Studies on the Wandering Leucocytes in Oral Cavity, with Special Reference to the Myeloid Series

By

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With two Figures and two Tables

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1. Introduction

The wandering leucocytes in oral cavity are called salivary corpuscles, and it has long been believed that the salivary corpuscles are nothing but lymphocytes, and that the polymorphonuclear leucocytes in saliva are derived from the lymphocytes while infiltrating the epithelium or wandering in oral cavity. Even in the recent text-books of histology, the salivary corpuscles are often explained as lymphocytes. However, there is no convincing evidence of its transformation into the polymorphonuclear leucocytes after a definite differentiation has occurred.

On the other hand, Renn (1912), Laquer (1912, '13, '18), also in Japan Horii and co-workers (1947-1950), and Mitsui (1949) pointed
out distinctly that the predominating cells of salivary corpuscles are not the lymphocytes, but the neutrophilic leucocytes. According to Maximow's text-book of histology, the salivary corpuscles may originate partly from lymphocytes, partly from heterophil leucocytes.

These statements, however, are not always in accord with each other depending on the methods employed or the persons examined. Unlike the blood, the formed elements of the saliva cannot be determined accurately, though the salivary corpuscles are derived largely, but not exclusively, from the neutrophils. Furthermore, up to now it remains to be determined in what way these neutrophils become the wandering cells in oral cavity.

It is generally believed that the neutrophils are always found in saliva, though it is not yet clear whether the neutrophilic leucocyte is the only salivary corpuscle or whether other cells such as eosinophils, basophils, may appear in all cases. Therefore, it should be of prominent interest to examine all kinds of salivary corpuscles on as many persons as possible by means of various staining methods.

I have attempted here first to find out the various kinds of salivary corpuscles with dry films, next to see the degeneration of them with supravital staining, and finally to observe the salivary corpuscles of some mammals. While attempting to examine the formed elements of saliva, some peculiar technics were worked out, which seemed even more simple and valuable in observing the character of the cells than any other method; these are Nas-Benzidine reaction and Iodine reaction of the items 6 and 7.

These studies were carried out on 866 persons including 789 healthy young persons, 5 healthy adults and 72 sick young persons, whose oral cavities showed inflammations in slight degree. The healthy person means a man whose oral cavity showed no pathological change at external appearance. The staining methods and remarks concerning cytological technics are described in each item respectively.

2. Eosinophilic Leucocytes in Saliva.

Staining Method:—

1) Swab the throat (soft palate), and make smears on several slides. If there are no eosinophilic leucocytes in smear, it is necessary to repeat to swab the throat after several days.

2) Fix the smears by methylalcohol.

3) Stain with Delafield's hematoxylin solution for 5 to 10 minutes.
4) Wash in tap water for 2 to 5 minutes.
5) Wash in 1% HCl-Alcohol solution (1 per cent hydrochloric acid in 70 per cent alcohol) for 5 seconds (Romeis § 502,505).
6) Wash in tap water.
7) Stain with a 0.5% eosin solution in 70% alcohol for a few seconds.
8) Wash in tap water, dry and examine.

This method stains eosinophilic leucocytes in saliva more distinctly than Giemsa or May-giemsa stain.

Saliva smears were made on healthy 789 young persons and 5 adults. Percentage of eosinophils in blood, absolute number of eosinophils in all salivary corpuscles, and percentage of eosinophils in all salivary corpuscles were calculated. The results are shown in Table I and II.

The essential points of these Tables are as follows:

a) Percentage of eosinophilic leucocytes in all salivary corpuscles.
   The maximum is 33.96% (No. A 132 in Table II), and the minimum is 0.025% (No. A 15 in Table II). The salivary corpuscles were not counted when strongly degenerated by saliva.

   The total number of salivary corpuscles on the smears shows a range of from 5,000 to 5; the former corresponds to No. A 116 (Table II), the latter to No. A 76 (Table II). In the case of No. A 116 eosinophilic leucocyte is only one and all salivary corpuscles are 5,000; while in the case of No. A 76 eosinophilic leucocyte is one and all salivary corpuscles are only five.

b) Relation between blood and saliva concerning the eosinophilic leucocytes.

