saliva because it is the most widely used method for quantitation of the serum immunoglobulins and saliva immunoglobulins (and other proteins) and is the accepted method for standardisation of reference sera (Sonnenworth & Jarett 1980).
It is also the method to which other methods are compared. This probably results both from its having been historically the earliest of the modern methods and even more from its apparent simplicity and lack of need for sophisticated or expensive laboratory equipment (Rose & Fridman 1976).

However, variables affecting accurate results were observed in a survey carried out by the United States Department of Health, Center for Disease Control, in 1974. Many of the difficulties were attributed to the difference in the antisera and reference sera employed. Therefore, it is of utmost importance that the same antisera and reference sera are used for the whole procedure. (Rose & Fridman 1976).

This radial immuno diffusion technique is also called the Fahey Technique and was employed in this study to quantify the Immunoglobulins A present in the saliva.

6.1.2 Principles of the Radial Immuno Diffusion Technique

One of the two immune reactions (usually antibody) is added and uniformly distributed in a layer of agar, or agarose gel, followed by introduction of the other reactant (usually antigen) into wells punched in the gel. The antigen diffuses radially into the gel antibody mixture, forming a visible ring of precipitate at a point dependent on antigen antibody stoichiometry (Sonnenworth & Jarett 1980).

As more antigen diffuses out, the precipitin ring dissolves in antigen excess and reappears at a greater distance from the well.
This increase in the diameter of the precipitin ring continues with time until the antigen, or antibody, completely reacts. 
(Davies & Monto 1976).

Certain quantitative relationships exist between the ring diameter and the concentration of the antigen, both while the ring is expanding and after it stops enlarging. 
(Fridenberg, Stites, Caldwell & Well 1978).

By using the Fahey Technique a plot of diameter squared ($D^2$) versus concentration is linear. In the former case (ie when the ring diameters are still growing), the plot is approximately linear when the logarithm of antigen concentration is plotted against the ring diameter. In practice, when quick results are required, timed diffusion might be used, and later, to improve the accuracy, the values could be rechecked after limit diffusion has been reached. However, limit diffusion can be done only if the correct antibody concentration is used; ie the concentration should be low enough to give measurable rings at low antigen concentration but high enough to be in antibody excess at high antigen concentrations. 
(Fridenberg et al 1978)
6.2 REAGENTS

For the experiment the following reagents as proposed by Davies and Monto (1976) were used:

i  Agarose (SIGMA)

ii Barbitone acetate buffer of pH = 8.6, ionic strength of 0.025

iii Seward BA208 antiserum - anti human IgA secretory component (2ml)

iv Seward BR08 reference standard serum for human IgA secretory component (1ml)

v Polyethylene glycol (PEG) 6000 (SIGMA)

vi Human saliva concentrate 20x

The Seward antiserum BA208 and the Reference standard serum BR08 were supplied from Unipath Ltd of U5 Viking Industrial Estate, Norse Road, Bedford of the United Kingdom.

The 3 percent polyethylene glycol 6000 used is a polymer that can enhance precipitation of the immunological complex. therefore, the diffusion ring can appear more prominently in the agar plate and is much more easier to be measured after staining.

The human saliva was concentrated 20x by using the Minicon B15 macrosolute concentrator no. 9033 which was supplied by the Amicon Corporation, Danvers, USA.
The Minicon - Clinical Sample Concentrator

The concentrator is a plastic container with eight isolated sample chambers. Each of the eight chambers can hold up to 5ml of sample. The inner surface of the chambers is a membrane of selective permeability, backed, in turn by absorbent pads which wick away water and permeating species.

Retained constituents are progressively concentrated in the chambers as sample volume diminishes.

Graduation lines at 5x, 10x, 25x, 50x and 100x indicating the concentration ratio are marked on the surface of the container.

About 100x level, the membrane has been made impermeable to prevent further concentration and inadvertent reduction to dryness.
6.3 LABORATORY PROCEDURES

1. Three millilitres of the saliva sample was pipetted out from the 5ml saliva sample collected from the subject undertaking the acupuncture stimulation.

2. The saliva sample was then transferred immediately to the Amicon concentrator. A nine inch Pasteur pipette was used so that the tip of the pipette could reach the bottom of the concentrator chamber. Saliva was released out while the tip of the pipette remained at the bottom of the chamber until the saliva reached the 5x level. This was to avoid trapping bubbles in the saliva content which would affect the accuracy in concentration level. Care was taken to avoid scratching the membrane surface during insertion of the pipette.

3. The sample was allowed to dry out undisturbed up to 100x. To avoid the sample from drying out beyond 100x level, it was necessary to check concentration level periodically and to remove the concentrate once it had reached 100x. Thus drying from 5x level to 100x level, the saliva sample was concentrated to 20x. The manufacturer's suggestion to keep the concentrator unit in a refrigerator after first use was followed.

4. The concentrated saliva sample was then recovered from the chamber using the 9 inch Pasteur pipette. It was also suggested by the manufacturer that the sample should be mixed by drawing it in and out of the pipette several times in order to improve recovery.

110
5. The extracted saliva sample was placed in a sterilised test tube which was then sealed immediately by parafilm and stored in -70°C (to avoid destruction of immunoglobulin) chamber until the Radial Immuno Diffusion Technique was carried out.

6. When all the saliva samples from the experiment were collected and concentrated they were removed from the -70 degree celcius chamber and warmed to room temperature.

7. Before making up the agarose, 3 percent polyethylene glycol 6000 polymer was dissolved in to the working barbitone buffer to enhance precipitation of the immuno sample.

8. The buffer was filtered through filter paper using a funnel.

9. The 1.5% of agarose was made up by adding 1.5gm of agarose to 1000gm of filtered barbitone buffer in a boiling water bath. Constant stirring of the mixture during heating is important until a clear solution results. It is important to avoid boiling the agarose solution.

10. The Radio Immuno Diffusion plates were poured using 20ml anti secretory IgA in 3ml agarose buffer.

11. When the agarose plate had cooled to room temperature, wells of 2.5mm were punched using a gel puncher.

12. Then, 5 μl of each control and concentrated saliva was delivered into wells using the capillary pipette. Care must be taken to avoid trapping of bubbles in the pipette, since this would affect the concentration of the sample.
13. The reacting samples were then left in the reacting wells for 48 hours to allow diffusion to occur. During the reaction the agar plate was stored in a glass chamber at room temperature to avoid contamination or evaporation by the air current produced by the air conditioner.

14. After the reaction had taken place, the agar plates were rinsed with distilled water and amide black staining solution was applied on the surface for 4 hours to stain the reacting rings. After staining, the agar plate was rinsed again with distilled water and allowed to dry on the bench. Room temperature was 25 degrees Centigrade.

15. Slides were then ready to measure the diameter of diffusion.
6.4 PHOTOGRAPHIC ILLUSTRATIONS OF EXPERIMENTAL AND LABORATORY PROCEDURES

Photographic illustrations of the experimental and laboratory procedures are presented in Figure 4.

Photograph Legends

1. The electro-acupuncture stimulator.
2. The stimulation in progress.
3. Close up view of the ear electrode.
4. The saliva sample in the test tube.
5. Saliva is pipetted into the saliva concentrator.
6. The saliva concentrator.
7. The concentrator saliva (20x) is sealed in the test tube before stored in the -70 degrees celous chamber.
8. After all of the samples are collected, they are defrosted on the bench.
9. Saliva is pipetted onto the agar plate.
10. After Ab-Ag reaction, the agar is laid out on slides.
11. The diffusion ring is measured by the precision viewer.
12. The precision viewer.
Figure 4. Photographs of Laboratory Procedures

1.

2.

3.
6.5 DATA MEASUREMENT

Measurement of the diameter of the diffusion rings on the agar plate was by means of the precision viewer which is a special designed device with magnifier and measuring apparatus that can measure up to 1/10 of a millimeter on the agar plate accurately.

In order to obtain a plot of linear relationship between the $D$ \(d^2\) versus antigen concentration, three reference antisera were added for each agar plate used to produce three reference points on the standard curve (Rose & Fridman 1976).

Three different concentration references antisera were made up according to the following combination:

- **High concentration** -
  
  \[\text{standard 1} = 260\text{mg}\% \ (10 \mu l \text{ neat std} + 10 \mu l \text{ buffer})\]

- **Medium concentration** -
  
  \[\text{standard 2} = 156\text{mg}\% \ (6 \mu l \text{ neat std} + 14 \mu l \text{ buffer})\]

- **Low concentration** -
  
  \[\text{standard 3} = 52\text{mg}\% \ (2 \mu l \text{ neat std} + 18 \mu l \text{ buffer})\]

It is from this standard curve that unknown values can be obtained by reading the diameter square values on the y axis and reading the concentration values on the x axis.
7  DATA ANALYSIS

7.1  SUBJECTS IN STUDY

For statistical studies, it is ideal to select samples at random within a selected population group. (Blalock 1960).

The selection of the test group and control group subjects in this experiment was at random from the author's record cards storage panel. They were telephoned and were asked to participate for the experiment. Out of the 225 patients appointed only 50 subjects were selected. The other 175 subjects were rejected, either due to medical reasons or due to co-operation problems.

A total of 12 male and 13 female test subjects were chosen and a total of 15 male and 10 female control subjects were chosen.

The ages ranged from the youngest at 18 years old to the eldest, a 52 year old. The over 60 and under 18 were not chosen due to co-operation reasons.

