In this country, innumerable studies have hitherto been made concerning the disease popularly called osteomalacia of domestic animals, from early years, but only a few pathological studies have been made.

Yamagiwa and Satoh made investigations on the histopathological changes of this disease. They proved that the disease known as "so-called osteomalacia" in this country should be diagnosed as "osteodystrophia fibrosa". The present author carried on biochemical studies in relation to attendant morphological changes under the guidance of Prof. Yamagiwa.

In the first part of this series, a careful examination was presented of the analytical methods which have been so far accepted for the biochemical study of bone diseases. The author attempted a new method as the chemical compositions of the bone were evaluated by per unit-volume. In the second part, the changes in bone composition of osteodystrophia fibrosa of horses were studied in relation to the histopathological examination, and a severe reduction of bone minerals was found to take place simultaneously with the reduction of bone matrix.

However, the chemical composition of the bones seems to be changeable not only under pathological but also under various other physiological conditions. The basic figures on such biochemical investigations hitherto made in this field were found to be very scant. This is the reason why the present author has proposed to make a study on certain influential conditions, e.g., varying ages, different districts as growth environment and the bone composition in different parts of the skeleton.

Materials and Methods

Source of materials and parts of skeleton used for the present study, as well as analytical and evaluation methods were already described in the first part.

Firstly the relation between the varying ages of the animals and bone composition was studied on the materials selected from Nos. 292-360. The materials of both os nasale and metacarpus were taken from such a stage as is to be classified as normal or slightly changed in the bone tissue. This stage was named “silent” small-sized hole formation (cf. Yamagawa and Satoh and Ushijima). Four grades in the ages of animals were taken as follows: up to 3 years in which the growth of bone seems to have been completed, over 15 years assumed as “old horse”, and those between 4 to 14 years being divided into two classes, viz., 4-8 years and 9-14 years. The mean values of bone composition were compared with 11 cases from each of these four age groups.

Secondly the bone composition of horses was compared using materials taken from horses (aged 4-14 years) kept in rice-paddy areas and in field-crop environmental areas. Materials selected for this comparison were 10 cases of os nasale and 5 cases of metacarpus from each of the two areas.

In order to make further comparisons as to the differences between the composition of os nasale at each pathological stage of this disease and those of metacarpus as a different part of the skeleton, six additional samples were selected from horses kept in the same environmental area.

RESULTS AND DISCUSSION

1. The Relation between the Ages of Animals and Bone Composition (Table 1)

Nakajima and Sugita found the increase of mineral substances accompanied by the ageing of rats. However, no study on this relation in horses is to be found in literature on the subject. Data resulting from the present study are shown in table 1. The mineral compositions except magnesium indicate a slight tendency to increase with ageing of horses while the water content in the bones was reduced. However, this tendency was so slight that statistically significant differences were found or occasionally not found. For example, the reduction of water and increase of ash were found in os nasale as well as in metacarpus, the increases of calcium and specific gravity were found only in os nasale, and the increases of phosphorus and calcium/nitrogen ratio were found only in metacarpus. The tendency in compositional changes was similar in the materials from both os nasale and metacarpus; they are considered as the most characteristic parts of the skeleton.

An interesting fact was noted in the results of total nitrogen in the bones. As shown in table 1, no change could be found in the amount of total nitrogen representing bone matrix, in spite of the increase of calcium and phosphorus composing the bone salt; these results agree with those of Baker et al. From this fact, it may be considered that the growth of bone matrix is completed in the early ages and the degree of calcification in the organic matrix increases with the age of the animals. The analytical results of examination of magnesium content and calcium/phosphorus ratio did not show the changes accompanying increase in age.

The comparison of bone composition between ages 4-8 and ages 9-14 did not show any statistically significant differences (Table 1).

