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GINGIVAL FLUID : ITS NATURE AND MEASUREMENT

A thesis embodying original work submitted in partial requirement for the degree of Master of Dental Science in the Faculty of Dentistry, University of Sydney.

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Department of Preventive Dentistry
University of Sydney.
March 1974.
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INTRODUCTION

The presence or absence of gingival fluid in the non-inflamed gingival sulcus may be of importance in the early pathogenesis of periodontal disease.\textsuperscript{131,207}

The presence of gingival fluid has been thought to play a defensive role against the invasion of bacteria, foreign particles\textsuperscript{30,71} and other injurious matter\textsuperscript{30} into the gingival sulcus and by bathing the sulcus with antibacterial components.\textsuperscript{29} The ability of the gingival fluid to enhance the attachment of the epithelium to the tooth has been thought to be an important function in preventing the initiation of periodontal disease.\textsuperscript{164}

On the other hand, the opposite point of view has been put forward implicating gingival fluid in the aetiology of periodontal disease. This detrimental effect is thought to occur by the fluid providing the source of nutrients needed for the growth of bacteria\textsuperscript{121,161} and the formation of calculus.\textsuperscript{1,29,90,131,136,186,206,207}

It is well established that gingival fluid exists when the gingival tissues exhibit an inflammatory change.\textsuperscript{15,26,28,29,31,48,89,120,137,143,159,181,191} However, if it is to play a role in the formation of periodontal disease whether this role is inhibiting or
aggravating, it must be present before any change occurs in the tissues. If it was not present beforehand any role it had would not be in the initiation of the change but rather would result from the change and be one of either exacerbating, reversing or maintaining the change.

In order to understand the role of gingival fluid in the biology of the gingival sulcus it is important to understand the exact nature of the gingival fluid and how this may change with disease of the tissue.

An understanding of the nature of gingival fluid lies in a knowledge of its relationship to the environment of the gingival sulcus. If gingival fluid is separated from its environment its whole nature is lost. Similarly, it is impossible to separate the gingival sulcus from gingival fluid and still maintain a complete understanding of it.

The whole nature of gingival fluid includes its composition, mechanism of formation as well as factors that affect its formation. The environment of the gingival sulcus is in effect the nature of the epithelial attachment - the epithelium-tooth interface. With an understanding of these together with a knowledge of the role gingival fluid plays in the diseased state, it may be possible to predict on a theoretical basis whether gingival fluid exists or not in the healthy
gingival sulcus. It may also be possible to predict the role that this fluid, if present, would play in the biology of its environment.

The ability to predict on any level of reality is a measure of the truth value of any scientific theory.

The structure of this thesis is necessarily divided into two parts, which may seem separate and even unrelated. However, the interrelation of each part is such that the second, experimental part, would not be meaningful without the first, theoretical part.

Part I of this thesis is a theoretical consideration on the whole nature of gingival fluid and its relationship to the gingival sulcus, including a consideration of the role that it plays in both health and disease. From this theoretical consideration a concept is arrived at and the prediction made that in the healthy state gingival fluid is essentially interstitial fluid which because of the nature of the epithelial attachment can escape into the gingival sulcus. In disease the nature of this fluid changes to that of an inflammatory exudate.

Therefore, theoretically speaking, gingival fluid should exist in definite but minute amounts in the healthy gingival sulcus. Part II of this thesis is concerned with an experimental attempt
to detect and quantitatively measure this fluid theoretically present in the healthy gingival sulcus. The method used in this experiment was the most refined available at the present time and is based on an understanding of the gingival sulcus.

It should be obvious therefore that to carry out the experimental procedure presented in Part II without the theoretical considerations of Part I would be meaningless and add nothing to the present knowledge of gingival fluid, the gingival sulcus and their significance in the early pathogenesis of periodontal disease.

To present the theoretical considerations after the results of the experiment is in the author's opinion contrary to basic aims of the Scientific Theory. Theories are distinguished from experimental laws by the fact that they contain concepts which cannot be defined by overt experimental procedures, but which can be held to describe some level of reality. The theory can predict and this prediction in turn is able to be tested by experiment.

Hence in this thesis the theory is presented in Part I and the experiment in Part II.
Throughout this thesis the terms "gingival sulcus" and "gingival crevice" will be used synonymously and the term "gingival fluid" will be taken as being synonymous with such terms as "gingival crevice fluid", "gingival sulcus fluid", "crevicular fluid", "sulcular fluid" and "pocket fluid", unless otherwise specified.
PART I

REVIEW OF THE LITERATURE

Theoretical considerations
on the nature of gingival fluid
and its relationship to the biology
of the gingival sulcus.
CHAPTER 1

HISTORICAL REVIEW

The presence of a fluid passing from the gingival crevice and bathing the teeth has been postulated for over 100 years.\textsuperscript{152} Serres,\textsuperscript{166} in 1817, implicated a secretion from gingival glands in the formation of calculus.

This concept of gingival glands persisted until the time of Black.\textsuperscript{16} He tried to show their presence histologically, in order to confirm his clinical observations of a secretion pouring into the 'gingival space'. Unable to do so he proposed that the fluid was of serumal origin and responsible for calculus formation. He also suggested that there was a normal fluid flow which increased during some form of 'excitation' of the gingiva or with inflammation of the tissues and that this increased fluid flow led to the deposition of an excessive amount of serumal calculus.\textsuperscript{17}

The first suggestion that this fluid may be beneficial came from Stillmann and McCall\textsuperscript{187} in 1922. They thought that a 'serous secretion' bathed and cleansed the gingival crevice and imparted an immunity to the formation of cervical caries.
Two years later, in 1924, McCall\textsuperscript{125} contradicted this idea saying that there is constantly exuded a serous fluid whose exact composition is unknown, but which is evidently, in large part, a transudation from the blood. In healthy tissue this exudate would have the same reaction as the blood, that is, alkaline, but in diseased tissue, the acidity of the inflammation would be passed into this transudate. The acidity, by itself, may not reach sufficient strength to cause caries, but may cause hypersensitivity in the vicinity of the gingival crest. Commenting further on the action of this fluid, McCall suggests that it may, in fact, enhance cervical caries rather than retard it. He based this theory on the concept that in inflammation, the fluid is acidic and this slight acidity may lead to the proliferation of acid producing bacteria and these, in turn, form smooth surface caries.

McCall\textsuperscript{125} also postulated that traumatic occlusion, although it did not cause inflammation, could lead to a localised congestion of blood vessels, which may become the source of a "perverted blood transudate".
Boedecker\textsuperscript{19} supported the idea of an inflammatory exudate. As histological examination of the gingival tissues showed the absence of all glandular structures, he suggested the fluid emanating from the gingival crevice was not a secretion, but an exudate resulting from an inflammatory condition of this tissue. Further, the exudate of the gingival crevice varied both quantitatively and qualitatively, depending on the condition of the surrounding soft tissue.

Like McCall, Boedecker\textsuperscript{19} believed the gingival fluid to be acidic, and that there was a relationship between this acid exudate and erosion occurring at the gingival margin. He inserted litmus paper into the gingival crevice, to determine the acidity of the fluid.

That the gingival fluid was acidic in nature was challenged by Box\textsuperscript{21} in 1940. His findings in inflammatory conditions tended to show a slight increase in alkaline reaction. He found that the pH of the fluid in normal conditions ranged from pH 7.3 to 7.5, with slightly higher values for fluid from crevices associated with chronic inflammation. Caries did not tend to spread under the
gingival margin but rather, a type of calculus, darker in colour than salivary calculus, was found in this region. He cited inflammation as being one of the agents responsible for this calculus formation. Box 21 indicated that the gingival fluid may be both beneficial in reducing caries and detrimental in leading to calculus formation.

This double role of gingival fluid was supported by Waerhaug. 205 He showed that Indian ink inserted into a healthy crevice is completely removed in about 48 hours. 205 From this he concluded that there was a continuous but minute flow of fluid from a healthy gingival crevice.

Even though this was a minute flow of fluid, Waerhaug postulated that the outward flow was faster than the inward diffusion of saliva. Using Methylene blue, Toluidine blue and Alizarin red, he showed that these dyes did not penetrate the gingival crevice. 205 He concluded that the source of mineral salts in subgingival calculus is the blood. 206
Waerhaug also suggested that there were two components of gingival fluid. There was the minute continuous flow from healthy tissue and this was supplemented by an exudate when the tissues became inflamed. He said the outward stream from the pocket is manifoldly increased when an irritant causes an exudation into the pocket$^{206}$ and this exudate helped to restore the crevice to its sterile condition.$^{206}$

We now have concepts that

(1) gingival fluid flushed bacteria$^{205}$ and particulate matter (Indian ink)$^{205}$ out of the crevice and prevented the inflow of saliva.$^{205,206}$

(2) gingival fluid contained the mineral elements essential to the formation of subgingival calculus.$^{17,21,206}$

Although the first of these concepts was supported by Arnim,$^4$ he also speculated that the outward flow of fluid supplied a continuous source of nutrients to the bacteria, thus assisting the maintenance of the chronic inflammatory condition.
Up to this stage all that could really be said about gingival fluid was

(1) There was a fluid emanating from the gingival crevice which increased in amount with inflammation

(2) The role of this fluid, although it was thought to have both detrimental (calculus formation and nutrient supply to bacteria) and beneficial (flushing) actions, was unknown.

The first comprehensive study of gingival fluid was carried out between 1958 and 1962 by Brill and his associates. Brill sought to discover the mechanism of formation of gingival fluid and thereby determine its composition and function. He used both humans and experimental animals in his study, which marked the beginning of serious investigations into gingival fluid per se and its use as an index of gingival physiology and pathology.
CHAPTER 2

THE COMPOSITION OF GINGIVAL FLUID

The action of any physiological fluid is due to the chemical composition of that fluid. So, too, with gingival fluid, where the contents of the fluid determine the role that it is to play in both health and disease. A greater knowledge, therefore, of the composition of gingival fluid would lead to a greater understanding of its formation and role in the gingival tissues.

The exact nature of the composition of gingival fluid is unknown. This is in part due to the difficulties of collection and variation in flow which have inhibited the collection of sufficient quantities of fluid for exact quantitative analysis.

The qualitative analysis of gingival fluid (table 2.1) tends to indicate that it is of similar composition to extracellular fluid. However, it should be noted that in the collection of gingival fluid an artificial environment is created which alters the flow of gingival fluid which in turn may also alter the composition.
Table 2.1. Composition of gingival fluid.

| Inorganic | Na\textsuperscript{+89} K\textsuperscript{+89} Ca\textsuperscript{+89} Mg\textsuperscript{2+88} Cl\textsuperscript{−208} P\textsuperscript{−} (inorganic)\textsuperscript{88} |
| Cellular  | Bacteria,\textsuperscript{47} Leukocytes,\textsuperscript{47} Desquamated epithelial cells.\textsuperscript{47} |
| Organic   | 1. Carbohydrates - glucose\textsuperscript{70}, hexuronic acid\textsuperscript{70}, hexosamine (and histamine\textsuperscript{149}, and serotonin\textsuperscript{149}) ribose\textsuperscript{69}, acid mucopolysaccharides\textsuperscript{190}, mucoproteins\textsuperscript{190}, glycoproteins\textsuperscript{190}, glycolipids\textsuperscript{190}, lipopolysaccharide (endotoxin)\textsuperscript{173} |
|           | 2. Lipids - lipopolysaccharide (endotoxin)\textsuperscript{173}, glycolipid\textsuperscript{190}, lipoproteins\textsuperscript{190} |
|           | 3. Free amino acids\textsuperscript{95} - Gly \textsuperscript{+}, Ala \textsuperscript{+++}, Val \textsuperscript{+}, Leu \textsuperscript{+}, Isoleu \textsuperscript{+}, Gal \textsuperscript{++++}, Lys \textsuperscript{+}, Arg \textsuperscript{±}, Cys \textsuperscript{++}, Ser \textsuperscript{+} |
4. Enzymes - Hyaluronidase

Lysosomal enzymes -
- Cathepsin -D
- β-glucuronidase
- acid phosphatase
- lysozyme
- alkaline phosphatase
- non-specific esterases
- β-D-galactosidase
- β-D-glucosidase
- aminopeptidase
- lactic dehydrogenase
- succinic dehydrogenase
- fibrinolysin

5. Proteins - Albumin

- α1-globulins - (orosomucoid)
- α2-globulins - (ceruloplasmin)
- β-globulin - (transferrin)
- γ-globulin - IgA
- IgG
- IgM
- IgE

Complement (C3)
Fibrinogen
glyco
lipo
proteins
muco

6. Urea
7. Lactic acid
8. Bradykinin.
Basically, two methods exist for the collection of gingival fluid for quantitative analysis:

(1) The use of filter paper strips, and

(2) Capillary tubes with and without suction.

In both methods, the tissue is isolated and dried and is left for 10-15 minutes in this dried-out state until enough fluid is formed to be collected. This dehydrated state is certainly not the normal situation and, as a result, the analysis obtained may not be representative of the norm.

If a filter paper method is used, further variables arise, in as much as the filter paper has to be weighed precisely before and after collection, and evaporation from the sides of the paper has to be accounted for, and, because of the minute amounts of fluid involved, this evaporation is extremely difficult to assess.

Again, if suction is used with a capillary tube method, this creates an extra force attracting fluid out of the tissue, which does not normally exist and as a result substances may be drawn up into the tube, which would not normally occur in gingival fluid. Certainly a higher cellular component would occur with this method.
In both methods the possibility of picking up the contents of the gingival sulcus is encountered. Thus the presence of bacteria in gingival fluid is observed. Furthermore, it is unknown whether some of the substances found in gingival fluid are derived from the gingival tissues per se or are breakdown products of cells and bacteria\(^{189}\) in the gingival sulcus.

It is known that if erythrocytes are left standing, K\(^+\) ions will pass out of the cell, thus altering the electrolytic content of the surrounding fluid.\(^{211}\) It is possible that the cellular components of gingival fluid could behave in a similar manner thus increasing the K\(^+\) ion concentration of gingival fluid. This is therefore indicative of how the contents of the gingival fluid may change and yet not reflect changes in the gingiva. The composition of gingival fluid has been shown to change with different times of the day (circadian rhythms),\(^{14}\) the presence or absence of pus or blood in the fluid,\(^{90}\) and with varying degrees of inflammation.\(^{89,90}\)
Because of these variations in the composition of gingival fluid, it is difficult to determine the basic composition of the fluid and, in turn, its source and exact nature. However, taking these variables into account, the mass of knowledge on the composition of gingival fluid tends to indicate that it is similar to a modified plasma or serumal exudate. These theoretical considerations on the basic composition will lead to more work and a better understanding of gingival fluid.
CHAPTER 3

THE FORMATION OF GINGIVAL FLUID

3.1 Introduction

The exact mechanism of the production of gingival fluid is unknown, although it is known that gingival fluid is not:

(1) A salivary product, since the high protein content is not seen in saliva, and an outward flow of the fluid has been demonstrated\(^{30, 71, 205}\)

(2) A glandular secretion, since no glands can be demonstrated in the gingivae\(^{17}\)

(3) A straight vascular transudate, as this would have an ionic composition similar to plasma\(^{89, 90}\)

Two theories have been postulated to explain the mechanism of fluid production. These are:

1. Inflammatory exudate, and
2. Specifically altered transudate.

The difference between an exudate and a transudate is empirical. Where a transudate becomes an exudate cannot be stated precisely, yet, qualitatively, the permeability of the capillaries to colloidal carbon is the deciding factor. Once colloidal carbon can escape from the vascular system, the outflow of fluid is termed an exudate.
It is important to consider the composition of gingival fluid in relation to normal body fluids: intracellular fluid, plasma and interstitial fluid, and also in relation to inflammatory exudate, if we are to consider the mechanisms of its production.

3.2 Cationic composition

Extra cellular fluid resembles sea water in ionic composition, with the major cation being Na\(^+\) and the major anion Cl\(^-\)\(^{211}\star\).

*Footnote: In any space, the number of cations must equal the number of anions to maintain electrical equilibrium. For this reason, all concentrations are expressed in milli-equivalents per litre:\(^{12}\)

\[
\text{meq/}l = \frac{\text{mg/litre} \times \text{valence}}{\text{atomic or formula weight}}
\]
The composition of plasma and interstitial fluid is almost the same except that interstitial fluid contains little protein whereas plasma contains about 7g/100mls. Consequently, the electrolytic composition varies slightly according to the Gibbs-Donnan equilibrium. 211*

*Footnote: For a system at equilibrium across a semi-permeable membrane which contains diffusable ions and protein ions with a net charge of P-

\[ \frac{Na^+}{Na^-}, \frac{Cl^-}{Na^+}, \frac{Cl^-}{b}, \frac{P^-}{b} \]

so \[ \left[ Na_b^+ \right] = \left[ Cl_b^- \right] + \left[ P_b^- \right] \] and \[ \left[ Na_a^+ \right] = \left[ Cl_a^- \right] \]

therefore, \[ \left[ Na_a^+ \right] \] is different from \[ \left[ Na_b^+ \right] \], as \[ P_b^- \] is on one side of the equation.

The concentration of diffusable ions have the relationship

\[ \frac{Na_a^+}{Na_b^+} = \frac{Cl_b^-}{Cl_a^-} \]

This is derived from the thermodynamic equation where the change in free energy is zero.

\[ \Delta F = 0 = RT \ln \frac{Na_a^+}{Na_b^+} + RT \ln \frac{Cl_b^-}{Cl_a^-} \]

which is solved to give

\[ \frac{Na_a^+}{Na_b^+} = \frac{Cl_b^-}{Cl_a^-} \]
Table 3.1. The ionic composition of extra- and intracellular fluid.

<table>
<thead>
<tr>
<th>Extracellular fluid</th>
<th>Intracellular fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cations</td>
<td>Anions</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>145</td>
</tr>
<tr>
<td>K$^+$</td>
<td>5</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>2</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>2</td>
</tr>
<tr>
<td>Organic acid</td>
<td>5</td>
</tr>
<tr>
<td>Protein</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
</tr>
</tbody>
</table>
For large amounts of plasma and interstitial fluid, the difference in electrolyte concentration is negligible; however, it may be significant for small amounts of fluid.

In intracellular fluid, the main cations are $K^+$ and $Mg^{2+}$ and the anions are organic phosphate and protein. 11

The difference between extracellular fluid and intracellular fluid in meq/litre is hard to explain, but this difference is maintained by the sodium pump mechanism. Where the metabolism of the cell is disrupted, $K^+$ is lost from the cell and $Na^+$ is taken up. This is seen, for example, in the loss of $K^+$ from the erythrocytes of stored blood. 11

The osmotic pressure within the cell is equal to that outside the cell and thus the cell's integrity is maintained. This is due to a preponderance within the cell of ions with more than one charge/molecule, for example, $Mg^{2+}$, $PO_4^{3-}$ and protein. 11 (Table 3.1)
fig. 3.1  Passage of interstitial fluid from the blood stream to the cells and back again.
Changes in extracellular fluid occur at different stages. From the blood stream to the interstitial spaces onto the cell and back again as nutrients are supplied to the cell and waste products removed (fig. 3.1).

Most of the work done on gingival fluid has been on the cation composition.\(^8^9,9^0,9^6\) Very little is known about the anions of gingival fluid.

Krasse and Egelberg\(^9^6\) studied the Na/K, Na/Ca and K/Ca ratios. This was repeated by Weinstein et al.\(^2^0^8\) However, the difficulty in comparing ratios is that it is unknown whether one or both components change in magnitude. For example, suppose we have a ratio of \(x:y = 10:1\), if both \(x\) and \(y\) increase by 3 times the ratio remains the same but the absolute values are 3 times as great. Now, suppose \(x\) increases by 3 and \(y\) decreases by 2, \(x:y = 30:\frac{1}{2} = 60:1\). This is the same as a 6-fold increase of \(x\), with \(y\) remaining constant.

Taking this into account, they found a ratio of 4:1 for Na:K in fluid from clinically healthy gingiva and 10:1 in fluid from chronically inflamed gingiva and compared this to 29:1 ratio for extracellular fluid.\(^9^6\) Weinstein et al.\(^2^0^8\) also found a ratio of 4:1 for Na:K from clinically healthy tissue and supported the observations of Krasse and Egelberg\(^9^6\) in as much as theorising that intracellular K is added to and/or Na withdrawn from the fluid as it passes into the gingival crevice.
Table 3.2. The ionic composition of inflammatory exudate. 132

<table>
<thead>
<tr>
<th></th>
<th>Mg/100 mls.</th>
<th>Meq/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>336.8</td>
<td>144.6</td>
</tr>
<tr>
<td>K⁺</td>
<td>36.0</td>
<td>9.2</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>16.44</td>
<td>8.1</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.61</td>
<td>1.3</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>-</td>
<td>98.7</td>
</tr>
<tr>
<td>Inorganic P⁻</td>
<td>7.49</td>
<td></td>
</tr>
</tbody>
</table>

(Serum = 4.02)
The concentration of inorganic ions in inflammatory exudates has been determined by Menkin\textsuperscript{132} (Table 3.2).

When these values are compared to those for extracellular fluid (plasma-interstitial fluid) we see that the $\text{K}^+$ is increased by about 2, whereas the $\text{Na}^+$ is approximately the same. This, then, gives us a $\text{Na}^+ / \text{K}^+$ ratio of 15.7:1, if meq/litre values are used.

Fuhrman\textsuperscript{59} also shows an increased $\text{K}^+$ level in the extracellular fluid of damaged tissue. He says that this is due to a failure of the $\text{Na}^+$ pump mechanism whereby the $\text{K}^+$ inside the cell comes out and $\text{Na}^+$ goes in.

Menkin\textsuperscript{132} cited the increase in glycolysis as being important for the increased $\text{K}^+$ released from the cell.

Krasse and Egelberg\textsuperscript{96} used their results to say that gingival fluid is an inflammatory exudate rather than a simple filtrate. In order to explain the anomaly of a higher ratio of Na/K from chronically inflamed tissue, than that from clinically healthy tissue, they theorised that it could be due to the increased vascularisation and ulceration of the crevice epithelium in chronically inflamed tissue, hence the fluid does not pass through as much tissue as in the healthy case, and would therefore agree better with the values from plasma.
Table 3.3. The ionic composition of gingival fluid. 88, 89, 90

<table>
<thead>
<tr>
<th></th>
<th>Gingival fluid</th>
<th>nearly normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>moderately inflamed</td>
<td></td>
</tr>
<tr>
<td>Na$^+$</td>
<td>137.8</td>
<td>88.3</td>
</tr>
<tr>
<td>K$^+$</td>
<td>17.5</td>
<td>17.4</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>17.9</td>
<td>9.9</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>174.00</td>
<td></td>
</tr>
</tbody>
</table>

Inorganic P - Inflammatory exudate G.F.(inflamed) Plasma

- 7.5 mg.%
- 4.2 mg.%
- 4.0 mg.%
These results obviously do not tell us anything except that there is an altered Na\(^+\)/K\(^+\) ratio compared to plasma.

Since Krasse and Egelberg\(^{96}\) suggest that the Na/K ratio is altered by the tissue, through which the fluid passes, it does not indicate that the fluid is an inflammatory exudate as they state, but rather that it could be a specifically altered transudate. They shed no light on whether the fluid initially is an exudate or transudate, but only that K\(^+\) is added and Na\(^+\) withdrawn from it.

Absolute values of the ions in fluid from both healthy and inflamed tissue would shed more light on the actual mechanism of gingival fluid production.

Kaslick et al\(^{88,89,90}\) measured the Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) concentrations in fluid from inflamed tissue and in fluid from nearly normal tissue. They could not collect fluid from healthy tissue. However, their results are more useful in the understanding of the nature of gingival fluid than the ratios of Krasse and Egelberg\(^{96}\) (Table 3.3).

From these results\(^{88,89,90}\) the variation of each ion with changes in the degree of inflammation can be seen (fig. 3.2).

That the K\(^+\) level remains constant and is about 3 times as great as in plasma and 1\(\frac{1}{2}\)-2 times as great as in inflammatory exudates and that as the degree of inflammation gets less, the Na\(^+\) level falls to about 2/3 that of plasma, is observed. If we calculate Na/K ratios
fig. 3.2  The concentration of inorganic cations in gingival fluid from nearly normal tissue and moderately inflamed tissue compared to normal serum.
From Kaslick et al. 89
with these values, we get for chronically inflamed tissue about 8:1 and for nearly normal tissue about 5:1, which agrees with Krasse and Egelberg's and Weinstein et al. However, we can now see that the variation from plasma is due to an increased $K^+$, without a significant reduction in $Na^+$ for inflamed tissue, but as the inflammation gets less, $Na^+$ ions are absorbed and the $K^+$ remains level and so we get a lower $Na/K^+$ ratio.

Therefore, Krasse and Egelberg's theory of the fluid passing through less tissue and therefore not picking up as much $K^+$ does not seem to be valid, since the changes seem to be primarily due to altered $Na^+$ concentrations.

To explain the higher $Na^+$ concentration in fluid from nearly normal tissue, Kaslick et al suggest that with increased inflammation, there is increased alveolar bone destruction, resulting in an increased Na in the extracellular fluid from the large quantity of $Na^+$ in bone; they go on to say that this theory does not answer the question of why is there a lower $[Na^+]$ in fluid from nearly normal tissue? Surely this should approximate even more closely that of "normal" interstitial fluid. Again, why is there an increase in the $[K^+]$ in nearly normal fluid?

The increased $[K^+]$ does not compensate for the decreased $[Na^+]$ to maintain electrical equilibrium.
Hence, the second theory, that is, that under normal circumstances, \( \text{Na}^+ \) is reabsorbed actively by the sulcus epithelium. It has been shown that intestinal, colonic and gastric mucosa actively resorb \( \text{Na}^+ \). A bioelectric potential exists across the gingival epithelium and is of a similar magnitude as the potential in intestinal, colonic and gastric mucosa. As this potential is reduced, the active reabsorption of Na is also reduced.

This reduction in potential can occur through the action of toxins and it is possible that the toxins which cause gingival inflammation can reduce this potential across the crevice epithelium.

A potential across a membrane exists when that membrane restricts the passage of certain substances through it. In the case of cells, the resting membrane potential is due to the 177 meq/l inside the cell and the 154 meq/l outside the cell and is maintained by the Na pump mechanism.
In gingival fluid from inflamed tissue, we have 174 meq/l which is very close to the 177 meq/l of intracellular fluid. Therefore, the potential across the membrane is markedly reduced. However, we cannot say whether the potential was reduced first (by the toxins) which leads to a failure of the active reabsorption of Na⁺ by the cells and a subsequent rise in meq/litre of the extracellular fluid, or whether the rise in [Na] of the extracellular fluid (for example, due to bone resorption) came first and the potential was reduced because of it.