All subjects were self-reported to be medically fit with no severe medical problems and no previous acupuncture experience. Their occupations were also recorded for reference.
<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Occupation</th>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>34</td>
<td>Mechanic</td>
<td>26</td>
<td>M</td>
<td>38</td>
<td>Engineer</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>32</td>
<td>Teacher</td>
<td>27</td>
<td>M</td>
<td>29</td>
<td>Technician</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>18</td>
<td>Student</td>
<td>28</td>
<td>M</td>
<td>26</td>
<td>Postman</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>56</td>
<td>House-wife</td>
<td>29</td>
<td>F</td>
<td>41</td>
<td>House-wife</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>27</td>
<td>Salesman</td>
<td>30</td>
<td>F</td>
<td>30</td>
<td>Shopkeeper</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>25</td>
<td>Clerk</td>
<td>31</td>
<td>M</td>
<td>42</td>
<td>Labourer</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>18</td>
<td>Student</td>
<td>32</td>
<td>F</td>
<td>34</td>
<td>House-wife</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>25</td>
<td>Shopkeeper</td>
<td>33</td>
<td>F</td>
<td>32</td>
<td>House-wife</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>46</td>
<td>House-wife</td>
<td>34</td>
<td>M</td>
<td>35</td>
<td>Teacher</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>20</td>
<td>Student</td>
<td>35</td>
<td>F</td>
<td>22</td>
<td>Clerk</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>30</td>
<td>Receptionist</td>
<td>36</td>
<td>M</td>
<td>38</td>
<td>Policeman</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>19</td>
<td>Student</td>
<td>37</td>
<td>M</td>
<td>28</td>
<td>Waiter</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>23</td>
<td>Typist</td>
<td>38</td>
<td>M</td>
<td>34</td>
<td>Accountant</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>26</td>
<td>Unemployed</td>
<td>39</td>
<td>M</td>
<td>43</td>
<td>Worker</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>52</td>
<td>House-wife</td>
<td>40</td>
<td>F</td>
<td>26</td>
<td>Messenger</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>28</td>
<td>Nurse</td>
<td>41</td>
<td>M</td>
<td>24</td>
<td>Waiter</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>22</td>
<td>Clerk</td>
<td>42</td>
<td>M</td>
<td>26</td>
<td>Cook</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>33</td>
<td>Manager</td>
<td>43</td>
<td>F</td>
<td>28</td>
<td>Cashier</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>33</td>
<td>Dentist</td>
<td>44</td>
<td>F</td>
<td>31</td>
<td>Worker</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>28</td>
<td>House-wife</td>
<td>45</td>
<td>F</td>
<td>32</td>
<td>Clerk</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>21</td>
<td>Dental Nurse</td>
<td>46</td>
<td>M</td>
<td>38</td>
<td>Clerk</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>29</td>
<td>Driver</td>
<td>47</td>
<td>M</td>
<td>43</td>
<td>Fireman</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>48</td>
<td>Businessman</td>
<td>48</td>
<td>M</td>
<td>40</td>
<td>Clerk</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>59</td>
<td>Unemployed</td>
<td>49</td>
<td>M</td>
<td>37</td>
<td>Worker</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>48</td>
<td>Unemployed</td>
<td>50</td>
<td>F</td>
<td>24</td>
<td>Shopkeeper</td>
</tr>
</tbody>
</table>
7.2 EXPERIMENTAL RESULTS

Each subject provided three samples of saliva being "Before Acupuncture", "Immediately after", and "24 hours after". After laboratory procedures previously described, the sample plates were left for 24 hours during which time the antibody diffuses out of the wells to form soluble complexes with the antigen. These continued to diffuse outwards, binding more antigen until an equivalence point is reached and the complexes precipitate in a ring. (Roitt et al 1985).

This ring was stained and measured by using the precision viewer. The area within the precipitin ring, measured as ring diameter squared in proportion to the antibody concentration. (Fridenberg et al 1978)

The diameter of each reaction ring was measured in units of 0.1mm. The diameter of the reaction is squared and the concentration in mg/dl can be read from the coordinate axis from the straight line graph which was plotted using the five known reference anti-sera. (Rose & Fridman 1976) The standard used is shown on Figure 5.

The exact concentration of sIgA can be obtained by dividing the concentration obtained by 20 because all the saliva samples had been concentrated 20X by the saliva concentrator before the reaction procedure.
Figure 5 Standard Curve for sIgA Concentration

sIgA Concentration
versus
Diameter\(^a\) of
Reaction Rings

<table>
<thead>
<tr>
<th>Reference</th>
<th>d(mm)</th>
<th>(d^a)</th>
<th>mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>1849</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>3025</td>
<td>104</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>4489</td>
<td>156</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>5929</td>
<td>209</td>
</tr>
<tr>
<td>5</td>
<td>87</td>
<td>7569</td>
<td>262</td>
</tr>
</tbody>
</table>
Individual sIgA Concentrations Found for Subjects

Table 7 presents the sIgA concentrations determined for subjects.

Samples 1-25 are test subjects and samples 26-50 are control subjects. Each subject donated 3 samples as described in the table as .1, .2, .3. Label .1 refers to base line level (i.e. at 11:00am), .2 refers to 11:20am, (in the case of test subjects, it means immediately after acupuncture stimulation) and .3 samples refer to 24 hours after. For the test and control subjects the .3 samples simply meant the saliva was collected at the same specific time but without any stimulation at all.

Also shown on the table are the diameter (mm) of the reaction, the diameter squared, the concentration in mg/dl read from the standard curve shown in Figure 5, and the concentration of sIgA in the saliva. Note that for some samples (marked *) the mucous in the saliva was thick and these samples were only concentrated 10X. The final concentrations were divided by a factor of 10 instead of 20.
## Table 7  sIgA Concentration Results for Subjects