   The eosinophils could be demonstrated from saliva not only in cases of increase, but also in cases with normal value or decrease in the eosinophils of blood. The percentage of eosinophils in the blood of 104 cases in which the eosinophils were demonstrated in the saliva, is as follows.

<table>
<thead>
<tr>
<th>Percentage of eosinophils in blood</th>
<th>Number of cases</th>
<th>Average percentage of eosinophils in saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.0—30.0</td>
<td>5</td>
<td>5.87</td>
</tr>
<tr>
<td>29.9—20.0</td>
<td>12</td>
<td>1.55</td>
</tr>
<tr>
<td>19.9—10.0</td>
<td>19</td>
<td>1.16</td>
</tr>
<tr>
<td>9.9—4.0</td>
<td>39</td>
<td>0.72</td>
</tr>
<tr>
<td>3.9—0.2</td>
<td>28</td>
<td>0.90</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>—</td>
</tr>
</tbody>
</table>

(excepting one case in which the eosinophils in blood are 4.39%, in saliva 33.96%. This is No. A 132 in Table II.)
Tabelle I

\(a = 7-11\) years old, \(A = 12-15\), \(s = 16-20\), \(SS = 40\)

<table>
<thead>
<tr>
<th>No. of examined persons</th>
<th>Percentage of eosinophils in blood</th>
<th>Absolute number of eosinophils in all salivary corpuscles</th>
<th>Percentage of eosinophils in all salivary corpuscles</th>
<th>No. of examined persons</th>
<th>Percentage of eosinophils in blood</th>
<th>Absolute number of eosinophils in all salivary corpuscles</th>
<th>Percentage of eosinophils in all salivary corpuscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>a 40</td>
<td>12.7</td>
<td>3/200</td>
<td>1.50</td>
<td>a 343</td>
<td>4.2</td>
<td>1/60</td>
<td>1.66</td>
</tr>
<tr>
<td>a 82</td>
<td>40.0</td>
<td>5/120</td>
<td>4.16</td>
<td>a 348</td>
<td>1.8</td>
<td>1/30</td>
<td>0.33</td>
</tr>
<tr>
<td>a 92</td>
<td>10.0</td>
<td>1/400</td>
<td>0.25</td>
<td>a 350</td>
<td>9.8</td>
<td>5/30</td>
<td>1.66</td>
</tr>
<tr>
<td>a 99</td>
<td>6.7</td>
<td>2/280</td>
<td>0.71</td>
<td>a 370</td>
<td>7.2</td>
<td>1/100</td>
<td>0.10</td>
</tr>
<tr>
<td>a 107</td>
<td>2.4</td>
<td>1/200</td>
<td>0.50</td>
<td>a 383</td>
<td>9.5</td>
<td>4/100</td>
<td>0.40</td>
</tr>
<tr>
<td>a 118</td>
<td>5.8</td>
<td>1/400</td>
<td>0.25</td>
<td>a 285</td>
<td>28.3</td>
<td>2/200</td>
<td>1.00</td>
</tr>
<tr>
<td>a 119</td>
<td>22.0</td>
<td>1/400</td>
<td>0.25</td>
<td>a 387</td>
<td>4.5</td>
<td>1/200</td>
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</tr>
<tr>
<td>a 123</td>
<td>1.1</td>
<td>1/2500</td>