The fact that resting crevice epithelium has a bioelectric potential indicates that it is acting as a semi-permeable membrane. The results of Browne support the theory of active reabsorption of Na by the crevice epithelium. He showed that from healthy gingival crevices in rabbits, diido fluorescein and serum albumin labelled with I¹³¹ could be consistently recovered after intravenous injection. However, Na²⁴ and I¹³¹ ions could not be recovered. Thus indicating selectivity of ion transport across the crevice epithelium.
If one of the metabolic functions of the crevice epithelial cells is the active reabsorption of Na\(^+\) ions, in order to maintain electrical equilibrium within the cell as well as a resting membrane potential, the intracellular K\(^+\) of these cells must be lower than the intracellular K\(^+\) of what is called normal intracellular fluid. Thus the normal interstitial fluid around these cells would have a higher K\(^+\) level than would normal interstitial fluid.

Since crevice epithelium is in contact with the outside as well as the underlying connective tissue, the passage of interstitial fluid from blood vessels to the cells and back again is not 100%. The interstitial fluid can also be lost outside into the crevice, together with the waste products of the epithelial cells (e.g. K\(^+\)). This gives rise to a possible third theory of gingival fluid production. That is: gingival fluid = interstitial fluid + inflammatory exudate.

The concentration of sodium and potassium in gingival fluid varies as to the time of day of collection.\(^9\) The flow rate of gingival fluid also follows a circadian rhythm, with the highest flow rate being at 2200 hours and the lowest at 0600 hours.\(^1\)
In samples taken in the morning, compared to those taken at noon, the variation of $[\text{Na}]$ in fluid from nearly normal tissue was $80.03 \text{ meq/l} - 61.05 \text{ meq/l}$.

The variation in $[\text{K}]$ in this fluid was $11.32 \text{ meq/l} - 21.58 \text{ meq/l}$.

In fluid from chronically inflamed tissue, the variation of $[\text{Na}]$:

- AM $127.65 \text{ meq/l}$ - NOON $105.69 \text{ meq/l}$

and in $[\text{K}]$:

- AM $13.48 \text{ meq/l}$ - NOON $21.64 \text{ meq/l}$.\(^{90}\)

It therefore appears that the $[\text{Na}]$ is more dependent upon the state of inflammation than on the time of day. Whereas variations in the $[\text{K}]$ are dependent upon the time of day.\(^{90}\)

However, fluid collection should be standardised to the time of day for consistent results.

However, in comparing Na/K ratios it appears that the time of day is the major influencing factor and the degree of inflammation is of only secondary importance.\(^{90}\)
Other factors which affect the $[K]$ are the presence of blood in the fluid sample. If erythrocytes are present in the fluid, high concentrations of $K$ are recorded. This is probably due to the release of $K$ into the fluid by the erythrocytes.

Krasse and Egelberg measured Na/Ca and K/Ca ratios but stated that because the $[Ca]$ was at the lower limit of the flame photometer these ratios were not reliable. However, they noted that, with increasing inflammation, the ratio approached that of extracellular fluid. These results could be misleading as they tend to indicate that the $[Ca]$ approaches normal plasma concentration with increasing inflammation. Kaslick et al., in determining the absolute value, found the opposite situation, that is, the $[Ca]$ rose with increasing inflammation to nearly 3 times that of plasma. The discrepancy is probably due to the fact that Krasse and Egelberg compared ratios which do not indicate the variations of the individual components and compounded this by using inaccurate measurements of the $[Ca]$.

In inflammatory exudate, Menkin found that the $[Ca]$ was not significantly different from that of serum. But when the exudate had a viscous, purulent appearance the content was markedly increased. Kaslick et al. found that, in gingival
fluid, a higher [Ca] occurred when a white sediment could be centrifuged from the whole fluid. This white sediment, they thought, reflected the presence of pus.

This increased [Ca], with increasing inflammation and the even higher [Ca] in the presence of pus, corresponds very well with the idea of gingival fluid consisting of two parts. The [Ca] fitting in with the inflammatory exudate component. It does not indicate that gingival fluid per se is an inflammatory exudate.

A preliminary study by Kaslick et al on the [Mg] in gingival fluid from moderately inflamed tissue has shown that it is lower than that of normal serum and is even lower than that of inflammatory exudate, as measured by Menkin.

The significance of a lower [Mg] could be in the fact that Mg acts as a co-factor in various intermediate phosphorylation reactions. Phosphorylase requires adenylic acid and Mg and it is this enzyme by which glycogen undergoes its initial breakdown in phosphorylation. Therefore the utilization of Mg may indicate the activity of phosphorylase in the gingival tissues. Glycogen is found in inflamed gingival tissue, but not in uninflamed gingiva.
If the activity of phosphorylase is increased, one would also expect to find an increase in the inorganic phosphorous concentration. This was noted by Weinstein et al\textsuperscript{208} in fluid from inflamed tissue, and also by Kaslick et al.\textsuperscript{88}

The values of inorganic phosphorous as found by Kaslick et al\textsuperscript{88} (4.2 mg\%) were higher than that of normal serum (3.5 mg\%) but not as high as that in inflammatory exudate as found by Menkin (7.2 mg\%).\textsuperscript{132}

3.3 Anionic composition

Although quantitative measurements have not been made, Weinstein et al\textsuperscript{208} have indicated a higher C1 content in gingival fluid of inflamed tissue than that of serum.

This increase in C1\textsuperscript{-} would maintain an electrical equilibrium within the gingival fluid.

Other anions in the gingival fluid, apart from inorganic phosphorous, have not been studied.
Degree of gingival inflammation.

fig. 3.3 The concentration of lactic acid in gingival fluid from tissue with varying degrees of inflammation.
From Hasegawa.
3.4 Lactic acid content

Besides a rise of phosphorylase activity an increase in glycolysis can also result in increased amounts of inorganic phosphorous. The end product of glycolysis (anaerobic metabolism) is lactic acid. An increase in the lactic acid content of the fluid would therefore indicate an increase in glycolysis. Hasegawa\textsuperscript{72} found that the total lactic acid content of gingival fluid varies according to the degree of inflammation. He was unable to demonstrate the presence of lactic acid in fluid obtained from clinically healthy tissue, but with severe inflammation, there was a marked increase in the total lactic acid content. However, the highest lactic acid concentration was in the fluid from mildly inflamed tissue with the lowest in fluid from moderately inflamed tissue, apart from that fluid from clinically healthy tissue. This same tendency has been noted with the oxygen tension in the tissues of varying degrees of inflammation.\textsuperscript{117} The explanation of this phenomenon is not clear; however, it may indicate that there is a rapid increase in glycolytic activity with inflammation which levels off so that with more inflammatory exudate the concentration of lactic acid decreases, but as the number of cells increases with increased mitosis of the epithelial cells in severe inflammation and a levelling off of the amount of exudate, the concentration of lactic acid increases again. (fig. 3.3)
In the same investigation, Hasegawa\textsuperscript{72} also demonstrated that the lactic acid content of the gingival fluid showed a statistically significant correlation to the lactic acid content in the gingival tissues, but there was no correlation between the lactic acid content of serum and that of gingival fluid nor that of gingival tissue.

It seems likely then, that with inflammation we could have an increase in glycolytic metabolism by the cells of the gingival tissue. With glycolysis the amount of energy produced is only about 1/5th of that released by aerobic metabolism (table 3.4).

If one of the normal functions of the epithelial cells of the gingiva is the active reabsorption of Na, this would require energy. Now, if the energy available to the cell was drastically reduced, it would be unable to carry out its normal functions. Therefore the active transport of Na across the cell membrane would be reduced if not completely stopped. With an increase in glycolysis with inflammation, the amount of energy available to the cell is drastically reduced, therefore we would expect to see failure of active Na reabsorption. This fits in with the observation of the Na concentration of the gingival fluid and the
<table>
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<th>Oxid P</th>
<th>Total</th>
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<tr>
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<td>2</td>
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<tr>
<td>(\rightarrow) pyruvate</td>
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Total for glycolysis = 8

2 x Pyr \(\rightarrow\) AcCoA
2 x Iso cit \(\rightarrow\) Oxal. Succ.
2 x $\alpha$-Keto glu $\rightarrow$ Succ. CoA

2 x Succ. CoA $\rightarrow$ Succ.

2 x Succ. $\rightarrow$ Fum

2 x Malate $\rightarrow$ Oxal. Acetate

NET $\rightarrow$ $\text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$

\[ \begin{array}{lll}
2 \times 3 \text{ NAD} & 6 \\
2 \times 2 \text{ FAD} & 4 \\
2 \times 3 \text{ NAD} & 6 \\
\hline \\
& \text{NET} & 38 \\
\end{array} \]

\[ \frac{\text{Ratio glycosis : Total Respiration}}{\text{Respiration}} = \frac{8}{38} = \frac{1}{5} \]
fig. 3.4  The concentration of urea in gingival fluid. 66

fig. 3.5  The concentration of nitrogen in gingival fluid compared to the degree of gingival inflammation.  
From Golub et al. 66
theory of gingival fluid consisting of two components. The fact that lactic acid was not found in the fluid or tissue of clinically healthy gingiva also supports this theory.

Although it must be realised that the metabolic pathway of phagocytosis is one of glycolysis and that the increase in lactic acid content could be due to the increased phagocytosis with inflammation, as well as to the increase in glycolysis of the gingival epithelium.

3.5 Urea content

The concentration of urea in gingival fluid has been shown to be inversely related to the degree of inflammation. In mildly inflamed gingiva, the urea concentration was much greater than that of serum but as the inflammation increased the levels approached that of serum, this decrease was exponential in character\(^6\) (fig. 3.4).

Golub et al\(^6\) point out that the total nitrogen concentration was only slightly affected by mild to moderate inflammation but was markedly increased with severe inflammation (fig. 3.5), with the urea nitrogen only contributing about 8% to the total nitrogen content.
This study\textsuperscript{66} indicates that there is some mechanism for the concentration of urea when the fluid flow is small. However, with inflammation this mechanism is destroyed.

The possible mechanisms were discussed by Golub, Borden and Kleinberg\textsuperscript{66} and these included that the passage of water from the tissue into the crevicular space was slower than urea. This seems unlikely, as water is a much smaller molecule than urea and the passage of water would be virtually unimpeded by the tissues. They go on to say that the next possibility is the active transport of urea and with inflammation this active transport mechanism would decrease. This fits in with the theory that the epithelial cells of the gingival crevice can actively transport substances across their membranes and that this active transport is stopped with inflammation. However, it is difficult to explain the higher urea content of gingival fluid from severely inflamed tissues (although of lower concentration) except that with increased number of cells we have increased urea.

The next mechanism that is discussed\textsuperscript{66} is that of water being reabsorbed by the intact epithelial cells of the gingival crevice. Since the movement of water through membranes is generally associated with Na\textsuperscript{+} ions, the active resorption of Na\textsuperscript{+}
ions by the epithelial cells would therefore lessen the water
content and lead to a more concentrated urea content of gingival
fluid. The higher concentration of urea in gingival fluid from
clinically healthy to mildly inflamed tissue, supports the concept
that gingival fluid is interstitial fluid (including products from
the cells) and with inflammation the inflammatory exudate is
the major component. The fact that the urea concentration
approaches that of serum with severe inflammation and that
the total nitrogen content and concentration is increased with
severe inflammation lends further support for this concept.

3.6 Protein content

The increase in nitrogen could be explained by an increase
in the protein content of the exudate over the interstitial (transudate)
fluid. Bang and Cimasoni\textsuperscript{9} have measured the total protein
concentration in gingival fluid from moderately to severely
inflamed gingival tissues and found that no statistically significant
difference existed between this gingival fluid and serum. This
indicates that the gingival fluid from moderately to severely
inflamed gingiva is comprised mainly of inflammatory exudate.
Mann and Stoffler\textsuperscript{130} again with gingival fluid from moderately
to severely inflamed tissue showed that it had a similar electro-
phoretic pattern to serum and that it contained fibrinogen. They
stated that this indicates that the gingival fluid in their study was essentially an inflammatory exudate.

The study by Paunio Brushn and Makinen on the azo-dye binding property of gingival fluid, indicates that the protein content of the fluid changes with inflammation.

Azo-dyes have the property to bind to various tissue proteins: haemoglobin, globin, albumin and other soluble proteins. Paunio, Brushn and Makinen found a correlation between the ability of the gingival fluid to bind the azo-dyes and the clinical degree of inflammation, thus indicating a larger concentration of these types of protein in fluid from more inflamed tissue. This is in accordance with the theory that the character of the fluid changes to that of an inflammatory exudate with increasing inflammation.

The theory is now put forward that in the healthy state the gingival fluid is essentially interstitial fluid in both composition and formation. However, with inflammation the nature of the gingival fluid changes to that of an inflammatory exudate and that this change is reflected in changes in the inorganic and organic composition of the fluid.
CHAPTER 4

VASCULAR PERMEABILITY

4.1. The formation of interstitial fluid

To show that the gingival fluid is of serumal origin, Salkind et al.\(^{158}\) followed the passage of proteins labelled with sodium fluorescein into the oral cavity. They showed that fluorescent material could be obtained from stimulated whole saliva and from gingival fluid. However, it could not be obtained from saliva collected at the orifices of the various salivary glands, thus indicating that all the fluorescent material came from the gingival fluid which, in turn, came from the serum.

Brill and Björn\(^{31}\) also showed fluorescent material in gingival fluid but not from other oral epithelium.

Under normal conditions a transudation occurs from the capillary to the surrounding tissue. This is a free passage of water and electrolytes with the amount of protein being limited. Thus we find that normal interstitial fluid has a lower protein content than plasma.
fig. 4.1 Starling's hypothesis. The formation of interstitial fluid.
From Bell, Davidson and Scarbrough.¹¹
The passage of fluid out of the capillaries occurs at the arteriolar end and resorption at the venous end (fig. 4.1). The capillary is not completely impermeable to proteins; however, the rate of leakage is dependent upon the molecular size, and the size of the capillary pores. 11

The passage of fluid back into the circulation occurs via the lymphatics. Once in the lymphatic vessels, this fluid is called lymph and has the same composition as interstitial fluid except for a higher protein concentration. 11 Obstruction of the lymphatics leads to the formation of oedema fluid, the composition of which is then the same as plasma.

It has been calculated that in man, half of the plasma proteins 'leak' from the capillaries per day and are returned to the circulation by the lymphatics. 11

With an increase in the permeability of the capillary there is a greater outflow of fluid including plasma proteins. Thus the colloidal osmotic pressure within the capillary is lowered, leading to a greater outflow and a reduced inflow of fluid. A vicious cycle is set up, leading to the formation of oedema fluid.
In the gingiva, this oedema fluid can escape from the tissues via the unkeratinised junctional epithelium.

Therefore, the amount of gingival fluid measured is governed by the permeability of junctional epithelium as well as the amount of oedema fluid.

The amount of oedema fluid depends upon the permeability of the capillary bed. Factors therefore which increase the surface area of capillary membrane or the permeability of the membrane will in turn lead to an increase in the amount of gingival fluid.

The metabolism of the cells of the junctional epithelium depend upon an intact capillary bed. Capillary permeability can change rapidly in attempts by the body to adapt to altered conditions. 184

4.2 Anatomy of the dento-gingival blood vessels

The morphological configuration of the terminal vascular supply of the gingiva conforms to the connective tissue - epithelial interface. 184 Thus the vessels of healthy junctional epithelium can be parallel to the enamel surface. 184 Egelberg, 50 using dog gingiva, notes that the characteristic loop formations found under
oral epithelium are not found under junctional epithelium but rather, there exists an anastomosing network of vessels in close relationship to the epithelium. This network has been termed the "crevicular plexus".

With inflammation there is proliferation of the epithelial cells and a change from the layered arrangement to loop-like formations, coinciding with the epithelial projections into the connective tissue. With this flat arrangement in healthy tissue, the arterioles and venules are associated in a more superficial relation to the epithelium, thus ensuring a good nutrient supply to the epithelial cells. 50

Egelberg 50 also noted that the vessels had a diameter larger than 7-8 microns and therefore should not be considered true capillaries, but rather as postcapillary venules. These venules have a greater tendency to increased permeability than true capillaries (fig. 4.1).

Thus the anatomy of the vascular bed of the junctional epithelium is conducive to the formation of gingival fluid. Venules are more susceptible to injury - haemorrhage thrombosis, and have
a greater tendency for increased permeability and therefore the formation of oedema fluid, and lying in close relationship with the epithelium the drainage of this fluid through the epithelium is less likely to be influenced by changes in the connective tissue.

4.3 Permeability of the dento-gingival blood vessels

To relate increased permeability of the blood vessels to increased flow of gingival fluid, Egelberg used carbon particles to label the blood vessels where the permeability had been increased. With an increase of permeability the endothelial cells of the capillaries become farther apart. Circulating carbon particles can pass through these widened intercellular spaces but cannot pass the basement membrane. In this way, the vessels of increased permeability become labelled and can be identified. In Egelberg's study, increased permeability was provoked by applying histamine to the tissues, massaging with a ball ended amalgam plugger and by scraping in the gingival crevice with a blunt instrument. The gingival fluid was measured by gently drying the tissues, placing a filter paper strip at the entrance to the gingival crevice for 3 minutes, and then staining with an 0.2% ninhydrin solution. The length of stain was measured, using an ordinary rule to the nearest half millimetre.
The experiment was repeated three days later, but this time vascular labelling was carried out. The results were similar for each method of irritation to the gingiva. That is, a rapid flow of gingival fluid initially which tapered off over a period (which was variable). The vascular labelling showed marked labelling initially over the entire crevicular plexus but predominantly in the wider vessels (venules). The amount of labelling tapered off over a period of time, the duration of which again depended upon the type of irritation used.

These results show that there is a direct relationship between the flow of gingival fluid and the permeability of the blood vessels of the dento-gingival junction.

Brill\textsuperscript{26} used plasma protein bonded Evans blue to study the effect of capillary permeability. He showed that the concentration of the dye collected at the gingival margins could be increased by (1) mechanical irritation - toothbrushing, (2) intravenously injected histamine and (3) inflammatory reactions.
This increased concentration of Evans blue indicates that the source of gingival fluid is initially the blood plasma since the Evans blue was bound to plasma proteins. It is probably true that the increase was due to increased permeability of the blood vessels; however, Brill's\textsuperscript{26} experiment did not show that there was any change in the blood vessel wall, unlike Egelberg's\textsuperscript{51} experiment. Using the same logic as Brill, his experiment could also be taken to show that there is an increase in the permeability of the junctional epithelium, thus letting more protein escape into the gingival sulcus.

Egelberg's\textsuperscript{51} experiment, on the other hand, does demonstrate that a change takes place in the capillary endothelium. If Brill had demonstrated an increase of concentration of Evans blue in the tissue following irritation and then increased concentration in gingival fluid, this would then indicate that a change had taken place in the permeability of the blood vessels.

In a biological system, the permeability of a membrane is proportional to its surface area and the pressure gradient across it. Vascular permeability cannot be measured by applications of a formula of these variables. The use of electron microscopy and vascular labelling with carbon particles has led to a greater understanding of vascular permeability.\textsuperscript{49}
fig. 4.2  Zonula adherens.

T = tonofilaments.
OL = outer layer.
ML = middle layer.
IL = intermediate line.

fig. 4.3  Zonula occludens.

C = cytoplasm.
IL = inner layer of cytoplasmic membrane.
ML = middle layer of cytoplasmic membrane.
OL = fused outer layer of cytoplasmic membrane.

IL = inner layer of cytoplasmic membrane and adjacent plate of fibrillar material.
Where the permeability of the capillary has been increased, the endothelial cells partially disconnect, allowing the carbon particles to pass between them. The carbon particles then are stopped by the basement membrane; thus labelling the capillary. Increased leakage of protein-bound dyes corresponds to this area of labelling in the capillary. 49

Anapole and Albright 3 found that in histologically inflamed rabbit gingiva, the separation between endothelial cells was of the order of 20-100 Å, and that the inter-endothelial junction appeared to be more of a zonular adherens than a zonular occludens (figs. 4.2, 4.3).

After the injection of saccharated iron oxide, it appeared at these inter-endothelial junctions and within the connective tissue.

The cytoplasm of the endothelial cells contain numerous plasma luminal vesicles which open on to both the luminal and connective tissue sides of the vessel wall. Vacuoles containing saccharated iron oxide were seen within the cytoplasm.
In chronically inflamed dog gingiva, Theilade, Egelberg and Attström found that the endothelial cells themselves took up the carbon particles and it was not until after histamine application that carbon particles were found between the cells. They interpreted this as indicating a change in the endothelial cells but not necessarily an increase in the capillary permeability of resting chronically inflamed gingiva. The permeability only increased with the application of histamine. The significance of this will be discussed in a subsequent section (section 7.4).

However, the important point here is that, although the inter-endothelial spaces widen, the basement membrane remains intact. So far, the author has been unable to find any literature relating to the structure, function or changes of the basement membrane of the capillaries of the periodontal ligament or gingiva. The function of this apparent amorphous layer is surely most important in any study of capillary permeability in both health and disease. A greater understanding of the basement membrane may lead to a greater understanding of the disease process in gingivitis and periodontitis.
1. Oral epithelium
2. Oral sulcular epithelium
3. Junctional epithelium

fig. 5.1 Anatomy of the gingival epithelium.
From Listgarten. 117
CHAPTER 5

EPITHELIAL PERMEABILITY

5.1 Anatomy of the gingival epithelium

In the passage of gingival fluid from the underlying connective tissue into the gingival sulcus, the epithelium of the gingival sulcus acts as a semi-permeable membrane. It is therefore important to understand the nature of the tooth-epithelial interface and the epithelial-connective tissue interface.

Listgarten\textsuperscript{117} divides the gingival epithelium into three portions, each with a different function (fig. 5.1):

(i) **Oral epithelium**, extending from the muco-gingival junction to the gingival margin;

(ii) **Sulcular epithelium**, lining the gingival sulcus. It consists of a coronal part which resembles oral epithelium and an apical part which resembles junctional epithelium;

(iii) **Junctional epithelium**, joining the epithelium to the tooth. The coronal part lines the floor of the gingival sulcus. It is from this surface that cell desquamation takes place.
Oral epithelium is keratinized, stratified, squamous epithelium, characterised by rete pegs extending into the underlying connective tissue.

Junctional epithelium is non-keratinized, stratified, squamous epithelium with its epithelial-connective tissue interface being straight. The intercellular spaces of the junctional epithelium are wider than in oral epithelium and account for 1/5th of the tissue volume. It is quite common for leukocytes to occur in the junctional epithelium, the number of which does not relate to the amount of inflammation of the underlying connective tissue.

In the case of sulcular epithelium the apical portion resembles junctional epithelium with wide intercellular spaces frequently containing leukocytes and is unkeratinized. The coronal part has narrow intercellular spaces which seldom contain leukocytes. It does not have the same degree of keratinization as does oral epithelium and the stratum corneum is irregular in cell shape and staining characteristics compared to the uniformity of the stratum corneum of oral epithelium.
5.2 Ultrastructure of the gingival epithelium

Since keratinized stratified squamous epithelium is not permeable to the passage of small ions,\textsuperscript{83,84} it is therefore not likely to allow oedema fluid in the gingival connective tissue to escape. For this reason, only the permeability of the unkeratinized junctional epithelium and sulcular epithelium will be discussed, in the following sections.

The ultrastructure of these epithelia may shed some light on the passage of molecules through them.

A. The Basement Lamina

The basement lamina joins the epithelium to the underlying connective tissue. It consists of two parts: the lamina densa - an electron dense finely fibrillar layer, approximately 350-600 Å wide, which is separated from the epithelium by the lamina lucida, an electron lucent layer 300-500 Å.\textsuperscript{117}

The basement lamina is formed by the epithelial cells and will form between epithelial cells and any inert substance on which they are growing. Fibroblasts do not form any similar structure.\textsuperscript{117}

It is chiefly comprised of protein, with a small amount of carbohydrate. The exact nature of the protein is unknown but it is susceptible to collagenase activity.\textsuperscript{117}
fig. 5.2 Hemidesmosomal attachment.


E = enamel
BL = basement lamina
EDL = electron dense layer
OL = outer layer
IL = inner layer
T = tonofilaments
C = cytoplasm
The basement lamina is only recognisable at the electron microscope level and should not be confused with the basement membrane which is seen in the light microscope, and has a high carbohydrate component.

The connection between the epithelium and connective tissue is enhanced by hemidesmosomes and anchoring fibrils.

(i) Hemidesmosomes

These consist of an attachment plaque 200 Å thick. The unit of the cytoplasmic membrane consists of an inner and outer layer each about 30 Å wide. The inner layer is more electron dense and better defined than the outer layer. There is an electron dense layer 35-60 Å wide in the lamina lucida, approximately 70 Å from the unit membrane (fig. 5.2).

(ii) Anchoring fibrils

These are connective tissue fibrils related to the basement lamina. They are short, curving fibrils 200-400 Å thick. They seem to cross the lamina densa as filaments near hemidesmosomes and anchor the basement lamina to the connective tissue.
B. Junctional Epithelium

The cells of the basal layer of the junctional epithelium have a well-developed golgi-apparatus and a rough surfaced endoplasmic reticulum. Desmosomal junctions between the cells are few. The cells of the supra basal layers are in a flattened plane parallel to the tooth surface. Intercellular connections are desmosomes (adherens) and tight junctions (occludens) (fig. 4.2, 4.3). There are fewer connections than in oral epithelium and as a result, wide intercellular spaces exist. 117

The junctional epithelial cells adjacent to the tooth surface have hemidesmosomes linking the epithelium to the tooth surface. The basement lamina, lining the tooth surface are different to that lining the connective tissue. It does not demonstrate a clearly defined lamina densa and lamina lucida although its width is about 1200 $\text{Å}^0$ which is similar to the combined widths of the densa and lucida of other basement laminae. 117

C. Sulcular Epithelium

The ultrastructure of sulcular epithelium is similar to that of junctional epithelium in the apical portion but is less uniform. The sulcular epithelium should be considered as a transitional epithelium between the junctional and oral epithelia.
JE = Junctional epithelium.
OE = Oral epithelium.
A = Desquamative surface of junctional epithelium.
B = Basal layer of junctional epithelium.
C = Basal layer of oral epithelium.
D = Desquamative surface of oral epithelium.
\( \frac{C}{D} = 174 - 7\cdot71 \)
\( \frac{B}{A} = 50 - 100 \) (estimated).

fig. 5.3  The desquamation surface area of junctional and oral epithelium.
From Listgarten. 117
D. **Cell Turnover**

All the cells of the junctional epithelium must desquamate into the gingival sulcus via the narrow zone at the bottom of the sulcus, since this is the only free zone of junctional epithelium. If the number of mitoses is the same in the basal layers of both junctional and oral epithelium, obviously the rate of cell desquamation (number of cells/unit area) will be much greater at the free surface of junctional epithelium. \(^{117}\) (fig. 5.3)

It has also been suggested that the rate of cell turnover is higher in junctional epithelium than oral epithelium \(^{117}, 177\) and that cell turnover increases with age. \(^{177}\)

The significance of the high turnover of cells is that damaged tissue is replaced rapidly \(^{117}\) and that leukocytes 'trapped' between two cells reach the surface very rapidly.