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Sample Number</th>
<th>Reaction Diameter</th>
<th>Diameter²</th>
<th>Concentration mg/dl</th>
<th>± 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>37</td>
<td>1369</td>
<td>30</td>
<td>1.5</td>
</tr>
<tr>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>70</td>
<td>4900</td>
<td>164</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>46</td>
<td>2116</td>
<td>58</td>
<td>2.9</td>
</tr>
<tr>
<td>2.2</td>
<td>60</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td>65</td>
<td>4225</td>
<td>138</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.1</td>
<td>46</td>
<td>2116</td>
<td>58</td>
<td>2.9</td>
</tr>
<tr>
<td>3.2</td>
<td>43</td>
<td>1849</td>
<td>48</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>41</td>
<td>1681</td>
<td>42</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.1</td>
<td>42</td>
<td>1764</td>
<td>56</td>
<td>2.8</td>
</tr>
<tr>
<td>4.2</td>
<td>50</td>
<td>2500</td>
<td>73</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.1</td>
<td>69</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
</tr>
<tr>
<td>5.2</td>
<td>40</td>
<td>1600</td>
<td>38</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>70</td>
<td>4900</td>
<td>164</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.1</td>
<td>41</td>
<td>1681</td>
<td>42</td>
<td>2.1</td>
</tr>
<tr>
<td>6.2</td>
<td>56</td>
<td>3136</td>
<td>98</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>6.3</td>
<td>65</td>
<td>4225</td>
<td>138</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.1</td>
<td>62</td>
<td>3844</td>
<td>130</td>
<td>6.5</td>
</tr>
<tr>
<td>7.2</td>
<td>68</td>
<td>4624</td>
<td>158</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>7.3</td>
<td>73</td>
<td>5329</td>
<td>183</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8.1</td>
<td>58</td>
<td>3364</td>
<td>113</td>
<td>5.7</td>
</tr>
<tr>
<td>8.2</td>
<td>64</td>
<td>4096</td>
<td>138</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>8.3</td>
<td>72</td>
<td>5184</td>
<td>117</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9.1</td>
<td>40</td>
<td>1600</td>
<td>50</td>
<td>2.5</td>
</tr>
<tr>
<td>9.2</td>
<td>52</td>
<td>2704</td>
<td>90</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>9.3</td>
<td>50</td>
<td>2500</td>
<td>83</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.1</td>
<td>58</td>
<td>3364</td>
<td>113</td>
<td>5.7</td>
</tr>
<tr>
<td>10.2</td>
<td>70</td>
<td>4900</td>
<td>168</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>10.3</td>
<td>78</td>
<td>6084</td>
<td>209</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11.1</td>
<td>65</td>
<td>4225</td>
<td>138</td>
<td>6.9</td>
</tr>
<tr>
<td>11.2</td>
<td>56</td>
<td>3136</td>
<td>98</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>11.3</td>
<td>40</td>
<td>1600</td>
<td>39</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>12.1</td>
<td>65</td>
<td>4225</td>
<td>142</td>
<td>7.1</td>
</tr>
<tr>
<td>12.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12.3</td>
<td>55</td>
<td>3025</td>
<td>92</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Subject Number</td>
<td>Sample Number</td>
<td>Reaction Diameter</td>
<td>Reaction Diameter²</td>
<td>Concentration mg/dl</td>
<td>20</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
<td>-------------------</td>
<td>--------------------</td>
<td>---------------------</td>
<td>----</td>
</tr>
<tr>
<td>13</td>
<td>13.1</td>
<td>60</td>
<td>3600</td>
<td>116</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>13.2</td>
<td>65</td>
<td>4225</td>
<td>142</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>13.3</td>
<td>65</td>
<td>4225</td>
<td>142</td>
<td>7.1</td>
</tr>
<tr>
<td>14</td>
<td>14.1</td>
<td>60</td>
<td>3600</td>
<td>116</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>14.2</td>
<td>55</td>
<td>3025</td>
<td>92</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>14.3</td>
<td>45</td>
<td>2025</td>
<td>56</td>
<td>2.8</td>
</tr>
<tr>
<td>15</td>
<td>15.1</td>
<td>55</td>
<td>3025</td>
<td>92</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>15.2</td>
<td>72</td>
<td>5184</td>
<td>126</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>15.3</td>
<td>54</td>
<td>2196</td>
<td>88</td>
<td>4.4</td>
</tr>
<tr>
<td>16</td>
<td>16.1</td>
<td>40</td>
<td>1600</td>
<td>39</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>16.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16.3</td>
<td>60</td>
<td>3600</td>
<td>116</td>
<td>5.8</td>
</tr>
<tr>
<td>17</td>
<td>17.1</td>
<td>39</td>
<td>1521</td>
<td>36</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>17.2</td>
<td>34</td>
<td>1156</td>
<td>22</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>17.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>18.1</td>
<td>41</td>
<td>1681</td>
<td>42</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>18.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>18.3</td>
<td>43</td>
<td>1849</td>
<td>48</td>
<td>2.4</td>
</tr>
<tr>
<td>19</td>
<td>19.1</td>
<td>68</td>
<td>4624</td>
<td>158</td>
<td>15.8*</td>
</tr>
<tr>
<td></td>
<td>19.2</td>
<td>70</td>
<td>4900</td>
<td>168</td>
<td>16.8*</td>
</tr>
<tr>
<td></td>
<td>19.3</td>
<td>51</td>
<td>2601</td>
<td>87</td>
<td>4.4</td>
</tr>
<tr>
<td>20</td>
<td>20.1</td>
<td>56</td>
<td>3136</td>
<td>106</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>20.2</td>
<td>68</td>
<td>4761</td>
<td>163</td>
<td>16.3*</td>
</tr>
<tr>
<td></td>
<td>20.3</td>
<td>60</td>
<td>3600</td>
<td>122</td>
<td>12.2*</td>
</tr>
<tr>
<td>21</td>
<td>21.1</td>
<td>50</td>
<td>2500</td>
<td>85</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>21.2</td>
<td>65</td>
<td>4225</td>
<td>144</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>21.3</td>
<td>60</td>
<td>3600</td>
<td>122</td>
<td>6.1</td>
</tr>
<tr>
<td>22</td>
<td>22.1</td>
<td>90</td>
<td>8100</td>
<td>280</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>22.2</td>
<td>56</td>
<td>3136</td>
<td>106</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>22.3</td>
<td>56</td>
<td>3136</td>
<td>106</td>
<td>5.3</td>
</tr>
<tr>
<td>23</td>
<td>23.1</td>
<td>43</td>
<td>2849</td>
<td>58</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>23.2</td>
<td>62</td>
<td>3844</td>
<td>130</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>23.3</td>
<td>64</td>
<td>4096</td>
<td>138</td>
<td>6.9</td>
</tr>
<tr>
<td>24</td>
<td>24.1</td>
<td>69</td>
<td>4761</td>
<td>163</td>
<td>16.3*</td>
</tr>
<tr>
<td></td>
<td>24.2</td>
<td>60</td>
<td>3600</td>
<td>122</td>
<td>12.2*</td>
</tr>
<tr>
<td></td>
<td>24.3</td>
<td>60</td>
<td>3600</td>
<td>122</td>
<td>12.2*</td>
</tr>
<tr>
<td>25</td>
<td>25.1</td>
<td>39</td>
<td>1521</td>
<td>36</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>25.2</td>
<td>40</td>
<td>1600</td>
<td>38</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>25.3</td>
<td>45</td>
<td>2025</td>
<td>56</td>
<td>2.8</td>
</tr>
<tr>
<td>Subject</td>
<td>Sample Number</td>
<td>Reaction Diameter</td>
<td>Diameter $^2$</td>
<td>Concentration mg/dl</td>
<td>$\pm$ 20</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>-------------------</td>
<td>--------------</td>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td>26</td>
<td>26.1</td>
<td>60</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
</tr>
<tr>
<td>26</td>
<td>26.2</td>
<td>58</td>
<td>3364</td>
<td>113</td>
<td>5.7</td>
</tr>
<tr>
<td>26</td>
<td>26.3</td>
<td>60</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
</tr>
<tr>
<td>27</td>
<td>27.1</td>
<td>49</td>
<td>2400</td>
<td>69</td>
<td>3.5</td>
</tr>
<tr>
<td>27</td>
<td>27.2</td>
<td>49</td>
<td>2400</td>
<td>69</td>
<td>3.5</td>
</tr>
<tr>
<td>27</td>
<td>27.3</td>
<td>50</td>
<td>2500</td>
<td>73</td>
<td>3.7</td>
</tr>
<tr>
<td>28</td>
<td>28.1</td>
<td>71</td>
<td>5041</td>
<td>170</td>
<td>8.5</td>
</tr>
<tr>
<td>28</td>
<td>28.2</td>
<td>70</td>
<td>4900</td>
<td>164</td>
<td>8.2</td>
</tr>
<tr>
<td>28</td>
<td>28.3</td>
<td>69</td>
<td>4701</td>
<td>156</td>
<td>7.8</td>
</tr>
<tr>
<td>29</td>
<td>29.1</td>
<td>38</td>
<td>1444</td>
<td>32</td>
<td>1.6</td>
</tr>
<tr>
<td>29</td>
<td>29.2</td>
<td>38</td>
<td>1444</td>
<td>32</td>
<td>1.6</td>
</tr>
<tr>
<td>29</td>
<td>29.3</td>
<td>37</td>
<td>1369</td>
<td>30</td>
<td>1.5</td>
</tr>
<tr>
<td>30</td>
<td>30.1</td>
<td>47</td>
<td>2209</td>
<td>55</td>
<td>2.7</td>
</tr>
<tr>
<td>30</td>
<td>30.2</td>
<td>47</td>
<td>2209</td>
<td>55</td>
<td>2.7</td>
</tr>
<tr>
<td>30</td>
<td>30.3</td>
<td>45</td>
<td>2025</td>
<td>56</td>
<td>2.8</td>
</tr>
<tr>
<td>31</td>
<td>31.1</td>
<td>38</td>
<td>1444</td>
<td>32</td>
<td>1.6</td>
</tr>
<tr>
<td>31</td>
<td>31.2</td>
<td>39</td>
<td>1521</td>
<td>36</td>
<td>1.8</td>
</tr>
<tr>
<td>31</td>
<td>31.3</td>
<td>40</td>
<td>1600</td>
<td>35</td>
<td>1.7</td>
</tr>
<tr>
<td>32</td>
<td>32.1</td>
<td>46</td>
<td>2116</td>
<td>58</td>
<td>2.9</td>
</tr>
<tr>
<td>32</td>
<td>32.2</td>
<td>44</td>
<td>1936</td>
<td>62</td>
<td>3.1</td>
</tr>
<tr>
<td>32</td>
<td>32.3</td>
<td>48</td>
<td>2304</td>
<td>76</td>
<td>3.8</td>
</tr>
<tr>
<td>33</td>
<td>33.1</td>
<td>68</td>
<td>4624</td>
<td>156</td>
<td>7.8</td>
</tr>
<tr>
<td>33</td>
<td>33.2</td>
<td>65</td>
<td>4225</td>
<td>142</td>
<td>7.1</td>
</tr>
<tr>
<td>33</td>
<td>33.3</td>
<td>69</td>
<td>4761</td>
<td>160</td>
<td>8.0</td>
</tr>
<tr>
<td>34</td>
<td>34.1</td>
<td>66</td>
<td>4356</td>
<td>146</td>
<td>7.3</td>
</tr>
<tr>
<td>34</td>
<td>34.2</td>
<td>66</td>
<td>4356</td>
<td>146</td>
<td>7.3</td>
</tr>
<tr>
<td>34</td>
<td>34.3</td>
<td>69</td>
<td>4761</td>
<td>160</td>
<td>8.0</td>
</tr>
<tr>
<td>35</td>
<td>35.1</td>
<td>37</td>
<td>1369</td>
<td>30</td>
<td>1.5</td>
</tr>
<tr>
<td>35</td>
<td>35.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>35.3</td>
<td>42</td>
<td>1764</td>
<td>56</td>
<td>2.8</td>
</tr>
<tr>
<td>36</td>
<td>36.1</td>
<td>41</td>
<td>1691</td>
<td>39</td>
<td>2.0</td>
</tr>
<tr>
<td>36</td>
<td>36.2</td>
<td>40</td>
<td>1600</td>
<td>38</td>
<td>1.9</td>
</tr>
<tr>
<td>36</td>
<td>36.3</td>
<td>40</td>
<td>1600</td>
<td>38</td>
<td>1.9</td>
</tr>
<tr>
<td>37</td>
<td>37.1</td>
<td>60</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
</tr>
<tr>
<td>37</td>
<td>37.2</td>
<td>60</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
</tr>
<tr>
<td>37</td>
<td>37.3</td>
<td>60</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
</tr>
</tbody>
</table>