<td>0.04</td>
<td>a 397</td>
<td>2.7</td>
<td>5/500</td>
<td>1.00</td>
</tr>
<tr>
<td>a 137</td>
<td>3.6</td>
<td>1/3000</td>
<td>0.03</td>
<td>a 400</td>
<td>3.0</td>
<td>1/500</td>
<td>0.20</td>
</tr>
<tr>
<td>a 154</td>
<td>7.0</td>
<td>7/300</td>
<td>2.33</td>
<td>a 403</td>
<td>3.6</td>
<td>1/2000</td>
<td>0.05</td>
</tr>
<tr>
<td>a 158</td>
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<td>1/300</td>
<td>0.33</td>
<td>a 405</td>
<td>16.5</td>
<td>1/300</td>
<td>0.33</td>
</tr>
<tr>
<td>a 167</td>
<td>15.0</td>
<td>1/70</td>
<td>1.42</td>
<td>a 409</td>
<td>2.7</td>
<td>3/20</td>
<td>15.60</td>
</tr>
<tr>
<td>a 196</td>
<td>1.3</td>
<td>2/200</td>
<td>1.00</td>
<td>a 415</td>
<td>15.2</td>
<td>1/200</td>
<td>0.50</td>
</tr>
<tr>
<td>a 197</td>
<td>9.8</td>
<td>1/700</td>
<td>0.14</td>
<td>a 420</td>
<td>3.7</td>
<td>1/500</td>
<td>0.20</td>
</tr>
<tr>
<td>a 201</td>
<td>0.2</td>
<td>5/400</td>
<td>1.25</td>
<td>a 429</td>
<td>1.3</td>
<td>1/100</td>
<td>0.10</td>
</tr>
<tr>
<td>a 202</td>
<td>12.0</td>
<td>4/203</td>
<td>2.00</td>
<td>a 435</td>
<td>8.0</td>
<td>1/1000</td>
<td>0.10</td>
</tr>
<tr>
<td>a 215</td>
<td>0.4</td>
<td>4/700</td>
<td>0.57</td>
<td>a 439</td>
<td>0.8</td>
<td>1/300</td>
<td>0.33</td>
</tr>
<tr>
<td>a 220</td>
<td>9.2</td>
<td>1/1000</td>
<td>0.10</td>
<td>a 454</td>
<td>2.0</td>
<td>1/200</td>
<td>0.50</td>
</tr>
<tr>
<td>a 276</td>
<td>4.4</td>
<td>1/800</td>
<td>0.12</td>
<td>a 463</td>
<td>5.2</td>
<td>1/22</td>
<td>4.54</td>
</tr>
<tr>
<td>a 288</td>
<td>4.8</td>
<td>1/900</td>
<td>0.11</td>
<td>a 468</td>
<td>3.4</td>
<td>2/1000</td>
<td>0.20</td>
</tr>
<tr>
<td>a 297</td>
<td>4.6</td>
<td>6/300</td>
<td>2.00</td>
<td>s 150</td>
<td>21.0</td>
<td>8/100</td>
<td>8.00</td>
</tr>
<tr>
<td>a 301</td>
<td>3.7</td>
<td>2/400</td>
<td>0.50</td>
<td>s 152</td>
<td>17.5</td>
<td>2/1000</td>
<td>0.20</td>
</tr>
<tr>
<td>a 304</td>
<td>22.0</td>
<td>1/25</td>
<td>4.00</td>
<td>s 32</td>
<td>14.4</td>
<td>2/1000</td>
<td>0.20</td>
</tr>
<tr>
<td>a 310</td>
<td>14.0</td>
<td>1/2000</td>
<td>0.05</td>
<td>s 94</td>
<td>1.2</td>
<td>1/1000</td>
<td>0.10</td>
</tr>
<tr>
<td>a 313</td>
<td>5.3</td>
<td>1/1500</td>
<td>0.06</td>
<td>SS 151</td>
<td>4.2</td>
<td>2/400</td>
<td>0.50</td>
</tr>
<tr>
<td>a 329</td>
<td>0.7</td>
<td>1/650</td>
<td>0.15</td>
<td>A 2</td>
<td>27.5</td>
<td>1/1000</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Tabelle II

(A = 12-15 years old)