E. **The Gingival Sulcus**

Histologically, the gingival sulcus is shallow, <0.5mm. in depth. It is bounded by the tooth, junctional epithelium and sulcular epithelium. The junctional epithelium is the most permeable and it is also the most easily damaged by such objects as periodontal probes, filter paper strips, etc. The damage usually occurs within the epithelium, not at the tooth-epithelium interface. The clinical depth of the gingival sulcus depends upon the thickness of the probe,
fig. 5.4  The comparison of the histological and clinical sulcus.
From Listgarten. 117
pressure applied and degree of leukocyte infiltration into junctional epithelium.\textsuperscript{117} (fig. 5.4).

Thus the gingival sulcus is not lined with a highly permeable epithelium as this in the intact state is connected to the tooth.

5.3 Permeability of gingival epithelium

A. The passage of leukocytes into the gingival sulcus.

The flow of gingival fluid into the gingival sulcus depends upon the permeability of the epithelium. From the anatomical and ultrastructural features of the epithelium just discussed, the passage of gingival fluid would be most likely to occur through the junctional epithelium and into the sulcus at its base.

Leukocytes are found in the gingival fluid of both healthy and chronically inflamed tissue.\textsuperscript{47} A differential count shows 95-97% neutrophils, 1-2% lymphocytes and 2-3% monocytes. This count does not change with inflammation\textsuperscript{6} although there is increased numbers of cells.\textsuperscript{7} This continuous migration of leukocytes occurs in the absence of increased vascular permeability as indicated by the absence of vascular labelling with colloidal carbon,\textsuperscript{49} and is probably due to the elaboration of neutrophil chemotactic substances by the plaque.\textsuperscript{112}
As already stated, one of the histological features of junctional epithelium is the presence of leukocytes in the widened intercellular spaces.\textsuperscript{117} Grant and Mulvihill\textsuperscript{61} describe this infiltration in dogs as occurring following a collection of leukocytes below the basement lamina. The leading pseudopodal extension of a neutrophil extends through the basement lamina and into the intercellular space forcing the epithelial cells apart. This pseudopod is devoid of cytoplasmic organelles with the nucleus between the leading part and the main bulk of the cell. The migration of neutrophils into the epithelium causes a break in the cell connections - desmosomes and tight junctions, and a widening of the intercellular spaces.

In these widened spaces, Grant and Mulvihill\textsuperscript{61} describe the presence of particulate matter as well as the leukocyte. This particulate matter was thought to be precipitated plasma protein, monoparticulate glycogen, lysosomes and cellular debris. This then provides a path for escape of tissue fluid from the underlying connective tissue. The neutrophils force the epithelium apart and a small pocket between the cells is formed. This pocket contains neutrophils and a small amount of tissue fluid. The
pocket advances towards the surface with the epithelial cells and is ultimately freed into the gingival sulcus with the desquamation of the epithelial cells.

In clinically healthy tissue, there is a high desquamation rate from junctional epithelium so that this path of escape could be of importance to the passage of gingival fluid in this tissue.

This theory is supported by the observation of Anapolle and Albright of a medium electron dense material in the widened intercellular space of inflamed rabbit gingival epithelium. This material resembles precipitated plasma protein and may therefore represent the presence of an inflammatory exudate. However, this path would assume a greater role in the non-inflamed state.

B. The passage of injected particles.

Weinstein et al. traced the path of saccharated iron oxide out of the capillaries between the endothelial cells and into the connective tissue. However, they were unable to trace it through the gingival epithelium although they could collect it on filter paper strips placed in the gingival sulcus.

Anapolle and Albright found increased intercellular spaces but were unable to find the saccharated iron oxide in these spaces. It did, however, collect below the basement lamina and was
phagocytosed by fibroblasts in the connective tissue. Further attempts to trace particles from the blood vessels through the epithelium and into the gingival crevice were done by Gavin. Here, intravenous colloidal thorium dioxide was used. The results showed that the thorium dioxide left the capillaries at the venous end between the endothelial cells and collected beneath the perivascular basement lamina. From here, the particles were released into the connective tissue where they were phagocytosed by cells of the connective tissue. Nowhere did the particles cross the epithelial basement lamina.

The size of saccharated iron oxide particles is between 80 and 100 Å, the size of thorium dioxide is 50-80 Å. These are large particles and recognised as foreign by the cells of the connective tissue, as a result, phagocytosis of these particles occurred. The fact that the basement lamina of the blood vessels acted as a semi-barrier, meant that only small numbers of particles were in the connective tissue at any one time. If they succeeded in passing through the connective tissue, they were further held up by the basement lamina of the epithelium, allowing the phagocytes to catch up.
That Weinstein et al.\textsuperscript{208} could demonstrate particles in the gingival crevice may have been due to the passage of the phagocytes out into the gingival crevice, taking with them the ingested particles.

Although these attempts at tracing the pathway of gingival fluid are inconclusive, the widened intercellular spaces of the junctional epithelium form the most likely pathway for the passage of both fluid and leukocyte together.

C. The passage of substances from the gingival sulcus into the tissues.

The passage of substances from the gingival sulcus into the underlying connective tissues has been demonstrated. Fine et al.\textsuperscript{57} showed carbon particles 1-3\textmu in diameter could penetrate both normal and pathological sulcus tissues and can be identified in the underlying connective tissue where they are phagocytosed. The path of penetration of these particles through the epithelium was not shown.

Schwartz et al.\textsuperscript{165} traced the passage of tritiated bacterial endotoxin through the sulcus epithelium to the underlying connective tissue in dogs, without signs of clinical inflammation. They showed the greatest density of substance occurred at the basal and parabasal cell layers. They go on to say that this indicates the selective permeability of the basement lamina acting as a temporary barrier leading to a damming up of substance which was then slowly released into the connective tissue.
Although they used a light microscope and could therefore not establish the precise location of the substance (intra- or extracellularly), they did note a slight widening of the intercellular spaces.

The grains entering the epithelium in this study appeared to do so at the coronal part of the epithelium. This is in line with the concept of a histological and clinical sulcus. What appears to be the coronal part of this sulcus corresponds to the base of the histological sulcus and the free surface of the junctional epithelium.

McDougall applied horse radish peroxidase (HRPO) to the gingival epithelium of rats. HRPO is a toxic protein of molecular weight of about 40,000 and a diameter of about 50 Å, it can be demonstrated histochemically and electron-microscopically. He showed that this protein failed to penetrate the keratinized epithelium but had spread throughout the intercellular spaces of the junctional epithelium, reaching the apical limit of the junctional epithelium in as little as 10-15 minutes. The HRPO then spread through the basement membrane and into the connective tissue. The uptake of the HRPO by the epithelial cells increased as the time of exposure of these cells to the HRPO increased. Once through the epithelium and into the connective tissue, macrophages and fibroblasts showed an uptake of the HRPO.
JE = junctional epithelium.
OSE = oral sulcular epithelium.
OE = oral epithelium.

fig. 5.5 The exchange of substances between the gingival sulcus and the connective tissue of the gingiva through the junctional epithelium.
From Listgarten. 117
The basement membrane offered no resistance to the passage of HRPO, $^{127}$ although it did offer resistance to the bacterial endotoxin used by Schwartz et al. $^{165}$

McDougall $^{127}$ also noted that the HRPO caused a widening of the intercellular spaces and stimulated neutrophilic migration. There was an inflow of HRPO despite the outflow of gingival fluid that was taking place at the same time.

The pathway of transport across the non-keratinized junctional epithelium appears to be via the intercellular space. Under certain circumstances the intercellular space may become enlarged, thus providing even less resistance to the passage of substances across the epithelium. This pathway seems to be associated with the junctional epithelium and not the sulcular or oral epithelium (fig. 5.5).

The flow of fluid through the epithelium probably results as a net product of hydrostatic and osmotic pressure differences between the connective tissue and the sulcus. $^{199}$ A concentration gradient also exists between the sulcus and underlying tissue $^{117}$ thus favouring the inward flow of substances in the gingival sulcus, with the outward flow as a result of the hydrostatic and osmotic pressures.
Tolo has put forward the hypothesis that the flow of fluid occurs in a step wise fashion. The tight junctions, cell membrane and basement lamina lead to compartmentalisation of the epithelium and the transport of fluid results as these compartments are disrupted with desquamation of the cells, thus freeing their contents. The flow of fluid from one compartment to the other can take place as a result of pressure or concentration gradients.

From this theory the inward flow of fluid would be restricted, compared to the outward flow of fluid; however, it would allow both movements of fluid to occur at the same time.

5.4 Effect of plaque on epithelial permeability

It has been suggested that enzymes which occur in dental plaque can contribute to an increase in the width of the intercellular spaces and thus allow an easier passage of bacterial products through the epithelium and thereby setting up an inflammatory reaction.

Leukocytes are attracted through the epithelium by chemotactic substances released by the plaque or by the presence of antigen in the sulcus not related to the bacteria (e.g. in germ free animals).
<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Enzymes produced</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci</td>
<td>Hyaluronidase</td>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td></td>
<td>Coagulase</td>
<td>Clotting of plasma</td>
</tr>
<tr>
<td></td>
<td>Gelatinase</td>
<td>Gelatine</td>
</tr>
<tr>
<td></td>
<td>Haemolysins</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>Streptococci</td>
<td>Hyaluronidase</td>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td></td>
<td>Streptokinase</td>
<td>Fibrin, Fibrinogen</td>
</tr>
<tr>
<td></td>
<td>Haemolysins</td>
<td>Red blood cells</td>
</tr>
<tr>
<td></td>
<td>( \beta )-Glucuronidase</td>
<td>Glucuronidic links</td>
</tr>
<tr>
<td></td>
<td>Proteases</td>
<td>Various proteins</td>
</tr>
<tr>
<td>Diptheroids</td>
<td>Hyaluronidase</td>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td></td>
<td>Chondroitinase</td>
<td>Chondroitin sulphate</td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>Proteases</td>
<td>Various proteins</td>
</tr>
<tr>
<td></td>
<td>Sulphatase</td>
<td>Aryl sulphates</td>
</tr>
<tr>
<td>B. melaninogenicus</td>
<td>Collagenase</td>
<td>Collagen</td>
</tr>
<tr>
<td>Spirochetes</td>
<td>Proteases</td>
<td>Various proteins</td>
</tr>
<tr>
<td>Gam-negative cocci</td>
<td>Proteases</td>
<td>Various proteins</td>
</tr>
</tbody>
</table>
The intercellular substance which cements the cells together is an acid mucopolysaccharide-protein complex. Enzymes which disrupt this complex include collagenases, hyaluronidase, chondrosulphhatase and $\beta$-glucuronidase as well as lysosomal enzymes.

Bacteroides melaninogenicus, an anaerobic organism found in plaque, will produce collagenase. Other organisms cultivated from plaque include Streptococcus sangiuis, Streptococcus mitis, Actinomyces, Clostridium, Fusobacterium and Veillonella. A list of some of the bacteria of plaque, the enzyme(s) produced and the substrate acted on is shown in table 5.1.

A study of this table reveals that there exists in plaque bacteria capable of producing enzymes which will lead to the breakdown of cell adhesion, a widening of the intercellular spaces and an increase in the permeability of the epithelium and basement lamina.

Lysosomal enzymes can result from the breakdown of leukocytes in the gingival crevice. These lysosomal enzymes are found in the gingival fluid and may contribute to the breakdown of cell adhesion of the junctional epithelium.
Endotoxin, a lipopolysaccharide of the bacterial cell wall, with the ability of acting as an antigen, has been cited as an aetiological agent in the formation of gingival inflammation. This occurs via an antigen-antibody reaction with the fixation of complement and the release of anaphylatoxin and the subsequent inflammation reaction. 133, 134, 179, 180

Endotoxin has also been shown to enhance the detachment of cells grown on a glass surface in vitro. 140

Endotoxin occurs in plaque 94 and has also been shown to occur in gingival fluid 173-176 and it appears that in the presence of endotoxin, the ability of the junctional epithelium to withstand mechanical forces is decreased. 140

Therefore the effect of plaque on the junctional epithelium is to increase the number of leukocytes migrating through the epithelium 134 to disrupt the cellular adhesion between the cells, leading to a widened intercellular space and therefore increasing the permeability 43 and to make the epithelium more susceptible to mechanical trauma. 140
CHAPTER 6

ROLE OF INFLAMMATION IN THE FORMATION OF GINGIVAL FLUID

6.1 Methods of collection of gingival fluid

It is well established that with inflammation of the gingival tissues there is a flow of gingival fluid. However, it is still controversial as to whether or not there is a fluid flow in clinically healthy gingival tissue.

Brill, using dogs fed on a soft, then hard diet, showed that intravenously injected sodium fluorescein could be recovered on filter paper strips inserted into the gingival crevice. The fluorescein could be recovered in large amounts when the tissue showed a degree of chronic inflammation (soft diet). When the tissues were returned to be clinically free of gingival inflammation (hard diet and polishing of teeth), the amount of fluorescein able to be recovered was much reduced. However, it could be recovered, thus indicating a reduced but definite flow of fluid from the clinically healthy tissue.

This method of fluid collection, where the filter paper strip is placed into the gingival crevice until gentle resistance is felt has been termed the intra-crevicular method. An extra-crevicular
fig. 6.1  
(a) The intracrevicular method of Brill.²⁹

(b) The extracrevicular method of Brill.²⁹
method was also used by Brill\textsuperscript{29} where the strips were placed over the gingiva and tooth (fig. 6.1).

Brill\textsuperscript{29} found that less fluid could be collected using the extra-crevicular method, but there was a definite fluid flow. This extra-crevicular method was found to be inconvenient and was discarded.

Other investigations\textsuperscript{15,48,159} using the intra-crevicular method, as described by Brill, have demonstrated the presence of gingival fluid in clinically healthy tissue. Using this same technique, differences in the composition have been reported between the fluid obtained from clinically healthy tissue and that obtained from chronically inflamed tissue.\textsuperscript{66,72,96,202}

A variation on this method was introduced by Mann.\textsuperscript{126} He used three filter paper strips inserted into the same sulcus side by side. After drying the middle strip, it was removed and replaced by a thinner strip, not in contact with the other two. This method was used to ensure that the sample was uncontaminated by saliva and also enabled a limited area of sulcus to be sampled (fig. 6.2)
fig. 6.2 The triple strip arrangement of Mann.\textsuperscript{129}
This variation is unnecessary, as saliva can be effectively removed from the area by isolation with cotton rolls and gentle drying with compressed air.

Furthermore, the two strips on either side of the sampling strip would be asserting a capillary force on the fluid which would tend to drain fluid away from the smaller sampling strip so that any reference to the total flow of fluid would be invalid.

Another variation on this intra-crevicular method of Brill's was described by Löe and Holm-Pedersen. This method was introduced to reduce irritation to the gingiva by not inserting the filter paper strip into the sulcus, but rather by placing it at the entrance to the sulcus (fig. 6.3).

Using this method, they were unable to demonstrate the presence of fluid in clinically healthy tissue. They stated that clinically healthy tissue did not have a flow of gingival fluid and therefore gingival fluid should be considered as an inflammatory exudate. Oliver et al., using this method, demonstrated a positive correlation between clinical scoring, fluid measurements and histological evaluation of inflammation. No fluid was detected from clinically healthy tissue.
fig. 6.3. The method of Løe and Holm-Pedersen. The filter paper strip at the entrance to the gingival sulcus. From Løe and Holm-Pedersen.
That there is no fluid flow in the absence of inflammation has been supported by Egelberg, Kaslick et al, and Rudin et al.

In their quantitative analysis of the inorganic cations of gingival fluid, Kaslick et al were unable to collect gingival fluid in clinically healthy tissue and therefore used fluid obtained from tissue which was nearly normal, but did display some clinical evidence of inflammation. These workers used a capillary tube moved to and fro at the entrance of the sulcus and slight finger pressure on the gingiva.

Rudin et al used the method of Loe and Holm-Pedersen and could not demonstrate a flow of fluid. However, traces of fluid which did appear, were attributed to a faulty technique, which led to a contamination with saliva or plaque.

The anatomy of the gingival sulcus is such that it is easily damaged by foreign bodies placed in it. Thus, if a filter paper strip is placed into the gingival sulcus until resistance is felt, damage will occur to the junctional epithelium at the base of the sulcus or free surface of the junctional epithelium (fig. 6.4).
fig. 6.4 Possible damage to junctional epithelium by insertion of filter paper strip into the gingival sulcus.
The amount of damage that will occur depends on the amount of pressure used, and the type of filter paper used. More pressure could be applied with a thicker, heavier paper, thus leading to more damage.

Also, using this intra-crevicular method, the area of filter paper in contact with the permeable junctional epithelium is greater than the area of junctional epithelium at the base of the sulcus. Therefore more fluid could be collected over the same time on the filter paper than is actually released into the sulcus (fig. 6.4).

The method of Löe and Holm-Pedersen overcomes these difficulties by not disrupting the junctional epithelium and by only collecting that fluid which is released into the sulcus.

Further support for the absence of fluid in clinically healthy tissue and the method of Löe and Holm-Pedersen comes from the studies of Egelberg. 52, 53

Using dogs, Egelberg 52 related gingival fluid and the extent of vascular labelling in clinically healthy tissue. The gingival fluid was measured according to the methods of Löe and Holm-Pedersen and vascular labelling carried out as previously described (section 4.3). The results showed that gingival fluid could not be detected;
however, there was an indication of a minor variation in the vascular permeability. Punctuate deposits of carbon were seen in the wider vessels (venules) pointing to a minor increase in the permeability of these vessels. Also the adhesion of leukocytes, which had an absorbed layer of carbon particles, was observed, thus indicating a response to a low grade irritant. When, however, the filter paper strips were inserted into the gingival sulcus, regular labelling of the vessels was observed, thus indicating a definite change in the permeability of the vessels.

In the next stage of his experiment, Egelberg used dogs with chronically inflamed gingiva. The gingival fluid was again measured according to Löe and Holm-Pedersen and vascular labelling carried out. Contrary to previous investigations in this experiment, Egelberg could not demonstrate a flow of gingival fluid from resting chronically inflamed tissue. Vascular labelling indicated loop formation of the blood vessels with punctuate deposits of carbon and adhesion of "carbon leukocytes". However, distinct labelling of carbon between the endothelial cells and the basement membrane was not seen. This indicated that a change had taken place in the blood vessels but an abnormal permeability was not present. These vessels, however, were susceptible to damage very easily and once damaged a flow of fluid could be detected.
Egelberg suggests that the changes seen in the blood vessels of chronically inflamed gingiva indicates an increase in exchange between the vessels and the tissue but that the increased permeability which leads to the formation of oedema in acute inflammation is not seen.

Cowley, using fluorescent protein tracing, showed an increased presence of labelled plasma protein in the crevicular epithelium and connective tissue adjacent to an inserted paper strip. He also observed a difference between healthy and chronically inflamed gingiva after the insertion of a paper strip. The presence of labelled plasma protein was more intense in the inflamed tissue.

These results would therefore indicate that there is no flow of fluid from clinically healthy tissue and the method of Löe and Holm-Pedersen is the method of choice.

However, investigations by Weinstein et al. using the method of Löe and Holm-Pedersen, have indicated a flow of gingival fluid in clinically healthy human gingiva. This has been supported by Wilson and McHugh who found that only one of 202 surfaces free of clinical inflammation had no detectable fluid flow, when measured by the method of Löe and Holm-Pedersen.
This divergence of results of different workers supposedly using the same technique may be due to uncontrollable differences in technique or a difference in criteria for "clinically healthy". Other investigators have used fluorescein as a marker while Løe and Holm-Pedersen have used ninhydrin stain. Weinstein et al found that fluorescein is 100 times more sensitive than ninhydrin. Thus minute amounts of fluid detected by fluorescein may not have been detected by ninhydrin.

That different criteria do exist for "clinically healthy" tissue has been shown by Alexander et al. What one examiner calls inflamed may be healthy to another. Alexander et al found that intra-examiner reliability using the PMA index and gingival index was achieved very rapidly. However, inter-examiner reliability could not give a consistent pattern until extensive training and clinical instruction was given by an experienced examiner.

McLendon and Suomi tried to arrive at a mathematical formula to cancel out the effect of examiner error in the measurement of marginal gingivitis. This formula had as many shortcomings and variables as exist without its use. Therefore it appears that unless extensive training by one examiner is given, the amount of inter-examiner reliability will be small.
Therefore, this factor probably plays a role in the differences observed in gingival fluid flow by different investigators.

Variations in techniques are bound to exist - the length of time between removal of the filter paper and staining, the time after staining before measuring, the drying time. These things can have an effect on the amount of fluid measured. Although both examiners might say that the paper is immediately stained and dried, immediately to one examiner might mean 30 seconds, while to the other, 1 - 2 minutes. Changes could occur in this time which would ultimately affect the result. The influence of these variables will be discussed in a subsequent section (Chapter 12). However, it is important to note that these variables do exist and are virtually impossible to eliminate.

To date, the conclusion, must be drawn that it has not been proven that gingival tissues, free of inflammation, do not exhibit a flow of fluid.

When it is considered that the junctional epithelium is a non-keratinized stratified squamous epithelium with a small exposed free surface, \(^{117}\) it must be realised that the cells close to the surface are in fact living cells. These cells depend upon a continuous flow of tissue fluid to provide nutrition in order to maintain their metabolic
functions (e.g., sticking to the tooth). Waste products must also be removed from these cells, in order to prevent toxic products building up inside the cells.

The movement of fluid through the intercellular spaces, as already described (section 5.3), provides for this supply of nutrients and removal of waste products. Some of this fluid would ultimately be expelled into the gingival sulcus with desquamation of the cells. In keratinized epithelium the fluid is reabsorbed into the vascular system via the lymphatics just below the basement membrane. However, in junctional epithelium with the high desquamation rate, some fluid would be expelled into the sulcus while some is reabsorbed via the lymphatics.

Hence, theoretically speaking, a flow of interstitial fluid into the gingival sulcus of healthy tissue would be expected. This flow would, of course, be minute. The methods of collection of gingival fluid used up to the present (filter paper and capillary tube) are very coarse, with the environment of the gingival sulcus being changed by the collection technique. The detection of the minute amounts of fluid theoretically present would be very difficult in any case and cannot be done with the present methods.
The inability to detect the presence of gingival fluid in the uninflamed state, \(^{120, 143}\) is not a proof that this fluid is not present. It does show, however, that the method used is limited, and may not be suitable for the detection of the minute amount of fluid theoretically present.

Those workers, \(^{159, 208}\) who do detect a flow of gingival fluid in the uninflamed state probably do so because the method used stimulates a flow of gingival fluid by altering the environment of the gingival sulcus which in turn leads to an increase in the permeability of the dento-gingival blood vessels.

In the uninflamed state this fluid, theoretically present, would be of a similar nature to interstitial fluid. With inflammation the nature of the fluid would change to that of an inflammatory exudate.

6.2 The correlation between gingival fluid measurements and the clinical and histological degree of inflammation

It is know that with increasing severity of inflammation the rate of flow of gingival fluid increases, \(^{28, 29, 48}\) and that the composition changes. \(^{89, 90, 91}\) That these changes in flow rate can be used to indicate the severity of the inflammation is under conjecture.
That this conjecture exists is, in the author's opinion, due in part to a failure of these workers to understand the nature of gingival fluid in its relationship to its environment and the inflammatory process. It is also due in part to a failure to recognise the limitations of the techniques used to measure gingival fluid and the error involved in trying to quantitate these measurements.

In all of these studies the amount of stain on the filter paper strips is assessed and it is inferred by these workers that this amount of stain reflects the volume of fluid absorbed by the strip and that this in turn may or may not reflect the degree of inflammation. The major error, of course, lies in the fact that the amount of stain may not reflect the volume of fluid absorbed. In which case the correlation between the amount of stain and the degree of inflammation does not tell us anything about the inflammation and may not even relate to it. Whereas, by inferring that the amount of stain reflected the volume of fluid, these workers were indirectly saying that the amount of stain was related to the volume of inflammatory exudate which, taken in turn, related to the inflammation.
Bearing in mind this failure to understand what they were doing, several studies have been undertaken to try and determine the relationship between the flow of gingival fluid and the degree of both clinical and histological inflammation.

Löe and Holm-Pedersen\textsuperscript{120} found a good correlation between gingival fluid measurement and clinical gingivitis using the gingival index of Löe and Silness.\textsuperscript{122} Other workers\textsuperscript{15, 115, 143} have corroborated this finding, stating that the flow of gingival fluid may be used to assess the effects of therapy on gingival inflammation\textsuperscript{157} by measuring the extent to which the inflammation has been resolved.

On the other hand, Wilson and McHugh\textsuperscript{212} and Orban and Stallard\textsuperscript{144} found a poor correlation between the gingival fluid flow and other methods of evaluating gingival inflammation, e.g. PMA index and gingival index.

In order to understand this difference, a closer look at these studies, their methods and results, is essential.

In their study, Löe and Holm-Pedersen\textsuperscript{120} found that gingival fluid flow increased with increasing inflammation. They also showed that with the development of gingivitis\textsuperscript{123} a flow of gingival fluid commenced before clinical signs of inflammation were evident and
<table>
<thead>
<tr>
<th>Score</th>
<th>State of section</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No inflammatory cells.</td>
</tr>
<tr>
<td>1</td>
<td>Sparse distribution.</td>
</tr>
<tr>
<td>2</td>
<td>Isolated dense areas with sparse distribution in others.</td>
</tr>
<tr>
<td>3</td>
<td>Dense aggregation throughout.</td>
</tr>
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</table>
that it continued after the clinical signs of inflammation had disappeared. They thought that the flow of gingival fluid therefore represented a state of subclinical inflammation.  

