RELATIONSHIP OF SALIVARY CORPUSCLES TO ORAL DISEASES AND THEIR SIGNIFICANCE

BY

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INTRODUCTION

The recognition that saliva has some clinical meaning dates back into antiquity. Hippocrates was a representative man of the scholars who considered its value from animals licking their wounds. Since Kölliker (1850) found that there were leukocytes in the saliva, the studies in this field of salivary corpuscles have been extended by many workers, and voluminous literatures, though baffling, have been produced. But this problem still remains with open questions.

On looking back upon the history of the previous works which are related much to the author's present work, however, few are the reports regarding the reciprocal relationship between oral diseases and salivary leukocyte count, and the majority of these reports, which will be shown below, dealt with examinations on the fixed and stained saliva specimens.

Orban and Weinmann (1939) examined the cellular elements of the saliva from the subjects with or without dental carieses. Their observations on the smear preparations of a loopful saliva from the buccal fold of the mandible without scraping the surface of the mucous membrane resulted in the fact that in the saliva of the caries-immune there were relatively more epithelial cells and fewer leukocytes than in the caries-susceptible. Müller (1940) examined the leukocyte count in four groups: the caries-immune (gingivitis-active), the caries-immune (gingivitis-free), the caries-susceptible (gingivitis-active) and the caries-susceptible (gingivitis-free), obtaining the same morphological results as Orban, though the relationship between leukocytes and epithelial cells was different from that by Orban. And he claimed that the condition of the oral soft tissue affected the change of the number of leukocytes and epithelial cells more considerably than that of dental carieses. Kumc (1944) examined the saliva from patients of various ages with or without periodontal inflammation by means of salivary leukocyte calculation. And he concluded that the number of leukocytes in the saliva might not depend upon the existence of dental carieses but upon that of gingivitis, and the salivary leukocytes might be chiefly contained in the pus discharged from the crevices of the pyorrhetic gingivae, since the leukocyte count in the case with alveolar pyorrhea of inflammatory
type was the highest. His fixed and stained preparation of saliva was made from a loopful saliva in the lower layer of 0.5 c.c. of saliva which was centrifuged. Miki (1950) examined the centrifuged saliva from patients with gingivitis or pyorrhea by means of salivary calculation per c.mm. According to his report the number of salivary corpuscles was the highest in the case with gingivitis ulcerosa (12,400 salivary corpuscles), that in the case with gingivitis marginalis was the same as that in the case with healthy gingiva (3,340 corpuscles), that in the case with pyorrhea of atrophic type was the lowest (2,780 corpuscles), that in the case with pyorrhea of inflammatory type was 3,480 corpuscles which was slightly more than that in the case with healthy gingiva. Wright and Jenkins (1950) compared the salivary leukocyte count of the caries-immune with that of the caries-susceptible by means of a hemocytometer glass under a phase microscope, finding that no admitted difference existed as to the total leukocyte count between the two, while many intact leukocytes were found surviving in the saliva of the caries-immune compared with that of the caries-susceptible. The count of both intact leukocytes and broken ones was mentioned as the total leukocyte count. Thus they claimed an active phagocytosis of salivary corpuscles. Their method of saliva collection was to collect the saliva in the oral cavity for five minutes just after cleaning the teeth with a toothbrush.

So far as the author knows there is yet no description regarding the reciprocal relationship between diseases of the oral soft tissue and salivary leukocyte count by the aid of phase microscopy.

The purpose of the present work is to investigate the reciprocal relationship between the diseases of the oral soft tissue and salivary leukocyte count without disregarding the conditions of dental caries under a phase microscope by means of examining the fresh saliva specimens obtained from subjects with or without oral diseases.

**Preliminary Examinations**

From the literatures on salivary corpuscles by the previous workers the following facts can be readily seen. (1) There existed two reciprocally opposite schools on the correlation between the total number of leukocytes in the peripheral blood and that in the saliva: one school claimed some reciprocal changes between them while another school emphasized no correlation between them. The latter has been insisted by Stephens and Johns (1934), Orban and Weinmann (1939); the former has been supported by Isaacs and Danielian (1927) and Comroe (1934). (2) It is generally believed that no leukocytes are found in the saliva as it appears at the opening of the duct of salivary glands, as pointed out by Laquer (1913), Jassinowsky (1925), Isaacs and Danielian (1927), Orban and Weinmann (1937).

In order to examine which of these opinions is correct, both salivary and peripheral blood leukocyte counts were examined, and comparison of the counts was made between patients with osteomyelitis purulenta acuta in a serious con-
dition and those in a convalescing state. As the result no admitted changes were seen in the salivary leukocyte count while striking differences were observed on the peripheral blood leukocyte count.

Careful examinations were made by the author under a phase microscope on the saliva specimens from healthy individuals which were collected with a special apparatus at the opening of the duct of the parotid gland so as not to have it mixed with the saliva saved in the oral cavity. From this examination it became certain that no leukocytes existed in the saliva itself.

It is reasonable to investigate the correlation between the salivary leukocyte count and the diseases of the oral soft tissues only if the salivary leukocytes are the product of the inflammatory oral tissues, and the healthy oral mucous membrane never permits leukocytes to emigrate into the oral cavity. It is reasonless, meanwhile, to elucidate the correlation between the salivary leukocyte count and diseases of the oral soft tissues if the blood leukocytes should emigrate into the oral cavity freely through the healthy oral mucous membrane. Therefore, one more examination was made concerning the possibility of the emigration of leukocytes through the oral mucous membrane.

The salivary leukocyte count was examined on completely anodont babies, edentulous healthy adults and severely alveolar pyorrheic patients. From this examination it became clear that no leukocytes were found in all of the saliva specimens from completely anodont babies and very few in those from the edentulous adults while numerous leukocytes were found in those from alveolar pyorrheic patients, despite the fact that saliva has some power to attract leukocytes as stated by Dietz (1939)². Thus it was revealed that the blood leukocytes in general never emigrate into the oral cavity through the healthy oral mucous membrane.