126
<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Sample Number</th>
<th>Reaction Diameter</th>
<th>Reaction Diameter$^2$</th>
<th>Concentration mg/dl</th>
<th>% 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>38.1</td>
<td>64</td>
<td>4096</td>
<td>138</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>38.2</td>
<td>62</td>
<td>3844</td>
<td>130</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>38.3</td>
<td>69</td>
<td>4761</td>
<td>160</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>39.1</td>
<td>56</td>
<td>3136</td>
<td>104</td>
<td>5.2</td>
</tr>
<tr>
<td>39</td>
<td>39.2</td>
<td>54</td>
<td>2916</td>
<td>88</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>39.3</td>
<td>56</td>
<td>3136</td>
<td>104</td>
<td>5.2</td>
</tr>
<tr>
<td>40</td>
<td>40.1</td>
<td>63</td>
<td>3969</td>
<td>133</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>40.2</td>
<td>64</td>
<td>4096</td>
<td>140</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>40.3</td>
<td>60</td>
<td>3600</td>
<td>116</td>
<td>5.8</td>
</tr>
<tr>
<td>41</td>
<td>41.1</td>
<td>59</td>
<td>3481</td>
<td>116</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>41.2</td>
<td>60</td>
<td>3600</td>
<td>122</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>41.3</td>
<td>58</td>
<td>3364</td>
<td>112</td>
<td>5.6</td>
</tr>
<tr>
<td>42</td>
<td>42.1</td>
<td>63</td>
<td>3969</td>
<td>134</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>42.2</td>
<td>46</td>
<td>2116</td>
<td>70</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>42.3</td>
<td>47</td>
<td>2209</td>
<td>72</td>
<td>3.6</td>
</tr>
<tr>
<td>43</td>
<td>43.1</td>
<td>57</td>
<td>3249</td>
<td>110</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>43.2</td>
<td>53</td>
<td>2809</td>
<td>92</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>43.3</td>
<td>55</td>
<td>3025</td>
<td>72</td>
<td>3.6</td>
</tr>
<tr>
<td>44</td>
<td>44.1</td>
<td>54</td>
<td>2916</td>
<td>98</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>44.2</td>
<td>60</td>
<td>3600</td>
<td>116</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>44.3</td>
<td>55</td>
<td>3025</td>
<td>105</td>
<td>5.3</td>
</tr>
<tr>
<td>45</td>
<td>45.1</td>
<td>48</td>
<td>2304</td>
<td>76</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>45.2</td>
<td>48</td>
<td>2304</td>
<td>76</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>45.3</td>
<td>50</td>
<td>2500</td>
<td>82</td>
<td>4.6</td>
</tr>
<tr>
<td>46</td>
<td>46.1</td>
<td>38</td>
<td>1444</td>
<td>32</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>46.2</td>
<td>38</td>
<td>1444</td>
<td>32</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>46.3</td>
<td>39</td>
<td>1521</td>
<td>36</td>
<td>1.8</td>
</tr>
<tr>
<td>47</td>
<td>47.1</td>
<td>61</td>
<td>3721</td>
<td>118</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>47.2</td>
<td>61</td>
<td>3721</td>
<td>118</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>47.3</td>
<td>62</td>
<td>3844</td>
<td>130</td>
<td>6.5</td>
</tr>
<tr>
<td>48</td>
<td>48.1</td>
<td>54</td>
<td>2916</td>
<td>88</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>48.2</td>
<td>54</td>
<td>2916</td>
<td>88</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>48.3</td>
<td>54</td>
<td>2916</td>
<td>88</td>
<td>4.4</td>
</tr>
<tr>
<td>49</td>
<td>49.1</td>
<td>52</td>
<td>2704</td>
<td>78</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>49.2</td>
<td>50</td>
<td>2500</td>
<td>73</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>49.3</td>
<td>52</td>
<td>2704</td>
<td>78</td>
<td>3.9</td>
</tr>
<tr>
<td>50</td>
<td>50.1</td>
<td>45</td>
<td>2025</td>
<td>56</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>50.2</td>
<td>46</td>
<td>2116</td>
<td>58</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>50.3</td>
<td>46</td>
<td>2116</td>
<td>58</td>
<td>2.9</td>
</tr>
</tbody>
</table>
7.3 STATISTICAL ANALYSIS

Four different groupings of data were selected for comparison and the 't' test was used to determine the significance levels between the sets of results.

1. The first grouping was selected to test the changes upon stimulation by the experimental factors for control and test groups. Baseline levels were compared with reference to each direction of change (ie either increased or decreased values), for each of the control and test groups, at the two different time intervals following stimulation (ie immediately after and at 24 hours after). Results are shown in 7.3.1 and Tables 8 & 9.

2. Changes following the experiment for all 50 subjects (Control and Test combined) were tested for samples at baseline versus "immediately after" and baseline "24 hours after" to evaluate their occurrence probability. Results are presented in 7.3.2 and Table 10.

3. The third grouping was used to test the 25 control versus 25 experimental subjects at baseline, immediate, and 24 hour levels. Results are presented in 7.3.3 and Table 11.

4. Comparison of Control versus Test groups by combining the "immediately after" with the "24 hours after" samples for both the control and test subjects. (7.3.4 and Table 12)

5. A replication of procedures was also carried out 12 months later for one of the original subjects. (7.3.5 and Table 13)
The sample size number (N) is important in the statistical tests carried in this section to determine the significance level of the results and the degree of freedom. Since the sample number is N, the degree of freedom for this test would be N-1 for all samples. (Blalock 1960)

The following equation was used to determine the 't' value of the results:

\[
't' = \frac{\bar{X}_1 - \bar{X}_2}{SE \, diff}
\]

\[
SE \, diff = \sqrt{\left(\frac{1}{N_1} + \frac{1}{N_2}\right) \left(\frac{SD_1^2 (N_1-1) + SD_2^2 (N_2-1)}{N_1 + N_2 - 2}\right)}
\]

Where \(SE \, diff\) = standard error of the difference between the 2 sets of samples.

\(N_1\) = number of the first set of samples

\(N_2\) = number of the second set of samples

\(SD_1\) = standard deviation of the first set of samples

\(SD_2\) = standard deviation of the second set of samples

\(\bar{X}_1\) = mean value of first set of samples

\(\bar{X}_2\) = mean value of second set of samples

If we use the null hypothesis that there is no difference between two sets of samples (e.g. before and after stimulation), thereby assuming that the experimental factor has no effect to the results obtained, we can simply apply the 't' test to the figure to evaluate the significant level of the results.
7.3.1 Changes after Stimulation in Control and Test Groups

a. Control Group ... Table 7 & Table 8

For the 25 control group, the "immediately after" samples consist of 6 samples with increase in S-IgA concentrations and 9 samples show decrease in S-IgA concentrations. There are 9 samples showing no changes and 1 missing.

The "24 hours after" samples consist of 12 samples with increase in S-IgA concentrations and 8 samples with decrease in S-IgA levels. Five samples showed no changes and no samples are missing.

The p values of all the control groups are well over the 0.05 level and therefore we can conclude that the null hypothesis is valid and there is no difference between the two sets of figures.

b. Test Group ... Table 7 & Table 9

From the table of results obtained, we can observe that out of the 25 test subjects, the "immediately after" samples consist of 14 samples that show an increase of S-IgA concentrations and 7 samples show decrease S-IgA concentrations. The remaining 4 samples are missing.

The "24 hours after" samples, consists of 15 samples with increase in S-IgA level. The missing samples are 2.

The missing data in the list are due to exclusions when the diffusion rings were not clear and obvious in the agar plate. The exact measurements of the rings were unable to be determined. This was caused by overlapping of the reaction rings on the agar plate or
the absence of an outline that could be defined. These data are ignored and hence the sample size was reduced accordingly. For instance, the total "immediately after" sample for the test group were 25, but the missing samples are 4, therefore the sample size has become 21.

Since we use the 0.05 level for significant level in the 't' test, the three test groups "immediate increase", "24 hours increase" and "24 hours decrease" all show p 0.05 and therefore we can decide to reject the null hypothesis and noting the direction of the difference, we can conclude that concentration of S-IgA levels after acupuncture is of scientific significance.

For the "immediate decrease" samples the p value is just over the 0.10 level and therefore it cannot be concluded to be significant as probability due to chance only is just above 10/100.
Table 8 Changes in S-IgA after Stimulation for Control Groups

Control Groups:
1. Baseline vs immediate increase (6 out of 15 samples)
2. Baseline vs 24 hour increase (12 out of 20 samples)
3. Baseline vs immediate decrease (9 out of 15 samples)
4. Baseline vs 24 hour decrease (8 out of 20 samples)

<table>
<thead>
<tr>
<th></th>
<th>Immediate increase</th>
<th>24 hrs increase</th>
<th>Immediate decrease</th>
<th>24 hrs decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>6</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>N2</td>
<td>6</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>X1</td>
<td>3.65</td>
<td>4.025</td>
<td>5.30</td>
<td>5.21</td>
</tr>
<tr>
<td>X2</td>
<td>3.97</td>
<td>4.55</td>
<td>4.66</td>
<td>4.39</td>
</tr>
<tr>
<td>SD1</td>
<td>2.37</td>
<td>2.23</td>
<td>2.59</td>
<td>2.21</td>
</tr>
<tr>
<td>SD2</td>
<td>2.53</td>
<td>2.33</td>
<td>1.79</td>
<td>1.99</td>
</tr>
</tbody>
</table>

't' values

<table>
<thead>
<tr>
<th></th>
<th>0.23</th>
<th>0.53</th>
<th>0.54</th>
<th>0.78</th>
</tr>
</thead>
</table>

degrees of freedom

<table>
<thead>
<tr>
<th></th>
<th>5</th>
<th>11</th>
<th>8</th>
<th>7</th>
</tr>
</thead>
</table>

confidence levels

<table>
<thead>
<tr>
<th></th>
<th>t&lt;0.70</th>
<th>t&lt;0.70</th>
<th>t&lt;0.70</th>
<th>t&lt;0.80</th>
</tr>
</thead>
</table>

p values

<table>
<thead>
<tr>
<th></th>
<th>p&gt;0.30</th>
<th>p&gt;0.30</th>
<th>p&gt;0.30</th>
<th>p&gt;0.20</th>
</tr>
</thead>
</table>
Table 9  Changes in S-IgA after Stimulation for Test Groups

Test Groups:
1. Baseline vs immediate increase (14 out of 21 samples)
2. Baseline vs 24 hours increase (15 out of 23 samples)
3. Baseline vs immediate decrease (7 out of 21 samples)
4. Baseline vs 24 hours decrease (8 out of 23 samples)

<table>
<thead>
<tr>
<th></th>
<th>Immediate increase</th>
<th>24 hrs increase</th>
<th>Immediate decrease</th>
<th>24 hrs decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>14</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>N2</td>
<td>14</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>X1</td>
<td>4.907</td>
<td>3.793</td>
<td>7.643</td>
<td>9.175</td>
</tr>
<tr>
<td>X2</td>
<td>7.443</td>
<td>7.053</td>
<td>4.629</td>
<td>4.725</td>
</tr>
<tr>
<td>SD1</td>
<td>3.377</td>
<td>3.793</td>
<td>5.062</td>
<td>4.989</td>
</tr>
<tr>
<td>SD2</td>
<td>4.074</td>
<td>2.533</td>
<td>3.438</td>
<td>3.051</td>
</tr>
</tbody>
</table>

't' values

|       | 1.794              | 3.940           | 1.303              | 2.152           |

degrees of freedom

|       | 13                 | 14              | 6                  | 7               |

confidence levels

|       | 0.95<t              | 0.975<t         | 0.80<t<0.90        | t<0.975         |

p values

|       | p<0.05              | p<0.03          | p>0.10             | p<0.03          |
7.3.2 Changes after Stimulation in All Subjects

When control and test data were combined the differences in the mean S-IgA between Baseline and Immediately After, and Baseline and 24 Hrs After were tested. The probability of the differences being due to chance alone are both greater than 30/100 and therefore the difference obtained cannot be accepted as significant.