That the flow of gingival fluid could be correlated to the state of histological inflammation was shown by Oliver et al. Here, the state of inflammation was assessed histologically as the density of inflammatory cell infiltration (table 6.1), while the flow of gingival fluid was measured according to the method of Löe and Holm-Pedersen. In this study, the degree of clinical inflammation was assessed using the Löe and Silness gingival index (GI) and was found to correlate strongly with the gingival fluid measurement. Whereas a not so strong correlation existed between the gingival index and histological inflammation and between the fluid flow and histological inflammation.

They were unable to explain the reason for the very close correlation between the gingival index and the flow of gingival fluid.

Although they did find a strong correlation between the gingival fluid measurement and the area of inflamed connective tissue.
Since the gingival index is a subjective index of clinical inflammation, the scorer must be influenced in his judgment by the area of the inflamed tissue. Therefore, the larger the area of inflammation the higher the subjective score. This may partially explain why the gingival index and gingival fluid showed a strong relation while only a weak one existed between these and the histological degree of inflammation.

On the other hand, Orban and Stallard \(^{144}\) assessed the histological inflammation according to the amount and extent of inflammatory cell infiltrate and gave a score of 0-10. The gingival fluid was measured by an intracrevicular method. A simplified oral hygiene index and Ramfjord's plaque index (after disclosing) were also performed. In this study a comparison between the biopsy score (histological inflammation) and gingival fluid showed an extremely poor correlation. There was a better correlation between the simplified oral hygiene index and the biopsy score and an even better correlation between the plaque score and the biopsy score. \(^{144}\)

These results therefore indicate that the flow of gingival fluid as measured by the intracrevicular method is a poor index of the degree of histological inflammation but may indicate the integrity of the junctional epithelium. \(^{144}\)
This poor correlation between biopsy scores and gingival fluid measurements was again demonstrated by Stallard et al. Although these workers recognised that with inflammation and an increase in vascular permeability the inflammatory exudate formed should be detectable as gingival fluid, they blamed the poor methods of gingival fluid measuring as giving unreliable results and thereby not reflecting the state of inflammation in the tissues.

Furthermore, the problem of comparing gingival fluid measurements with histological sections lies in the fact that two different aspects of the inflammatory response are being compared. Gingival fluid measurements are in effect a measure of the amount of plasma exudation while histological sections give a measure of inflammatory cell infiltration. These two different aspects vary as to the type and extent of the inflammatory response. Factors which cause an outflow of plasma from the blood vessels may not necessarily be chemotactic for inflammatory cells. In acute inflammation the increase in capillary permeability is due to histamine, 5-hydroxytryptamine and kinins (e.g. bradykinin). In chronic inflammation other mediators maintain the increased permeability. These mediators are products of sensitised lymphocytes and are called lymphokines.
It has also been reported that with chronic inflammation, oedema fluid is not formed, but there is an increased exchange between the tissues and the blood vessels;\textsuperscript{53} these blood vessels are also more susceptible to damage.\textsuperscript{54} If this is the case then certainly a comparison of inflammatory oedema and inflammatory cell infiltration would not correlate.

Even though gingival fluid measurements are a measure of the plasma exudation the quantitative assessment given is to the amount of stained\(\alpha\)-amino acids on the filter paper strip and not to the volume of fluid absorbed. Therefore, a quantitative relationship between the amount of stain and the degree of inflammation is not a comparison between the amount of inflammatory exudate and the inflammation, and should not be taken as such.

Therefore, the use of a quantitative measure of gingival fluid (the amount of stain on a filter paper strip) as an epidemiological index of inflammation may be invalid. Its use as such has been questioned by Orban et al\textsuperscript{145} and Wilson and McHugh.\textsuperscript{212}

Orban\textsuperscript{145} compared various indices to the extent of histological inflammation. He found that Ramfjord's plaque index gave the best result, whereas the gingival fluid measurement gave a poor result.
Furthermore, if a relationship does exist between the amount of stain and the amount of inflammatory oedema, the value of comparing this to the degree of histological inflammation is still questionable. In using epidemiology, it is important to know what is being measured - inflammatory cell infiltration or inflammatory oedema. If one is interested to find out how much inflammatory oedema exists in the gingiva of a population, gingival fluid measurements may be highly significant. As a measure of clinical inflammation, gingival fluid measurements may be significant, \(^{143}\) as oedema is of a clinical significance in gingivitis.

Using a different approach to assess histological inflammation, Rüdin et al.\(^ {157}\) found a correlation between gingival fluid and the area of inflammation in the histological section. This is in line with the findings of Oliver et al.\(^ {143}\) The area of inflammation would combine both the amount of oedema and inflammatory cell infiltration and this is why a correlation would exist with gingival fluid measurements.

Orban et al.\(^ {145}\) did not include any measure of the extent of inflammatory oedema in their criteria of histological inflammation and this could explain the differences between the two conflicting views.
Mann found a positive correlation between gingival fluid and depth of a periodontal pocket but cited the severity of inflammation as having a greater effect than the depth of the pocket. By relating the depth of the periodontal pocket Mann is in effect comparing the clinical equivalent of the histological area of inflammation to the gingival fluid flow, and is therefore relating the area of inflammation to the flow of gingival fluid.

The results of all these studies indicate that the flow of gingival fluid with inflammation of the tissues depends upon the number and permeability of the dento-gingival blood vessels. The area of inflammation in effect relates to the number of blood vessels involved as well as to the area of permeable junctional epithelium. Whereas the severity of inflammation, assessed clinically, relates to the degree of permeability of the blood vessels. The conclusion can therefore be drawn that with inflammation the nature of the gingival fluid is essentially that of an inflammatory exudate.

However, despite this, the use of a quantitative measure of gingival fluid flow as an index of the degree of inflammation may not be valid, as any quantitative measure only measures the contents of the fluid and not the fluid itself.
Furthermore, Wilson and McHugh\textsuperscript{212} found that a gingival fluid index did not correlate to the gingival index (Löe and Silness) for the whole mouth; however, for an individual tooth there was a highly statistical significant correlation between the two. They came to the conclusion that because of the great number of variables involved in measuring gingival fluid and because it did not give a measure of gingivitis in the mouth as a whole, the use of gingival fluid as an epidemiological index is not justified, despite its freedom from examiner bias.\textsuperscript{212}

As well as the variables inherent to the technique used to measure gingival fluid, Holm-Pedersen and Löe\textsuperscript{76} found that inflammation in the mesial and distal papilla with the labial gingiva free of inflammation increased the gingival fluid measurement of the labial tissue. Therefore, any use of a quantitative measure of gingival fluid as an index of inflammation is further questioned.

In summation, these results corroborate the theory that the flow of gingival fluid reflects the permeability of the dento-gingival blood vessels and the permeability of the junctional epithelium, and that with inflammation of the tissues, the nature of gingival fluid is essentially that of an inflammatory exudate.

However, any attempt to use a quantitative measure of gingival fluid as an index of inflammation is not justified.
CHAPTER 7

FACTORS AFFECTING THE FLOW OF
GINGIVAL FLUID

7.1 Introduction

The theory has been put forward by the author that the flow of gingival fluid reflects the permeability of the junctional epithelium and the dento-gingival blood vessels. In the healthy state gingival fluid is essentially interstitial fluid which, because of the nature of the epithelial attachment, can escape into the gingival sulcus. With inflammation of the tissues the nature of gingival fluid changes to that of an inflammatory exudate.

Based on this theory anything that affects the permeability of the junctional epithelium or the dento-gingival blood vessels should also have an effect on the flow of gingival fluid and so, in turn, have an effect on the measurement of the gingival fluid.

7.2 The effect of inflammation

As already discussed (section 6.2), the flow of gingival fluid increases with increasing severity\(^ {120}\) and extent\(^ {157}\) of gingival inflammation. This together with the changes in composition of the gingival fluid with increasing inflammation\(^ {89, 90}\) has led to the formulation of the above theory.

An understanding of the possible mechanisms of inflammation of the gingiva in relation to gingival fluid flow would be of benefit in the understanding of the nature of gingival fluid.
Inflammation of the gingival tissue is caused by the build-up of plaque. Broadly speaking, there are two mechanisms by which the plaque, or rather the micro-organisms in it, can initiate an inflammatory reaction. These mechanisms may be classified as non-immune and immune. However, both these mechanisms are complex and interrelated and it is impossible to separate the effects, due to the virulence of the organism (non-immune) from those due to the antigenic nature (immune) of the bacteria and/or their products.

The non-immune systems chiefly consist of the enzymes produced by the bacteria. These enzymes and metabolic end products of the bacteria lead to an increase in the permeability of the junctional epithelium and can gain entry into the tissues. Once the permeability of the junctional epithelium has been increased, antigenic material from the bacteria can gain entry to the tissues. This antigenic material can then elicit an antibody response with the subsequent formation of inflammation.

That there can be an increase in the flow of gingival fluid without alteration of the inflammatory reaction has been shown by Awwa. An increase in the flow of gingival fluid was caused by the application of hyaluronidase to the tissue. This enzyme occurs in plaque and can break down the intercellular cementing substance, thus causing an increase in the epithelial permeability.
These enzymes produced by the bacteria in plaque can also lead to an increased capillary permeability by affecting the ground substance of the connective tissue and the basement membrane of the capillaries. Therefore an increase in the flow of gingival fluid would be elicited.

Another non-inflammatory mechanism leading to an increased vascular permeability is the cleavage of the fifth factor of complement C5 to produce a molecule of molecular weight of 15,000 (termed C5a) by proteinases which occur in plaque. This C5a has the important biological property of anaphylatoxin, that is, it increases vascular permeability and is chemotactic for mononuclear leukocytes. Therefore the increased vascular permeability initiated by the bacterial enzymes that destroy the intercellular matrix is further enhanced by the action of C5a. Plasma cells are also attracted to the area by C5a and it is these cells that can produce antibodies to an antigenic stimulus.

Complement is a series of nine factors occurring in serum and together with immunoglobulins IgG, IgM and IgA has been shown to occur in gingival fluid.

The immune mechanisms of inflammation require an antigen-antibody reaction and may or may not involve the fixation of complement. These immune mechanisms would follow the initial widening of the intercellular spaces of the junctional epithelium by the enzymes and metabolic end-products produced by the bacteria of plaque.
PLÁQUE

(i) LOW M. W. METABOLITES
(ii) ENZYMES WHICH DESTROY INTERCELLULAR MATRIX

PROTEINASE → INCREASE IN EPITHELIAL PERMEABILITY → INCREASE IN VASCULAR PERMEABILITY → INCREASE IN GINGIVAL FLUID CONTAINING IgG + IgM AND COMPLEMENT

ANAPHYLATOXIN

C5a → CHEMOTACTIC PMN's + MN's

ENDOTOXIN (antigen) → ACTIVATION OF REGIONAL LYMPH NODES

PLASMA CELLS → ANTIBODIES

fig. 7.1 Formation of an antigen-antibody complex in the gingival tissues following the action of plaque. From Mergenhagen et al. 134
Mergenhagen \textsuperscript{134} and Snyderman \textsuperscript{179} have shown that the endotoxin or lipopolysaccharide occurring in the cell wall of gram negative bacteria can act as an antigen. This endotoxin diffuses away from the cell wall and through the now-widened intercellular spaces of the junctional epithelium to the underlying tissue, \textsuperscript{165} thereby eliciting an antibody response of the plasma cells and activation of the regional lymph nodes \textsuperscript{197} to produce more plasma cells. Once the antibodies are produced an antigen-antibody complex is formed. This is summarised in fig. 7.1.

The formation of this antigen-antibody complex can then lead to the fixation of complement and the formation of an inflammatory response (fig. 7.2). The important factors in the fixation of complement are the cleavage of C3 to produce C3a and of C5 to produce C5a. Both these molecules are anaphylatoxins and lead to an increase in vascular permeability and therefore an outflow of oedema fluid and subsequently the formation of gingival fluid. This increase in vascular permeability brought about by C3a and C5a is due to the liberation of histamine. Activated complement can also lead to the activation of lecithenase to form a prostaglandin-like substance which can again mediate an increase in vascular permeability. \textsuperscript{65}

This Arthus type immediate hypersensitivity reaction just described is not the only type of reaction which could lead to an inflammatory reaction in the gingiva. The anaphylactic type of
Fig. 7.2 The development of inflammation due to the fixation of complement following the formation of an antigen-antibody complex.
From Mergenhagen et al. 134
immediate hypersensitivity may also be important. This type of response depends on IgE antibody. This antibody has been found in gingival tissues and in the gingival sulcus. The amount of IgE increases with inflammation. In this type of response, reaction with an antigen leads to the degranulation of mast cells with the liberation of vasoactive amines. Complement is not thought to play a part in this type of reaction.

The vasoactive amines released (histamine and 5-HT), cause an increase in vascular permeability and therefore an increased flow of gingival fluid.

It is thought that IgE is produced locally in the tissues and that it enters the gingival sulcus via the gingival fluid. High levels of IgG, IgM and IgA have been found in the gingival tissue. With inflammation, the amount of these immune globulins in the tissue increase and IgG has been reported between the epithelial cells in the widened intercellular spaces.

Shillitoe and Lehner found high levels of IgG, IgA and IgM and complement in gingival fluid but in proportions less than that of serum. They interpreted their results as indicating complement dependent immune reactions favouring the IgG class of antibody occurring in the gingival crevice.
Lindström and Folke\textsuperscript{116} and Holmberg and Killander\textsuperscript{74} found no secretory piece IgA in gingival fluid and therefore indicated that it was of serum origin.

The increase in IgA in the saliva of patients with periodontal disease has been attributed to the increase in flow of gingival fluid with its non-secretory IgA.\textsuperscript{116}

Other immune mechanisms cited as playing a part in gingival inflammation include the cell-mediated delayed type hypersensitivity reaction.\textsuperscript{99} This reaction would lead to the release of lymphokines including skin reactive factor which increases the capillary permeability\textsuperscript{154} and would therefore lead to an increase in gingival fluid flow.

Therefore, with inflammation an increase in gingival fluid flow is brought about by an increase in vascular permeability and an increase in the permeability of the junctional epithelium.

The changes in composition of gingival fluid with inflammation reflect not only the results of the inflammatory process, but also the causes and the mechanism of the inflammatory reaction.

Endotoxin has been cited as a possible aetiological factor leading to immune mechanisms of inflammation.\textsuperscript{134, 179}
A statistically significant correlation between the endotoxin content of gingival fluid and the severity of inflammation has been shown.\textsuperscript{167} This correlation between endotoxin content of gingival fluid and inflammation has been shown to occur at the 1\% level of confidence for the degree of clinical inflammation,\textsuperscript{174} and at the 5\% level of confidence for histological inflammation.\textsuperscript{175}

There is also a correlation between the number of anaerobic gram-negative rods and the endotoxin content of gingival fluid and the clinical degree of inflammation.\textsuperscript{176}

Changes in the connective tissue due to the inflammatory response are also seen in the gingival fluid. In inflammation there is a decrease in the pH of the tissues. Reconstituted collagen has an acid soluble fraction\textsuperscript{210} and it is possible that part of the collagen breakdown involves dissolution of this fraction.\textsuperscript{210} Further collagen breakdown may be due to collagenase activity of \textit{Bacteroides melaninogenicus}\textsuperscript{196} and the release of lysosomal collagenases (cathapsin D)\textsuperscript{81} by breakdown of neutrophilic leukocytes. This breakdown of collagen is manifested by an increase in the hyproxproline containing components in gingival fluid with inflammation.\textsuperscript{146}
Fig. 7.3 The increase in the flow of gingival fluid following the release of vasoactive enzymes. From Schultz-Haudt and Solna. 163
With inflammation there is also an increase in the enzyme content of gingival fluid. The major proportion of most of these enzymes is due to breakdown of leukocytes and bacteria within the gingival sulcus.\textsuperscript{10, 58, 81, 189} The role of these enzymes in periodontal destruction has been emphasised as it appears that these enzymes destroy the connective tissue of the periodontium.\textsuperscript{81, 148, 213}

If the connective tissue is destroyed, the integrity of the basement membrane of the capillaries is disrupted and an increase in gingival fluid flow would occur (fig. 7.3).

Another factor in leading to increased capillary permeability in inflammation may be the destruction of the vasoconstrictors - adrenaline and noradrenaline. Activation of at least two enzyme systems causing accelerated breakdown of these amines takes place during inflammation.\textsuperscript{163}

Therefore, by the action of plaque and the mechanisms of inflammation both immune and non-immune, as well as the results of inflammation, the integrity of the junctional epithelium and the dento-gingival blood vessels is disrupted and an increased flow of gingival fluid results.
It is clearly seen, therefore, that with inflammation of the tissues, the nature of the gingival fluid is that of an inflammatory exudate and its flow is related to the permeability of the junctional epithelium and the dento-gingival blood vessels. This is in agreement with the theory already put forward by the author.

7.3 Influence of sex hormones

It is known that inflammation of the gingiva is exaggerated during pregnancy.\textsuperscript{38, 39, 122, 128, 171, 172, 217} The fundamental mechanism of this inflammation is unchanged from that occurring in non-pregnant individuals. The initiating and maintaining factor being the collection of plaque.\textsuperscript{128, 171, 172} However, the response in the tissue to the plaque is enhanced by the pregnancy. This increased response could be due to an altered metabolism of the tissues during pregnancy, a hypervascularisation of the tissues,\textsuperscript{114} or to connective tissue\textsuperscript{60} or epithelial changes\textsuperscript{216} during pregnancy.

It has also been reported that the gingival changes similar to those that may occur in pregnancy may also occur in females taking an oral contraceptive.\textsuperscript{105, 142}

Similarly, gingival changes occurring during menstruation have also been reported.\textsuperscript{97} These changes include an increase in severity of a pre-existing gingivitis during menstruation, a greater
tendency to bleeding from the gingival tissues which is thought to be due to an increased vascularity and an alteration in capillary fragility.\footnote{97} The overall response is thought to be similar in nature to that occurring in pregnancy.\footnote{97}

Prout and Happs\footnote{151} also reported a change in the oral bacteria throughout the menstrual cycle with a subsequent rise in salivary hyaluronidase during menstruation. They thought that this was due to changes in the gingival tissue creating favourable conditions for the growth of bacteria, capable of producing hyaluronidase.

Sex hormones therefore do have an effect on the gingival tissues. This effect should theoretically alter the flow of gingival fluid. If the severity of inflammation is increased, then the flow of gingival fluid should also be increased.

Lindhe et al.\footnote{103,104} studied the influence of sex hormones on the gingival fluid flow of female dogs with and without gingivitis. They demonstrated that, using the intracrevicular method of Brill,\footnote{29} an increased flow of gingival fluid could be detected following intra-muscular injection of 1 mg. of oestrogen and 25 mg. of progesterone in the gingivitis-free group.\footnote{103} However, at the end of hormonal administration, the flow of gingival fluid returned to pre-experimental
values. $^{103}$ This increase in the flow of gingival fluid could not be detected using the extracrevicular method$^{120}$ of placing the strip across the entrance of the gingival sulcus. $^{103}$ This extracrevicular method does not cause any irritation to the dento-gingival blood vessels.

In dogs with a chronic gingivitis$^{104}$ administration of the hormones resulted in a marked increase in the flow of gingival fluid. Termination of the hormones led to a marked and rapid decrease in the flow of gingival fluid as measured by the intracrevicular method of Brill. $^{29}$

These experiments indicate that the permeability of the dento-gingival blood vessels is increased so that with irritation (e.g. paper strip) the flow of fluid is markedly increased. However, without irritation, the fluid flow cannot be detected (e.g. with the extracrevicular method).

The increased permeability of the dento-gingival blood vessels by sex hormones has been further demonstrated by Lindhe et al$^{115}$ in rats using intravenously injected trypan blue. Furthermore, if chronically inflamed gingiva is considered as resembling epithelialised granulation tissue, $^{80}$ then the results of Lindhe and his associates $^{107, 109, 110}$ must be considered. These investigations showed that it is possible to induce hypervascularisation and vascular proliferation of immature granulation tissue in the rabbit's ear by
increasing the level of female sex hormones. \(^{107, 109, 110}\) By increasing the level of oestrogen alone \(^{110}\) there was no effect on vascular proliferation. These results were in part corroborated by Nyman \(^{142}\) who found that progesterone caused an increased vascularity and enhanced exudation whereas progesterone combined with oestrogen led to a decreased vascularity and reduced exudation. However, he concluded his experiments by noting that the administration of progesterone or progesterone combined with oestrogen, had a profound influence on the microvascular system of healing granulation tissue.

The flow of gingival fluid during healing has been shown to be influenced by the administration of sex hormones. \(^{79, 80, 114}\) In these experiments, Hugoson and Lindhe \(^{79, 80, 114}\) found that after the first four days, the flow of fluid decreased in the controls and those treated with oestrogen alone. However, there was no such decrease with the progesterone or progesterone/oestrogen treated animals. This was taken to indicate that progesterone rendered the dento-gingival blood vessels more susceptible to irritation, thus leading to an increase in the flow of gingival fluid.

Histologically, no difference was found between the epithelium of the treated and control dogs, \(^{79}\) indicating that oestrogen had no effect on the gingival epithelium. This is contrary to the results
of Ziskin et al., Ziskin and Nesse and Maier and Orban, who found that in pregnancy the epithelium is characterized by loss of keratin, proliferation of the basal cells and increased glycogen content. These changes are probably not specific to pregnancy as they can be seen in non-pregnant patients with a chronic marginal gingivitis.

There was no difference in the fibroblastic activity between the treated and control animals. However, in the progesterone treated animals, an increase in the number of neutrophils in the gingival sulcus was seen. This is in agreement with Attström and Egelberg who found an increasing gingival fluid flow with increasing numbers of neutrophils in the gingival sulcus.

Connective tissue changes during pregnancy have been demonstrated by Gans et al., who found a change in the density of colloidal charge which was due to disruption of the glycoprotein complexes of the ground substance, thus altering its permeability.

The flow of gingival fluid during pregnancy in humans has been studied by Holm-Pedersen and Løe and Hugoson.
Using the modified intracrevicular method, Holm-Pedersen and Løe found no statistically significant difference in the flow of gingival fluid from clinically healthy gingiva during pregnancy and post partum. This was taken to indicate that despite the increased water content of the connective tissue during pregnancy, there is no leakage through the epithelium and into the gingival sulcus. Hugoson corroborated these results for healthy tissue and also found that with inflamed tissue the fluid flow reached a maximum during the last trimester and fell to a minimum 20 weeks post partum. This rise and fall of the fluid flow was also associated with a rise and fall of the gingival index, thus indicating the relationship between the severity of inflammation and the flow of gingival fluid.

Hugoson supports the hypothesis that the aggravation of gingivitis, with subsequent rise in gingival fluid flow, during pregnancy is caused mainly by a high level of progesterone and its effect on the permeability of the dento-gingival blood vessels.

The flow of gingival fluid throughout the menstrual cycle has been studied by Holm-Pedersen and Løe. These workers found that for clinically healthy tissue there was no alteration of gingival fluid flow with menstruation. They also found that where there was a pre-existing gingivitis this was increased with menstruation with a subsequent rise in gingival fluid flow.
M-M = Menstrual phase.
0 = Ovulatory event.

Fig. 7.4 The flow of gingival fluid related to the menstrual cycle.
From Lindhe and Attstrom. 102
These results were obtained using the modified method of Löe and Holm-Pedersen\textsuperscript{120} where no irritation is applied to the gingival sulcus. Lindhe and Attström\textsuperscript{102} using the intracrevicular method of Brill\textsuperscript{29} did detect variations in gingival flow over the menstrual cycle. This variation consisted of minimum flow of fluid at the time of menstruation, with a maximum flow of fluid at the time of ovulation.(fig. 7.4).

If this flow of gingival fluid is related to the hormonal changes in a menstrual cycle, we find that at the time of ovulation, oestrogen production is at its highest, with progesterone reaching a peak 4-6 days after ovulation. There is then a gradual reduction of hormones, reaching a minimum at the time of menstruation. The flow of fluid closely follows this pattern, with the least fluid flow at periods of minimum hormone production.

These results disagree with those of Holm-Pedersen and Löe\textsuperscript{76} but fit in with the observed effects of sex hormones on the tissues.\textsuperscript{142}

In order to explain the difference, the work of Egelberg\textsuperscript{49} demonstrated that the increased permeability of the dento-gingival blood vessels only exhibit a flow of gingival fluid when filter paper strips are inserted into the gingival sulcus. Therefore, the method
used by Holm-Pedersen and Löe\textsuperscript{76} would not traumatize the blood vessels and therefore would not elicit a flow of gingival fluid. The intracrevicular method used by Lindhe and Attström\textsuperscript{102} would lead to a flow of fluid due to trauma to the blood vessels, the permeability of which has been increased by the hormone levels. Thus, the study of Holm-Pedersen and Löe\textsuperscript{76} does tell us that there is not an increased flow of fluid into the gingival sulcus at any stage during a menstrual cycle, but Lindhe and Attström show that at the time of ovulation there is an alteration in the dento-gingival blood vessels which makes them more susceptible to trauma.

Lindhe and Björn\textsuperscript{106} have shown that the regular use of hormonal contraceptives leads to increasing amounts of gingival fluid obtainable by the intracrevicular method of sampling. These results were confirmed by Lindhe et al\textsuperscript{105} who also showed that the gingival fluid flow during menstruation was statistically higher when the cycle was under the influence of a contraceptive. This indicates that the contraceptive passes its greatest effect onto the flow of gingival fluid when the production of ovarian progesterone is low.

It therefore seems that the influence of sex hormones on the flow of gingival fluid is due primarily to the effect of progesterone on the dento-gingival blood vessels. The blood vessels are rendered more susceptible to trauma by progesterone and therefore an
exaggerated response to bacterial toxins ensues. The flow of gingival fluid is therefore increased with this altered permeability due to the progesterone. This is in agreement with the theory put forward by the author that the flow of gingival fluid reflects the permeability of the junctional epithelium and the dento-gingival blood vessels.

7.4 The effect of histamine

The effect of histamine on the flow of gingival fluid is related directly to the increased capillary permeability caused by the histamine. Brill, using the intracrevicular method, showed a marked increase in the flow of gingival fluid following intravenous injection of small concentrations of histamine (0.1 mm.) in dogs. However, Brill also found that in large concentrations (1.0 mg.), the effect of the histamine was not appreciable. The reason for this difference in effect between small and large concentrations of histamine was not discussed by Brill.