**Material and Method**

The specimens of saliva examined were chiefly from the patients who visited the dental clinic of Kônodai Branch Hospital of Tokyo Medical and Dental University, partly from the pupils and students of Kônodai Branch School of Tokyo University of Education and from other healthy individuals.

There seems to be many factors which exert an influence upon the number of leukocytes in the saliva. And the number may vary during the day even in the specimens from the same individual. It may be influenced by diet, condition of oral or general health and also by physical or chemical changes in the saliva as pointed out by Watanabe (1951)¹. Furthermore, the method of collecting saliva seems to affect considerably the number of leukocytes in the preparation. In this investigation, therefore, the time of collecting the saliva specimens was limited regularly between meals in the forenoon and the patient was ordered to spit out the saliva on a watch glass before he rinsed the mouth. The specimen was then mixed thoroughly by a simple mechanical stirring device. One drop of thoroughly mixed saliva was then placed as quickly as possible on the counting chamber of a hemocytometer slide by the aid of a
pipette and a cover glass was placed over it. The counting chamber then was examined under a phase microscope. Only intact leukocytes were counted.

It was impossible to distinguish lymphocytes from polymorphonuclear leukocytes on Thomas's slide glass for hemocytometer under a lower magnification of a phase microscope, because the cover glass of Thomas's slide glass was too thick for high magnification.

The counting method adopted was designed to use 144 large squares—each equivalent to 1/25 of a square millimeter—obtaining the number of leukocytes per cubic millimeter by a simple calculation (Fig. 1, 2).

The saliva specimens of a suckling was obtained on a watch glass by sucking the saliva saved in the oral cavity with the aid of a pipette and then placing on the counting chamber by the same procedure described above. Since the saliva specimen of edentulous adults and that of completely anodont babies contained so few leukocytes that very often no leukocytes could be found in the counting chamber, the existence of leukocytes was followed by counting five additional hemocytometer slides prepared from the same saliva specimen.

Besides calculation some of the saliva specimens were observed in a living state under a higher magnification of a phase microscope and some were examined with a light microscope as fixed and stained preparations by Giemsa's method.

Results

The results of the present examination will be shown under the classification of the twelve following groups. Opinions on the classification of alveolar pyorrhea are baffling according to scholars. Therefore, a classification which is supported by a relatively great number of the current scholars is submitted: inflammatory type—a case with a predominant pus discharging symptom; mixed type—a case with inflammatory and degenerative symptoms; and atrophic type—a case accompanied by horizontal atrophy of alveolar bones.

1. Correlation between salivary leukocyte count and oral diseases without regard to the existence of dental carieses.

In the case with alveolar pyorrhea of inflammatory type the leukocyte count value was the highest and in the case free of diseases of the soft tissue it was the lowest. The minimum value (122 corpuscles) is probably due to the fact that one of the cases of this group has only thirteen remaining teeth (Table 1).

The case with gingivitis ulcerosa acuta showed a relatively low value compared with that of gingivitis marginalis chronica. However, only three cases with gingivitis ulcerosa acuta possessed complete gingivitis spreading to the entire jaw, while nine cases possessed incomplete gingivitis ulcerosa, in which the range of the inflammation was limited to one-fourth or one-half of the entire jaw (Table 1).
Table 1. Correlation between Salivary Leukocount and Oral Diseases without Regard to the Existence of Dental Caries.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value of salivary leukocyte count (average cases)</th>
<th>Value of salivary leukocyte count range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar pyorrhea (inflammatory type)</td>
<td>364 (22)</td>
<td>122-877</td>
</tr>
<tr>
<td>Alveolar pyorrhea (mixed type)</td>
<td>136 (57)</td>
<td>44-266</td>
</tr>
<tr>
<td>Gingivitis ulcerosa acuta</td>
<td>94 (12)</td>
<td>44-133</td>
</tr>
<tr>
<td>Gingivitis marginalis chronica</td>
<td>76 (99)</td>
<td>22-160</td>
</tr>
<tr>
<td>Alveolar pyorrhea (atrophic type)</td>
<td>66 (5)</td>
<td>55-88</td>
</tr>
<tr>
<td>Gingivitis aphthosa acuta</td>
<td>66 (1)</td>
<td>66-66</td>
</tr>
<tr>
<td>Cases with full teeth free from diseases of the soft tissues</td>
<td>29 (113)</td>
<td>22-88</td>
</tr>
</tbody>
</table>

2. Correlation between salivary leukocyte count and oral diseases of the oral soft tissue in case of the caries-free.

The leukocyte count value in this group was almost the same as in group I, though the value in each case was somewhat high compared with that in group 1 (Table 2).

Table 2. Correlation between Salivary Leukocount and Diseases of Oral Soft Tissues in Caries-free Cases.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value of salivary leukocyte count (average cases)</th>
<th>Value of salivary leukocyte count range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar pyorrhea (inflammatory type)</td>
<td>367 (9)</td>
<td>122-877</td>
</tr>
<tr>
<td>Alveolar pyorrhea (mixed type)</td>
<td>179 (10)</td>
<td>122-288</td>
</tr>
<tr>
<td>Gingivitis ulcerosa acuta</td>
<td>96 (4)</td>
<td>44-133</td>
</tr>
<tr>
<td>Gingivitis marginalis chronica</td>
<td>76 (26)</td>
<td>22-144</td>
</tr>
<tr>
<td>Gingivitis aphthosa acuta</td>
<td>66 (1)</td>
<td>66-66</td>
</tr>
<tr>
<td>Cases free from diseases of oral soft tissues</td>
<td>31 (29)</td>
<td>11-66</td>
</tr>
</tbody>
</table>

3. Correlation between salivary leukocyte count and diseases of the oral soft tissue in case of the caries-low-active.

“The caries-low-active” signifies cases with one or two dental caries. No admitted differences were seen between groups 2 and 3 (Table 2 and 3.)