This means that by combining the test and control subjects together, the changes were not significant as there was little variation in the control groups.

Table 10 Changes in S-IgA after Stimulation in All Subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline vs Immediately after</th>
<th>Baseline vs 24 hours after</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 Baseline</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>N2</td>
<td>45</td>
<td>48</td>
</tr>
<tr>
<td>X1</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>X2</td>
<td>5.33</td>
<td>5.38</td>
</tr>
<tr>
<td>SD1</td>
<td>3.31</td>
<td>3.31</td>
</tr>
<tr>
<td>SD2</td>
<td>3.38</td>
<td>2.66</td>
</tr>
</tbody>
</table>

't' values

<table>
<thead>
<tr>
<th>degrees of freedom</th>
<th>49</th>
<th>49</th>
</tr>
</thead>
<tbody>
<tr>
<td>confidence level</td>
<td>0.70 &gt; t &gt; 0.60</td>
<td>0.60 &gt; t &gt; 0.50</td>
</tr>
<tr>
<td>p values</td>
<td>p &gt; 0.30</td>
<td>p &gt; 0.40</td>
</tr>
</tbody>
</table>
7.3.3 Control versus Test Groups

When the mean S-IgA figures for the Control and Test subjects were compared at Baseline there was no statistically significant difference shown since the probability due to chance was 0.5.

However, the differences between the Control and Test groups for both the "immediately after" and 24 hour after samples are significantly different at the p < 0.05 level.

Table 11  S-IgA in Control and Test Subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Immediately after samples</th>
<th>24 hrs after samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>25</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>N2</td>
<td>25</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>X1</td>
<td>4.604</td>
<td>4.279</td>
<td>4.592</td>
</tr>
<tr>
<td>X2</td>
<td>5.396</td>
<td>6.501</td>
<td>6.224</td>
</tr>
<tr>
<td>SD1</td>
<td>2.137</td>
<td>2.126</td>
<td>2.053</td>
</tr>
<tr>
<td>SD2</td>
<td>4.174</td>
<td>4.196</td>
<td>3.008</td>
</tr>
</tbody>
</table>

'\( t \)' values

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.845</td>
<td>2.274</td>
<td>2.238</td>
</tr>
</tbody>
</table>

degrees of freedom

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
<td>23</td>
<td>24</td>
</tr>
</tbody>
</table>

confidence level

<table>
<thead>
<tr>
<th></th>
<th>t=0.50</th>
<th>t&gt;0.975</th>
<th>t&gt;0.975</th>
</tr>
</thead>
</table>

p values

<table>
<thead>
<tr>
<th></th>
<th>p=0.50</th>
<th>p&lt;0.03</th>
<th>p&lt;0.03</th>
</tr>
</thead>
</table>

135
7.3.4 Control versus Test Group after Stimulation

After combining the S-IgA results for the samples taken immediately after stimulation with those taken after 24 hours the mean figures for the Test group were found to be higher than those found for the Control group.

Since $p < 0.05$, the null hypothesis is rejected and we can conclude that the difference is significant.

Table 12 Control vs Test S-IgA after Stimulation
Combined Immediate After & 24 hour Samples

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Test Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 25 &quot;Immediately after&quot; samples</td>
<td></td>
<td>11. 25 &quot;Immediately after&quot; samples</td>
</tr>
<tr>
<td>25 &quot;24+ hours after&quot; samples</td>
<td>25 &quot;24+ hours after&quot; samples</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>49</td>
<td>44</td>
</tr>
<tr>
<td><strong>$\bar{X}$</strong></td>
<td>4.4565</td>
<td>6.368</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>1.994</td>
<td>3.582</td>
</tr>
</tbody>
</table>

$t = 3.039$

degrees of freedom = 48

confidence level = $t > 0.975$

$p$ value = $p < 0.03$
7.3.5 Replication of Procedures After 12 Months for 1 Subject

Patient No. 2 was called in 12 months later to repeat the acupuncture stimulation and collection of saliva following the exact procedures as in the acupuncture experiment one year previously.

The results obtained from the repeated experiment for subject No.2 show little deviation from the original results.

This result not only demonstrates little change induced by experimental method or patient variation but also it could show that the memory effect of antibody secretion upon stimulation by acupuncture was not existing after a period of one year.

Table 13 Replication of Procedures After 12 Months for 1 Subject

<table>
<thead>
<tr>
<th>Samples</th>
<th>Reaction Diameter</th>
<th>Reaction Diameter²</th>
<th>Concentration sIgA mg/dl</th>
<th>Conc./20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>46</td>
<td>2116</td>
<td>58</td>
<td>2.9</td>
</tr>
<tr>
<td>2.2</td>
<td>60</td>
<td>3600</td>
<td>115</td>
<td>5.8</td>
</tr>
<tr>
<td>2.3</td>
<td>65</td>
<td>4225</td>
<td>138</td>
<td>6.9</td>
</tr>
<tr>
<td>Recall Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>47</td>
<td>2209</td>
<td>55</td>
<td>2.7</td>
</tr>
<tr>
<td>2.2</td>
<td>62</td>
<td>3844</td>
<td>130</td>
<td>6.5</td>
</tr>
<tr>
<td>2.3</td>
<td>64</td>
<td>4096</td>
<td>138</td>
<td>6.9</td>
</tr>
</tbody>
</table>
Immunoglobulins and other proteins in mixed saliva are derived from three principle sources, the major salivary glands, the minor salivary glands and crevicular fluid. In addition, contributions to the total immunoglobulin content of mixed saliva may be made by direct passage across epithelium, particularly if this is inflamed (Lehner & Cimasoni 1980).

The source and function of immunoglobulins in the oral cavity is currently of great interest, particularly with regard to dental caries and periodontal disease (Lehner 1986). The tooth surface is unique in that it is exposed to both the secretory immune system, as represented by saliva, and the systemic humoral system via antibodies in crevicular fluid which are assumed largely to be derived from serum (Arnold, Mesticky & McRhee 1976).

Theoretically either or both of these systems may play a role in protection against dental caries and periodontal disease. The area of the tooth surface which is exposed primarily to saliva may be different from that exposed to crevicular fluid, and the terms salivary domain and crevicular domain have been proposed to distinguish between these areas (Lehner et al 1976).

The large number of gram positive and negative bacteria and their products, such as LPS, lipopolysaccharides, dextrins and levans enable most of the immunological mechanisms so far examined to be activated. Both complement pathways are activated, lymphocytes are stimulated, lymphokines are released and macrophages are activated (Gibbsons & Houte 1975).
The development of dental caries requires:

(a) the presence of cariogenic bacteria that are capable of rapidly producing acid below the critical pH required for dissolving enamel; and

(b) a sugar in the diet that favours colonisation of these bacteria and that can be metabolised by the bacteria to form acid. This process can be interfered with by the presence of an effective immune response (Figure 6).

In man, serum IgA, IgG, and IgM antibodies, as well as cell mediated immunity to strep mutans can be correlated with the DMF index of caries (Lehner 1980).

Secretory IgA antibodies are also found to have such a correlationship (Taubman & Smith 1974).

Involvement of salivary IgA antibodies is probably induced by immunisation of the gut associated lymphoid tissue, from where sensitised B cells may home to salivary glands. Salivary antibodies may prevent strep mutans from adhering to the tooth surface and thereby prevent caries (Gibbsons 1980).

Accumulation of dental bacterial plaque and the associated gingival inflammation have been correlated with an increase in lymphocyte transformation and release of macrophage migration inhibition factor (MIF) (Lehner 1980).

Dental plaque can induce increased DNA synthesis of lymphocytes which have been previously sensitised to some of the plaque antigens. Both T and B lymphocytes respond to plaque antigens, as
indeed they respond to different components of single bacteria; T lymphocytes respond to the protein fraction, whereas B lymphocytes respond to the lipoprotein of veillonella. There is a significant correlation between the proliferative response of lymphocytes stimulated by dental plaque, as compared with single organisms, such as Veillonella alcalescens, or Actinomyces viscosus (Lindhe & Hellden 1973).

Sensitized lymphocytes also respond to dental plaque by the release of soluble mediators or lymphokines. These are released by both T and B cells, though some lymphokines, such as mitogenic and chemotactic factors, are released predominantly by T cells and others, such as osteoclast activating factor (OAF), by B cells. A release of macrophage migration inhibitory factor might localise macrophages to the site of lymphocyte activation. Another mediator, lymphotoxin, is cytotoxic for human gingival fibroblasts which are concerned in laying down collagen in the periodontal membrane. OAF is also released by activated lymphocytes and causes bone resorption, so that it may cause destruction of the supporting alveolar bone (Horton, Oppenheim & Mergenhagen 1974).