Egelberg studied the effect of intravenously injected histamine on the permeability of the dento-gingival blood vessels in clinically healthy and chronically inflamed gingiva in dogs. He showed that the administration of 0.01 mg./kg. body weight led to an increase in vascular permeability of chronically inflamed tissue but had no effect on the clinically healthy. This was shown by the vascular labelling method with carbon particles and by the flow of gingival fluid as measured by the modified intracrevicular method of Löe and Holm-Pedersen.
Coming back to Brill's study, we find that he used two dogs for a series of experiments in which he stimulated gingival fluid flow by mechanical means, allowed inflammation to develop in one dog, and also studied the effect of intravenously injected histamine. It is interesting to note that the large flow of gingival fluid following systemic administration of histamine occurred in the dog which had been allowed to develop gingivitis and the dog on which the large concentrations of histamine had no effect had never been allowed to develop gingivitis. Although Brill states that in the histamine experiments only clinically healthy tissue was used, the extent of sub-clinical inflammation was not assessed.

Löe and Holm-Pedersen showed that a flow of gingival fluid occurred prior to the development of gingivitis and continued for some time after the disappearance of clinical gingivitis.

Taking into account the observations of Egelberg and Löe and Holm-Pedersen, the difference noted by Brill might have been due to the fact that in the dog where a large flow of fluid occurred, the blood vessels still had an increased permeability due to an inflammatory state which was not now present clinically. The vessels would thus be susceptible to trauma and the effect of histamine would be to cause an outflow of fluid, similar to that noted by Egelberg.
fig. 7.5 The rise and fall of the body temperature with varying periods of the day. The shaded areas are times when the patient is sleeping.
From Poppel.¹⁵⁰
for chronically inflamed gingiva, whereas in the dog that had never had gingivitis the blood vessels would be in an intact state and even the large concentration of histamine could not alter their permeability. This is in agreement with Egelberg's\(^\text{41}\) observation for clinically healthy gingiva.

The effect of topically applied histamine was shown by Egelberg.\(^\text{51}\) Here there was a marked and immediate flow of gingival fluid which rapidly decreased in the following 15 minutes and could not be detected 60-90 minutes after application. The extent of vascular labelling followed the same pattern as the flow of gingival fluid.

The effect following the application of histamine is to be expected if it is considered that the flow of gingival fluid is a reflection of the permeability of the dento-gingival blood vessels.

7.5 The influence of circadian rhythms

Circadian rhythms are the variations in bodily functions according to the time of day. Body temperature follows a rise and fall pattern reaching a minimum when the person is asleep (fig. 7.5).

Physiological functions also follow this pattern, e.g. urinary excretion of potassium, 17-OHCS (corticosteroids) and catecholamines. However, the maximum and minimum of these functions do not necessarily coincide, e.g. the body temperature peaks at the end
The flow of gingival fluid and the variation in oral temperature according to the time of day.
From Bissada et al. 14
of activity; however, the excretion of 17-OHCS (corticosteroids) occurs early in the morning. The peak for potassium excretion is a little later with the catecholamines following another pattern. Thus the body is in varying states of physiological equilibrium throughout the 24 hours.\textsuperscript{141,150} This circadian periodicity has also been observed in the secretion of calcium and phosphate in human parotid and sub-mandibular saliva.\textsuperscript{56}

Circadian rhythms have also been noted in the flow rate\textsuperscript{14} and composition\textsuperscript{90} of gingival fluid. Bissada et al.\textsuperscript{14} studied the effect of circadian rhythms on the flow of gingival fluid from clinically healthy tissue using sodium fluorescein and the intracrevicular method of Brill.\textsuperscript{29} These studies showed that a circadian rhythm and the flow of gingival fluid did exist with the highest flow rate occurring at about 2200 hours. They also showed that this peak in flow rate of gingival fluid followed the peak in body temperature by about 4 hours. The minimum flow of gingival fluid occurred at 0600 hours\textsuperscript{14} (fig. 7.6).

This circadian rhythm of gingival fluid flow may represent variations in vascular permeability and/or variations in epithelial function or permeability.
Bissada et al.\textsuperscript{14} point out that circadian rhythms represent different physiological states and is not a time of day phenomena. These physiological states will vary with different bodily functions but will change only gradually, thus affecting the state of well being.

Bissada et al.\textsuperscript{14} also point out that the circadian rhythm pattern of gingival fluid flow should be taken into account in studies involving quantitative measurements. It is not necessarily true that everybody has the same physiological patterns and therefore the same circadian rhythms. Therefore, in comparing gingival fluid of two individuals, differences in circadian rhythms may account for differences in the flow of fluid. There is great variation between individuals.\textsuperscript{14}

Kaslick et al.\textsuperscript{90} found variations in the cation concentrations of gingival fluid according to the time of collection. The $[\text{Na}^+]$ was found to be lower at noon (12 - 2 p.m.) than in the morning (8 - 10 a.m.) while $[\text{K}^+]$ was found to be higher at noon than in the morning. The differences in $[\text{K}^+]$ due to circadian rhythms is much larger than differences in $[\text{Na}^+]$.\textsuperscript{90}

In comparing fluid from moderately inflamed gingiva to that from nearly normal gingiva, the sodium ion concentration was statistically significantly higher in the moderately inflamed fluid for samples taken at noon (12 - 2 p.m.) and morning (8 - 10 a.m.). However the $[\text{K}^+]$ does not vary according to the degree of inflammation but is more dependent upon the time of day.\textsuperscript{90}
But when comparing $[\text{Na}^+]$ from moderately inflamed fluid taken at noon to $[\text{Na}^+]$ from nearly normal fluid taken in the morning, there is statistically significant differences. Therefore, for quantitative analysis of gingival composition, fluid must be standardised to the circadian rhythm.  

Therefore, it appears as if circadian rhythms are the major factor affecting $[\text{K}^+]$ and $\text{Na}^+/[\text{K}^+]$ ratios but has only a minor influence on $[\text{Na}^+]$.  

That the flow rate and composition of gingival fluid is influenced by circadian rhythms illustrates that gingival fluid is subject to the same physiological variations as other body fluids. This is in agreement with the concept put forward by the author that gingival fluid is essentially the fluid medium of the gingiva which because of the nature of the epithelial attachment can escape into the gingival sulcus.

7.6 The effect of age

Sandalli and Wade\textsuperscript{159} used the extracrevicular method of Brill\textsuperscript{29} in their study on gingival fluid flow in children 3 - 5 years old. In this study it was found that the children with clinically healthy gingiva had a small but definite flow of gingival fluid and that this flow increased when gingivitis was present. In the presence of gingivitis, a statistical correlation existed between the depth of the gingival sulcus and the flow of gingival fluid. This correlation did not exist for clinically healthy tissue. It must be emphasised, however, that the intracrevicular
fig. 7.7 The difference in gingival fluid flow between different age groups and the severity of inflammation.

From Björn et al.¹⁵
method as used in this study causes irritation to tissue and therefore
elicits a flow of gingival fluid. The same factors that affect the flow
of fluid in the adult dentition probably affect the flow of fluid in the
fully erupted deciduous dentition.

Granath found that in a total survey of 164 gingival areas
in children, there was a statistically significant correlation between
gingival fluid flow and the state of inflammation. However, this
correlation did not exist for individual cases. The reason for this
discrepancy was thought to lie in the difficulty of assessing gingivitis
in the primary dentition. In the primary dentition, some areas are
normally thickened and difficulty was found in assessing the amount
of swelling due to inflammation.

It was concluded that gingival fluid measurements were not
a reliable indication of gingival health in the primary dentition.

Björn et al. found a highly statistically significant difference
in the fluid flow between an 11 - 12 age group and a 25 - 36 years age
group. In both these groups there was a statistically significant
correlation between the gingival fluid flow (as measured by the intra-
crevicular method) and the state of inflammation (gingival index).
The difference between the age groups was less marked when a score
of 3 was recorded, indicating severe inflammation (fig. 7.7).
This difference between the ages was thought to be due to different sulcus depths and degrees of inflammatory change. This explanation is rather flimsy, as in the younger age group the depth of the gingival sulcus would be deeper than in the older age group. This is due to an 'eruption cuff' still being present around the teeth. What Björn et al. could have indicated was whether or not the filter paper strip went deeper into the sulcus in the older group. The tightness of fit of the gingiva around the tooth may limit the introduction of a filter paper strip. In the younger group, even though this 'eruption cuff' may be present, the tightness of it may only allow a shallow penetration of the filter paper strip compared to the older group. This could then explain the differences observed.

7.7 The effect of mechanical stimulation

Brill found that mechanical stimulation of healthy gingival sulci in dogs led to an increased flow of gingival fluid. The mechanical stimulation of the gingiva was due to the brushing of the teeth and gingiva using the Stillman method of toothbrushing, while the flow of fluid was measured by the intracrevicular method, using protein-bound Evans blue as the indicator. The effect of different toothbrushing techniques on the flow of gingival fluid was studied by Brill and Krasse who found that the Stillman method produced a larger flow of fluid than when the Charters method or a modified roll method was used. However, all three methods produced an increased flow of fluid.
As already stated, the flow of gingival fluid into the gingival sulcus depends upon the permeability of the junctional epithelium as well as the capillary permeability. It is therefore obvious that the Stillman method of toothbrushing, where the bristles are pointing apically and are even allowed to enter the gingival sulcus, would disrupt the junctional epithelium more so than either the Charters or modified roll method. This would then lead to a greater flow of gingival fluid. In Brill's first experiment, he used protein-bound Evans blue and thought that because this was recovered in greater quantities following the Stillman method, that this reflected an increase in capillary permeability. This increase in capillary permeability does occur. However, Brill's experiment does not show this. If the Evans blue was measured in the tissues before and after stimulation, the effect on capillary permeability could then be assessed. However, by measuring the Evans blue outside the tissues, the integrity of the junctional epithelium is also added to the measurement. Brill could therefore only say that more fluid is escaping through the junctional epithelium following stimulation and not that more is released from the capillaries.

Brill and Krasse and Brill studied the effect of chewing on the flow of gingival fluid. These results indicate that in dogs the chewing of a rubber bone increased the flow of fluid and in humans the chewing of a large volume (20 cc.) of paraffin wax for ten minutes increased the flow of fluid. The significance of these results is hard
to understand. Brill\textsuperscript{27} says that therefore vigorous chewing will increase the flow of fluid. However, one must consider how closely the holding of 20 cc. of paraffin on one side of the mouth and chewing for ten minutes resembles normal chewing. Brill\textsuperscript{27} states that smaller pieces of wax did not give the desired results in a pilot study and therefore small pieces were not used. Certainly, a large volume of wax would contact the gingival tissue and the muscular movements during chewing would lead to rubbing of the wax against the gingiva. This probably does not happen with smaller pieces of wax, nor does it probably happen with normal chewing. To what extent the healthy gingiva is stimulated by the contact of food during normal chewing is unknown; however, the results of Brill\textsuperscript{27} do not give any indication of gingival stimulation during normal chewing.

Brill and Krasse\textsuperscript{33} also found that internal and external 'massage' of the gingiva using the convex side of the blade of a straight elevator lead to an increase in the flow of gingival fluid. The internal stimulation was due to gently inserting the elevator into the gingival sulcus. The external stimulation was due to gentle rubbing with the convex side of the blade of the elevator on the external surface of the gingiva. The internal stimulation led to a greater increase in the flow of gingival fluid than the external methods. However, in both methods used, the flow rate returned to normal after ten minutes.
fig. 7.8 The flow of gingival fluid following drying of the gingiva with compressed air.

From Egelberg. 53
The effect of massage was further studied by Egelberg\textsuperscript{51} who found that the flow of fluid was initially increased and that it decreased slowly over a period and was still able to be recovered in small amounts after 60 - 90 minutes. This tendency was also noted using vascular labelling with colloidal carbon. Distinct labelling was still present after 60 - 90 minutes.

Egelberg\textsuperscript{51} used a ball burnisher to apply external stimulation and a dental explorer to scrape in the gingival sulcus. These experiments indicated that mechanical stimulation altered the permeability of the dento-gingival blood vessels and that this alteration followed a different pattern to that obtained following the topical application of histamine. The effect of antihistamines inhibited the response to histamine by 78\% whereas the response to mechanical stimulation was only inhibited by 7\%.\textsuperscript{51} This suggests a different mechanism or mediator of the capillary response following mechanical stimulation.

In chronically inflamed tissue, the effect of drying the tissues with compressed air leads to an initial and marked flow of gingival fluid. This decreases over a period of time and only a minute flow can be detected after 30 minutes using the method of L\öe and Holm-Pedersen\textsuperscript{53} (fig. 7, 8).
A similar response after drying was observed using vascular labelling.  

In clinically healthy tissue, no flow of fluid can be obtained. This difference could be due to difference in tonus between healthy and inflamed tissue, as well as differences in susceptibility of the vessels to irritation from the compressed air.  

The introduction of paper strips into the gingival sulcus has been shown by Egelberg to cause distinct changes in the permeability of the blood vessels and thus leads to an increased amount of gingival fluid recovered by the intracrevicular method.  

Thus the effect of mechanical stimulation on the flow of gingival fluid should be considered in light of these findings. That is, by drying the tissue and inserting a paper strip into the sulcus, a rapid and marked increase in gingival fluid can be obtained. However, in clinically healthy tissue and using the modified method of Løe and Holm-Pedersen, these variables can be, to a large extent, eliminated. In fact, Løe and Holm-Pedersen found that in patients with clinically healthy tissue, the chewing of 8 gms. of paraffin wax did not lead to an increase in the flow of gingival fluid as measured by their method. These findings corroborate those of Egelberg who could only recover fluid following irritation to the tissue in both clinically healthy and chronically inflamed tissue.
Continuous orthodontic forces capable of producing tooth mobility have been shown by Bowles and Mühlemann\textsuperscript{20} not to lead to an increase in gingival fluid. No fluid could be collected using the method of Löe and Holm-Pedersen,\textsuperscript{120} and there was no increase in the amount of fluid collected by the intracrevicular method of Brill.\textsuperscript{29} It therefore appears that traumatic occlusion, sufficient to cause tooth mobility, does not affect the dento-gingival blood vessels nor the junctional epithelium and therefore does not affect the flow of gingival fluid.\textsuperscript{20}
The gingival fluid as the fluid medium of the gingiva and gingival sulcus. Its flow reflects the permeability of dento-gingival blood vessels and the junctional epithelium.

CT = Connective tissue.

Fluid medium of gingiva.

Fluid medium of gingival sulcus.

JE = Junctional epithelium.

Gingival Sulcus.

Oral environment.
CHAPTER 8

THE ROLE OF GINGIVAL FLUID

8.1 The fluid medium of the gingiva and gingival sulcus

In any discussion on the nature of gingival fluid a consideration of the role that this fluid plays in the biology of the gingival sulcus is essential.

The theory so far put forward by the author is that gingival fluid whether in health or disease is the fluid medium of the gingiva which, because of the nature of the epithelial attachment, can escape into the gingival sulcus. In this respect gingival fluid should also be considered as the fluid medium of the gingival sulcus. Therefore we now have a fluid system which is separated by a semi-permeable membrane - the junctional epithelium. The amount and composition of this fluid system depends upon the permeability of the dento-gingival blood vessels which is the original source of the fluid and the passage from one compartment to the other depends upon the permeability of the junctional epithelium. This compartmentalisation of the gingival fluid system is seen in fig. 8.1.

From this compartmentalised concept of gingival fluid the role of this fluid in the environment of the gingival sulcus can be predicted. The most important prediction essential to the concept is the presence of fluid in the gingival sulcus in the non-inflamed state.
In this system the overall movement of fluid is from the dento-gingival blood vessels through the junctional epithelium into the gingival sulcus and then into the oral environment. However, a two-way passage of ions and molecules via concentration and osmotic pressure gradients allows for the passage of ions and molecules from the gingival sulcus through the semi-permeable junctional epithelium and ultimately into the blood vessels and lymphatics of the gingiva.

This system therefore leads to an ultimate shedding of the desquamated junctional epithelial cells into the oral environment. Similarly, foreign particles, e.g. carbon particles or silver alloy particles, as well as bacteria experimentally inserted into the gingival sulcus would be expected to be "washed out" of the sulcus and into the oral environment. This is in effect what happens; Waerhaug showed that Indian ink particles inserted into the gingival sulcus were completely removed, whereas Brill used charcoal particles and Harvey silver alloy particles and obtained similar results. Brill also inserted bacteria into the gingival sulcus and found that these were also "washed out". Brill also found that the best results were obtained when the flow of gingival fluid was stimulated by mechanical means. This is also to be expected from the above theory.
That the passage of biological molecules into the oral environment occurs via the gingival fluid is in effect demonstrated by the presence of non-secretory IgA in saliva. IgA is the immunoglobulin of external secretions, and has a "secretory piece" attached during secretion. In plasma it exists in its non-secretory form, i.e. without the attached secretory piece. Lindström and Folke\textsuperscript{116} and Holmberg and Killander\textsuperscript{74} found no secretory piece IgA in gingival fluid and therefore indicated that the IgA present was of serumal origin. The increase of IgA in the saliva of patients with periodontal disease has been attributed, by Lindström and Folke,\textsuperscript{116} to the increase in flow of gingival fluid with its non-secretory IgA.

On the other hand, the passage of biological molecules from the gingival sulcus and into the gingival tissues is seen by the passage of endotoxin\textsuperscript{165} and horse radish peroxidase (HRPO)\textsuperscript{127} into the gingival tissues. The pathway of this penetration is through the junctional epithelium.\textsuperscript{127} The endotoxin can then lead to activation of the regional lymph nodes to produce plasma cells by being absorbed via the lymphatics of the gingiva.\textsuperscript{13, 197}

This concept does not allow for any diluting effect\textsuperscript{22} by the gingival fluid on bacterial products or tissue damaging substances present in the gingival sulcus. The passage of these substances depends upon the concentration gradient between the gingival sulcus and the connective tissue and the permeability of the junctional
fig. 8.2  Lack of gingival sulcus in absence of gingival fluid.

fig. 8.3  Maintenance of gingival sulcus by the presence of gingival fluid.
epithelium. In fact, the content of endotoxin in gingival fluid has been shown to correlate to the degree of clinical\textsuperscript{174} and histological\textsuperscript{175} inflammation. This is to be expected if endotoxin is important in the aetiology of periodontal disease. The resulting increase in the amount of gingival fluid due to the ensuing inflammation would in fact be due to the endotoxin and not a defensive mechanism against it.

The presence of a small amount of fluid in the healthy gingival sulcus would be necessary for the presence of the sulcus. If no fluid was present the healthy gingiva would present with zero sulcus depth, the tissue coming away from the tooth at the apex of the junctional epithelium (fig. 8.2). The presence of a fluid in the sulcus would ensure a force of attraction between the sulcular epithelium and the tooth surface and thus maintain a positive sulcus depth. This attraction would be due to hydrogen bonding and Van der Waal's forces balanced by the electrostatic repulsion between the tooth surface and the epithelium. As the film of fluid becomes thicker the forces of attraction would become weaker and the tissue would come away from the tooth (fig. 8.3). However, a small but definite sulcus would be present. This should not be confused with the attachment of the junctional epithelium to the tooth surface. This type of attraction just described is similar to that proposed by Schultz-Haudt et al\textsuperscript{164} and would allow for the desquamation of the epithelial cells. Schultz-Haudt et al\textsuperscript{164} proposed a similar mechanism
for the attachment of junctional epithelium. However, since the presence of hemidesmosomes and a basement lamina have been shown to exist in the attachment of junctional epithelium, this balance between attraction and repulsion is not likely to exist. However, it may be important between the sulcular epithelium and the tooth surface, in maintaining a positive sulcus depth.

The presence of proteins and fibrin in the gingival fluid would form a layer on the tooth surface which would enhance the stickiness of the tooth surface.

Therefore the physical properties of gingival fluid - flushing and sticking epithelium to tooth, can be explained and even predicted by the concept put forward by the author.

However, the largest flow of gingival fluid exists with inflammation and its role as an inflammatory exudate should also therefore be considered.

The presence of bacteria in the healthy gingival sulcus has been shown by Gavin and Collins. However, variations in this bacterial content have been shown to occur in various parts of the mouth. Egelberg and Cowley showed that bacteria were found twice as frequently in the mesial, distal and palatal regions than in the buccal regions. This difference was attributed to ease of tooth-brushing and not the flow of gingival fluid.
Furthermore, Monhart et al. suggested that because of the presence of H₂S in pockets 3mm. or deeper, that the metabolism of the bacteria differed in periodontal pockets as compared to healthy gingival sulci.

Gingival fluid contains antibodies of the IgG, IgM and IgA classes and also neutrophils and lymphocytes. Other antibacterial substances also occur such as lysozyme. This enzyme breaks down a certain polysaccharide component of the bacterial cell wall with the resultant protoplasm being lysed. Cells coated with IgE antibody have also been found in the gingival sulcus.

The quantity of cell antimicrobial factors increases with the severity of inflammation and increased flow of gingival fluid. Because of the presence of these substances, one would expect that the gingival fluid had a high antimicrobial action. However, Collins and Gavin found that strips of filter paper containing gingival fluid did not inhibit the growth of oral bacteria on an agar plate. Rather, the growth of the bacteria was enhanced by the gingival fluid. This was attributed to the presence of nutritional factors in the gingival fluid, e.g. amino acids.

In order to explain this apparent contradiction, the origin of the various components of the gingival fluid and their mechanism of action should be considered.
Holmberg and Killander\textsuperscript{74} found the concentrations of IgG, IgA and IgM in gingival fluid to be so close to those in serum that serum should be considered as the likely origin. Although they did consider the local production of IgG but could not determine what fraction of the total IgG was of local origin. Brandtzaeg\textsuperscript{23} also found similar concentrations of IgG, IgM and IgA in gingival fluid and serum.

Before an immune reaction can take place an antigen must gain entry to the tissues. This antigen then stimulates specific antibody production which is initially of the IgM class and then later of the IgG class. An antigen-antibody reaction then follows.

With gingival fluid the presence of antibodies does not necessarily mean that these play a part in the defence of the gingival tissues. These circulating antibodies of serumal origin may not have been formed due to the antigen in the gingival sulcus and therefore would not react with it.

However, Brandtzaeg and Kraus\textsuperscript{25} found many cells in clinically healthy tissue containing IgG, a few containing IgA and occasionally one containing IgM. Genco and Krygier\textsuperscript{64} also found cells containing IgG, IgA and IgM in clinically healthy tissue. The number of those cells increased with inflammation,\textsuperscript{25,64} together with an increase in extracellular IgG in the connective tissue and between the epithelial cells.\textsuperscript{64} Genco and Krygier\textsuperscript{64} also found
that it was impossible to determine the proportion of cells containing IgG, IgA and IgM in inflamed tissue, due to the intense staining of extracellular IgG.

These results show that these antibodies may be produced locally in the gingival tissues and that their production is due to stimulation by antigen in the gingival tissues. This local production of antibodies is supported by Berglund et al\textsuperscript{13} who showed that after intramucosal injection of endotoxin in rabbits the regional lymph nodes showed specific antibody production to the antigen and that a heavy infiltrate of plasma cells and lymphocytes appeared at the site of injection and that antibody to the endotoxin was being produced by the cells in this local inflammatory reaction.

Thonard and Dalbow\textsuperscript{197} showed that antigen is carried by the reticuloendothelial system to lymphoid centres and then the transfer of lymphocytes and plasma cells back to the site of initial injection. The necessity of the reticuloendothelial system has been questioned by Thonard\textsuperscript{198} who found that in splenectomized and cortisone treated rats, the immune competence of the gingival tissue remains intact.
1. Recognition limb
   - Humoral Antibody
     - IgA
     - IgD
     - IgE
     - IgG
     - IgM
   - Cell mediated (sensitized lymphocytes)

2. Effector limb
   - Complement
   - Kinin
   - Clotting
   - Lymphotoxin M.I.F. etc.
   - Polymorphonuclear leukocytes
   - Activated macrophages

*fig. 8.4  Limbs of the immune system.*
*From Snyderman.*
Therefore some of the antibodies found in gingival fluid would be of local origin as a result of antigen stimulation from the bacterial products (e.g. endotoxin) of the gingival sulcus.

That these antibodies do play a part in the defence of the gingival tissues has been supported by Shillito and Lehner$^{169}$ who found that the concentration of complement (C3) in gingival fluid as well as the concentration of IgG, IgA and IgM was lower in gingival fluid than in serum. They interpreted their results as indicating a complement dependent immune reaction favouring the IgG class of antibody occurring in the gingival sulcus.

Genco and Krygier$^{64}$ found complement (C3) localised in the connective tissues and blood vessels of normal and inflamed gingiva. This was also observed at the connective tissue/epithelial boundary and between the epithelial cells of inflamed tissue.

The immune system can be divided into two compartments, the recognition limb and effector limb, the recognition limb discriminates "self" from "non-self" and is due to cell mediated and humoral antibody responses. To eliminate the "non-self" the second functional limb is activated. These are the immune effectors, which eliminate the "non-self" by mediating the inflammatory process (fig. 8.4).
Therefore, the ultimate effect of the immunological response due to the presence of specific antibodies as seen in gingival fluid would be to elicit an inflammatory process, which is in essence a defensive reaction on the part of the body.

Obviously then, the presence of immunoglobulins in gingival fluid would not be of any effect in an in vitro experiment such as performed by Collins and Gavin, since the antimicrobial effect of the immunoglobulins is via the inflammatory process.

The immunoglobulin IgE has been found in cells of inflamed gingiva. The number of cells containing IgE has been shown to increase with increasing severity of inflammation. Nisengard et al also found IgE coating filamentous bacteria in the gingival sulcus whereas bacteria in supragingival plaque did not show evidence of IgE. They suggest that locally produced IgE enters the gingival sulcus via the gingival fluid and coats in vivo specific bacteria. Localised anaphylactic type immediate hypersensitivity to the IgE coated bacteria may then occur, resulting in inflammation of the periodontal tissues. This is supported by the observations of Zachrisson and Shelton and Hall that the mast cell population in inflamed gingiva is decreased. The gingival fluid should therefore be considered as the fluid medium by which locally produced antibodies can enter the gingival sulcus and bacterial antigens can gain entry to the tissues to stimulate this antibody production.
Other factors in the gingival fluid which are antimicrobial include the cellular component of the fluid. Egelberg \textsuperscript{47} has shown that gingival fluid contains neutrophils and lymphocytes and that the numbers of these cells increase with inflammation. These cells have been shown to migrate through the widened intercellular spaces of the junctional epithelium in inflamed gingiva, \textsuperscript{61} and that the migration of these cells occurred in resting healthy and chronically inflamed tissue in the absence of vascular labelling.\textsuperscript{6, 7} The presence of these cells may be due to chemotactic substances in the plaque \textsuperscript{73, 112} or the result of complement fixation and the release of chemotactic substances.