<table>
<thead>
<tr>
<th>No. of examined persons</th>
<th>Percentage of eosinophils in blood</th>
<th>Absolute number of eosinophils in all salivary corpuscles</th>
<th>Percentage of eosinophils in all salivary corpuscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 4</td>
<td>18.0</td>
<td>1/50</td>
<td>2.00</td>
</tr>
<tr>
<td>A 10</td>
<td>4.9</td>
<td>10/1000</td>
<td>0.50</td>
</tr>
<tr>
<td>A 11</td>
<td>22.5</td>
<td>5/1000</td>
<td>0.20</td>
</tr>
<tr>
<td>A 13</td>
<td>3.9</td>
<td>2/1000</td>
<td>0.20</td>
</tr>
<tr>
<td>A 14</td>
<td>3.7</td>
<td>2/1000</td>
<td>0.025</td>
</tr>
<tr>
<td>A 15</td>
<td>1.7</td>
<td>1/4000</td>
<td>6.00</td>
</tr>
<tr>
<td>A 17</td>
<td>13.8</td>
<td>3/50</td>
<td>1.60</td>
</tr>
<tr>
<td>A 21</td>
<td>17.9</td>
<td>8/500</td>
<td>0.03</td>
</tr>
<tr>
<td>A 22</td>
<td>13.0</td>
<td>1/3000</td>
<td>0.10</td>
</tr>
<tr>
<td>A 27</td>
<td>5.8</td>
<td>1/1000</td>
<td>0.08</td>
</tr>
<tr>
<td>A 31</td>
<td>7.5</td>
<td>4/5000</td>
<td>0.20</td>
</tr>
<tr>
<td>A 36</td>
<td>15.5</td>
<td>1/500</td>
<td>0.20</td>
</tr>
<tr>
<td>A 38</td>
<td>1.5</td>
<td>2/1000</td>
<td>0.08</td>
</tr>
<tr>
<td>A 40</td>
<td>4.3</td>
<td>1/1200</td>
<td>0.30</td>
</tr>
<tr>
<td>A 42</td>
<td>1.8</td>
<td>3/1000</td>
<td>5.00</td>
</tr>
<tr>
<td>A 43</td>
<td>30.1</td>
<td>5/100</td>
<td>1.20</td>
</tr>
<tr>
<td>A 44</td>
<td>9.5</td>
<td>12/1000</td>
<td>0.10</td>
</tr>
<tr>
<td>A 47</td>
<td>33.1</td>
<td>1/1000</td>
<td>1.20</td>
</tr>
<tr>
<td>A 67</td>
<td>5.2</td>
<td>3/250</td>
<td>1.00</td>
</tr>
<tr>
<td>A 72</td>
<td>5.3</td>
<td>1/100</td>
<td>1.00</td>
</tr>
<tr>
<td>A 76</td>
<td>40.0</td>
<td>1/5</td>
<td>20.00</td>
</tr>
<tr>
<td>A 79</td>
<td>20.0</td>
<td>4/650</td>
<td>0.61</td>
</tr>
<tr>
<td>A 85</td>
<td>17.7</td>
<td>1/150</td>
<td>0.66</td>
</tr>
<tr>
<td>A 89</td>
<td>29.9</td>
<td>1/50</td>
<td>0.66</td>
</tr>
<tr>
<td>A 92</td>
<td>30.1</td>
<td>1/800</td>
<td>0.12</td>
</tr>
<tr>
<td>A 94</td>
<td>8.6</td>
<td>3/100</td>
<td>3.00</td>
</tr>
</tbody>
</table>
Therefore, I think that the more eosinophils are found in blood, the more the eosinophils can be demonstrated in saliva, namely, there is a rough, if not complete, parallelism between them. However, I wish especially to point out that the eosinophils can often be demonstrated in saliva, though the percentage of eosinophils in blood is below 3.9 (see Table I, II).

c) The leucocytes counts of the blood indicated normal values even in the cases where the eosinophils were found in saliva; therefore, the absolute counts of eosinophils in blood did not differ from the normal value.

d) The granules of eosinophilic leucocyte in saliva could be stained red by eosin, orange yellow by Orange G, and yellow by picric acid.

e) Further experiments were planned to observe the degeneration of eosinophils in saliva by adding one drop of fresh bloods to five drops of clearly centrifuged saliva, of which pH value was 7.3. Smears were made of this mixture. The test showed that the cytoplasm and nucleus degenerated in one hour, while the eosinophilic granules well stained with eosin even degenerated in twenty-four hours. It is of interest that even when the cell suffers such toxic injury leading to nuclear fragmentation, the granules appear unchanged.

f) Bacteria in the eosinophilic leucocytes in saliva.