The involvement of secretory IgA antibodies in periodontal disease has been summarised by Lehner (1983) in the illustration presented in Figure 7.
Figure 6 Requirements for Dental Caries
(Lehner 1983)
Figure 7 Defense Mechanism of Oral Cavity
(Bear & Morris 1977)
The result of the acupuncture experiment conducted in this treatise shows that there are significant changes in secretory IgA concentration levels both in the samples collected immediately after the electro-acupuncture stimulation and the samples collected 24 hours after the stimulation.

Out of the 25 test subjects, 8 patients show suppression of IgA concentration effect but the remaining 17 test subjects showed significant rise in IgA secretion levels after acupuncture stimulations.

Although the reason for the drop of IgA concentrations in the 8 subjects are not known, the main objective of this treatise is to conduct a pilot study to find out is there any effect on immunological response in human saliva upon stimulation by electro-acupuncture. That is to say, we are more interested in looking at the effect of changes in saliva IgA levels rather than just interested in looking at the exact amount of increase or decreased concentration levels.

From the statistical point of view, the null hypothesis proposed in this treatise can be rejected, the assumption that no relationship exists between acupuncture and salivary IgA antibody levels is not valid. The change of sIgA concentrations from the test group can be said to have significant values, whereas the changes in the control group had no significant difference.

The science in acupuncture research and studies has been quickly developed both in the East and the West during recent years. The medical principles and the physiological basis of this ancient art
of healing is gradually being revealed and has gained the increased confidence of modern medical workers (Advance in Acupuncture & Acupuncture Anaesthetic 1979).

The principle of the pain killing effect of acupuncture has obtained favourable supporting evidence, but the physiological phenomenon behind the healing effect of acupuncture as yet requires further investigations.

Up to the present time only a few experimental studies or data were available about the use and effect of acupuncture on the immunological status of man (Sabolovic & Michon 1978). The researches starting with Omura (1974) and then in China (Advances in Acupuncture and Acupuncture Anesthesia 1979) and works of Ding et al (1983), were mainly concentrated on the T & B cells responses that acupuncture could have on the human immunological system. The change in composition of T & B and the phagocytic activity of the reticular endothelial system of animals have been observed (Sin 1983). The anti-inflammatory effect by acupuncture has been established by different workers. (Ding et al 1983) (Bi & Gao 1979) (Ma & Chang 1979).

Yet for the effect of acupuncture on immunoglobulins responses little information is available from studies carried out. The findings from Li (1979), Chen et al (1979) and the reports from the Festering Moxibustion Research Group in China (1979) gives some introduction to the antibody levels following stimulation by acupuncture. These together with the observation by Rogers and Bossy (1981) of increased specific and non-specific antibody in
subjects after acupuncture stimulation provides a stepping stone in this research.

Shimura et al (1981) have reported that dental caries of rats was prevented by acupuncture but the exact mechanisms were not known. Whether this effect of caries prevention was due to antibody effect as yet requires further investigation. But, this information does stimulate interest to caries researchers on the immunological role of acupuncture in dental decay.

It would appear that in this present study that salivary sIgA antibody has been studied for the first time following stimulation by electric acupuncture.

The results found in this experiment not only provide some valuable information relating acupuncture to the defense mechanism of the oral cavity, but also it should stimulate medical researchers in acupuncture to study the healing effect that acupuncture could have for the human body.

It could be concluded that further investigations in this field are warranted and the following notes are suggested by the writer if any similar type of experiment is carried out in the future.

1. The total number of test subjects and control subjects should be increased.

2. The use of the ear acupuncture electrodes should be improved since the elastic copper electrodes used in this experiment tend to move or slip away from the ear point if the patient turned his head and thus may affect the accuracy of the stimulation.
3. During collection of saliva, some patients complained of difficulty in spitting 5ml of saliva three times because of dry mouth. This may be able to be overcome by the use of freshly cut lemons, the smell of this sour fruit may help to increase saliva secretions.

4. The presence of food debris in the saliva samples would cause problems in concentration accuracy determinations and thus could affect the final results. A professional dental prophylaxis is suggested for each patient before saliva collection in each case.

5. Punctuality is important for each patient’s stimulation appointment to avoid time variation factors. If the patient is late for the appointment, he should be advised to return the next day. The third saliva sample after 24 hours, could be collected by the patient at home if he is unable to attend the clinic exactly at the appointment time.

6. Storage and delivery of the samples to the laboratory should be specially considered. It is advised to keep the sample in dry ice during delivery and inside -70 degrees Celsius chamber immediately after arrival to the laboratory. They should be kept in the chamber until they are ready to carry out reactions on the agar plate. This precaution is to avoid protein degradation due to hot temperature and climate.

7. The distance between reaction wells in the agar plate should be increased to a minimum of 2cm away from the next well so as to avoid overlapping or interference of the reaction rings.
CONCLUSIONS

1. Acupuncture, the ancient Chinese healing art, has existed for centuries and is now being used in many countries, both in the East and the West. It has gained acceptance in many countries for its role in curing different diseases and for relief of pain.

2. The principles behind this healing art are now being studied and modern scientific appraisal of this 'mysterious phenomenon' has indicated promising results from its use.

3. The analgesic effect of acupuncture has been explored by many authors. Among these authors, the theories on the endorphin concept and the 'Gate' theory are most popular.

4. The healing effect of acupuncture is a new field that interests many scientists. The theory that acupuncture can affect antibody formation and secretion has been reviewed in the literature and much supporting evidence is available.

5. The immunological basis of the major oral diseases, dental caries and periodontal disease, has been reviewed. The involvement of secretory IgA antibodies in saliva has been shown to have demonstrable effects on these oral diseases.

6. An experiment was designed to find out if there are any changes in saliva secretory IgA antibodies in human subjects with acupuncture stimulation. The results show that changes can be observed.
7. The null hypothesis proposed in section 1.4 was rejected and we can assume that there is a relationship existing between the acupuncture stimulation conducted in this experiment and the level of sIgA concentration in saliva obtained from the subjects.

8. The study indicates that acupuncture stimulation could have some effect on the concentration level of IgA secretion in human subjects. This may imply that a "relationship" exists between this ancient art of acupuncture and the immunological science of the oral cavity.

9. Further study and investigation of the inter-relationship of these two subjects is strongly suggested for both the medical and dental professional workers.
ADVANCE IN ACUPUNCTURE AND ACUPUNCTURE ANAESTHETIC (1979)
Abstracts of papers presented at National Symposium of Acupuncture,
Beijing January 1-5 1979.
Beijing, China: The Beijing Medical Publishing House.

AI M, RU L, LUO Q (1979)
Influence of electroacupuncture on cholinesterase activity in thalamus
of rat.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 443.

ALEXANDER RE (1973)
Acupuncture: Ancient art modern enigma.

ANDERSON SA (1979)
Pain control by sensory stimulation.
In: Bonica JL, Liebskind JC, Albefassard DG eds. Advances in pain
research and therapy.
New York: Raven. 569-585.

ANDERSON SA, ERICSON T, HOLMGREN E, LINDQUIST G (1973)
Electro-acupuncture effect on pain threshold measured with electrical
stimulation of teeth.
Brain Res 63:393-396.

ARNOLD RP, MERTERKY J, McGHEE JR (JR) 1976
Naturally occurring secretory IgA antibody to s mutan in human
colostrum and saliva.
Infect Immun 14:355-357.

ATTSTROM R (1975)
The roles of gingival epithelia and phagocytosing leukocytes in
gingival defence.

ATTSTROM R, EGELBERG J (1970)
Emigration of blood neutrophils and monocytes into gingival
crevices.

BALDRY P (1986)
Changing attitude to acupuncture among oriental and western

BARBER J, MAYER D (1977)
Evaluation of the efficacy and neural mechanism of a hypnotic
analgesia procedure in experimental and clinical dental pain.
BAER PN, MORRIS ML (1977)
Textbook of periodontics.
Philadelphia: JB Lippincott. 96-129.

BEECHER CR (1974)
Acupuncture: some considerations for the control of pain in dentistry.
J Prosthet Dent 31:441-450.

BERGLUND SE (1971)
Immunoglobulins in human gingiva with specificity for oral bacteria

BI S, GAO J (1979)
Effect of acupuncture on adapting defense resistance of organism.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 509.

BI S, XU J, GAO J (1979)
Studies on the anti inflammatory effect of acupuncture.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 99.

BLALOCK HM (1960)
Social Statistics.

BOWEN WH, COHEN B, COLE MF, COLMAN G (1975)
Immunisation against dental caries.

BRANDTZAEG P (1971)
Human secretory immunoglobulin concentration of parotid IgA and
other secretory protein in relation to the rate of flow and
duration of secretory stimulus.

BRANDTZAEG P (1983)
Secretory immune system with special emphasis on its relation to
dental caries.

BRATTHALL D, GIBBONS RJ (1975)
Changing agglutination activities of salivary immunoglobulin. A
preparation against oral streptococci.
Immunol 11:603-606.

CAI L, XU C (1979)
A study on the relationship between autonomous nervous system and
acupuncture analgesia by determining dopamine - B - hydroxylase
cholinesterase activities in blood.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 458.

CARLSSON J, KRASSE B (1968)
Inhibition of streptococcal dextran sucrase by sera of rabbits
infected with s. sanguis.
CEBRA JJ, CRAIG SW, JONES PP (1974)
Cells contributing to the biosynthesis of SIgA.
In: Masteecky J, Lawton AR eds. The immunoglobin A system.
Advance in Experimental Medicine and Biology Vol 45.

The secretory IgA system of the gut.

CHALLACOMBE SJ, LEHNER T (1980)
Salivary antibody responses in Rhesus monkeys immunised with streptococcal mutants by the oral submucosal or subcutaneous routes.
Arch Oral Biol 24:917-919.