The presence of lysozymes in the gingival fluid is thought to be due to the disintegration of these leukocytes.\textsuperscript{22, 24, 58, 81} The phagocytosis of bacteria may be enhanced by lysozyme which leads to agglutination of the bacteria, thus rendering them more susceptible.

Other enzymes of the gingival fluid include the fibrinolytic system.\textsuperscript{68} This may have a protective activity by counteracting the formation of fibrin from fibrinogen present in the gingival fluid.\textsuperscript{23} The formation of fibrin clots would lead to stagnation of gingival fluid and may protect bacteria from immune factors by forming coatings of fibrin around them.\textsuperscript{22} The fibrinolytic enzymes would prevent this from occurring.
Therefore the role of the gingival fluid in inflammation of the tissues is the one that could be predicted from the theory presented by the author. Its role is that of the fluid medium of the whole inflammatory reaction. It is the medium in which the reaction takes place containing the causative agents, the mediators and results of the reaction.

8.2 Formation of subgingival calculus

There is general agreement that plaque is a precursor to calculus formation, and that the bacteria themselves can form a stroma in which calcification can take place. However, the presence of bacteria is not a necessary condition for the formation of calculus, as calculus has been observed in germ-free animals.

The differences in colour and hardness between supra- and subgingival calculus and the differences in composition may be taken to indicate difference in the source of constituents for the two different types of calculus.

Chemically, subgingival calculus has a higher potassium, magnesium and sodium concentration than does supragingival calculus. These high levels of inorganic ions suggest that the
subgingival calculus is derived from the gingival fluid. Gingival fluid has a higher potassium concentration than does serum or saliva and before the removal of subgingival calculus the gingival fluid has a higher sodium and calcium concentration than does normal serum. The sodium in supragingival calculus is derived from the saliva as are the other mineral elements of supragingival calculus.

Little et al. suggested that an increased sodium content of saliva could initiate calculus formation, and that Na/K ratios were important in the production of calculus. The increasing Na/K ratio of gingival fluid with increasing degrees of inflammation may be of some significance in the formation of subgingival calculus.

It appears therefore that the source of minerals in the formation of subgingival calculus is the gingival fluid.  

The presence of bacteria in the healthy gingival crevice has been demonstrated by Gavin and Collins and Egelberg and Cowley and in the deeper periodontal pocket by Monhart et al. Monhart et al. have also found increasing numbers of bacteria with increasing depth of the pocket, and because of the presence
of \( \text{H}_2\text{S} \) in the deeper pockets (\( > 3 \text{ mm.} \)) they suggested that these bacteria had a different metabolism.

Larger numbers of leukocytes and desquamated epithelial cells are found in the gingival fluid of inflamed tissue. \(^{47}\) Together with this increased desquamation rate of the epithelium, a greater quantity of intercellular cementing substance would be released. This intercellular cementing substance is a muco-polysaccharide complex. \(^{139,200}\)

Histochemical studies \(^{190}\) have indicated the presence of muco- and glyco-proteins in the gingival fluid. The reactions of these muco-polysaccharides were very similar to serum but were different to saliva. Histochemical reactions were weak or absent in saliva. \(^{190}\) Other carbohydrates have been demonstrated in gingival fluid. \(^{69,70}\)

The matrix for the formation of calculus is therefore present in the gingival crevice and is derived, except for the bacteria, from the gingival fluid.

Supra- and subgingival calculus are probably related by a common mechanism of formation rather than by a common source. \(^{36,136}\)
Various theories on the mechanism of calcification of calculus have been put forward. However, none of these mechanisms explain the whole phenomena and to greater or lesser extent, all the different mechanisms may play a part in calculus formation. These theories include:

(i) Bacterial, in which the mineralization depends upon specific metabolic activities of the bacteria. The bacteria provide a stroma for the precipitated salts and stimulate their precipitation by enzymatic activity on the salivary proteins, or in the case of subgingival calculus, on the proteins of the gingival fluid. This leads to a colloidal dispersion capable of maintaining a super saturated solution of calcium and phosphate ions.

(ii) The production of ammonia by bacterial breakdown of proteins, leading to an increase in the pH. According to the Henderson-Hasselbalch equation* this increase in pH would favour the formation of calculus.

* Henderson-Hasselbalch equation \[ \text{pH} = \text{pKa} + \log \frac{[\text{salt}]}{[\text{acid}]} \]

\[ \therefore \text{an increase in pH leads to greater concentration of salt. Which in this case is Ca}_3(\text{PO}_4)_2\text{ while the acid is HPO}_4^{2-}. \]
(iii) The loss of CO$_2$ leading to a decrease in the CO$_2$ tension (pCO$_2$), thus altering the dissociation of carbonic acid as seen in the following equation:

$$\text{H}^+ + \text{HCO}_3^- \xrightleftharpoons{\text{decrease pCO}_2} \text{H}_2\text{CO}_3 \xrightarrow{\text{increase p CO}_2} \text{H}_2\text{O} + \text{CO}_2$$

The loss of CO$_2$ shifts the equation to the right and therefore effectively removes H$^+$ ions from the solution. Thus the pH is increased and, as already seen from the Henderson-Hasselbalch equation, an increase in pH favours the deposition of calculus.

(iv) The epitactic or local factor theory of calcification.

In this theory the mucopolysaccharide molecule selectively removes calcium and phosphate ions from the solution. The end amino acid of the protein component attracts and combines a phosphate ion while the carbohydrate component combines with the calcium ion. The mucopolysaccharide therefore acts as a chelating agent forming a mucoid chelate. This then in turn serves as a nucleus for the further removal of calcium and phosphate ions and the progression of calcification.
In the formation of supragingival calculus the active bacterial concept is refuted on the basis that it depends on the stagnation of saliva and that stagnation is least likely to occur in areas of greatest salivary flow which are the areas of greatest calculus formation. The participation of this mechanism in the formation of subgingival calculus can be refuted on the same grounds. For this mechanism to be active the gingival fluid would have to stagnate. The work of Brill and Harvey has shown that gingival fluid is continuously moving out of the crevice and that the movement of fluid is greatest with factors that stimulate the flow of gingival fluid. It is generally agreed that the flow of gingival fluid is greatest with severe inflammation, and it is also agreed that the greatest amounts of subgingival calculus occur in areas of greatest inflammation.

Therefore this concept can be dismissed from playing a major role in the calcification of subgingival calculus.

The exact pH of gingival fluid is unknown, although Kleinberg and Hall found a high pH (overall average of 8.34) in crevices of average depth (0.7mm.). To explain this high pH, Golub et al cited the presence of urea in the gingival fluid which may be hydrolysed by the bacteria to form ammonia. The presence of ammonia in the gingival crevice was not demonstrated.
With increasing inflammation the nature of the gingival fluid changes to that of an inflammatory exudate. The pH of inflammatory exudates is usually considered to be acidic. However, Menkin points out that this acidic nature of inflammatory exudates takes time to develop. Initially an inflammatory exudate is an outpouring of serum with a pH similar to that of serum, the pH then falls and as neutrophils cannot survive in an acid environment, they are replaced by macrophages. At a pH below 6.5 all leukocytes die or are severely injured. In chronic inflammatory periodontal disease we have a constant outpouring of serum and this is being lost via the gingival fluid and being replaced by a further outpouring of serum, a continuum of formation, loss, formation exists. Therefore the pH of this exudate would not be expected to fall much below that of serum. Furthermore, within the gingival crevice there is a layer of neutrophils between the epithelium and the plaque thus indicating that the pH of the crevice is not significantly lowered. Menkin alos points out that with increased vascularity (as is seen in gingival inflammation) the pH of the exudate may tend to become either neutral or even slightly alkaline.
Theoretically, therefore, severe inflammation of the gingiva
does not produce an environment which would inhibit calculus
formation.

On the other hand, with increased inflammation, we have
an increase in glycolysis and a subsequent rise in lactic acid.
This increase in lactic acid content of gingival fluid, with increasing
inflammation, has been shown by Hasegawa. However, Hasegawa
also showed that the greatest concentration of lactic acid did not
occur with severe inflammation but rather with mild inflammation.
Therefore the effect of lactic acid in decreasing the pH may be
negligible with moderate to severe inflammation.

Therefore there is no conclusive evidence that an increase
in pH within the gingival crevice leads to the formation of sub-
gingival calculus. With severe inflammation, where the greatest
deposits of subgingival calculus are seen, theoretically an
environment is not produced which would inhibit calcification.

Further work in determining the pH of gingival fluid with
varying degrees of inflammation should be carried out before
any conclusion can be reached.
The loss of CO$_2$ and subsequent rise in pH depends upon the HCO$_3^-$/$H_2CO_3$ buffering system. The concentration of HCO$_3^-$ in gingival fluid is not known and neither is the buffering capacity of gingival fluid. The rise in inorganic phosphate$^{88}$ over that of serum may indicate that the PO$_4^{3-}$/HPO$_4^{2-}$ system may be important. The increased glycolysis with inflammation and higher desquamation rate could theoretically lessen the pCO$_2$ in the gingival crevice. This would then lead to an increase in the pH only if HCO$_3^-$/$H_2CO_3$ is the major buffering system.

Before the loss of CO$_2$ theory, a very attractive theory for the formation of supragingival calculus, can be applied to the formation of subgingival calculus, further work on the buffering capacity and buffering systems of the gingival fluid would have to be carried out. Furthermore, as Kleinberg and Hall$^{93}$ have pointed out, a relationship between the pH within the crevice and the pH of supragingival plaque may exist, the buffering capacity of gingival fluid may be of importance in the development of smooth surface caries around the gingival margin.

The epitactic theory is perhaps the most attractive theory for the formation of subgingival calculus. This theory arose out of histochemical analysis of calculus matrix, revealing it to be largely mucopolysaccharide in nature and possessing similar reactions (decreasing periodic acid Schiff reaction with calcification,
and increasing metachromasia with calcification) to other forms of calcification, for example, bone and renal calculi. 36

The gingival fluid contains all the elements for this form of calcification,¹ and it does not suffer from the drawbacks of other mechanisms. This is an active mechanism which does not depend upon stagnation and the role of bacteria can be explained in terms of their chemical composition rather than their metabolic or enzymatic activity. Bacterial cell walls are composed of almost 25% mucopolysaccharide as are their nuclear membranes. It is this mucopolysaccharide component which mediates both intra- and extracellular calcification processes. Crystal formation has been noted in both the body of the bacterial cell and its cell wall. 36

However, it must be emphasised that the other mechanisms of calcification should not be completely dismissed but, depending on the local environmental factors, they may assume a greater importance and contribute significantly to the calcification process.

In conclusion, then, subgingival calculus should be considered as deriving its mineral salts from gingival fluid as well as most of its matrix. It occurs in areas of greatest flow of gingival fluid, i.e. in areas of gingival inflammation. Since here the gingival fluid is considered to be an inflammatory exudate, i.e. a product of the inflammation, so, too, subgingival calculus
should be considered as a result of the inflammatory process and not as a cause of it.

Further work needs to be carried out on the physical properties (pH and buffering systems) of gingival fluid before the mechanism of calcification can be fully understood.

8.3 Growth of bacteria

It is generally agreed that gingival fluid provides ingredients necessary for bacterial growth and that the presence of serum proteins and ions provide an excellent media for this bacterial growth. 121, 207

A source of nitrogen is essential for bacteria to synthesise protein and to undergo mitosis. 44 The gingival fluid containing a wide range of free amino acids 95 as well as serum proteins 9, 29 could theoretically be considered as being a source of nitrogen to the bacteria of subgingival plaque.

Again, if gingival fluid is considered as an interstitial fluid in the non-inflamed state, that is, a source of nutrients to the cells of the junctional epithelium, it can also be theorised that with desquamation of these epithelial cells and the release of this interstitial fluid that nutrients would be made available to the bacteria of the gingival sulcus. The gingival fluid could
therefore be said to be a source of nutrients for the bacteria.

In the inflamed state, bacteria are always present in the gingival sulcus and/or periodontal pocket. Since saliva does not enter the periodontal pocket it cannot be considered as the source of nutrients for these bacteria. The gingival fluid which in this case is considered as an inflammatory exudate must be thought of as providing the medium for the continued growth of the bacteria. In this case, as Egelberg points out, the bacteria must survive in co-existence with the anti-microbial factors of the gingival fluid, including the neutrophils of the gingival sulcus.

Critchley and Saxton state that rapidly growing organisms in vitro are morphologically different from the main body of organisms in supragingival plaque. However, they cite the work of Schroeder and De Boever as suggesting that these in vitro organisms do resemble those of subgingival plaque. Thus indicating that the micro environment of subgingival plaque (i.e. gingival fluid) is a good medium for the rapid growth of bacteria, but because of the anti-microbial properties of the gingival fluid, this rapid growth may be self-limiting.

That gingival fluid is a good medium for bacterial growth has not definitely been shown. Collins and Gavin showed that it did not inhibit growth of bacteria, in vitro, but they did not
demonstrate that gingival fluid per se enhanced or supported the growth of bacteria.

The role of gingival fluid in the development of plaque has been suggested. Indeed Saxton established a correlation between gingival health and the time of deposition of the organisms of plaque. This, together with the aggregations of bacteria adjacent to the gingiva was taken as an indication that gingival fluid plays an important part in the colonization of the tooth surface.

In this study, Saxton cleaned the tooth surface with pumice and at varying intervals replicated the tooth surface and used a scanning electron microscope to study the development of the plaque. The gingival health was assessed by the gingival index of Löe and Silness. He found that those subjects with the most severe inflammation exhibited the earliest plaque formation and that in the absence of inflammation the time elapsing before the appearance of bacteria exceeded the time period of the experiment (3 hours). The number of subjects used was not sufficient to statistically analyse the results but a trend is definitely recognisable.
Saxton completely ignores the presence of subgingival plaque in this experiment so that it is unknown whether or not the subgingival plaque was removed during the initial prophylaxis, so that the correlation may not be between gingival health and the development of plaque but rather between the presence or absence of subgingival plaque and the development of supragingival plaque. In this case the gingival fluid would be involved only to the extent of supporting the growth of the subgingival plaque.

The colonization by bacteria of the tooth surface is preceded by the development of the acquired pellicle. An analysis of this pellicle indicates that it is chiefly a glycoprotein layer formed from the saliva. No muramic acid nor diaminopimelic acid has been detected, in the pellicle, thus indicating that it is not of bacterial origin. This pellicle occurs rapidly on exposure to saliva and appears to be due to the selective absorption of acid proteins onto the tooth surface.

Serum is known to contain a complex of these glycoproteins and an analysis of gingival fluid has shown it to contain free glucose and hexosamines. The presence of these carbohydrates may be due to breakdown products of bacteria and from the action of enzymes in the inflamed gingival connective tissue. Histochemical studies have shown the presence of glycoproteins in gingival fluid. The staining of these glycoproteins was similar to serum, however, the staining was weak
or absent in samples of saliva. These results demonstrate that there exist in the gingival fluid the elements for the formation of the acquired pellicle. Other elements in the gingival fluid - mucoproteins, serumal proteins, fibrin, etc., are capable of forming layers on the tooth surface. (section 8.1).

This, together with the observation that plaque generally begins to form at the dento-gingival junction (and not 1, 2 or 3 mm. above it) may indicate that the gingival fluid is important in the formation of the acquired pellicle in this region. With inflammation, therefore, and a greater flow of gingival fluid, this pellicle would be formed much more quickly and therefore allow colonization to take place more rapidly. The results of Saxton may indicate that the pellicle formed from gingival fluid is colonised much more quickly than that formed from saliva.

The relationship, therefore, between the gingival fluid and the formation and subsequent colonization of the acquired pellicle requires further work. It may emerge that the greatest significance of gingival fluid lies in this relationship to the early development of plaque.
8.4 Summary

The role that gingival fluid plays in the biology of the gingival sulcus whether in health or disease can be explained by the concept that gingival fluid is the fluid medium of both the gingiva and gingival sulcus.

Gingival fluid contains the causes of inflammation, the mediators and the results of the inflammatory reaction. In the pathogenesis of periodontal disease, therefore, gingival fluid should be considered as the fluid medium in which the whole reaction is taking place and its composition reflects stages of the entire reaction.

In the formation of subgingival calculus both the mineral elements and the matrix are derived from the gingival fluid. This is in agreement with the concept of the gingival fluid being the fluid medium of the gingival sulcus which, with inflammation of the gingiva, has the nature of an inflammatory exudate. Although the exact mechanism of calcification is not known, the microenvironment of the inflamed gingival sulcus is such so as to lead to the formation of subgingival calculus.
That in the non-inflamed state gingival fluid is essentially interstitial fluid, providing a supply of nutrients to the cells of the junctional epithelium, it is conceivable therefore that it should support the growth of bacteria.

Furthermore, its role in the formation of the acquired pellicle may be of some importance in the early development of plaque.

Therefore, by an understanding of the nature of gingival fluid, its composition, mechanism of formation and factors that affect its formation, a concept has been arrived at by which it is possible to begin to understand the biology of the gingival sulcus.

The most essential part of this concept is the presence of a minute but definite amount of fluid in the non-inflamed gingival sulcus. If this fluid was not present, the concept put forward by the author would be invalid. Therefore, the second part of this thesis is concerned with the suitability of the most refined method available at present to detect and quantitatively measure this fluid.
PART II

ORIGINAL WORK

Experimental procedure based on
a concept of the nature of gingival fluid.
CHAPTER 9

AIM OF THE EXPERIMENT

From the preceding review of the literature the theory has been put forward by the author that the flow of gingival fluid reflects the permeability of the dento-gingival blood vessels and the permeability of the junctional epithelium. In the healthy state this flow is essentially interstitial fluid which because of the nature of the epithelial attachment escapes into the gingival sulcus. With inflammation, the nature of the gingival fluid changes to that of an inflammatory exudate containing the causative agents, inflammatory mediators and the results of this inflammatory process.

In essence, the gingival fluid, whether in health or disease, should be considered as the fluid medium of the gingiva and gingival sulcus.

The flow of gingival fluid has been taken to reflect the degree of inflammation of the gingiva. Although this has been disputed, the measurement of gingival fluid has still been used in the study of periodontosis, orthodontic treatment and tooth mobility as an index of gingival inflammation.
In the investigation of periodontosis with periodontitis, Kaslick et al.\textsuperscript{91} used a modified strip method\textsuperscript{87} to divide the periodontitis group into "moderately inflamed" and "nearly normal" groups and the periodontosis group into those with minimal superimposed inflammation and those with a moderate inflammation superimposed.

Using the method of L\öe and Holm-Pedersen,\textsuperscript{120} Kaslick et al.\textsuperscript{87, 91} left the filter paper strips in position for 3 minutes, then removed and stained them with a solution of 0.2% ninhydrin. The length of the stained area was measured to the nearest 0.5 mm. The tissue was considered "nearly normal" if the length of the stained area was less than 4 mm. and "moderately inflamed" if greater than 5.5 mm.\textsuperscript{87, 91}

This quantitative method to determine the degree of gingival inflammation has been supported by Tersin.\textsuperscript{192} He found that for a L\öe and Silness gingival index (G.I.) of less than 2, the fluid measurement was 3.11 mm. or less and for a G.I. of 2 or greater than 2, the fluid measurement was 6.5 mm. or more. This is in agreement with the measurement of Kaslick et al.\textsuperscript{87, 91} However, other workers,\textsuperscript{15, 20, 120, 137, 157, 159, 160, 181} have failed to obtain such high values of fluid measurements.
Sandalli and Wade\textsuperscript{159} found that the mean gingival fluid measurement in children varied from 0.35 mm. in healthy gingiva to 1.28 mm. in inflamed gingiva. In obtaining these values, Sandalli and Wade\textsuperscript{159} measured the length of stain down the middle of the strip, whereas Tersin\textsuperscript{192} measured the mean length of the stained area, as judged on a visual criteria.

From resting inflamed gingiva (G.I. = 2) L\öe and Holm-Pedersen\textsuperscript{120} found that the fluid measurements varied from 0 mm. in one case to 6.4 mm. in another, with all except one being less than 4.0 mm. Furthermore, Björn et al\textsuperscript{15} found that the greatest flow of fluid occurred with the greatest degree of clinical inflammation and that the mean measurement of this fluid flow was 3.7 mm. in adults and 2.9 mm. in children.

In these studies it is implied that the length of stain reflects the volume of fluid absorbed by the strip and that this in turn reflects the degree of inflammation. However, these studies indicate the great variation that does exist in the measurement of gingival fluid and actually cast doubt on the accuracy and relevance of measuring the length of stained area to give a value of the flow of gingival fluid.
The group system of measuring gingival fluid.
From Bissada et al. 14
Granath,\textsuperscript{67} and Wilson and McHugh\textsuperscript{212} measured the surface area of stained material to give an index of gingival fluid flow. Both these workers magnified the strips by 35 and 30 times respectively and divided the strips into squares and counted the number of squares occupied by the stained area.

The measurement of surface area would give a more accurate assessment of the stain than measuring the length, as variations in the width of the strip and irregular staining patterns would be accounted for by this system.

The accuracy of measuring the surface area of stain and its relevance to the clinical inflammation has yet to be demonstrated. Granath\textsuperscript{67} states that caution should be exercised in evaluating the significance of small variations in measurements of gingival fluid, while Wilson and McHugh\textsuperscript{212} state that the gingival fluid flow is no more reliable than the simpler clinical indices of gingivitis.

Bissada et al\textsuperscript{14} used a group system to classify the flow of gingival fluid without giving an actual quantitative measurement (fig. 9.1). This system allows for variations to exist within the one group and is much simpler than the quantitative approach of measuring the length or surface area of stain.
The present study was undertaken in order to determine the suitability of the Löe and Holm-Pedersen method of measuring gingival fluid, as a means of detecting and quantitatively measuring the amount of gingival fluid theoretically present in the healthy gingival sulcus. The method of Löe and Holm-Pedersen was chosen as it represents, in the author's opinion, the most refined method available at present for the measurement of gingival fluid. It is based on an understanding of the epithelial attachment and is the method least likely to alter the environment of the gingival sulcus.

In this study the gingival fluid flow from clinically healthy gingival sulci was assessed on both a subjective and quantitative basis. The validity of a quantitative measurement of gingival fluid flow was evaluated.
Fig. 10.1: The method of obtaining filter paper strips for use in the experiment.

Filter paper circle.
CHAPTER 10

MATERIALS AND METHODS

In this study strips of filter paper (Munktell, Sweden, No. 3), cut to 1.5 mm. wide and 10 mm. long, were used. The strips were cut from the filter paper circle by means of a modified scalpel which enabled two blades to be mounted in parallel at a distance of 1.5 mm. (Plate 1). In this way strips of filter paper 1.5 mm. wide could be cut from the circle. Subsequently, the resulting strips could be cut into sections 10 mm. long using a photographic guillotine (Plate 2); these were then used in the experiment (fig. 10.1).

During the cutting and subsequent handling of the filter paper strips sterilised (autoclaved) instruments were used, thus avoiding contact with the skin, which may have subsequently altered the staining of the strip.

The areas from which the gingival fluid was obtained were the facial sulci of the maxillary right and left incisors. These areas were chosen for ease of access and thereby eliminating the variables which may be encountered by sampling more difficult areas. All measurements were made between 1400 and 1500 hours to reduce the influence of circadian rhythms.
fig. 10.2  Placement of the filter paper strip at the entrance to the gingival sulcus and therefore avoiding damage to the junctional epithelium.
The teeth and surrounding tissues were isolated by means of cotton rolls placed in the facial vestibular sulcus. The patients were in a horizontal position to further reduce the possibility of contamination with saliva. The isolated teeth and tissues were dried by a gentle blast of compressed air perpendicular to the tooth surface.

The method used to sample the gingival fluid was the modified method of Loe and Holm-Pedersen. The strips were guided, as parallel as possible to the long axis of the tooth, to the entrance of the gingival crevice (fig. 10.2).

Starting on the upper left lateral incisor, the strips were placed at 10 second intervals around to the upper right lateral incisor. The strips were left in position for 3 minutes, were then removed and placed on a watch glass.

Each strip was then stained with a 0.2% solution of alcoholic ninhydrin which stains α-amino acids a bluish-red colour. This is a very sensitive test for the detection of α-amino acids such that 0.1 micromole of α amino acid gives a colour intensity which is reproducible to a few per cent (fig. 10.3).
fig. 10.3  The ninhydrin reaction. From Roberts and Caserio. 155
The staining procedure was carried out within 1 minute of the strips being removed from the gingival sulci and was achieved by dropping the solution onto the paper strip on the watch glass. After wetting of the strips the watch glass was covered and the strips dried. The drying was done by means of a Phillips "Infraphil" infra-red heat lamp shining directly on the covered watch glasses.

Upon completion of the drying procedure the strips were photographed within 60 minutes.

The experiment was divided into two parts. In both parts 3 dental students were used: 2 males and 1 female. The females were not menstruating and had not ovulated in their present cycles - the sampling took part on day 8. This was to reduce the influence of the menstrual cycle by standardising the day on which the gingival fluid was sampled. The females used were not taking an oral contraceptive and thus the effect of sex hormones was avoided. None of the students was taking any drugs and there were no significant medical histories.

The influence of chewing and foodstuffs on the flow of gingival fluid was avoided with the students having nothing to eat for two hours prior to the sampling.
<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
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<tbody>
<tr>
<td>0</td>
<td>Absence of inflammation.</td>
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<td>1</td>
<td>Mild inflammation - slight change in colour and little change in texture.</td>
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<td>2</td>
<td>Moderate inflammation - moderate glazing, redness, oedema and hyperplasia. Bleeding on pressure.</td>
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<tr>
<td>3</td>
<td>Severe inflammation - marked redness and hyperplasia. Tendency to spontaneous bleeding. Ulceration.</td>
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</table>
Thus all extrinsic influences on the flow of gingival fluid were avoided or at least standardised.

The gingiva of each student was assessed as clinically healthy, having a Löe and Silness gingival index of 0 (table 10.1). This then eliminated the effect of overt inflammation on the flow of gingival fluid.

All variables therefore were intrinsic to the experimental procedure carried out.