In saliva, bacteria can be found very often in the neutrophils; while in the eosinophils bacteria seldom appear. Out of 866 cases including sick persons, I found two cases in which the cocci in small numbers appeared in the eosinophils in saliva. Such an eosinophilic leucocyte was demonstrated only one in each of these two cases. It is said that the motility and phagocytosis of eosinophils are less active than neutrophils, but I cannot determine whether this phenomenon of the eosinophils on saliva smears could be accepted as absolute phagocytosis.

g) As above described, eosinophils in saliva were demonstrated in many healthy persons. I, however, must admit that the eosinophils could not always be demonstrated in healthy persons, viz., out of 794 healthy persons, 104 were positive and 690 were negative. Some of the positive 104 persons had no eosinophilic leucocytosis in blood, while some of the negative 690 persons had distinct eosinophilic leucocytosis in blood. And in the cases where the eosinophils were seen in saliva, the total number of salivary corpuscles was not always higher than that of general healthy persons. I found one extreme case (No. a 409 in Table I), in which the percentage of eosinophils in blood was 2.7, total number of salivary corpuscles on smear was only 20 and as many
as three of them were eosinophils.


Staining Method:—
1) Swab the throat (soft palate) and make smears on several slides.
2) May-Giemsa staining in the usual manner.
3) Differentiate from other cells.
   i) Freifeld's method. (Fuchsin, Methylene blue).
      Toxic granules are stained with this, while basophilic granules are not.
   ii) One per cent hydrochloric acid in 70 per cent alcohol.
      Cocci in leucocyte are insoluble, while basophilic granules are soluble.

Renn (1912) already described that the mast cells and plasma cells could be found in the crypts of palatine tonsil, but not in saliva.

I examined 866 cases of both healthy and sick persons to find out the basophils in saliva. Out of these 866 cases I happened to find two cases where the basophils were seen in saliva. Their palatine tonsils had been removed and they showed slight inflammations in the oral cavities. Besides this, the eosinophils were also found in saliva in both cases.

The results of the two cases are as under:—

Case I. (7-year-old girl)

a) Per cent of basophils in blood ............................. 0.8
b) Per cent of basophils in saliva ............................. 0.015
c) Absolute number of all salivary corpuscles on smears... 26,000
d) Absolute number of basophils on saliva smears ......... 4
e) White count per cmm. in blood ............................ 6,600
f) Average number of nuclei of neutrophils in blood ..... 2.37

Case II. (15-year-old boy)

a) Per cent of basophils in blood ............................. 1.56
b) Per cent of basophils in saliva ............................. 0.026
c) Absolute number of all salivary corpuscles on smears... 7,800
d) Absolute numbers of basophils on saliva smears ........ 2
e) White count per cmm. in blood ............................ 5,300
f) Average number of nuclei of neutrophils in blood ..... 1.99

Showing such activity as to wander into oral cavity, the basophils may possess a somewhat motility, though in slight degree.

I, however, could not find again the basophils and eosinophils in
saliva after the patients recovered from the inflammations in the oral cavities.

4. Monocytes.

Staining method is the same as in case of basophils. It is an absolute fact that some monocytes may occur in saliva, but not in all cases. In some cases monocytes occur in small numbers; while in other cases they are entirely absent. I examined 43 healthy young persons and 77 sick young persons with inflammations in mild degree of oral cavities to find out the monocytes from saliva. The percentage of monocytes in all salivary corpuscles is as under:

<table>
<thead>
<tr>
<th>Healthy</th>
<th>Sick</th>
</tr>
</thead>
<tbody>
<tr>
<td>39 persons</td>
<td>56 persons</td>
</tr>
<tr>
<td>4 persons</td>
<td>20 persons</td>
</tr>
<tr>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>10%</td>
<td></td>
</tr>
</tbody>
</table>

Thus, the percentage of monocytes in saliva ranges 0–1.5, excepting one case of 10%. Most of other salivary corpuscles on smears are neutrophils.

There was no marked monocytosis in blood even in cases where the monocytes were found in saliva. The nuclei of monocytes in saliva are round, oval or horse-shoe shaped, and take a pale bluish violet stain. By May-Giemsa stain, the cytoplasm is deep blue in heavily stained smears, and by Freifeld's fuchsin methylene blue stain, it is dark brown. The margins of the monocytes are thin or often irregular, and occasionally one or more large non-refractile vacuoles may appear in cytoplasm. The monocyte with phagocytic activity for bacteria was not seen in all cases.

Sometimes questionable epithelium cells may occur, which are so small that it is difficult to distinguish them from monocyte by means of May-giemsa stain. In such a case, the Iodine-reaction and peroxidase stain are recommended as later described. In Iodine-reaction, the monocyte stains less intensively than epithelium cells of oral cavity, and by peroxidase stain, no positive granule occurs in epithelium cells.