From these findings, it might be said that the age factor in horses does somewhat
### Table 1. Relation between Age and Bone Composition

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>AGE (Years)</th>
<th>NOS. OF CASES</th>
<th>WATER % in wet-bone</th>
<th>ASH</th>
<th>P</th>
<th>Ca</th>
<th>Mg</th>
<th>TOTAL N</th>
<th>Ca/N</th>
<th>Ca/P</th>
<th>S.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 ~ 3</td>
<td>11</td>
<td>25.0</td>
<td>85.5</td>
<td>14.8</td>
<td>35.2</td>
<td>0.23</td>
<td>7.78</td>
<td>4.55</td>
<td>2.38</td>
<td>1.45</td>
</tr>
<tr>
<td>Os nasale</td>
<td>4 ~ 8</td>
<td>11</td>
<td>19.3</td>
<td>88.7</td>
<td>15.3</td>
<td>35.5</td>
<td>0.17</td>
<td>7.83</td>
<td>4.55</td>
<td>2.86</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>9 ~ 14</td>
<td>11</td>
<td>18.0</td>
<td>91.9</td>
<td>15.4</td>
<td>37.1</td>
<td>0.16</td>
<td>7.80</td>
<td>4.69</td>
<td>2.37</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>Over 15</td>
<td>11</td>
<td>19.7</td>
<td>90.8</td>
<td>16.1</td>
<td>38.9</td>
<td>0.27</td>
<td>7.90</td>
<td>4.80</td>
<td>2.36</td>
<td>1.63</td>
</tr>
<tr>
<td>Metacarpus</td>
<td>1 ~ 3</td>
<td>11</td>
<td>16.8</td>
<td>119.5</td>
<td>21.3</td>
<td>47.6</td>
<td>0.29</td>
<td>8.29</td>
<td>5.96</td>
<td>2.23</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td>4 ~ 8</td>
<td>11</td>
<td>13.1</td>
<td>124.0</td>
<td>22.3</td>
<td>50.6</td>
<td>0.28</td>
<td>8.15</td>
<td>6.24</td>
<td>2.26</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td>9 ~ 14</td>
<td>11</td>
<td>12.8</td>
<td>190.1</td>
<td>23.7</td>
<td>51.3</td>
<td>0.31</td>
<td>8.05</td>
<td>6.40</td>
<td>2.18</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>Over 15</td>
<td>11</td>
<td>13.8</td>
<td>129.3</td>
<td>22.4</td>
<td>50.9</td>
<td>0.18</td>
<td>7.70</td>
<td>6.50</td>
<td>2.27</td>
<td>1.90</td>
</tr>
</tbody>
</table>

****: Significance in 1% level  
*: Significance in 5% level  
S.G.: Specific gravity of dried bone
**TABLE 2. Relation between the Different Districts of Growth Environment in Respect to Bone Composition**

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>THE DISTRICT AS GROWTH ENVIRONMENT</th>
<th>NOS. OF CASES</th>
<th>WATER % in wet-bone</th>
<th>ASH</th>
<th>P</th>
<th>Ca</th>
<th>Mg</th>
<th>TOTAL N</th>
<th>Ca/N</th>
<th>Ca/P</th>
<th>S.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Os nasale</td>
<td>Rice-paddy area</td>
<td>10</td>
<td>18.7</td>
<td>91.1</td>
<td>15.3</td>
<td>36.4</td>
<td>0.12</td>
<td>8.0</td>
<td>4.59</td>
<td>2.39</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>Field-crop area</td>
<td>10</td>
<td>16.9</td>
<td>96.8</td>
<td>16.5</td>
<td>37.8</td>
<td>0.21</td>
<td>8.3</td>
<td>4.76</td>
<td>2.38</td>
<td>1.63</td>
</tr>
<tr>
<td>Metacarpus</td>
<td>Rice-paddy area</td>
<td>5</td>
<td>13.2</td>
<td>128.2</td>
<td>21.6</td>
<td>48.7</td>
<td>0.33</td>
<td>7.8</td>
<td>6.3</td>
<td>2.22</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td>Field-crop area</td>
<td>5</td>
<td>12.6</td>
<td>131.4</td>
<td>23.5</td>
<td>52.8</td>
<td>*</td>
<td>0.27</td>
<td>7.4</td>
<td>6.68</td>
<td>2.28</td>
</tr>
</tbody>
</table>

