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BIOCHEMICAL STUDIES ON SO-CALLED OSTEOMALACIA
(*OSTEODYSTROPHIA FIBROSA*) IN HORSES III.
SOME FACTORS RELATING TO CHANGES IN
THE COMPOSITION OF THE BONE

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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 part¹⁾ 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 part²⁾, 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 part¹⁾.

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. YAMAGIWA and SATOH⁵⁾ and USHIJIMA⁴⁾). 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*

MATERIAL	AGE (Years)	NOS. OF CASES	WATER	ASH	P	Ca	Mg	TOTAL N	Ca/N	Ca/P	S. G.
			% in Wet-bone	g/100 cc							
<i>Os nasale</i>	1 ~ 3	11	25.0	85.5	14.8	35.2	0.23	7.78	4.55	2.38	1.45
	4 ~ 8	11	19.3	88.7	15.3	35.5	0.17	7.83	4.55	2.36	1.55
	9 ~ 14	11	18.0	91.9	15.4	37.1	0.16	7.80	4.69	2.37	1.62
	Over 15	11	19.7 **	90.8 **	16.1	38.9 *	0.27	7.90	4.80	2.36	1.63 **
<i>Metacarpus</i>	1 ~ 3	11	16.8	119.5	21.3	47.6	0.29	8.20	5.86	2.23	1.86
	4 ~ 8	11	13.1	124.0	22.3	50.6	0.23	8.15	6.24	2.28	1.91
	9 ~ 14	11	12.8	130.1	23.7	51.3	0.31	8.05	6.40	2.18	1.96
	Over 15	11	13.8 **	129.3 *	22.4 *	50.9	0.13	7.70	6.50 *	2.27	1.90

** : 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*

MATERIAL	THE DISTRICT AS GROWTH ENVIRONMENT	NOS. OF CASES	WATER	ASH	P	Ca	Mg	TOTAL N	Ca/N	Ca/P	S.G.
			% in wet-bone								
<i>Os nasale</i>	Rice-paddy area	10	18.7	91.1	15.3	36.4	0.12	8.0	4.59	2.39	1.56
	Field-crop area	10	16.9	96.8	16.5	39.6	0.21	8.3	4.76	2.38	1.63
<i>Metacarpus</i>	Rice-paddy area	5	13.2	126.2	21.6	43.7	0.33	7.8	6.3	2.22	1.87
	Field-crop area	5	12.6	131.4	23.5	52.8*	0.27	7.4	6.68	2.28	1.93

*: 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*

STAGE OF ILLNESS	HISTOLOGICAL CHANGES	NOS. OF CASES	MATERIAL NO.	MATERIAL	WATER	ASH	P	Ca	Mg	TOTAL N	Ca/N	Ca/P	S.G.
					% in wet-bone	g/100 cc							
Stage I	"Silent" small-sized hole formation	2	Nos. 295 & 338	<i>Os nasale</i>	17.5	103.0	17.7	41.9	0.17	8.35	5.0	2.36	1.70
				<i>Metacarpus</i>	12.8	125.0	22.1	50.3	0.34	7.39	6.8	2.28	1.91
Stage II	"Progressive" small-sized hole formation	2	Nos. 309 & 349	<i>Os nasale</i>	32.8	79.7	13.6	33.6	0.22	7.64	4.5	2.47	1.40
				<i>Metacarpus</i>	15.2	120.3	22.2	49.7	0.33	8.50	5.9	2.25	1.88
Stage III	"Progressive" large-sized hole formation	1	No. 345	<i>Os nasale</i>	47.6	71.9	12.6	27.1	0.20	7.10	3.8	2.15	1.25
				<i>Metacarpus</i>	14.2	129.0	22.4	51.5	0.37	8.50	6.1	2.30	1.93
Stage IV	Large-sized hole formation accompanied by hyperplasia of new bone tissue	1	No. 344	<i>Os nasale</i>	48.1	46.0	7.9	18.2	0.13	6.00	3.0	2.30	0.92
				<i>Metacarpus</i>	.	107.0	18.0	40.4	0.40	6.54	6.2	2.26	1.54

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 *osteodystrophia 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 II⁴). 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 progress. 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*.

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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 periosteal proliferation of new bone tissue, while in the lower part, old bone tissues only remained as pieces.

No. 344, ♂, 8 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.