These results suggested to me that the monocytes may often appear in saliva when the inflammation is observed in oral cavity.
5. Lymphocytes in Saliva.

As already stated, it has long been believed that the salivary corpuscles are nothing but the lymphocytes in saliva, and that the granular leucocytes in saliva are the cells which are derived from the lymphocytes while infiltrating the epithelium or wandering in the oral cavity.

But as far as the wandering cells in oral cavity are concerned, this theory is denied to-day by many investigators, because most of the cells are practically the neutrophils which can be determined by special staining method.

I, however, cannot think that the lymphocytes are entirely absent in saliva. Indeed, the neutrophilic leucocyte is not the only salivary corpuscle. We can often find the lymphocytes infiltrating the epithelium of oral cavity, especially that of palatine tonsils, and they can be occasionally demonstrated in saliva smears, though in small numbers as compared with neutrophils. However, the cases where only neutrophils occur in saliva smears, are not unusual.

I examined 41 healthy and 53 sick young persons to find out the lymphocytes in saliva. In both healthy and sick persons, about one-seventh of them have no lymphocyte, while six-sevenths have the lymphocytes with figures below 0.4 or above 17.0 per cent besides neutrophils.

By May-giemsa stain, the cytoplasm of lymphocyte is deep blue in heavily stained smears, and by Freifeld's fuchsin methyleneblue stain, it is dark brown. The lymphocyte shows no granule with peroxidase stain, whether it may be fresh or degenerated in saliva.

It is clear that most of the salivary corpuscles are not the lymphocytic series, but the neutrophils which can be easily determined by peroxidase stain as later described.


As above described, the lymphocytic and monocytic series may appear occasionally in saliva; while the myeloid series, excepting eosinophils and basophils, are always found in saliva in large numbers. The percentage of neutrophils in all salivary corpuscles is often one hundred, or eighty-three at least.

The neutrophils can be determined by May-giemsa stain unless they are degenerated by saliva. However, if the neutrophils are degene-
rated and the nuclei become mononuclear or the vacuoles appear in the cytoplasm, it is difficult to distinguish them from other cells. In such cases the peroxidase stain is of great value because the lymphocytes never show granules with the stain; while the myeloid and monocytic series can be stained unless strongly degenerated.

For this peroxidase stain I used Nas-Benzidine method, which has already been published by Mitsui and myself at the 55th annual Japanese Anatomical Society, July, 1950.

The staining method is as follows:

1) Preparation of Nas-Benzidine solution (pH=5.8)

\[
\begin{align*}
\text{Neutral distilled water} & \quad \ldots \quad 100 \text{ cc.} \\
\text{Benzidine (E. Merck)} & \quad \ldots \quad 0.5 \text{ Gm.} \\
3 \text{ per cent hydrogen peroxide} & \quad \ldots \quad 10 \text{ gtt. (Sahli)} \\
1 \text{ per cent aqueous solution of Nickel-Ammonium Sulphate} & \quad \ldots \quad 5.26 \text{ cc.}
\end{align*}
\]

This mixture will keep for a long time if stored in the dark.

2) The fresh dry saliva smear is covered with the Nas-Benzidine solution for 3 to 5 minutes. (If blood smears, 45 seconds-2 minutes).

3) Pour off the Nas-Benzidine solution and wash gently in water.

4) As counterstain carbolfuchsin or safranine red may be used.

5) The staining must be carried out at room temperature.

By this stain the myeloid and monocytic series show bluish granules in the cytoplasm. A majority of salivary corpuscles can be stained by this, because they are neutrophils in saliva. But the peroxidase positive granules vary in size, form and color. If fresh neutrophils, the cytoplasm is full of fine or large deep blue granules, and in proportion as the degree of degeneration the granules become coarser, not uniform in size, fewer in number, less well defined, mixed with brown granules, though they are myelogenous.