The experimental procedure followed in Part I was:

1. Measurement of gingival fluid as already described.

2. Staining of plaque on the teeth with a 2.0% mercurochrome solution.

3. Prophylaxis of the teeth with a rubber cup and a pumice and glycerine mixture.

4. Measurement of the gingival fluid immediately following the prophylaxis.

5. Measurement of the gingival fluid 15 minutes after completion of the prophylaxis.

6. Measurement of the gingival fluid 45 minutes after completion of the prophylaxis.

During the prophylaxis care was taken not to damage the gingiva.
fig. 10.4. The placement of filter paper strips in Part II and II(b) of the experiment.
One of the male students who took part in part I of the study also participated in part II of the study. In part II the students refrained from all forms of oral hygiene for 3 days while still maintaining a normal diet. After 3 days a visible quantity of plaque had accumulated on all surfaces of the teeth although the gingiva was still assessed as clinically healthy (GI = 0).

The same procedure was again carried out as in part I. However, a second procedure was also carried out (part IIb). This involved a second filter paper strip which was guided as parallel as possible to the long axis of the tooth so that it did not touch the strip measuring the gingival fluid and ending approximately 1 mm. short of the gingival sulcus (fig. 10.4).

This double strip arrangement was carried out before prophylaxis of the teeth as well as at times 0 minutes, 15 minutes and 45 minutes after completion of the prophylaxis.

The strips used in both parts of the experiment were photographed in black and white on a standardised macrophotographic set up.

Subsequent to this a third experiment was carried out in which 2 measurements of gingival fluid were obtained from 3 patients and the stained strips photographed in colour.
A pilot study was initially conducted to determine the feasibility of using photographs to measure the amount of stain on each filter paper strip. This proved to be a highly sensitive technique for with the magnifications obtained variations in the pattern and intensity of staining could be observed (plate 3). The photographs also allowed a "blind" scoring of the filter paper strips to be performed, thus reducing the effect of examiner bias.

The amount of stain on each strip was assessed twice. Firstly, on a purely subjective visual basis and secondly, by a subjective assessment of the stain intensity together with a quantitative assessment of the area of stain.

In the first system used, each strip was given a score of 0, +, ++, +++ based on a visual assessment of the area and intensity of stain (plates 4, 5, 6 and 7). This allowed great variation to exist within each group as well as the occurrence of overlapping of groups. Plate 7(a) shows the maximum amount of staining obtained in the experiment.

In this system each strip was scored twice by the author and once by an independent observer. Variations existed in the scoring of 5 of the 137 strips studied. In these cases the score that predominated was taken (table 10.2). An example is seen in plate 8 where a score of ++ was given twice and a score of + was given once. In this case the score ++ was taken. In no case were three different scores given.
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<td>Score Taken</td>
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</tr>
<tr>
<td>2.3(b)</td>
<td>UR2</td>
<td>Before 0</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>45</td>
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</tr>
<tr>
<td></td>
<td>UR1</td>
<td>Before 0</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<td></td>
<td>45</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>
fig. 10.5 Grid 5 mm. x 5 mm. used to measure the area of stain.
In the second system used, each strip was given an intensity score of 0 1 2 3 or 4. The criteria used was a purely subjective visual assessment of the degree of greatest intensity of stain on each strip (plates 9, 10, 11, 12 and 13). This meant that if a strip had a spot of degree 4 intensity in a large area of degree 2 or 1, it was still given an intensity score of 4 (plate 14). Overlapping of groups occurred in the middle ranges, with the score 0 and 4 being the extremes.

In the second part of this system, the area of greatest intensity, i.e. that which determined the intensity score, as well as the total area of stain on the strip were measured quantitatively. This quantitative assessment was obtained by placing a grid divided into squares 5 mm. x 5 mm. (fig. 10.5) over the entire photograph and counting the number of squares in which staining occurred.

This size grid was used as it allowed the stain to be seen through the grid. A grid of square size 2 mm. x 2 mm. (fig. 10.6) was tried but despite the greater accuracy that would have been obtained, the stained area, especially those of light intensities, could not be clearly seen through it.
fig. 10.6  Grid 2 mm. x 2 mm. which was unsuitable for the experiment.
The magnification obtained by the photographic method was of the order of 15. Therefore the squares 5 mm. x 5 mm. measured an actual area of 0.1 mm.² on each strip.

In order to test reading errors, 13 strips were randomly selected and re-evaluated. Contaminated strips (plate 15) were not used and strips were considered unmeasurable if the staining pattern was uncertain or was outside the bounds of the photograph (plate 16).

A statistical comparison of the results of each part of the experiment as well as a comparison between the subjective and quantitative assessments was carried out. This was done by using both the chi squared and student "t" tests.
### Table 11.1

No. of Strips

<table>
<thead>
<tr>
<th></th>
<th>Part I of experiment</th>
<th>Part II</th>
<th>Part II(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stain</td>
<td>40</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>No Stain</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>44</td>
<td>45</td>
</tr>
</tbody>
</table>

### Table 11.2

No. of Strips - Part I

<table>
<thead>
<tr>
<th></th>
<th>Before prophylaxis</th>
<th>0 min.</th>
<th>15 min.</th>
<th>45 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stain</td>
<td>12</td>
<td>9</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>No Stain</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
CHAPTER 11

RESULTS

1. Subjective assessment

Staining occurred on 129 of the 137 strips studied while 8 exhibited no staining. The 8 strips which had not stained areas were all from part I of the experiment and occurred following the prophylaxis. All of the strips studied from part II and part II(b) showed the presence of stain (tables 11.1 and 11.2).

The results of part I are shown in table 11.3. In this part 12 areas were studied, all of which exhibited stain before the prophylaxis. The tendency following the prophylaxis appears to be an overall reduction in the amount of stain obtainable on the filter paper strips (plate 17). Only one area showed an increase in the amount of stain while one remained the same throughout all stages of the experiment (plate 18).

Immediately following the prophylaxis (0 minutes), 3 strips exhibited no staining while another 4 showed less staining, 4 remained the same and 1 an increase in the amount of stain.

At the 15 minutes mark 3 strips had no stain, 5 showed less than before the prophylaxis, 3 showed the same amount and 1 an increase in the amount of stain. Two strips showed an increase between 0 and 15 minutes but remained less than pre-prophylaxis values.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Tooth</th>
<th>Before</th>
<th>0</th>
<th>15</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>UR2</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>UR1</td>
<td>++</td>
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<td>UL1</td>
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<td>+</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>UL2</td>
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<td>+</td>
</tr>
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<td>++</td>
<td>++</td>
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<td>UL2</td>
<td>++</td>
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<td>++</td>
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<tr>
<td>*1.3</td>
<td>UR2</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
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<tr>
<td></td>
<td>UR1</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>0</td>
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<td>UL2</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>
At the end of the experiment, 45 minutes after the prophylaxis, all but 2 strips showed an overall decrease in the amount of stain.

The results of the second part of the experiment are seen in tables 11.4 and 11.5. With table 11.4 showing the results of those strips which were in contact with the gingiva at the entrance to the gingival sulcus (part II).

Staining occurred on all strips taken before the prophylaxis. At 0 minutes immediately following the prophylaxis, 3 strips showed an increase in the amount of stain while 3 showed a decrease and 5 remained the same. One strip was contaminated between the staining and photographing and was not included in the pre-prophylaxis group, therefore it is unknown whether or not this area showed an increase or remained the same immediately after the prophylaxis. In all probability it remained the same although if an increase did occur it was maintained throughout the experiment. Of the 5 that remained the same, one was +++ and could not be given a higher score. The tendency immediately after the prophylaxis seemed to be one of a slight increase which over the 45 minutes of the experiment returned to pre-prophylaxis values or slightly lower. At the 45 minute mark, 5 strips showed a lower score than at the beginning (plate 19) while 5 were the same (plates 20 and 21) and 2 showed a higher score (plate 22). Plates 20 and 21 demonstrate the variation that can exist within the same group.
Table 11.4.

<table>
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<tr>
<th>Patient</th>
<th>Tooth</th>
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<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
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<td>+++</td>
<td>+++</td>
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<tr>
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<td>UR1</td>
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<tr>
<td>*2.2</td>
<td>UR2</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
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<tr>
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<td>UR1</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>++</td>
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<td>+</td>
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<td>++</td>
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<tr>
<td>2.3</td>
<td>UR2</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>UR1</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<td>UL2</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

*Same patient.
The results of those strips which did not contact the gingiva (part II(b)) are seen in table 11.5. All strips obtained prior to the prophylaxis shown an area of stain which generally decreased following the prophylaxis. No strips either prior to or following the prophylaxis showed absence of stain.

As seen in plates 23, 24 and 25, the degree of staining was generally at its lowest 15 minutes after the prophylaxis which then increased slightly at the 45 minute mark. Although the amount of staining has visibly increased between 15 and 45 minutes, this increase is not sufficient to alter the score.

The general pattern of staining is one extending along the edges of the strip with the main area of stain being at the tip (plates 23, 24 and 26). In one case observed, the stain differed on one side of the strip to the other, depending upon which side was in contact with the tooth surface (plate 27).

A visual comparison between those strips which did not contact the gingiva (part II(b)) and those strips which did (part II) in the second part of the experiment reveals a similarity in staining patterns (plates 28 and 29).
<table>
<thead>
<tr>
<th>Patient</th>
<th>Tooth</th>
<th>Before</th>
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<th>15</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>UR2</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
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<td></td>
</tr>
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<tr>
<td>2.3</td>
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<td>UL2</td>
<td>+</td>
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<td>+</td>
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</tbody>
</table>
The difference between the first and second parts of the experiment in the one patient that participated in both is seen in table 11.6 (plate 30). The scores prior to the prophylaxis are similar in both parts; however, in the second part of the experiment there was an increase in staining following the prophylaxis which returned to approximately pre-prophylaxis values after 45 minutes. Whereas in the first part there was a decrease in staining following the prophylaxis which was maintained till the end of the experiment 45 minutes later. The strips which did not contact the gingiva show a similar pattern, i.e. the decrease following the prophylaxis being maintained throughout the experiment.

2. Quantitative assessment

A total of 10 strips were considered as being of unmeasurable intensity (table 11.7). All of these except one came from part II(b) of the experiment (table 11.8).

The area of greatest intensity (A.G.I.) was unmeasurable on 15 strips while 2 were of questionable assessment (table 11.9). In part II(b) of the experiment, the area of greatest intensity was unmeasurable in 12 strips (table 11.9). Of these, 5 were before the prophylaxis and 7 after the prophylaxis (table 11.10).
<table>
<thead>
<tr>
<th>Tooth</th>
<th>Subjective Score</th>
<th>Part I</th>
<th>Before</th>
<th>Subjective Score</th>
<th>Part II</th>
<th>Same patient</th>
<th>Subjective Score</th>
<th>Part II(b)</th>
<th>Same patient</th>
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Table 11.8.

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<td>45</td>
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<tr>
<td>UM</td>
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### Table 11.9.
No. of Strips with unmeasurable area of greatest intensity (A.G.I.)

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<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Part II(b)</td>
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### Table 11.10.
Unmeasurable A. G. I. - Part II(b)

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Table 11.11.

No. of Strips with unmeasurable Total Area (T.A.)

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<th>Total</th>
</tr>
</thead>
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<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
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<td>4</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Part II(b)</td>
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<td>5</td>
<td>3</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
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<td>4</td>
<td>3</td>
<td>29</td>
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Table 11.12.

No. of strips with ? unmeasurable T.A.

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<th>Total</th>
</tr>
</thead>
<tbody>
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<td>Part I</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Part II</td>
<td>0</td>
<td>0</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Part II(b)</td>
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<td>2</td>
<td>1</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>15</td>
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</tbody>
</table>
An assessment of the total area (T.A.) shows that 17 of 45 strips in part II(b) of the experiment were considered unmeasurable, with a further 8 of questionable evaluation. Whereas of the 92 strips in parts I and II, 12 were unmeasurable with a questionable result in a further 7 (tables 11.11 and 11.12).

All of those strips with an unmeasurable intensity were also of an unmeasurable A.G.I. and T.A., and those with an unmeasurable A.G.I. also had an unmeasurable T.A. Therefore, of the 137 strips studied, 93 were able to be quantitated.

The trend with the unmeasurable strips seems to be one of a higher proportion in part II(b) of the experiment with the greater number occurring before the prophylaxis which then decreases following the prophylaxis.

The results of the intensity scoring are seen in tables 11.13, 11.14 and 11.15.

The assessments of the area of greatest intensity are shown in tables 11.16, 11.17 and 11.18. Whereas the results of total area evaluations are seen in tables 11.19, 11.20 and 11.21.
### Table 11.13

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<td>2</td>
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<td>L2</td>
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</tr>
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<td>4</td>
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<td></td>
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Table 11.15.

Intensity Score - After accumulation of plaque + Strip not in touch with gingiva - Part II(b)
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*Same patient.*
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Table 11.17.

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<td>9</td>
<td>12</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>
### Table 11.21.

**Total Area - After plaque accumulation + strip not in contact with gingiva - Part II(b)**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tooth</th>
<th>Before</th>
<th>0</th>
<th>15</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>R2</td>
<td>UM</td>
<td>9</td>
<td>22(UM)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>R1</td>
<td>10(UM)</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>UM</td>
<td>UM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>22(UM)</td>
<td>19(UM)</td>
<td>8</td>
<td>14(UM)</td>
</tr>
<tr>
<td>2.2</td>
<td>R2</td>
<td>UM</td>
<td>15</td>
<td>9</td>
<td>16(UM)</td>
</tr>
<tr>
<td></td>
<td>R1</td>
<td>UM</td>
<td>13</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>UM</td>
<td>10</td>
<td>10</td>
<td>7(UM)</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>5</td>
<td>8(UM)</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>R2</td>
<td>14</td>
<td>14</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>R1</td>
<td>UM</td>
<td>UM</td>
<td>UM</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>18</td>
<td>UM</td>
<td>UM</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>UM</td>
<td>UM</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

*Same patient.*
Table 11.22. Re-evaluation and Results

<table>
<thead>
<tr>
<th>Sample Strip No.</th>
<th>1st Reading</th>
<th>2nd Reading</th>
<th>1st Reading</th>
<th>2nd Reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>7</td>
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<tr>
<td>2</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>10</td>
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<td>3</td>
<td>7</td>
<td>7</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>8</td>
<td>UM</td>
<td>UM</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>8</td>
<td>UM</td>
<td>UM</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>4</td>
<td>19(UM)</td>
<td>UM</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>3</td>
<td>16(UM)</td>
<td>16</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>3</td>
<td>7(UM)</td>
<td>UM</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>13</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>
The result of the re-evaluation of every tenth strip is seen in table 11.22. Since the difference in scores was not greater than ±1 in any case except those 3 total areas in which the first scoring was of questionable assessment, the reading error of the A.G.I. and T.A. was ignored. 80

3. Statistical analysis

A total of 93 strips were used. Those strips that were considered as unmeasurable were not used in this analysis (table 11.23).

The relationship between the subjective score and the part of the experiment is seen in table 11.24. The difference in the subjective score between each part is statistically significant (p < 0.01); however, no trend can be observed. If part I is compared to part II (table 11.25) a movement to the right is observed which is statistically significant (p < 0.01). This movement is due primarily to the extremes of the subjective range, i.e. 0 and ++.

The difference between each part and the intensity score (table 11.26) is also statistically significant (p < 0.01). With the comparison between part I and part II showing a statistically significant (p < 0.01) increase in the intensity score in part II. This increase is due to the greater number of 0's in part I and 4's in part II (table 11.27).
<table>
<thead>
<tr>
<th>Part I</th>
<th>Intensity Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 1 1 1 1 1 2 2 2 2 2 2 3 3 3 3 3 3 3 3 4 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjective Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + + + + + + + + + + + + + + + + + + + + + + + + +</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 5 5 6 3 4 4 10 17 18 20 9 12 16 8 14 9 10 23 9 25 13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A.G.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 5 5 6 3 4 4 14 8 3 3 7 6 3 6 6 8 4 9 4 3 5 15</td>
</tr>
<tr>
<td>Table 11.23 (cont'd.)</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td><strong>A.G.I.</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>43</td>
</tr>
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</tr>
<tr>
<td>9</td>
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<tr>
<td>3</td>
</tr>
<tr>
<td>10</td>
</tr>
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<td>11</td>
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<tr>
<td>4</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Part II (cont'd.)</th>
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</thead>
<tbody>
<tr>
<td><strong>Subjective Score</strong></td>
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<td>+ + + + + + + + + + + + + + + +</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intensity Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4</td>
</tr>
</tbody>
</table>
## Part II(b)

<table>
<thead>
<tr>
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<th></th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+</td>
<td>10</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>9</td>
<td></td>
<td>9</td>
</tr>
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<td>14</td>
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<td>14</td>
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<td>14</td>
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<td>13</td>
</tr>
<tr>
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<td>+</td>
<td>3</td>
<td></td>
<td>10</td>
</tr>
<tr>
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<td>+</td>
<td>5</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>2</td>
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<td>10</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>7</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>2</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>3</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Subjective Score No. / %</td>
<td>Part I</td>
<td>Part II</td>
<td>Part II(b)</td>
<td>Total</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>---------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>18</td>
<td>13</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>18.5%</td>
<td>33.33%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>53%</td>
<td>32.33%</td>
<td>14.7%</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>20%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>93%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>
As the intensity score increases so does the subject score (table 11.28) and this increase is also statistically significant ($p < 0.01$). Again, the difference is greater at the extremes of the scoring range.

If the average area of greatest intensity for each part of the experiment is compared (table 11.29), we find that the difference between part I and part II falls just short of significance at the 0.05 level ($t = 1.86$). A "t" of 1.96 is required for significance.

A similar comparison between the average total areas of part I and part II (table 11.29) shows that this difference is also not significant at the 0.05 level ($t = 1.6$).

Table 11.30 shows the average area of greatest intensity and the average total area for part I and part II of the experiment. These values are the same as the average A.G.I. and T.A. respectively in part II(b) of the experiment and therefore, the same as the total average A.G.I. and T.A.

The relationship between the subjective score and the average A.G.I. and average T.A. is seen in table 11.31. As the subjective score increases, both the average A.G.I. and T.A. are also observed to increase.
<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Part I</strong></td>
<td>8</td>
<td>18</td>
<td>13</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>20.5%</td>
<td>46.27%</td>
<td>33.33%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Part II</strong></td>
<td>0</td>
<td>18</td>
<td>11</td>
<td>5</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>53.0%</td>
<td>32.3%</td>
<td>14.7%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>8</td>
<td>36</td>
<td>24</td>
<td>5</td>
<td>73</td>
</tr>
</tbody>
</table>

$p < 0.01$
The difference between + and ++ subjective score is significant at the 0.01 level for both A.G.I. \( (t = 5.92) \) and T.A. \( (t = 4.17) \). The difference between ++ and +++ is significant at the 0.05 level \( (t = 2.08) \) for the A.G.I.; however, for the T.A. the difference falls just short of 0.05 level of significance \( (t = 1.85) \).

The increased level of significance for the A.G.I. and the absence of significance for the T.A. between ++ and +++ is due to the small number with a score of +++ . With a larger number the significance level may have been maintained.

No relationship is observed between the average A.G.I. and the intensity score nor between the average T.A. and intensity score (table 11.32).

If for the average A.G.I. and T.A. the part of the experiment is compared to the subjective score, we see that the relationship between each part and the subjective score is significant at the 0.01 level (tables 11.33 and 11.34). The relationship between part I and part II is also statistically significant \( (p \ 0.01) \). The prime region of this relation being at the end of the subjective range: score +++ in parts I and II as well as in the + score of part II(b).
<table>
<thead>
<tr>
<th>Table 11.26.</th>
<th>Intensity Score No./%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Part I</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>20.5%</td>
</tr>
<tr>
<td>Part II</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Part II(b)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 11.27</th>
<th>Intensity Score No./%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Part I</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>20.5%</td>
</tr>
<tr>
<td>Part II</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Total No.</td>
<td>8</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>
Tables 11.35 and 11.36 show the relationship between the different parts of the experiment and the intensity score for the average A.G.I. and T.A. respectively. This relationship is also statistically significant at the 0.01 level; however, no trends can be observed. This 0.01 level of significance is also observed in the relationship between parts I and II and the intensity score for both the average A.G.I. and T.A.

When the subjective score and intensity score are compared for the average A.G.I. and T.A., the 0.01 level of significance is maintained (tables 11.37 and 11.38). However, here the trend is clearly evident that as the intensity score increases, so too does the subjective score.
<table>
<thead>
<tr>
<th>Subjective Score</th>
<th>Intensity Score</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
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<td>+</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>+</td>
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<td>0</td>
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</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total No.</td>
<td>8</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>31</td>
</tr>
</tbody>
</table>

p < 0.01
<table>
<thead>
<tr>
<th>Part</th>
<th>A.G.I.</th>
<th>T.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ = 4.5</td>
<td>$\bar{x}$ = 8.3</td>
</tr>
<tr>
<td></td>
<td>SE = $\pm 0.52$</td>
<td>SE = $\pm 1.04$</td>
</tr>
<tr>
<td></td>
<td>N = 39</td>
<td>N = 39</td>
</tr>
<tr>
<td>Part II</td>
<td>$\bar{x}$ = 7.1</td>
<td>$\bar{x}$ = 11.3</td>
</tr>
<tr>
<td></td>
<td>SE = $\pm 1.30$</td>
<td>SE = $\pm 1.56$</td>
</tr>
<tr>
<td></td>
<td>N = 34</td>
<td>N = 34</td>
</tr>
<tr>
<td>Part II(b)</td>
<td>$\bar{x}$ = 5.75</td>
<td>$\bar{x}$ = 9.75</td>
</tr>
<tr>
<td></td>
<td>SE = $\pm 0.82$</td>
<td>SE = $\pm 0.88$</td>
</tr>
<tr>
<td></td>
<td>N = 20</td>
<td>N = 20</td>
</tr>
<tr>
<td>Total</td>
<td>$\bar{x}$ = 5.73</td>
<td>$\bar{x}$ = 9.71</td>
</tr>
<tr>
<td></td>
<td>SE = $\pm 0.56$</td>
<td>SE = $\pm 0.75$</td>
</tr>
<tr>
<td></td>
<td>N = 93</td>
<td>N = 93</td>
</tr>
<tr>
<td>Subjective Score</td>
<td>$\bar{x}$</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>4.7</td>
<td>8.3</td>
</tr>
<tr>
<td>++</td>
<td>7.2</td>
<td>13.0</td>
</tr>
<tr>
<td>+++</td>
<td>19.6</td>
<td>25.0</td>
</tr>
<tr>
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CHAPTER 12

DISCUSSION OF RESULTS

The use of a filter paper method for detecting and measuring the flow of gingival fluid is as pointed out by Leirskar,\textsuperscript{101} either descending or ascending paper chromatography, depending upon whether measurements are taken from the upper or lower jaw. Therefore, factors which affect the movement of solutions over filter paper should also affect the movement of gingival fluid over the filter paper strips.

Leirskar\textsuperscript{100} in an in vitro experiment, showed that the concentration of various components of exudates was important in the movement of these exudates across the filter paper. He showed that a cellular component did not change the mobility of the solution; however, the mobility of protein fractions was inversely related to the concentration of the solutions. He goes on to question the reliability of quantitative measurements of gingival fluid in light of these findings.

The results of the present study indicate that the in vitro effects noted by Leirskar\textsuperscript{100} may also apply to the in vivo measurement of gingival fluid. This is seen in plates 31 and 32 where the variation of staining intensities is seen. The most intense stain
occurs at the bottom of the strip and extends up along the sides. The reason for this variation in staining intensities is a concentration gradient of stainable \(\alpha\)-amino acid along the strip. The smaller free amino acid molecules move quickly across the filter paper while the larger protein molecules move more slowly, thus giving a higher concentration of stainable material at the bottom of the strip. The results of the third part of the study where the filter paper strips were photographed in colour reveal this concentration gradient more vividly.

The movement along the edges of the strip adds a further variable to the use of filter paper strips in the measurement of gingival fluid. The strips used were cut to be 1.5 mm. wide and evaporation from these very narrow strips is likely to be high and is very difficult to assess.\(^8^9\) For this reason, the use of filter paper strips for the quantitative analysis of the cationic concentration of gingival fluid was rejected by Kaslick et al.\(^8^9\) This evaporation from the strips takes place from the edges, thus creating a greater force for the movement of fluid along the edges. Therefore, the fluid would move more rapidly so that a higher concentration of stainable material would exist in this region as opposed to the centre of the strip which has less evaporation and a slower movement of fluid.
Another factor which would influence the intensity of stain of the filter paper strip would be variations in the amino acid and protein concentrations of fluid from different gingival areas. These variations may account for similar areas of stain but of different intensities on the strips taken from different areas (plate 34).

Leirskar showed again by an in vitro experiment that different solutions moved 2.5 - 2.9 times faster on Munktell No. 3 filter paper than on Whatman No. 1 filter paper, thus indicating that the type and quality of the paper may be important. He also showed that the mobility was 30 - 50% higher in descending chromatography as compared to ascending chromatography, thus indicating that gingival fluid measurements may be influenced by the direction of flow.

In the present study the effect of gravity on the movement of the fluid across the strip was eliminated by having the patient in a horizontal position with the strip also being as horizontal as was possible. However, other characteristics of the filter paper, which theoretically could alter the movement of fluid across it, could not be accounted for.

It is generally agreed, in paper chromatography, that the direction of the fibres in the filter paper is important in the rate of flow of solutesacross it. During rolling of the filter paper the
fibres take up a preferred orientation\(^{41,86}\) which, unless realised, can lead to what would be unexplained variations in the flow rate.

In studies on gingival fluid the filter paper strips used are cut randomly from circles of filter paper\(^{75,204}\) with no attention being paid to the direction of the fibres. In this way some strips result with the predominant orientation of the fibre parallel with the long axis of the strip (plate 35) while in others the fibres orientation is predominantly perpendicular to the long axis (plate 36). Therefore, the rate of flow of the fluid over different filter paper strips would be different and for an equal period of time the amount of fluid absorbed by those strips with fibres parallel to the long axis would be greater than that absorbed by those strips with fibres perpendicular to the long axis. Therefore, the variations in amount of fluid absorbed may not reflect variations in the amount of fluid produced by the tissue.

Furthermore, using the double bladed scalpel to cut the strips it is conceivable that the fibres along the edges of the strips are dragged by the blades to be parallel with the long axis (plate 37), thus allowing for a more rapid absorption of fluid along the edges of the strip than in the centre.
The passage of the fluid across the filter paper strip occurs along the fibres of the filter paper and not in a uniform front up the strip. This is seen in plate 38. This pathway along the fibres leads to variations in the staining patterns of different strips—depending upon the orientation of the fibres.

It should now be becoming evident that a great many variables exist in using filter paper to absorb and measure gingival fluid, and that these variations are inherent to the filter paper itself and would be extremely difficult if not impossible to assess.