*: Significance in 5% level

S.G.: Specific gravity of dried bone
### Table 3. Comparison between the Bone Composition of Os nasale and Metacarpus in Each Stage of the Pathological Changes of Osteodystrophia fibrosa

<table>
<thead>
<tr>
<th>Stage of Illness</th>
<th>Histological Changes</th>
<th>Nos. of Cases</th>
<th>MATERIAL</th>
<th>WATER % in wet-bone</th>
<th>ASH g/100 cc</th>
<th>P mg/l00 cc</th>
<th>Ca g/l00 cc</th>
<th>Mg g/l00 cc</th>
<th>TOTAL Ca/N</th>
<th>Ca/P</th>
<th>S.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>“Silent” small-sized hole formation</td>
<td>2 Nos. 295 Os nasale &amp; 338 Metacarpus</td>
<td>17.5</td>
<td>103.0</td>
<td>17.7</td>
<td>41.9</td>
<td>0.17</td>
<td>8.35</td>
<td>5.0</td>
<td>2.36</td>
<td>1.70</td>
</tr>
<tr>
<td>Stage II</td>
<td>“Progressive” small-sized hole formation</td>
<td>2 Nos. 309 Os nasale &amp; 349 Metacarpus</td>
<td>32.8</td>
<td>79.7</td>
<td>13.6</td>
<td>33.6</td>
<td>0.22</td>
<td>7.64</td>
<td>4.5</td>
<td>2.47</td>
<td>1.40</td>
</tr>
<tr>
<td>Stage III</td>
<td>“Progressive” large-sized hole formation</td>
<td>1 No. 345 Os nasale Metacarpus</td>
<td>47.6</td>
<td>71.9</td>
<td>12.6</td>
<td>27.1</td>
<td>0.20</td>
<td>7.10</td>
<td>3.8</td>
<td>2.15</td>
<td>1.25</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Large-sized hole formation accompanied by hyperplasia of new bone tissue</td>
<td>1 No. 344 Os nasale Metacarpus</td>
<td>48.1</td>
<td>46.0</td>
<td>7.9</td>
<td>38.2</td>
<td>0.13</td>
<td>6.00</td>
<td>3.0</td>
<td>2.30</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Notes: Figures in Stages I and II are mean results.
S.G.: Specific gravity of dried bone
influence bone composition, and these changes appear mainly in mineral substances. However, in adult ages (4—14 years), such a change is not recognized.

2. The Relation between the Different Districts as Growth Environment and Bone Composition (Table 2)

In the samples of both os nasale and metacarpus taken from horses kept in field-crop areas, it was found that the water content decreases somewhat, while the contents of bone ash, calcium, phosphorus, and the ratios of calcium/nitrogen and specific gravities slightly increase, the contents of magnesium and total nitrogen and the ratio of calcium/phosphorus showing no changes.

From these results, it may be said that the degree of calcification is slightly higher with animals kept in field-crop areas rather than with those in rice-paddy areas, even though the histological findings of both are found to show a normal structure.

3. Differences in Bone Composition between Os nasale and Metacarpus (Tables 1, 2 and 3)

The contents of the chemical constituents in metacarpus are markedly different from those of os nasale (Tables 1 and 2). Mineral substances contained in metacarpus are approximately 40 per cent more than those in os nasale, while total nitrogen is up only an approximate 3 per cent. The figures of calcium/nitrogen ratio which may represent the degree of calcification are higher in materials of metacarpus by approximately 35 per cent and figures of the specific gravity are also higher by approximately 23 per cent than those of os nasale. These facts may indicate that there are only slight differences in organic matrix between metacarpus and os nasale, while the degree of calcification of metacarpus is much higher than that of os nasale. Calcium/phosphorus ratio and content of magnesium in both bones have not shown any noticeable findings.

Similar comparisons in bone composition were made between os nasale and metacarpus using materials affected by osteodystrophy fibrosa (Table 3). The materials used for this comparison were selected from animals of the same age and from those of the same environmental district; samples of metacarpus and os nasale were taken from the same horse. In this way only 6 horses remained to be taken for this study.