Further experiments were planned to determine the influence of saliva on the neutrophils by adding five parts of centrifuged saliva to one part of the oxalated blood in test tube. The results showed that the peroxidase positive granules in the cytoplasm disappeared entirely after three hours; while the controls, in which the physiological salt solution was added to the blood in the same proportion as in case of saliva, showed the distinct positive granules even after three hours. The pH value of saliva in test tube was 7.3.

In some saliva smears I could find fresh lymphocytes in small numbers, which were determined by this stain. Therefore, this sug-
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gested to me also that the neutrophilic leucocyte is not the only salivary corpuscle.

Fig. 1. Nas-Benzidine reaction of salivary corpuscles (neutrophils).
\( n = \text{nucleus}, \ g = \text{bluish peroxidase positive granules}, \ v = \text{vacuoles indicating degeneration of the cell.} \)

7. Iodine Reaction of Salivary Corpuscles.

The iodine is a non-metallic element, which, when heated to 114°C, melts, giving off violet colored vapors. The liquid boils at 183°C. This violet colored vapors were applied to stain the neutrophils, which were the predominating cells of salivary corpuscles.

About 0.4 Gm. of iodine is slowly heated in a heat-resisting Petri dish, which is soon saturated with the violet colored iodine vapors, then put the smears into the dish and let stand about twenty minutes. But it is necessary to stain the nuclei previously with hematoxylin or safranine red solution before iodine reaction, since the iodine vapors are not enough to identify the neutrophils and distinguish them from other cells. For the purpose of staining nuclei, hematoxylin seems to be better than safranine red, while fuchsin and methylenblue are entirely unfit for it. The iodine reaction should be carried out at 100°–120°C of Petri dish, and the extreme care must be taken so that the iodine vapors are always slowly volatilizing in the dish.

By this iodine stain, the normal neutrophils in blood show a homogenous yellow or light brownish tinge in the cytoplasm. If the smears of blood are too thick, the cells show a little darker brown tinge. In cases of saliva, neutrophils are variously stained by iodine vapors. Most of the neutrophils in saliva show the same color as in blood, while some of them may show the following results:

a) Cytoplasm with homogenously dark brown tinge,
b) Large or small brown granules existing in the center of cytoplasm.

c) Fine brownish granules at the extreme margins of the cells.

d) Brownish cytoplasm around vacuoles in cytoplasm.

e) Fine brownish granules around the nucleus.

f) Less intensely stained cytoplasm.

I suppose these results will indicate more or less degenerations of neutrophils in saliva, and in what way these granules are seen remains to be determined.

Some experiments were carried out in order to examine the influence of saliva on the neutrophils. Five parts of centrifuged saliva were added to one part of the oxalated blood, and the results showed that the neutrophils indicated neither iodophilic granules nor yellowish cytoplasm when the cells were strongly degenerated by saliva. The pH value of saliva in test tube was 7.3.

8. Supravital Staining With Neutral Red and Janus Green of Salivary Corpuscles.

It has generally been confirmed that the form of leucocyte in saliva differs from that in blood, and that the form in dry film also differs from that in supravitaly stained film.

The salivary corpuscles were supravitally stained with the mixture of neutral red and Janus green. First, prepare a saturated solution of neutral red and Janus green in absolute alcohol separately. For use
add 40 drops of the saturated solution of Janus green and 4 cc. of the saturated solution of neutral red to 10 cc. of absolute alcohol. This solution of combined stains is a little thicker than those of staining leucocytes in blood.

Prepare slides with solution of the combined stains in the usual manner. After gargling thoroughly a drop of fresh saliva is placed in the center of a cover slip. With this cover slip mount the saliva on the slide as in making an ordinary fresh preparation, next rim with vaseline in order to prevent evaporation. Examine this within 15 to 20 minutes with the oil lens.

The supravital staining of salivary corpuscles showed the following results on 7 persons.

a) Only neutrophils were seen in saliva.
b) The forms of fresh neutrophils are generally round, and the cytoplasm contains fine granules which are less intensely stained with neutral red and show Brownian movement.
c) The nuclei are polymorphonuclear or mononuclear, although the cells are not degenerated. The nuclei do not stain at all unless degenerated by saliva.
d) The granules of neutrophils in saliva do not seem to stain as well as in blood, although they are fresh.
e) In proportion as the cells are degenerated, the cytoplasm becomes swollen, showing an irregular margin and fragments, and no Brownian movement of granules is seen.
f) No cell with distinct mitochondria, which can be stained with Janus green such as histiocytes and others, was found in all cases.
g) If strongly degenerated, the nuclei stain brown or bluish brown with neutral red or Janus green.
h) Brownian movements of granules are seen even in the case in which the nucleus was brownish stained with neutral red, while in the case in which the nucleus became bluish stained with Janus green, the granules never show Brownian movement.