CHAPMAN CR (1974)
Acupuncture - Some considerations for the control of pain in dentistry.

CHAPMAN CR, GEHRIG JD, WILSON ME (1975)
Acupuncture compared with 33% nitrous oxide for dental analgesia.
Anaesthesiology 42:532-537.

CHAPMAN CR, MURPHY TM, BUTLER SH (1973)
Analgic strength of 33 percent nitrous oxide: A signal detection theory evaluation.
Science 179:1246-1248.

CHEN D (1979)
A clinical investigation in the prevention and treatment of hypertension by moxibustion therapy.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 57.

CHEN Y, SOU M, LIU F, WU S, MENG X, SONG X (1979)
A research test for the effect of the regular acupuncture moxibustion therapy device and moxibustion on the immune function of rabbits.
In: Advance in Acupuncture and Acupuncture Anaesthetics. 540.

CHENG RSS, POMERANZ BH (1979)
Electro-acupuncture analgesia could be mediated by at least two pain relieving mechanisms: Endorphin and non endorphin systems.

CHENG RSS, POMERANZ BH (1980)
Electro-acupuncture analgesia is mediated by stereospecific opiate receptors and is reversed by antagonists of type I reporters.
Life Science 26:631-638.

COGAN RB, ROSEMAN JM, AL-JOUBURI W, LOWE WC, ACTON CT, BARGER BO,
GO RCP, RASMUSSEN RA (1986)
Host factors in juvenile periodontitis.
COUNCIL ON DENTAL RESEARCH (1974)
Use of acupuncture for dental therapy and analgesia.

CRADDOCK CG, LONGMIRE R, McMILLAN R (1971)
Lymphocytes and the immune response.

CRAWFORD JM, TAUBMAN MA, SMITH DJ (1978)
The natural history of periodontal bone loss in germ free and
geticobiotic rats infected with periodontopathic microorganism.

CUTTRESS TW, HUNTER, HOSKINS DIH (1986)
Comparison of the Periodontal Index (PI) and Community
Periodontal Index of Treatment Needs (CPITN).

DANNENBERG AM (1975)
Macrophage in inflammation and infection.

DAVIES NC, MONTO H (1976)
American Society of Microbiology. 4-8.

DEMBEK Z (1972)
A contribution towards the comprehension of acupuncture and its
physiological basis.
Willimantic, USA: Eastern CT Stage College. 2-12.

DESCHEPPER L (1985)
Acupuncture for the practitioners.
Hong Kong: Alpha Educational Ltd. 5-15.

DIMOND EG (1971)
Acupuncture anaesthesia, western medicine and chinese traditional
medicine.

DING V, ROATH S, LEWIS GT (1983)
The effect of acupuncture on lymphocytes behaviour.

DOLBY AF (1969)
Recurrent aphthous ulceration: Effect of sera and peripheral blood
upon epithelial tissue culture cells.
Immunology 17:709-710.

DUBNER R (1976)
Efficacy and possible mechanisms of action of acupuncture anaesthesia:
Observation based on a visit to the People's Republic of China.
EBERSOLE JL, TAUBMAN MA, FREY DE, SMITH DJ (1980)
An ELISA for measuring serum antibodies to Actinobacillus
actinomycetemcomitans.

EBERSOLE JL, TAUBMAN MA, SMITH DJ, SOKRANSKY SS (1982)
Humoral immune response and the diagnosis of periodontal disease.

EBERSOLE JL, TAUBMAN MA, SMITH DJ, HAFFAJEE AD (1985)
Effects of subgingival scaling on systemic antibody responses to
oral microorganism,

EBERSOLE JL, FREY DE, TAUBMAN MA, HAFFAJEE AD, SOKRANSKY
SS(1987)
Dynamics of systemic antibody responses in periodontal disease

EVANS RT, GENCO RJ (1973)
Inhibition of glucosyltransferase activity by antisera to known
serotypes of streptococcus mutans.

EVANS RT, EMMINGS FG, GENCO RJ (1975).
Prevention of streptococcus mutans infection of tooth surfaces by
salivary antibody in Irus monkeys. (Macaca fascicularis).

FESTERING MOXIBUSTION RESEARCH GROUP(1979)
Observation of immunological variation in the festering moxibustion
treatment of bronchial asthma.
In: Advance in Accupuncture and Accupuncture Anaesthetics. 168.

Bone resorption in advanced cases of periodontitis.

FRIDENBERG HH, STITES DP, CALDWELL JL, WELL JV (1978)
Basic and clinical immunology. 2nd ed.
California: Lange Medical Publisher Co. 205-216.

FULLMER HM, GIBSON W (1966)
Collagenolytic activity in gingivae of man.

Host responses in periodontal disease

GENCO RJ, MASHIMO PA, KRYGIER G, ELLISON SA (1974)
Antibody mediated effects on the periodontium.
Systemic immune response to oral anaerobic organism
In: Lambe DW, Genco RJ, Mayberry Carson KJ eds.

GIBB JWG (1981)
Acupuncture: Its role in general practice medicine.
Patient Management 9: 77-79.

GIBBONS RJ (1980)
Adhesion of bacteria to surface of mouth.
In: Berkeley RCW, Lynch JM. Microbial adhesion and surfaces.
Chichester: Ellis Horwood. 35-36.

GIBBONS RJ, HOUTE J (1973)
On the formation of dental plaques.
J Periodont 44:347-349.

GIBBONS RJ, HOUTE J (1975)
Dental caries.
Ann Rev Med 26:121-123.

GROSS MA, MORSE DR (1976)
Acupuncture and endodontics, a review and preliminary study.

GUAN X, YU B, WANG D, WANG CY, LIU XC (1979)
The role of cholinergic nerves in electro-acupuncture analgesia.
Influence of eserine, acetylcholine and hemicholinium-3 on the electro-
acupuncture analgesia.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 441-442.

GUUGENHEIM B (1980)
Effect of immunisation on periodontal disease and caries in
gnotobiotic rats associated with actinomyces viscosus.
In: Lehner T, Cimasoni G. The border between caries and periodontal
disease.

HAMADA S, SLADE HD (1980)
Biology, immunology and cariogenicity of s mutans.

HAN JS, TERENIUS L (1982)
Neurochemical basis of acupuncture analgesia.

HAN J, GUAN X, XU J (1979)
The study of turnover rate of CNS norepinephrine during acupuncture
analgesia in the rat.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 452.
HATAI B, HASHIMOTO T, ISHIZUKA H, TANY M (1977)
Immune response in animal lymph nodes by electroacupuncture stimulation.
Am J Acupuncture 5:229-238

HAUPTMAN SP, TOMASI TB (1975)
The mechanism of IgA polymerisation.

HOLMBERG K, KILLANDER J (1971)
Quantitative determination of immunoglobulin IgA, IgG, IgM and identification of IgA type in the gingival fluid.

A role of cell mediated immunity in the pathogenesis of periodontal disease.

HUHIGES J, SMITH TW, KOSTERLITZ HW, FOTHERGILL LA, MORGAN BA, MORRIS HR (1975)
Identification of two related penta-peptides from brain with potent opiate agonist activity.
Nature 258:577-578.

HUSBAND AJ, MUNIE HJ, GOWANS JL (1977)
The natural history of the cells producing IgA in the gut
North Holland New York. 29-30.

IVANYI L, LEHNER T (1971)
Lymphocyte transformation by sonicates of dental plaque in human periodontal disease.
Arch Oral Biol 16: 1117-1118.

IVANYI L, LEHNER T (1978)
The relationship between caries index and stimulation of lymphocytes by s mutans in mothers and their neonates.

KAADA B, JORUM F, SAGVOLDEN T, ANSETHWOEN TE (1979)
Analgesia induced by trigeminal nerve stimulation (electro-acupuncture) abolished by nuclei raphe location in rats.
Acupuncture Electro Ther Res 4:221-234.

KAGAN JM (1980)
Local immununity to bacteriodic gingivalis in periodontal disease.

KRASSE B (1977)
Microbiology of the gingival plaque
LALLY ET, BAEHNI PC, McARTHUR W (1980)
Local immunoglobulin synthesis in periodontal disease.
J Period Res 15:159-164.

LALLY E, ROSENBERG E, EVIAN C (1982)
Studies on the specificity of local antibody produced in
periodontal disease.

LEHNER T (1977)
Progress report: Oral ulceration and Behcet's syndrome.
Gut 18:491.

LEHNER T (1980)
Future possibilities for the prevention of caries and periodontal
disease.

LEHNER T (1982)
Cellular immunity in periodontal disease: an overview.
In: Gency RJ, Mergenhagen SW, eds: Host parasites interaction
in periodontal disease.

LEHNER T (1983)
Immunology of oral diseases. 2nd ed.
Oxford: Blackwell Scientific. 96-152.

LEHNER T (1986)
Antigen presenting contra-suppressor human T cells.
Immunology Today 7:87-88.

LEHNER T, CIMASONI G (1980)
The borderland between caries and periodontal disease II.

LEHNER T, WILTON JMA, SHILLITOE EJ (1975)
Immunological basis for latency, recurrences and putative oncogenicity
of Herpes Simplex Virus.

LEVIN JD, GORDEN NG, FIELD HL (1979)
The role of endorphins in placebo analgesia.
In: Bonica JJ, Liebskind DG. Advance in pain research and therapy.
New York: Raven. 547-551.

LI S (1979)
Electric needle and its effect on peripheral blood picture and
immune function.
In:Advance in Acupuncture & Acupuncture Anaesthetic. 96.

LINDHE J, HELLDEN L (1973)
Enhanced emigration of crevicular leucocytes mediated by factors in
human dental plaque.
LISTGARTEN MA, LAI CH, EVIAN CI (1981)
Comparative antibody titers to Actinobacillus,
actinomycetemcomitans in juvenile periodontitis,
chronic periodontitis, and periodontally healthy subjects.
J Period 8:155-164.