Most workers in measuring the amount of gingival fluid have measured the length of stain on the filter paper strip, \(^5\), \(^{49}\), \(^{111}\), \(^{120}\), \(^{143}\), \(^{144}\), \(^{157}\), \(^{159}\), \(^{160}\), \(^{192}\) while others have measured the area of stain. \(^{67}\), \(^{212}\)

Egelberg, \(^{49}\) in the only experiment performed to show the relationship between volume and length of stain, used serum expelled from micropipettes of one or two microlitres onto one end of a filter paper strip, then stained and measured the length. From this experiment he concluded that differences in the length of stained areas reflected true differences in volumes of fluid.

This in vitro experiment is not representative of the in vivo situation where fluids of varying composition are being measured, \(^{89}\), \(^{90}\), \(^{147}\) a fluid of continuous formation is being measured, and
especially from non inflamed tissue smaller volumes are being measured.

Furthermore, Egelberg did not take variations in the filter paper itself into account. Therefore, doubt is cast on the validity of Egelberg's experiment as applied to the in vivo situation and on the validity of the measurement of length in general.

For the length of stain to bear any relationship to the volume of fluid it is paramount that each strip is of exactly the same width. In cutting the strips to 1.5 mm. variation in the width of the strips does occur (plate 39). In the present study the range of variations in width was 0.15 mm. Thus, any relationship between length and volume would be invalid.

Because of variations in the staining patterns between the various strips some criteria must be established before any measurement of length can be carried out. Sandalli and Wade measured the length up the middle of the strip to the nearest 0.1 mm. Tersin also measured to the nearest 0.1 mm.; however, he measured the mean length of the stained area as judged by a visual criteria. On the other hand, Rudin et al used a mathematical formula to arrive at the mean length.

Once a criteria is established the validity of the measurement depends upon the validity of the criteria. The measurement of stain
up the middle of the strip does not account for the more rapid flow rate up the edges of the strip. Plates 40, 41 and 42 show variations in staining patterns and as can be seen in plate 40(a), any measurement up the middle of the strip would not give any indication of the volume of fluid compared to that in plate 40(b). Furthermore, the mathematical formula of Rudin et al.\textsuperscript{157} does not account for the fact that the fluid can travel up one edge (plate 40(b)) or up both edges (plate 40(a)) nor does it account for the non-uniformity of stain on the bottom of the strip (plate 41). Nevertheless, the method of Rudin et al.\textsuperscript{157} does give a better indication of the amount of stain on the filter paper strip than does the method of Sandalli and Wade.\textsuperscript{159}

The visual criteria used by Tersin\textsuperscript{192} to judge the mean length of stain would probably give an even better indication of the amount of stain. However, it does lose the complete objectivism of the other criteria. Difficulty is encountered with this method when staining occurs along the edges of the strip (plate 42).

In none of these criteria is any regard paid to the different intensities of stain, that is, the fact that a concentration gradient may exist along the strip, is completely ignored. Therefore, any relationship between length and volume would be invalid. Furthermore, that the strips vary in width would make any relationship between length and volume invalid.
The measurement of the area of stain would overcome some of the drawbacks of the length measurement and would give a better correlation to the volume of fluid. Variations in the width of the strips would not matter and extension of the stain along the edges could be accurately included in the measurement. Differences in the staining patterns would not be of any major significance as this could be easily included in the measurement.

Wilson and McHugh\textsuperscript{212} examined the strips under a microscope with an eye piece graticule at a magnification of x 30. The number of squares of the graticule in which stain occupied more than 50\% were counted.

On the other hand, Granath\textsuperscript{67} used a magnification of x 35 and an actual square size of 0.04 mm.\textsuperscript{2}; however, he did not set the criteria of only counting those squares in which stain occupied more than 50\%.

By using this 50\% or more criteria, Wilson and McHugh\textsuperscript{212} would be underestimating the actual area of stain. However, if the squares in which any stain occurred were counted an over-estimation of the area would be obtained.
Neither Granath\textsuperscript{67} nor Wilson and McHugh\textsuperscript{212} noted the presence of varying intensities of stain which would have been obvious at the magnifications used. Plate 43 shows a strip at a magnification of x 20: here the variation in intensities is quite obvious and even at this magnification difficulty in determining the presence or absence of stain in the regions of light intensities is experienced.

Furthermore, in the movement of the fluid across the filter paper various fronts are set up according to the flow rate of the various components,\textsuperscript{41, 86} so that the faster moving amino acids create a front ahead of the slower moving proteins, and thereby set up the concentration gradient of stainable material. It is conceivable that a front containing smaller molecules or ions exists ahead of the amino acid front. Such a front was observed by the author using filter paper strips 3 cm. x 15 cm. inserted into a 0.01 M. solution of alanine. The results of this experiment are not presented; however, the observation was made that the fluid front moving up the strip was ahead of the amino acid which was subsequently stained with a 0.2% alcoholic solution of ninhydin.
With this mind, the area of stain may not reflect the area of fluid absorbed and therefore would not reflect the volume of fluid absorbed.

Certainly the underestimation of stained area by Wilson and McHugh\textsuperscript{212} would not give a true indication of the volume of fluid absorbed.

An overestimation by counting all the squares in which staining occurred may give a better relationship to the volume of fluid. However, difficulty in assessing the actual front of fluid makes the testing of this relationship impossible.

Therefore, the measurement of the area of stain at high magnification is certainly more accurate than the measurement of length of stain at low magnification. However, any relationship between the area of stained amino acids and the total volume of fluid is questionable.

Because of this questionable relationship, any reference to the volume of fluid should be avoided. So that all reference should be confined to the amount or area of stained material. For this reason the presence of a concentration gradient of stained material along the strip should be taken into account.
In the quantitative assessment used in this study the strips were first given an intensity score depending upon the degree of greatest intensity of stain present on each strip. The area of this intensity was then measured using a grid placed over the photograph as well as measuring the total area of stain visible through the grid. A square was counted if staining occurred anywhere in that square and this resulted in an overestimation of the areas rather than an underestimation.

In this way a criteria was established which included both amount and area of stained material.

In the subjective assessment used in this study, the criteria was a visual one based on the area and intensity of stain, such that a small area of high intensity may be given a similar score as a larger area of lower intensity.

Using this subjective criteria for the assessment of stain, several trends became evident.

In part I of the study, the effect of the prophylaxis seemed to be a general reduction in the amount of stainable material absorbed onto the strip. Whereas in part II of the study, the effect immediately following the prophylaxis seemed to be one of a slight increase in
in the amount of stain which then decreased to pre-prophylaxis values or slightly lower by the end of the experiment.

Brill\textsuperscript{27} found that chewing and other mechanical stimulation of the gingiva\textsuperscript{29,33} led to an increased flow of gingival fluid as measured by the intracrevicular method. This increased flow returned to normal 15 minutes following the stimulation.\textsuperscript{29,33}

On the other hand, L\öe and Holm-Pedersen\textsuperscript{120} found that chewing or cleaning of the tooth surface with a rubber cup did not lead to an increase in the flow of fluid from clinically healthy gingiva using their extracrevicular method. They also showed that gingival fluid was formed before clinical signs of inflammation were present and stated that this represented a state of subclinical inflammation.\textsuperscript{120}

Egelberg\textsuperscript{49} found that gingival fluid could not be detected from resting chronically inflamed tissue but the dento-gingival blood vessels were more susceptible to injury in this tissue. He also found that drying the tissues and inserting a paper strip into the gingival sulcus constituted an injury to the tissues which increased the permeability of the dento-gingival blood vessels.\textsuperscript{49}
With this in mind the results observed in part I and part II of the study can be explained. In part I the tissue was healthy and was not damaged by the rubber cup, therefore the permeability of the dento-gingival blood vessels was not increased and as a result, an increase in the flow of fluid was not noted. In fact, a decrease was noted, this may indicate that the cleaning of the tooth surface removed any material from that surface which may have contained stainable \( \alpha \)-amino acid, e.g. plaque, which would have altered the amount of stain on the strip.

However, following the accumulation of plaque (part II) the tissue may be in the state of subclinical inflammation as described by Løe and Holm-Pedersen.\(^\text{120}\) In this state the dento-gingival blood vessels would be more susceptible to injury\(^\text{49}\) and whereas in part I, the prophylaxis did not affect their permeability in this state, the trauma from the rubber cup was sufficient to cause an increase in the outflow of fluid.

Assuming that the rubber cup removed any source of stainable material from the tooth surface, this increase in permeability of the dento-gingival blood vessels was sufficient to lead to an increased score immediately following the prophylaxis. That by the end of the experiment the amount of stain was at pre-
prophylaxis values or slightly lower, may indicate that this increased permeability was maintained throughout the experiment, that is, assuming that the plaque present on the tooth surface pre-prophylaxis added to the amount of stain on the paper strip.

These results are in agreement therefore with the results of Loe and Holm-Pedersen\textsuperscript{120} and Egelberg\textsuperscript{49} but differ from those of Brill\textsuperscript{29,33} in as much as in the healthy state the mechanical cleaning of the tooth surface did not lead to an increase in the amount of stain. Also in the case of subclinical inflammation, the increased permeability of the dento-gingival blood vessels did not return to normal in 15 minutes as described by Brill\textsuperscript{29,33} but rather was maintained even after 45 minutes following stimulation.

However, these results differ from those of Loe and Holm-Pedersen\textsuperscript{120} on the point that in part I of the study where the tissues were healthy, stainable material was obtained on all strips prior to the prophylaxis and on 28 of 36 strips after the prophylaxis.

Loe and Holm-Pedersen\textsuperscript{120} state that clinically healthy gingiva does not exhibit a flow of gingival fluid. If this were so then we must assume that all the stainable material picked up on the filter paper strips prior to the prophylaxis in this present study, was due to the presence of that substance on the tooth surface. Therefore, following
removal of this substance from the tooth surface by means of a rubber
cup no stainable material should be picked up by the filter paper strips.
However, staining occurred on 28 of the 36 strips, now if this staining
was due to an increased flow of gingival fluid due to damage to the
dento-gingival blood vessels, thereby increasing their permeability,
it is difficult to explain the fact that 3 strips had absence of stain
immediately following the prophylaxis, of which one showed staining
at 15 and 45 minutes while the other two showed staining at 45 minutes.
And that of the two strips which showed absence of stain at 45 minutes,
both showed staining immediately following the prophylaxis while only
one showed staining at 15 minutes.

Even if these individual cases are explained in terms of
damage to the dento-gingival blood vessels and the removal and
reformation or incomplete removal of stainable material from the
tooth surface, the number of variables that exist with the technique
is apparent.

On the other hand, if these results are explained by the
presence of gingival fluid in the healthy state, the lack of refinement
of the technique is still apparent, in as much as it detects the presence
of fluid in some cases and not in others. Furthermore, the number
of different states of both the tooth surface and the gingiva in the
"clinically healthy" condition, add more variables which increase
the lack of refinement of the technique.
Therefore, the use of this technique to test the concept of the nature of gingival fluid put forward in part I of this thesis is questioned. In order to test the effect of the tooth surface on the measurement of gingival fluid part II(b) of the experiment was carried out.

In this experiment the filter paper strip did not contact the gingiva but was left short of the gingival margin by approximately 1 mm.

Prior to the prophylaxis, the amount of stain on the strips was similar to that in parts I and II, thus indicating that the placement of the strip in parts I and II did not damage the tissue, and thereby elicit a flow of gingival fluid. It also shows that the presence of stain on the strips may not be entirely due to the flow of gingival fluid.

Following the prophylaxis the amount of stain on the strips was lower than pre-prophylaxis values but nevertheless was still present. The general pattern of the stain was one extending along the edges of the strip with the main area of stain being at the tip (plates 23, 24 and 26).
fig. 12.1 The bathing of the tooth surface with gingival fluid and its subsequent absorption onto the filter paper strip 1 mm short of the gingiva.
Remembering that this experiment was carried out on those patients that had allowed plaque to accumulate on the tooth surface, the following explanation of these results is offered.

Prior to the prophylaxis the presence of plaque had a substantial influence on the staining properties of the strips. Immediately following the prophylaxis there was an increase in the permeability of the susceptible dento-gingival blood vessels with the subsequent increase in flow of gingival fluid. At this stage, all possible sources of stainable material had been removed from the tooth surface. However, with this flow of gingival fluid from the gingival sulcus the tooth surface itself became bathed in gingival fluid which, when a filter paper strip was placed on the tooth surface, was absorbed onto the strip, not only at the tip but also to a lesser extent at the sides of the strip (fig. 12.1).

That this may in fact take place is enhanced by the observation that the amount of stain differed on one side of the strip to the other depending upon which side was in contact with the tooth surface (plate 27).
That the tooth surface or at least substances on the tooth surface can alter the staining characteristics of the filter paper strips further highlights the lack of refinement of this technique. Furthermore, if gingival fluid can be absorbed from the sides of the strip as well as at the tip, any attempt to relate a measurement of length to amount of fluid would be made more difficult if not invalidate the relationship altogether.

When the amount of stain on each strip used in the present study was assessed quantitatively, it was found that 32% were unmeasurable, and of these unmeasurable strips 57% were from part II(b) of the experiment. This high proportion of unmeasurable strips further highlights the lack of refinement of this filter paper method of measuring gingival fluid. That most of these unmeasurable strips occurred in part II(b) indicates that this filter paper method is so unrefined that it would be unsuitable as a method to detect the presence of the small amounts of fluid theoretically present in the healthy gingival sulcus.

The results of the re-evaluation of every tenth strip showed that once a criteria had been established, accuracy of measurement within the bounds of that criteria was of a high order. However, as already stated, the validity of the measurement depends upon the validity of the criteria used to make that measurement.
The results of the statistical analysis carried out revealed that the differences between each part was statistically significant when the subjective score and the intensity score were used, but when the area of greatest intensity and the total area were measured, the differences were not statistically significant.

These results indicate that if a quantitative measure of the amount of stain is to be made in order to detect slight differences between two groups, the degree of intensity of stain must be taken into account.

Therefore, the criteria of measuring only the area of stain in order to detect differences between different groups is questioned.

The results of the present experiment showed that the relationship between the subjective score and the intensity score was statistically significant. A statistically significant relationship between the subjective score and the average area of greatest intensity as well as between the subject score and average total area was also shown.

These relationships were to be expected as the visual criteria set up to determine the subjective score was based on both the area and intensity of staining.
Although the intensity score alone showed statistically significant differences between each part of the experiment the reasons for these differences did not become evident until the area of staining was taken into account in the subjective score.

Therefore it appears that the most sensitive criteria to detect differences in amounts of staining would have to include both the area and intensity of stain.

The subjective score used in this study loses the complete objectivism of a purely quantitative measurement.

Therefore, a quantitative measurement of the area and intensity of stain would be desirable. This measurement should not only include the area of greatest intensity as was used in this study but also the areas of each grade of intensity. Since an almost infinite number of intensity grades would occur on the one strip an accurate measurement of the area of each grade would be impossible visually. Another method must therefore be found.

One such method could be the use of a travelling photo densitometer passing over the strip and the readings being recorded on a cathode ray oscilloscope. In this way, an accurate assessment of the area and intensity of stain may be made. This method would require the use of sophisticated equipment and its practicality is questionable.
Another, simpler method would involve the elution of the stain material from the filter paper strip and the resulting colour densities being compared using a spectrometer. Again this would be time consuming and its practicality is questionable.

Siegel et al.\textsuperscript{170} in an in vitro experiment found that the rate of movement of serum across filter paper strips depended upon the thickness of the filter paper and stated that a thin paper with a fast movement of fluid across it is the most desirable in measuring the flow of fluid. These workers\textsuperscript{170} also found that the use of an alcoholic solution of ninhydrin as opposed to a water solution, the use of heat to dry the strips and drying strips before staining all reduced the amount of diffusion past a set end point.

The present study which indicates that concentration gradient of stainable material exists along the strip would contradict the conclusion of Siegel et al.\textsuperscript{170} Siegel et al\textsuperscript{170} thought that by reducing the amount of diffusion that the measurement of length would more accurately reflect the volume of gingival fluid. In fact, by reducing the amount of diffusion the measurement of the length would probably be more accurate; however, this would certainly, because of the concentration gradient, not reflect the volume of fluid absorbed. By allowing maximum diffusion to take place the \(\alpha\)-amino acid front
would more closely coincide with the fluid front and a more even distribution of stainable material would occur. Therefore, if a filter paper method is used to measure the volume of gingival fluid it would be most desirable to use a filter paper with a fast flow rate and to allow maximum diffusion to take place between removal of the strip from the gingival sulcus and staining. However, care must be taken during staining not to wash the fluid along the strip.

The results of the present study show that the number of variables inherent to the filter paper, the technique, as well as to the state of the gingiva and the tooth surface, preclude the use of the Löe and Holm-Pedersen filter paper method as a method of detecting the presence of the minute amounts of fluid theoretically present in the healthy gingival sulcus.

Furthermore, the results cast doubt on the accuracy of previously accepted methods of quantitatively measuring the volume of gingival fluid and indicate that all reference to volume should be avoided. Reference should be made only to the amount of stain on the filter paper strips, where small volumes of fluid are involved.

The statistical analysis indicates that for these small amounts of stain a subjective visual criteria is more accurate than any of the quantitative measurements carried out, in detecting differences between different groups.
As only gingiva which was considered clinically healthy was used in this study, the results cannot be taken to be indicative of all gingival fluid measurements where both healthy and inflamed gingiva is involved. However, based on the present findings, care must be taken in establishing a criteria for the quantitative assessment of the amounts of stain on any filter paper strip.

Such a criteria would have to involve both the area and intensity of stain and as this would necessarily be time consuming and may require sophisticated equipment, the practicality of using such a system is questionable.

Again, because of the inherent variables, which may not be of such importance with large volumes but would still exist, complete accuracy would be impossible.

For these reasons, the quicker, more simple, clinical indices of gingivitis may be just as accurate as a gingival fluid index. Therefore, the use of a gingival fluid index as an epidemiological index of gingivitis may be unnecessary.
CHAPTER 13

CONCLUSIONS

From the review of the literature presented in part I of this thesis, the theory has been put forward by the author that in the healthy state gingival fluid is essentially interstitial fluid which because of the nature of the junctional epithelium, and epithelial attachment, escapes into the gingival sulcus, and that with inflammation the nature of gingival fluid changes to that of an inflammatory exudate.

In essence, the gingival fluid, whether in health or disease, should be considered as the fluid medium of the gingiva and gingival sulcus.

A study of the factors which affect the flow of gingival fluid and the known role of this fluid support this concept.

However, of paramount importance to the concept is the presence of gingival fluid in the healthy gingival sulcus.

The results of the present study have indicated that the number of variables which exist using the method of Loe and Holm-Pedersen, preclude the use of this method as a method of detecting the presence of the small minute volumes of fluid theoretically present in the healthy gingival sulcus.
These variables are due to the filter paper strip itself, the state of the gingiva and the tooth surface as well as to the general lack of refinement of the technique. These variables may not be of major importance in the detection of large volumes of fluid present with inflammation of the tissues; however, they are significant in the detection of the minute amounts of fluid in the healthy sulcus, even to the extent that they preclude the use of the technique itself.

Furthermore, the results of the present experiment have shown that in using a filter paper method to measure the flow of gingival fluid a concentration gradient of stainable material may exist along the filter paper. For this reason any reference to the volume of fluid absorbed should be avoided and reference only made to the amount of stain on the filter paper.

Any criteria established to quantitatively measure this amount of stain should include both the area and intensity of stain. The statistical analysis carried out in this study showed that small differences between groups could be more easily detected using a criteria which included both the area and intensity of stain as opposed to one which included area alone.
An objective quantitative measure of the area and intensity of stain would be difficult and time consuming and for these reasons it is suggested that the use of a gingival fluid index of gingivitis may be unnecessary as the simpler, quicker clinical indices may be just as accurate. This is in agreement with the conclusions reached by Wilson and McHugh but in disagreement with that reached by Egelberg.

Egelberg stated that despite the many variables that do exist, as no other quantitative method of measuring the degree of gingivitis exists, the use of gingival fluid measurements will probably remain of great value.

If gingival fluid measurements are to be of any value, based on the present findings, future work should be aimed at either refining the present techniques or at finding a new method to detect and measure its flow. However, the author believes that the greatest value of any new method to measure gingival fluid should lie in its ability to detect the presence of the minute amounts of fluid theoretically present in the healthy gingival sulcus, and so be able to test the concept of gingival fluid being the fluid medium of the gingiva and gingival sulcus.

If this concept is true, future work need not be concerned with the volume of fluid but rather should be aimed at obtaining a greater knowledge of its composition which would in turn lead to a greater
understanding of the biology of the healthy gingiva and gingival sulcus. In the inflamed state a greater knowledge of the composition of gingival fluid would lead to a better understanding of the mechanism and result of the inflammatory process and so lead to a better understanding of the pathogenesis of periodontal disease.

Furthermore, the present study indicates that gingival fluid leaving the gingival sulcus bathes the tooth surface. The role that this fluid plays in the formation of the acquired pellicle and the early colonisation by bacteria of this pellicle is not understood. Future work should be aimed at a greater understanding of this role which in turn would lead to a greater understanding of the formation of plaque. Once the formation of plaque is understood, it may be an easy matter to then prevent its formation.

Therefore, the present study has shown that a concept, based on an understanding of the present knowledge of gingival fluid and the nature of the epithelial attachment, cannot be tested with the most refined of the methods available at present to measure the flow of gingival fluid.

Doubt has been cast on the reliability of present quantitative methods of measuring gingival fluid and areas of future research concerning gingival fluid have been suggested.
SUMMARY

In part I of this thesis the literature was reviewed concerning the history, composition and formation of gingival fluid. A theory, which was based on this knowledge as well as on an understanding of the nature of the dento-gingival blood vessels and epithelial attachment, was presented. This theory was that the flow of gingival fluid reflects the permeability of the dento-gingival blood vessels and the permeability of the junctional epithelium. In the healthy state this flow is essentially interstitial fluid which, because of the nature of the epithelial attachment, can escape into the gingival sulcus and that with inflammation, the nature of the gingival fluid changes to that of an inflammatory exudate.

This led to the concept of gingival fluid, whether in health or disease, being considered as the fluid medium of the gingiva and gingival sulcus.

The literature concerning factors which affected the flow of gingival fluid was reviewed in light of this concept, as well as the literature concerning the role that gingival fluid played in the biology of the gingival sulcus in both health and disease.

The literature concerning the role of gingival fluid added support to the concept put forward by the author.
In part II of this thesis, the method of Loe and Holm-Pedersen was used in an attempt to detect and measure the presence of this minute amount of fluid in the healthy gingival sulcus, as predicted by the theory put forward in part I.

However, because of the number of variables which were found to exist with the method, the method was found to be unsuitable as a means of detecting minute amounts of fluid.

The results of the study furthermore cast doubt on the reliability of quantitative methods used to measure gingival fluid.

Areas of future research based on the concept presented in part I and the lack of refinement of the methods available at present, were suggested.
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Plates 3 - 32 and 34 - 42 have a magnification of x 15.
Plate 3. (a) and (b). Filter paper strips photographed allowing intensity and pattern of staining to be observed.

(Magnification x 15)
Plate 17. Staining on a series of strips obtained from part I of the experiment.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 18. Staining on strip remaining the same throughout all stages of part I of the experiment.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 19. Staining on strips from part II of the experiment showing a lower score after the prophylaxis than before the prophylaxis.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 20. Staining on strips from part II of the experiment showing increase in staining immediately following the prophylaxis with a similar staining pattern at 45 minutes as before the prophylaxis.

(a) before the prophylaxis;

(b) Immediately following the prophylaxis;
Plate 21. Staining on strips from part II of the experiment showing increase in stain following the prophylaxis which returned to pre-prophylaxis values at 45 minutes.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 22. Staining from part II showing increase in stain at the end of the experiment compared to that of pre-prophylaxis at the beginning of the experiment.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 24. Staining from part II(b) of the experiment.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 25. Staining from part II(b) of the experiment.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 25.  
(cont'd.)  
(c) 15 minutes following the prophylaxis;  
(d) 45 minutes following the prophylaxis.
Plate 26.  Staining from part II(b) of the experiment showing staining along the edges of the strips.

(a) before the prophylaxis;
(b) immediately following the prophylaxis;
Plate 27. Difference in staining pattern depending upon which side of the strip was in contact with the tooth surface.

(a) side not in contact with tooth surface;
(b) side in contact with tooth surface.
Plate 29. Stain from part II(b) of the experiment showing similar staining pattern as in part II seen in plate 28.

(a) before the prophylaxis;

(b) immediately following the prophylaxis;
Plate 30. Comparison between part I and part II of the experiment in the one patient who participated in both parts.

(a) part I - before the prophylaxis;
(b) part II - before the prophylaxis;
Plate 31. (a) and (b). Variations in staining intensities along the strip. Indicating concentration gradient of stainable material.
Plate 32. (a) and (b). Variations in staining intensities indicating concentration gradient of stainable material. Also showing stain extending along the edges of the strips.
Plate 33. (a) and (b). Results of the third part of the experiment. Variations in colour indicating concentration gradient of stainable material along the strip.
Plate 33. (c) and (d). Results of third part of experiment. Variation in colour indicating concentration gradient of stainable material along the strip.
Plate 33. (e) and (f). Results of third part of experiment. Variation in colour indicating concentration gradient of stainable material along the strip.
Plate 34. Showing similar areas of stain but of different intensities.

(a) light intensity;
(b) dark intensity.
Plate 35. (a) and (b) Strips with fibres predominantly parallel to the long axis of the strip.
Plate 36. (a) and (b). Strips with fibres predominantly perpendicular to the long axis of the strip.
Plate 37. (a) and (b). Strips with fibres along the edge being predominantly parallel to the long axis of the strip. Stain is seen to occur in these regions.
Plate 38. (a) and (b). Strips showing staining along the fibres of the filter paper.

Thus indicating the passage of fluid along these fibres.
Plate 39. Variation in width of strip.

(a) wider strip of width 1.57 mm.;

(b) narrow strip of width 1.42 mm.
Plate 40. Variations in staining patterns.

(a) stain occurring on corners of the strip;

(b) stain predominantly on one side.
Plate 41. Variations in staining patterns.

(a) dark stain in corners of the strip;

(b) band of dark stain across the bottom of the strip.
Plate 42. Variations in staining patterns.

(a) dark stain at bottom and along the edges of the strip;

(b) lighter stain extending along the edges of the strip.
Plate 43. Strip of magnification x 20 showing

(i) staining along edges;
(ii) staining along fibres;
(iii) Variations in intensity of stain indicating a concentration gradient of stainable material.