The classification of these cells was already planned by Nishimura and Morikawa. For the purpose of morphological classification of salivary corpuscles, this supravital staining is more valuable than using dry film, because the latter causes often an artificial deformation of the cells.

I studied on the saliva of horse, rabbit, pig, dog, cat and raccoon-dog in order to see what kind of blood cells would appear in saliva, and it was confirmed that the predominating cells in the saliva were neutrophils or pseudoeosinophilic leucocytes. These cells were also examined with peroxidase stain.

1) Horse. In one case only neutrophils were seen.
2) Rabbit. In all cases of three rabbits only pseudoeosinophilic leucocytes were seen.
3) Pig. In one case most of salivary corpuscles were neutrophils, and the eosinophils were also found though in smaller numbers. The percentage of eosinophils in blood was 2.9. In another case only neutrophils were seen.
4) Dog. In one case the neutrophils were seen in large numbers (99.9%), while the eosinophils were only 0.1%. The percentage of eosinophils in blood was 6.9. In another case only neutrophils were seen.
5) Cat. In one case only neutrophils were seen.
6) Raccoon-dog. In one case only neutrophils were seen.

10. Summary and Conclusion.

The results of these studies made on 866 persons and some mammals are concluded as follows:

1) These 866 persons included 789 healthy young persons, 5 healthy adults, and 72 sick persons whose oral cavities indicated inflammations in mild degree. Excepting supravital staining, dry films were prepared from all cases.

2) The predominating cells of salivary corpuscles were neutrophils in both healthy and sick persons. However, the neutrophilic leucocyte is not the only salivary corpuscle, namely, other blood cells such as lymphocytes, monocytes, eosinophilic, and basophilic leucocytes, may be found in saliva if examined precisely.

3) The eosinophils in saliva were found in 104 persons (13.1%) out of 794 healthy persons, and I have made it clear that there was a rough, if not complete, parallelism between blood and saliva concerning the percentage of eosinophils. However, eosinophils may often appear in saliva, although the percentage of blood eosinophils is below 3.9 and
the total number of salivary corpuscles is not higher than that of general healthy persons.

4) Out of 866 cases, I happened to find two cases of young persons in which the basophilic leucocytes were found in saliva, though in small numbers. But these two cases indicated inflammations of oral cavity in mild degree.

5) Monocytes may appear in saliva, though in small numbers. However, most of healthy persons indicated the absence of monocyte in saliva. The cells, if present, showed a range of from 0.4 to 1.5 per cent, up to 10 per cent in a rare case with inflammations of oral cavity.

6) Lymphocytes never occurred in saliva in one-seventh of all cases. But the cells, if present, showed a range of from 0.4 to 17.0 per cent. This case of 17 per cent corresponded to that of inflammations in oral cavity.

7) Nas (Nickel-Ammonium Sulphate)-Benzidine reaction is of great value to determine the sorts of salivary corpuscles. The lymphocytes never show granules with the peroxidase stain.

8) Iodine or Hematoxylin-iodine reaction can often indicate the degenerations of salivary corpuscles.

9) The supravital staining with neutral red and Janus green also proved that the predominating cells were the neutrophils, and this staining method is recommended to observe the degree of degenerations of the cells.

10) The salivary corpuscles of some mammals were examined similarly, and most, if not all, of the cells were neutrophilic or pseudo-eosinophilic leucocytes. In the saliva of a dog and pig, the eosinophils were found distinctly, though in small numbers.

11) These studies suggested to me that the myeloid, monocytic, and lymphocytic series existing in blood, may be demonstrated in saliva under certain circumstances.

Out of this paper, the items of eosinophils and of some mammals have already been published in October, 1949, and those of basophils, Nas-Benzidine reaction and Iodine reaction in November, 1950 at the Japanese Anatomical and Dental Society.

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