Table 3. Correlation between Salivary Leukocount and Diseases of Oral Soft Tissues in Caries-low-active Cases.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value of salivary leukocyte count (average cases)</th>
<th>Value of salivary leukocyte count range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar pyorrhea (inflammatory type)</td>
<td>400 (9)</td>
<td>166-711</td>
</tr>
<tr>
<td>Alveolar pyorrhea (mixed type)</td>
<td>190 (30)</td>
<td>55-266</td>
</tr>
<tr>
<td>Gingivitis marginalis chronica</td>
<td>72 (32)</td>
<td>22-166</td>
</tr>
<tr>
<td>Alveolar pyorrhea (atrophic type)</td>
<td>66 (3)</td>
<td>55-88</td>
</tr>
<tr>
<td>Cases free from diseases of oral soft tissues</td>
<td>41 (36)</td>
<td>22-88</td>
</tr>
</tbody>
</table>
To the author’s regret, a case with gingivitis ulcerosa acuta was unable to be dealt with in this section, since in the materials no caries-low-active case simultaneously with gingivitis ulcerosa acuta was found.

4. Correlation between salivary leukocyte count and diseases of the oral soft tissue in cases of the caries-high-active.

“The caries-high-active” signifies cases with more than six dental carieses. Among all the cases examined a case with sixteen carieses was the highest in the number of carieses. No admitted differences were found between groups III and IV; the low leukocyte count value in the inflammatory alveolar pyorrheic cases deserves notice, though it is in the same range as that in groups 1, 2 and 3 (Table 1, 2, 3, and 4). It is no wonder that such a low value is present in this group, because only one case was found in the materials that could fulfil simultaneously both conditions: inflammatory alveolar pyorrheic and caries-high-active.

Table 4. Correlation between Salivary Leukocyte Count and Diseases of Oral Soft Tissues in Caries-high-Active Cases.

<table>
<thead>
<tr>
<th>Disease Description</th>
<th>Value of salivary leukocyte count</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar pyorrhea (inflammatory type)</td>
<td>177 (1)</td>
<td>177-177</td>
</tr>
<tr>
<td>Alveolar pyorrhea (mixed type)</td>
<td>150 (7)</td>
<td>88-244</td>
</tr>
<tr>
<td>Gingivitis marginalis chronicis</td>
<td>89 (11)</td>
<td>44-166</td>
</tr>
<tr>
<td>Cases free from diseases of oral soft tissues</td>
<td>37 (20)</td>
<td>22-100</td>
</tr>
</tbody>
</table>

5. Correlation between salivary leukocyte count and dental caries in cases free of diseases of the oral soft tissue.

Some admitted differences, though slight, were found among the three respective cases (Table 5). It seems that the more the number of the dental caries which belongs to the case, the higher becomes the leukocyte count value; however, it deserves notice that in the case with numerous dental carieses the remaining tooth root with a caries was often accompanied by a periodontal inflammation, even in a case free of diseases of the oral soft tissue. Thus it is understood to be probably due not to the dental caries itself but due to the decayed tooth root with periodontal inflammation (Table 5).

Table 5. Correlation between Salivary Leukocyte Count and Dental Caries in Cases free from Diseases of Oral Soft Tissues.

<table>
<thead>
<tr>
<th>Caries Type</th>
<th>Value of salivary leukocyte count</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free from caries</td>
<td>31 (29)</td>
<td>11-66</td>
</tr>
<tr>
<td>Caries-low-active</td>
<td>41 (36)</td>
<td>22-88</td>
</tr>
<tr>
<td>Caries-high-active</td>
<td>37 (20)</td>
<td>22-100</td>
</tr>
</tbody>
</table>
6. *Correlation between salivary leukocyte count and dental caries in cases with gingivitis marginalis chronica.*

On the whole there were no admitted differences to be seen among the three cases; however, such a relatively high value seen in the case of the caries-high-active as in group V is understood to be probably due to the presence of decayed tooth root with periodontal inflammation (Table 5 and 6).

Table 6. Correlation between Salivary Leukocyte and Dental Caries in Cases with Gingivitis Marginalis Chronica.

<table>
<thead>
<tr>
<th></th>
<th>Value of salivary leukocyte count average (cases)</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free from caries</td>
<td>76 (26)</td>
<td>22—144</td>
</tr>
<tr>
<td>Caries-low-active</td>
<td>72 (32)</td>
<td>22—166</td>
</tr>
<tr>
<td>Caries-high-active</td>
<td>89 (11)</td>
<td>44—166</td>
</tr>
</tbody>
</table>

7. *Correlation between salivary leukocyte count and dental caries in cases with alveolar pyorrhrea of mixed type.*

In the above group 5 the decayed tooth root with periodontal inflammation, which was often encountered, was considered likely of exerting some influence upon the salivary leukocyte count in the case of the caries-high-active. Nevertheless it should not be considered that such an interpretation may not hold true in the case of this group; even numerous dental carieses do not have entirely any relation with the salivary leukocyte count as seen in this group. Most of the cases in this group may not have possessed any decayed tooth root with periodontal inflammation in spite of the numerous dental carieses.

In a word, from the investigation on this group it seems to be clear that the dental caries never plays a part upon the increase of the number of leukocytes in the saliva (Table 7).

Table 7. Correlation between Salivary Leukocyte and Dental Caries in Cases with Alveolar Pyorrhrea of Mixed Type.