LINDSTROM F, FOLKE L (1973)
Salivary immunoglobulin A in periodontal disease.

LOVELACE EM, THOMPSON JJ, YUKNA RA (1982)
Evidence for the local immunoglobulin synthesis in periodontitis.
J Perio 53:626-630.

LU GD, NEEDHAM J (1978)
A history and rationale of acupuncture and moxabustion.
London: Cambridge University Press. 194-263.

LU HC (1975)
A complete textbook of auricular acupuncture. 2nd ed.

LU Z, CHENG J (1979)
The role of histamine in acupuncture analgesia.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 472.

MA Z, CHANG M (1979)
Experimental observation on cell immune function under influence of
acupuncture.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 511.

MA R, ZHANG F, LU E, LIN J (1979)
Clinical observation on the correlation between the propagated
sensation along channels and acupuncture analgesia.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 502-503.

MAN PL, CHEN CA (1973)
Acupuncture: a dental case report.

MANN F (1973)
Acupuncture - the ancient chinese art of healing. 2nd ed.
London: Heinemann Medical Books. 25-34.

MANN F (1978)
Acupuncture - the ancient chinese art of healing. 3rd ed.

MAYER DJ, PRICE DD, RAFII A (1977)
Antagonism of acupuncture analgesia in man by the narcotic antagonist
naloxone.
Brain Res 121:368-372.

MELZACK R, WALL PD (1965)
Pain mechanism: a new theory.
Science 150:971-979.
MELZACK R, STILLWELL DM, FOX EJ (1977)
Trigger points and acupuncture points for pain: correlations and implications.
Pain 3:3-23.

MERGENHAGEN SE (1973)
A role for complement in host resistance.
In: McPhee IT ed. Host resistance to commensal bacteria. The response to dental plaque.
London: Churchill Livingstone. 100-110.

MESSING RB, LYTLE LD (1977)
Serotonin containing neurones: possible role in pain and analgesia.

MICHALEK SM, McGhee JR (1977)
Effective immunity to dental caries: passive transfer to rats of antibodies to streptococcus mutans elicits protection.
Infect Immun 17:644-646

Ingestion of streptococcus mutans induces secretory immunoglobulin A and caries immunity.
Science 192:1238-1240.

MIHIE D, BINKERT B (1978)
Is placebo analgesia mediated by endorphins?
Pain Abstr 1:19-21.

MONEFELDT K, TOLLEFSEN T, ASSEV S, ROLLA G (1986)
Increased serum IgG antibodies reactive with lipoteichic acid in subjects with gingivitis.

Serum antibodies to oral Bacteroides asaccharolyticus (Bacteroides gingivalis): relationship to age and periodontal disease.

NEWMAN HN (1980)
Dental Plaque: the ecology of flora on human teeth.
Springfield, Illinois: CC Thomas. 54-56.

NISENGARD RJ (1977)
The role of immunology in periodontal disease.
J Period 48:505-516.

NISENGARD RJ, BEUTNER EH (1970)
Relation of immediate hypersensitivity to periodontitis in animals and man.
OMURA Y (1974)
Effect of acupuncture on blood pressure leukocyte serum lipids and lipoproteins in essential hypertension
Fed Proc 33:3.

OMURA Y (1976)
Pathophysiology of acupuncture effects, ACTH and morphine-like substances, pain, phantom sensations, brain microcirculation, and memory.

PAGE RC, SCHROEDER HZ (1982)
Periodontitis in man and other animals.

PALENSTEIN-HELMERMAN WH (1981)
Microbial etiology of periodontal disease

Lymphoproliferative response to oral bacteria in humans with varying severities of periodontal disease.

PLATT D, CROSSBY R, DALBOW M (1970)
Evidence for the presence of immunoglobulins and antibodies in inflamed gingiva.

POMERANZ B, CHIU D (1976)
Naloxone blocks acupuncture analgesia and causes hyperalgesia: endorphin is implicated.
Life Science 19:1575-1582.

POMERANZ B, CHENG R, LAW R (1977)
Acupuncture reduces electrological and behavioural responses to noxious stimuli: pituitary is implicated.

PRICHARD JP (1979)
The diagnosis and treatment of periodontal disease in general dental practice.

REN M, TU Z, HAN J, (1979)
The effect of hemicholine, choline, eserine and atropine on acupuncture analgesia in rats.

ROBERTSON PR, MACKLER BF, WRIGHT TE, LEVY BM (1980)
Periodontal status of patients with abnormalities of immune system. Observations over a two year period.
J Period 51:70-73.


SELDEN HS (1978)
Pain perception modification with acupuncture - a clinical study.

SHIMURA N, NAKAMURA C, HIRAYAMA Y (1981)
The preventive effect of acupuncture on rat dental caries.

SIN YM (1983)
Effect of electric acupuncture and moxibustion on phagocytic activity of the reticulo endothelial system of mice.

SIN YM, WEDGEWICK AD, MACKAY AR, BATES MB, WILLOUGHBY DP (1983)
Effect of electric acupuncture stimulation on acute inflammation.

SJOLUND B, ERIKSSON M (1979)
The influence of naloxone on analgesia produced by peripheral conditioning stimulation.

SMITH DJ, GADALIA LM, EBERSOLE JL, TAUBMAN MA (1985)
Gingival crevicular fluid antibody to oral microorganisms.

SONNENWORTH AC, JARETT L (1980)
Gendohol's clinical laboratory methods and diagnosis. Vol 2. 8 ed.

SPIEGEL CA, HAYDUK SE, MINAH GE, KRYWOLAP GN (1979)
Black pigmented Bacteroides from clinically characterized periodontal sites.

SQUIER CA, JOHNSON NW, HOPPS RM (1976)
Human oral mucosa development, structure and function.

TANNER ACR, DZINK JL, SCORANSKY SS, DES ROCHES CL (1986)
Diagnosis of periodontal disease using rapid identification of activity related gram negative specimen.

TAUBMAN MA (1974)
Immunoglobulins of human dental plaque.
TAUBMAN (1982)
Association between systems and local antibody and periodontal disease.
In: Genco RJ, Mergenhagen SZ eds. Host parasites interaction in periodontal disease.

TAUBMAN MA, SMITH J (1974)
Effect of local immunisation with streptococcus mutans on induction of salivary immunoglobulin A.
Int Immunol 9:1079-1082.

TAUBMAN MA, SMITH DJ (1974)
Effects of local immunization with s mutans on induction of salivary IgA antibody and experimental dental caries in rats.

THYLSTRUP A, FEJERSKOV O (1986)
Textbook of Cariology.
Copenhagen: Munksgaard. 29-43, 167-178.

TOLO K, SCHENCK K (1985)
Activity of serum immunoglobulins G, A and M to six anaerobic oral bacteria in diagnosis of periodontitis.

TOMASI TB, BIENSTOCK J (1965)
Characteristics of an immune system common to certain external secretions.

TOMASI TB, HAUPTMAN SP (1975)
The mechanism of IgA polymerisation.

VEITH I (1966) Translator: Huang Ti Nei Ching Su Wen (The yellow Emperor's classic of internal medicine)

WAN X, ZHANG D, WANG X, GU X (1978)
The relationship between acupuncture analgesia and concentrations of calcium and phosphorus in blood.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 470.

WANG C, YU B, LIU X (1979)
The influence of acupuncture in the acetylcholine level in various regions of rat brain.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 442.

WALL PD, SWEET WH (1967)
Temporary abolition of pain in man.
WHITE D, MAYRAND D (1981)
Association of oral Bacteroides with gingivitis and adult periodontitis.

WHO (1984)
Standard acupuncture nomenclature.
Manila: WHO Regional Office for Western Pacific. 1-5.

WILLIAMS RC, GIBBSONS RJ (1972)
Inhibition of bacterial adherence by secretory immunoglobulin A: mechanism of antigen disposal.
Science 177:697-699.

WILTON JMA (1982)
Polymorphonuclear leukocytes of the human gingival crevice, clinical and experimental studies of cellular function in humans and animals.
In: Genco RJ, Mergenhon SE eds. Host parasites interaction in periodontal disease.

XIONG X, ZHU C, ZHENG D, JIANG Z, HONG Z, DENG D (1979)
Histochemical observations on AChE and ATP in different brain areas under acupuncture analgesia in rats.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 445-446.

XU R, HOU X (1979)
A study of auricular acupuncture.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 120.

XU S, LI W, SHENG M, ZENG D, ZHANG L (1979)
The cyclic AMP level in caudate perfusate during acupuncture analgesia.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 449.

ZAMBON JJ, REYNOLDS HS, SLOTS J (1981)
Black pigmented bacteroides in human oral cavity.
Infect Immunol 32:198-203.

ZHANG R, YANG Y, ZHANG Y (1979)
The influence of moxibustion on immunity of experimental rabbits.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 513-514.

YE W, FENG X, ZHAO D, ZHANG J (1979)
Effect of electrical stimulation of acupuncture point "medium spinal" on the contents of 5HT and 5-HIAA in substantia nigra of midbrain in rabbits.

ZHAO J, WANG Z (1979)
Experimental study of effect of electro-acupuncture on cell mediated immune responses of rabbits.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 510-511.
ZHOU CY, CAO Z (1979)
Preliminary studies effect of moxibustion on the phagocytotic activities of the mononuclear phagocytic in man.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 512-513.

ZHOU L, CHEN Y (1979)
Changes is plasma prostaglandin level among patients under acupuncture analgesia.
In: Advance in Acupuncture and Acupuncture Anaesthetic. 497-499.

ZHU S, JIANG J, WEN T (1979)
Changes in localisation of 3H-5-hydroxytryptamine content in mid brain raphe nuclei in the process of acupuncture analgesia by means of micro-autoradiography.