The descriptions of the histopathological changes in this disease are omitted as they have already been noted in report II9. As shown in table 3, though the general changes in the composition show the same tendency between os nasale and metacarpus, the degree of changes in composition is markedly different in two cases as the pathological condition progresses. For example, in the case of os nasale, bone ash is reduced to 46 per cent in the advanced stage (classified as large-sized hole formation accompanied by hyperplasia of new bone tissue) from the almost normal stage (classified as "silent" small-sized hole formation), while in the case of metacarpus, this component is found to be up to 83 per cent in the same stage. Similar facts are also detected in other components. In the histopathological findings, the changes in all materials taken from metacarpus should be classified in the slight stage ("silent" small-sized hole formation) as compared with those of os nasale (Figs. 1, 2, 3 and 4). From these facts, it may be said that the severe changes found in os nasale through the microscope and those found by the chemical analysis might
not occur with the same severity in metacarpus, though if such were to be detected they would be very slight.

Summary

Few biochemical studies on the bone of domestic animals have so far been made, and fundamental figures which are necessary for this kind of study are difficult to be found in literature. Even though the bone is apparently seen as normal, its chemical composition may be physiologically changed by various conditions. The present report deals with investigations on some influential conditions respecting the bone composition.

1. The Age of the Animal and Bone Composition (Table 1)

The analytical results of 44 samples from each of os nasale and metacarpus, which are divided into 4 age groups by origin, 1-3, 4-8, 9-14 and over 15 years, indicated the reduction of water, the increase of bone ash, and the slight increase of phosphorus, calcium, calcium/nitrogen ratio and specific gravity of dried bones. However, the content of total nitrogen, magnesium and the ratio of calcium/phosphorus did not show any changes in company with ages of animals. It may be considered that the growth of organic matrix takes place in relatively young years and that the degree of calcification increases with the age of animals.

2. Different Districts as Growth Environment and Bone Composition (Table 2)

Comparison was made employing 30 cases of horses which had been kept in rice-paddy areas and field-crop areas. From the results of analyses, it might be said that the degree of calcification of the bones in animals kept in field-crop areas is somewhat higher than in cases from rice-paddy areas, but without showing statistically significant differences except for calcium in metacarpus.

3. Comparison of Bone Composition between Os nasale and Metacarpus (Tables 1, 2 and 3; Figs. 1, 2, 3 and 4)

The contents of mineral substances, except magnesium, are markedly higher in metacarpus than in os nasale. However, the content of total nitrogen indicates scarcely any differences between the two bones. As shown in calcium/nitrogen ratios, the degree of calcification in metacarpus seems to be markedly higher than that in os nasale.

Further comparisons were made between bone composition of these two parts of the diseased bone affected by osteodystrophia fibrosa. The degree of compositional changes in this disease is distinctly different in these two bones even though the general tendency of the changes is similar. In spite of the severe reduction of bone salt in os nasale, the reduction in metacarpus remains slight. These facts may indicate that the degree of calcification in metacarpus is markedly
higher than that in *os nasale*; moreover, in comparison with *os nasale*, this disease does not manifest itself as distinctly in *metacarpus*.

The author wishes to express his gratitude to Prof. *Yamagiwa* of the Department of Veterinary Pathology for his kind direction and review of this study, and to his assistant Mr. *Satoh* for kind cooperation.

The author further acknowledges his debt to Prof. *Ito* and members of the Department of Biochemistry for their advice.

**REFERENCES**


**EXPLANATION OF PLATE**

Fig. 1. × 100.

*Os nasale*; it is observed as apparently normal, though "silent" small-sized holes can be occasionally seen.

No. 295, ♂, 9 yrs.

Fig. 2. × 100.

*Metacarpus* of the same case as Fig. 1; it is observed to be similar to the findings in *os nasale*.

Fig. 3. × 32.

*Os nasale*; advanced case of the bone destruction, the upper part above the cementing lines is the periostal proliferation of new bone tissue, while in the lower part, old bone tissues only remained as pieces.

No. 344, ♂, 5 yrs.

Fig. 4. × 115.

*Metacarpus* of the same case as Fig. 3; the progress of the disease is hardly observed in comparison with that in *os nasale*, though the "silent" small-sized holes are occasionally observed in normally lamellar structure.