<table>
<thead>
<tr>
<th></th>
<th>Value of salivary leukocyte count average (cases)</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free from caries</td>
<td>179 (10)</td>
<td>122—288</td>
</tr>
<tr>
<td>Caries-low-active</td>
<td>160 (30)</td>
<td>55—266</td>
</tr>
<tr>
<td>Caries-high-active</td>
<td>150 (7)</td>
<td>88—244</td>
</tr>
</tbody>
</table>

8. *Correlation between salivary leukocyte count and dental caries in cases with alveolar pyorrhrea of inflammatory type.*

The presence of numerous dental carieses seems not to exert an admitted influence upon the value of salivary leukocyte count also in the case of this group as in that of group VII. However, definite conclusion may be difficult to be drawn from the cases of this group, since in the materials of the present work there was only one case which fulfilled both conditions: inflammatory
alveolar pyorrhoic and caries-high-active. It is due to this fact that most pyorrheic patients generally have good teeth while those with numerous dental caries seldom suffer from inflammatory pyorrhea (Table 8).

Table 8. Correlation between Salivary Leukocyte count and Dental Caries in Cases with Alveolar Pyorrhea of Inflammatory Type

<table>
<thead>
<tr>
<th>Category</th>
<th>Value of salivary leukocyte count average (cases)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free from caries</td>
<td>367 (9)</td>
<td>122–877</td>
</tr>
<tr>
<td>Caries-low-active</td>
<td>400 (9)</td>
<td>166–711</td>
</tr>
<tr>
<td>Caries-high-active</td>
<td>177 (1)</td>
<td>177–177</td>
</tr>
</tbody>
</table>

9. Correlation between salivary leukocyte count and oral diseases in cases with completely edentulous jaws free of oral diseases.

In cases with completely edentulous jaws but with no dentures put on no leukocytes were usually found. However, in the saliva from the cases with full dentures put on some leukocytes, though of a very small number, could be always found under careful examination (Table 9).

Table 9. Correlation between Salivary Leukocyte count and Oral Diseases in Cases with Completely Edentulous Jaws Free from Oral Diseases.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Value of Salivary leukocyte count per c.mm</th>
<th>Salivary leukocyte in a given 5 c.mm</th>
<th>With or without denture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>♂</td>
<td>42</td>
<td>0</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>2</td>
<td>♂</td>
<td>52</td>
<td>0</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>3</td>
<td>♂</td>
<td>55</td>
<td>11</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>4</td>
<td>♂</td>
<td>56</td>
<td>11</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>5</td>
<td>♂</td>
<td>60</td>
<td>0</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>6</td>
<td>♂</td>
<td>62</td>
<td>0</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>7</td>
<td>♂</td>
<td>62</td>
<td>0</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>8</td>
<td>♂</td>
<td>63</td>
<td>0</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>9</td>
<td>♂</td>
<td>64</td>
<td>0</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>10</td>
<td>♂</td>
<td>66</td>
<td>11</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>11</td>
<td>♂</td>
<td>69</td>
<td>0</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>12</td>
<td>♂</td>
<td>71</td>
<td>11</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>13</td>
<td>♂</td>
<td>71</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>14</td>
<td>♂</td>
<td>71</td>
<td>11</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>15</td>
<td>♂</td>
<td>78</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

10. Correlation between salivary leukocyte count and oral diseases in cases with incompletely edentulous jaws free of dental caries.

The case with alveolar pyorrhea of mixed type showed the highest leukocyte count value, that with gingivitis marginalis chronica the next and that
free of diseases of oral soft tissues the lowest. It was almost the same as in
those of groups 1 and 2. These values, however, were far low compared with
those in groups 1 and 2, due likely to the small range of inflammation (Table
1, 2 and 10).

Table 10. Correlation between Salivary Leukocyte count and Oral Diseases in Cases
with Incompletely Edentulous Jaws free from Dental Caries.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Oral disease</th>
<th>Value of salivary leukocyte per c.mm</th>
<th>Number of remaining teeth</th>
</tr>
</thead>
</table>
| 1    | ♂️  | 55  | pyorrhea
      |         | (mixed type)            | 88                                    | 6                         |
| 2    | ♂️  | 53  | do.                     | 66                                   | 7                         |
| 3    | ♂️  | 65  | do.                     | 55                                   | 7                         |
| 4    | ♂️  | 66  | do.                     | 55                                   | 9                         |
| 5    | ♂️  | 53  | gingivitis
      |         | (marg. chr.)            | 55                                   | 5                         |
| 6    | ♂️  | 43  | do.                     | 44                                   | 7                         |
| 7    | ♂️  | 40  | do.                     | 33                                   | 7                         |
| 8    | ♂️  | 34  | do.                     | 33                                   | 3                         |
| 9    | ♂️  | 52  | do.                     | 22                                   | 2                         |
| 10   | ♂️  | 63  | do.                     | 22                                   | 1                         |
| 11   | ♂️  | 58  | disease-free            | 22                                   | 9                         |
| 12   | ♂️  | 62  | do.                     | 22                                   | 4                         |
| 13   | ♂️  | 67  | do.                     | 22                                   | 2                         |
| 14   | ♂️  | 63  | do.                     | 11                                   | 6                         |
| 15   | ♂️  | 41  | do.                     | 11                                   | 7                         |

11. Correlation between salivary leukocyte count and oral diseases in cases with
complete anodontia free of oral diseases.

Fifteen cases of complete anodontia of two to seven months old, eleven
males and four females were examined. All the cases proved to have no leuko-
cytes in the saliva under repeated careful examinations. On the other hand,
in a completely edentulous case one or two leukocytes could be found always
in the saliva if further examination was made on several other slides from the
same saliva specimen even when a case was encountered where no leukocyte
was found in the saliva of a given slide. However, in the cases of this group
11 any favorable attempt did not turn out as a success in finding it.

12. Correlation between salivary leukocyte count and oral diseases in cases with
erupted tooth under the age of thirteen months.

Eleven cases of seven to thirteen months old were examined; seven male
and four female babies with erupted teeth, ranging from one to six. Cases
with many erupted teeth always showed a relatively high value, though the
value was under eleven leukocytes per c.mm, compared with those with a few
erupted teeth. In this group there were seven cases whose leukocyte count
value was zero. However, in all of these leukocytes could be found in the saliva by further careful examination of several other slides from the same specimen.

From the examination on groups 11 and 12 it seems to be clear that the beginning of the tooth eruption coincided with the appearance of salivary leukocytes in the oral cavity.

**Additional Experiments and Results**

From the clinical examination it was clarified that the appearance of leukocytes into the oral cavity coincided with the stage when the tooth eruption started, and at the same time it became doubtful if the leukocytes originated from the gingival crevice of the erupting tooth. Thus an observation was made on the hematoxylin-eosin stained sections from the rats before and after tooth eruption. From this observation it seemed that leukocytes appear into the oral cavity only by way of the unkeratinized epithelium which lies at the gingival attachment of the erupting tooth, since one could find unkeratinized epithelium in the gingival attachment of the erupting tooth while completely keratinized epithelium covered all the surface of the mucous membrane of the oral cavity of the rats with unerupted tooth.

From the present experiment it became clear that there is no correlation between the salivary leukocyte count and dental carieses; however, at the same time it became doubtful whether the salivary leukocytes have an active phagocytic property against the oral flora.

To clarify this point, each drop of the saliva specimen from the patients was placed as quickly as possible on the slide by the aid of a pipette and a cover glass was placed over it and sealed with fluid paraffin, and then observed under the phase microscope for a long period of time in order to prove bacterial phagocytosis at a temperature of 37°C.

Judging from the result of the observation on the living saliva preparation no phagocytosis was found. Meanwhile, the granules in the salivary leukocyte showed a brownian movement, which became more and more vigorous with the lapse of time, and at the same time the swelling of the leukocyte increased gradually and was finally followed by cell disintegration.

Since observation only by phase microscopy was thought to be somewhat hazardous, further morphological observation was carried out in regard to the leukocytes on the ten saliva specimens not only with the aid of Giemsa’s staining but also with peroxidase staining by Ritter and Oleson’s method.10

From the examination on these preparations it became clear that most of the cells presumably derived from lymphocytes gave positive peroxidase reaction. Though some of them possessed cytoplasm which was stained slightly reddish purple by peroxidase stain with hematoxylin post-staining, their nucleus was larger than that of large lymphocytes. Occasionally cells with pseudopodia, though the cytoplasm of which stained light red, were found, while sometimes cells with cytoplasm stained blue and whose peroxidase reaction was negative were encountered.
However, it is a difficult problem to be solved whether the peroxidase reaction-positive mononuclear cells were from lymphocytes or from polymorphonuclear leukocytes, since the cells which resembled the lymphocytes were always swollen by the influence of hypotonic saliva and possessed a larger nucleus than that of lymphocytes, and they always contained neutrophile granules which were never found in lymphocytes.

In order to search how neutrophile granules in mononuclear cells in the saliva appeared and how their positive peroxidase reaction were acquired, an experiment was made on the spleen of rats in vitro.

The spleen dissected from the rat was transferred to Gey's balanced salt solution and then cut into pieces. The roller tube method with cover-slip technique and the hanging drop method with Maximow's slide were used. Cultures were made in a medium consisting of equal parts of heparinized rooster plasma and extract derived from eight-day chick embryos. The nutrient fluid contained fifty per cent horse serum, forty-five per cent Gey's balanced salt solution and five per cent chick embryo extract. The initial pH of the fluid nutrient was approximately 7.7 as shown by phenol red at 1:50,000.

The experiment was made on two groups: in one group the nutrient fluid mentioned above was used while in another group a medium consisting of equal parts of saliva and Gey's balanced salt solution was used instead of the nutrient fluid mentioned above. Concerning the salivary medium the saliva collected directly by a special apparatus at the opening of the duct of the parotid gland was added to the equal amount of Gey's balanced salt solution and then purified by Seitz's sucking filtration apparatus.

Period of cultivation ranged from one to three days. Half of the preparations were fixed in the solution consisting of ninety-five per cent ethyl alcohol 90, thirty-seven per cent formalin 10 and 0.1N sodium hydroxide 1, and then stained by Ritter and Oleson's peroxidase reaction method with hematoxylin post-staining; the rest of the preparations was fixed and stained by Giemsa's method (Fig. 3).

No admitted difference was seen between the two groups except that in the saliva-added group the leukocytes were swollen and the cytoplasm was stained reddish purple: no aspect was visible revealing the mechanism of transition of peroxidase reaction in the lymphocytes. From this experiment it seems that transition of the peroxidase reaction of lymphocytes into positive cannot be acquired by the influence of saliva only.

Discussion

It was noted that there are physiologically irregular variations in the salivary leukocyte count even in the same individual at different times or diurnal fluctuation in the same individual, varying over a considerable range as pointed out by Watanabe and Hammerschlag. However, under a stable condition the individual salivary leukocyte count shows anyway a relatively constant value. This has been also demonstrated by Jassinowsky (1929). It deserves
notice that there are some discrepancies between the values from the author’s examination and those from Miki’s. However, it might probably be due chiefly to the fact that far more numerous leukocytes were collected actively than under the usual conditions, since in most cases the patient strongly sucked the oral mucous membrane in order to obtain quickly as much saliva as possible, and partly due to the very act of centrifugation of saliva. Because considerably many salivary leukocytes with broken cytoplasm, though the nuclei remained intact, were usually found in the preparations obtained by means of centrifugation.

It is a matter of question whether leukocytes could pass through the healthy oral mucous membrane. With due consideration regarding the movement of leukocytes by the aid of the reports by the previous workers, most of them except Recklinghausen (1863)\textsuperscript{15} and Renn (1912)\textsuperscript{16} admitted that only lymphocytes could emigrate through the epithelium of the mucous membrane, while the polymorphonuclear leukocytes could not under a normal state, even if they appeared in the mucous tissue from the capillary nets in order to pass through the epithelium under the influence of stimuli.

Describing in detail, furthermore, regarding the emigration of lymphocytes, there seems to be two schools: one is that by Gulland (1891)\textsuperscript{17}, Bloch (1899)\textsuperscript{18}, Briers-Görecke (1901)\textsuperscript{19}, and Lindt (1908)\textsuperscript{20} advocating the inability of the lymphocytes to emigrate through the epithelium, lacking the active power of emigration, though it it may be possible for them to emigrate passively under some conditions; another opinion is that by von Ribbert (1897)\textsuperscript{21}, Hirschfeld (1901)\textsuperscript{22}, Almkvist (1902)\textsuperscript{23}, Rosin and Bibelgeil (1902)\textsuperscript{24}, Gött (1908)\textsuperscript{25}, Klatsoho (1913)\textsuperscript{26}, Lewis and Webster (1921)\textsuperscript{27}, and Nishiyama (1931)\textsuperscript{28} claiming the ability of the lymphocytes to emigrate freely and actively through the epithelium. In fact tissue culture work by Maximow (1924)\textsuperscript{29}, Bloom and others (1927)\textsuperscript{30} has proven that lymphocytes are ameboid cells.

It is generally believed that the epithelium of the oral mucous membrane is considerably different from that of other organs, because the oral cavity is doomed to be placed under incessant mechanical and chemical stimuli from the outside. These stimuli, therefore, bring about the constant intercellular existence of lymphocytes in the epithelium of the healthy oral mucous membrane, as examined by von Ebner (1902)\textsuperscript{31} and Cattoni (1951)\textsuperscript{32}. The lymphocytes in such a situation may be able to appear in the oral cavity passing through the unkeratinized epithelium. Regarding this, it has been supported by Zachinsky (1954)\textsuperscript{33} that polymorphonuclear leukocytes are unable to emigrate through the oral epithelium so long as any ulcers do not exist in it, though there is no detailed description concerning the behavior of lymphocytes.

According to T. Kitamura (1957, 1958)\textsuperscript{34}, in the newborn human babies with no erupted teeth all the epithelia of the oral cavity are free from keratinization, while in the old edentulous the entire epithelium of the oral cavity is completely keratinized. In mature humans some portions of the oral epithelium still remain half keratinized, though that of the back of the tongue, hard palate and gingivae is completely keratinized. Therefore, the portions of the half keratinized epithelium may permit the emigration of lymphocytes into the oral
cavity under stimulation.

In spite of these pertinent theories, from the author’s examination it was revealed that neither lymphocytes nor polymorphonuclear leukocytes emigrate into the oral cavity through the healthy oral epithelium under a normal state.

There are, however, two further difficulties to be surmounted: one is the record insisting the emigration of the lymphocytes into the oral cavity from the follicles of the tongue and tonsils, as stated by Frey (1862), Recklinghausen (1863) and Weidenreich (1808); another is the author’s finding that no leukocyte was found in the oral cavity of completely anodont babies while relatively many leukocytes were found in that of babies with two or three erupted teeth. Regarding the latter it seems to be more reasonable to consider that the appearance of leukocytes is probably due to direct or indirect injuries of the opposite mucous membrane caused by the erupted teeth. Because from the author’s examination on the fixed and stained preparations from the rats before and after tooth eruption it became clear that one could find the unkeratinized epithelium of the gingival attachment in the rats with erupting teeth while completely keratinized epithelium covered all the surface of the mucous membrane of the oral cavity in the rats with unerupted tooth. Such a finding is, however, not a matter of significance, because in rats there exists unkeratinized epithelium in other portion of the oral cavity except that of the gingival attachment, and furthermore in man it is usual that the whole epithelium of the gingiva, even after tooth eruption, is still free from keratinization for the time being as demonstrated by Orban.

In cats, according to NishiYama, the development of the lymphatic germ center of the sublingual gland begins one year after birth. According to Flemming (1885) some of the lymphocytes are produced in the lymphatic germ center of the sublingual gland. However, the development of the lymphatic germ center of the sublingual gland in man remains still unknown. It can never be thought, therefore, that the lymphatic germ center of the sublingual gland in man will be completed before tooth eruption starts, even if it is applicable to the case of man that the lymphatic germinal center of the sublingual gland of the cat begins one year after birth. Therefore, the cells found in the oral cavity of human babies after tooth eruption might not be from the lymphatic organs but from the capillary nets in the submucosa.

However, the record insisting the emigration of the lymphocytes into the oral cavity from the follicles of the tongue and tonsils is somewhat hazardous, since the result of the author’s experiment proved that very few leukocytes existed in the saliva of the edentulous; and it has been supported also by Laquer’s experiment (1912) that bilateral tonsillectomy exerted no influence upon the number of leukocytes in the saliva.

It is likely to be true that numerous lymphocytes from the submucosa emigrate into the oral cavity with polymorphonuclear leukocytes through the injured portion of the oral epithelium; nevertheless it is generally almost impossible to find lymphocytes of a typical form in the saliva smear preparations from the patients with a severe oral disease by means of Giemsa’s staining.

According to Maximow (1925) and Lewis (1926) lymphocytes or
polymorphonuclear leukocytes are likely to transform themselves into mononuclear leukocytes.

Not only the observation by the phase microscope on fresh saliva from the oral cavity but also the examination by the light microscope on fixed and stained saliva preparations could generally catch neither the typical image of polymorphonuclear leukocytes nor that of lymphocytes as seen in blood smears. However, typical lymphocytes were occasionally found by the author in saliva preparations from dental and oral disease-free subjects, in spite of the descriptions by ORBAN and WEINMANN (1939), MÜLLER (1940) and KUME (1941) that lymphocytes are seldom found in the saliva. These typical lymphocytes in the saliva will probably be very fresh ones having appeared with blood in the oral cavity from the submucosa because they always gave a negative peroxidase reaction (Fig. 4). Besides these typical lymphocytes there existed some monocytes which were of medium size and shape between lymphocyte and polymorphonuclear leukocyte in saliva preparations. They mostly gave a positive peroxidase reaction, but occasionally negative (Fig. 5: a, b).

How should that fact be interpreted that such salivary monocytes always gave a positive peroxidase reaction and possessed neutrophile granules, though they resembled closely the swollen lymphocytes (Fig. 6)?

According to WEIDENREICH, STÖHR (1884) and HAMMERSCHLAG (1920) lymphocytes transform themselves into polymorphonuclear leukocytes in the saliva. And according to LAQUER, KAMMERER and MEYER (1909) polymorphonuclear leukocytes transform themselves into monocytes in the saliva. However, in the present experiment no aspect of transformation could be observed in the leukocytes in the saliva. The result of the author’s observation indicates that lymphocytes might perform transformation to acquire a positive peroxidase reaction not in the saliva but in the submucosa.

The fact that relatively many polymorphonuclear leukocytes were generally seen in the saliva while very few lymphocytes were encountered and yet numerous monocytes were always observed suggests that the transformation from lymphocytes into monocytes were performed very quickly while that from the polymorphonuclear leukocytes into monocytes were less quickly done. This coincides with the fact that the salivary corpuscles generally consisted of three types of cells: polymorphonuclear leukocytes with nuclei of strong segmentation, that with nuclei of weak segmentation and monocyte with neutrophile granules (Fig. 6, 7: a, b).

According to NISHIYAMA (1935) salivary corpuscles are not of blood origin but of lymphatic origin, and the same with the description by STÖHR (1884). It is, however, almost impossible to distinguish salivary leukocytes of blood origin from those of lymphatic origin under the microscope; it is easily understood from previous records how furiously it has been discussed whether the salivary corpuscles are derived from the blood or from the lymphatic organ by many a worker.

On the other hand, according to HARTWELL (1955) mononuclear blood cells, particularly the lymphocytes, provide the fiber-producing material in the repair of connective tissue in wound, since mitoses are not found generally in
the subepithelial areas while they are often encountered in the areas adjacent to the normal epithelium or to the extension membrane composed of out-growing epithelial cells in regard to the healing mechanism of the wound surgically made.

Carrel and Ebeling (1922) demonstrated by cell culture that mononuclear leukocytes could transform themselves into fibroblast-like cells. Also in in vitro culture Maximow (1927, 1928) and Bloom (1928, 1937) demonstrated the transformation of lymphocytes into granulocytes or fibroblasts. Furthermore, Lewis (1926) emphasized that polymorphonuclear leukocytes possessed the possibility of transformation into epithelial cells by rat blood culture. Andrew and Andrew (1949) described the possible transformation of lymphocytes into epithelial cells in the normal epidermis, while Cattoni (1951) reported that the lymphocytes in the epithelium seemed to have had increased in size compared with those in the connective tissue though he was sceptical about the conclusion as to the lymphocytes being transformed into epithelial cells.

Salivary monocytes seem to be primarily derived from lymphocytes infiltrating the oral submucosa, since they had always neutrophile granules which blood monocytes never possess.

Thus it is not altogether reasonless to think that the course which the lymphatic organs adjacent to the oral cavity follow as their fate will be displayed chiefly as infiltration in the submucosa contiguous to wounds which almost always exist in the oral cavity, while the course from within the lymphatic organ through its epithelium directly into the oral cavity, as insisted by Nishiyama, will be rather a special case.

Wright and Jenkins (1953) claimed active phagocytosis of salivary leukocytes in a comparative examination on the saliva specimens from two groups: the caries-active and the caries-free. They obtained the result that the count of intact leukocytes in the saliva from the caries-free was approximately four times greater than that from the caries-active while the count of total leukocytes showed no admitted difference between the two.

However, in regard to the condition of the oral soft tissue in each case it seems to be inadequate that the conclusion was obtained from the records of their experiment which were lacking in sufficient observation, though abundant in detailed descriptions on the aspects of the caries. Furthermore it remains open to discussion that the salivary leukocytes collected by their method from the saliva saved in the oral cavity for a short period of time just after the cleaning of the teeth with a toothbrush may be far different from the ordinary ones. Because it is evident that a great deal of blood components appeared possibly into the oral cavity as the result of the abnormal condition raised in the oral mucous membrane by an artificial stimulus as tooth brushing.

An active phagocytosis of salivary leukocytes has been emphasized not only by Wright and Jenkins but also by Appleton (1933), Bibby, Hine and Clough (1939), Hammond and Weinmann (1942) and Petzold (1952).

Provided that salivary leukocytes possess a strong phagocytosis and appear into the oral cavity with the purpose of diminishing the oral flora, as insisted by the previous workers, the existence of the dental caries must exert a con-
siderable influence upon the salivary leukocyte count in the oral cavity with inflammation or wound, but this was not the case.

However, the actual state of phagocytosis of salivary leukocytes is still unknown and has never been observed by any of the previous workers. The salivary leukocytes which were observed by the author under the phase microscope were generally swollen due to the hypotonic property of the saliva and in all the leukocytes the appearance of brownian movement of granules was observed. Therefore they are likely to be incompetent or extremely weak in phagocytosis, as stated by de Robertis (1955). WRIGHT and JENKINS' conclusion is lacking in the ground of having observed the very act of phagocytosis in a living state, in spite of their emphasis of phagocytosis. The phagocytosis of salivary leukocytes cannot be affirmed only by the reason that bacteria were found in some of the salivary leukocytes in the fixed and stained saliva preparations, though only such a method has been employed uniformly by all the previous workers (Fig. 7:a, b, c). We have no way to ascertain whether the bacteria were captured before or after the appearance of leukocytes into the oral cavity, in other words, in the inflammatory tissue or in the saliva saved in the oral cavity. From the cytological standpoint mentioned above it seems to be reasonable to think that the bacteria found in the salivary leukocytes might have been captured before they appeared in the oral cavity.

It seems, therefore, to be meaningless if the consideration had not been given to the comparison of the salivary leukocyte count between the caries-free and the caries-active, both of which are free from gingivitis, pyorrhea or stomatitis.

Regarding the reciprocal relationship between the salivary leukocyte count and oral diseases, the author's examination showed that the leukocyte count depended rather upon the condition of the oral soft tissue than that of the dental caries. This conclusion is almost the same as that by Kume (1941).

ORBAN, WEINMANN and MULLER described that there was some correlation between the salivary leukocyte count and dental caries; however, such a result could not be obtained from the author's present work.

Moreover, MULLER referred to the fact that numerous leukocytes were found in the saliva of a case with full dentures or with nearly full dentures while few leukocytes were found in that of babies. The author's experimental results, however, did not show at all such a view that numerous leukocytes existed in the saliva of the edentulous adult, though they coincided with his on the view that few leukocytes existed in the saliva of babies.

From the present work it is apparent that the appearance of leukocytes never occurs so long as any epithelial defect does not exist, since no leukocyte was found in the saliva of completely anodont babies. This fact suggests that the leukocytes having infiltrated under the oral epithelium partly with the purpose of preventing wounds from infection and partly with that of taking part in fiber production in wound repair fell into the oral cavity due to a weak attraction of saliva.

The power of the saliva to attract leukocytes was demonstrated by KARSNER
and Merrill (1929), describing that the emigration of leukocytes in a medium with a pH of normal saliva was weak, while it was strong in an alkaline medium; and this has also been supported by Hugenschmidt (1896) and Feringa (1922) and Dietz (1939).

Conclusion

These considerations from various viewpoints mentioned above make it reasonable to think that the salivary corpuscles are composed of mononuclear leukocytes with neutrophile granules, polymorphonuclear leukocytes and lymphocytes; their appearance in the saliva is likely due to the fact that the cells into which blood leukocytes in the oral submucosa had transformed themselves or having transformed themselves fell into the oral cavity through the defect of the oral epithelium due to the weak attraction of saliva.

The salivary corpuscles are incompetent or extremely weak in phagocytosis and the salivary leukocyte count value depends not upon the dental caries or uncleanliness of the oral cavity but upon the condition of the oral soft tissue, and it depends not upon the degree of inflammation under the oral mucous membrane but upon the condition of the epithelial loss caused by the oral disease or by the chemical or mechanical agent.

Summary

1. The salivary leukocyte count was examined with the aid of Thoma’s glass for hemocytometer under a phase microscope on the saliva specimens obtained directly from the oral cavity.
2. The specimens were collected from the individuals with or without oral disease ranging from anodont babies to edentulous adults.
3. The salivary leukocyte count showed the highest value in the case with benign alveolar pyorrhea; higher value in the inflammatory type and lower value in the mixed type, while the case with malignant alveolar pyorrhea, i.e. the atrophic type, showed a relatively low value. The case with gingivitis marginalis chronic showed a considerably high value, gingivitis acuta comparatively low; the case free of diseases of the oral soft tissue showed a very low value, while an edentulous adult extremely a low value and a completely anodont baby did not. It deserves notice that the case with osteomyelitis purulent acuta had almost the same value as that of the case free of disease.
4. No noticeable correlation was found between the salivary leukocyte count and the number of dental caries.
5. It seems to be interesting that the beginning of the tooth eruption coincided with the appearance of leukocytes in the oral cavity.
6. The salivary leukocyte count varies to some extent even in cases with the same disease after a variety of conditions such as individual quantitative discrepancy of salivary secretion, circumstances of diseases, etc. The number of leukocytes in the saliva, therefore, may play a supplementary rôle in diagnos-
ing the degree of the disease of the oral soft tissue, though it might not take a conclusive part.

7. With regard to phagocytosis and transformation of leukocytes in the saliva or in the nutrient media, further studies with continuous observation by cinematography technique will be needed.

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REFERENCES

3) Muller, A. H.: Salivary Cell Types in Gingivitis and Caries. Northwestern Univ. Bulle-
tin, 17: 19, 1940.
16) Renn, P.: Zur Funktionsfrage der Gaumenmandel. Cytodiagnostische und histopatho-

**EXPLANATION OF FIGURES**

Fig. 1, 2. Photomicrographs of hemocytometer preparations of saliva from a subject with alveolar pyorrhea of mixed type (Fig. 1) and from a subject free from dental and oral diseases (Fig. 2). Phase contrast microscopy ×100, reduced to 2.7/2.9

Fig. 3. Rat spleen in 3-day culture with saliva-added nutrient, Giemsa's staining ×1000

Fig. 4. Typical lymphocyte in saliva, Giemsa's staining ×1000

Fig. 5. Transforming patterns of lymphocyte in saliva, Peroxydase staining ×1000
   (a) Peroxydase reaction positive
   (b) Peroxydase reaction negative

Fig. 6. Monocytes in saliva, Peroxydase staining ×1000

Fig. 7. Polymorphonuclear leucocytes in saliva, Giemsa's staining ×1000
   (a), (c) Bacteria are found in cytoplasm
   (b) No bacteria are found in cytoplasm
**List of Abbreviations**

- s: salivary leukocyte
- l: lymphocyte
- n: neutrophile leukocyte
- m: monocyte