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BIOCHEMICAL STUDIES ON SO-CALLED OSTEOMALACIA
(*OSTEODYSTROPHIA FIBROSA*) IN HORSES II.
THE RELATION BETWEEN HISTOLOGICAL FINDINGS
AND CHEMICAL COMPOSITION OF THE BONE

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Osteomalacia in horses which was reported by KINTNER and HOLT and other workers has been found in a sporadic or enzootic form all over the world. In Japan, since TOKISHIGE (1897) reported the first occurrence, many affected horses have been found in various districts, and innumerable studies have been made in view of the fact that the animal husbandry industry has been affected. Especially, investigations by NIIMI and co-workers¹⁵⁻¹⁷⁾, SHIMAMURA and HAYASHI, IIZUKA and co-workers^{5,6)}, CHIBA,²⁾ and NAKAJIMA and co-workers¹⁰⁻¹³⁾, have greatly contributed to the advance in understanding the causal genesis of the disease. However, pathological investigation has hardly been reported except for studies by CHIBA³⁾, TAJIMA and OHSHIMA. In fact in this country, the study on the true character of this disease has so far seemed to be disregarded.

According to THEILER, though many cases of *osteomalacia* in horses have been reported, all records of the abnormal skeletal condition in equines commonly termed *osteomalacia* probably refer to "*osteodystrophia fibrosa*". In Japan, this disease has generally been named "so-called *osteomalacia*" because it has a different pathological condition compared with *osteomalacia* found in human. TAJIMA and OHSHIMA agree with THEILER in their opinion that "*osteomalacia*" in horses found in Japan should probably be considered *osteodystrophia fibrosa*.

YAMAGIWA and SATOH performed pathological investigation on the bone affected by so-called *osteomalacia* in domestic animals. In the investigation they studied the pathogenesis of the disease using many cases clinically so diagnosed and using also the bones obtained indiscriminately from slaughterhouses in various districts. They were the first to show the initial lesion in the bone tissue and its development, which initial lesion seems to be the change of the earliest stage of this disease. From their observations, YAMAGIWA and SATOH concluded that the disease named as so-called *osteomalacia* in horses and cattle observed in Japan should be diagnosed as *osteodystrophia fibrosa*. The present author has

carried out a biochemical study as a part of their pathological investigation.

Biochemical studies on the bone in osteodystrophic disease found in animals have also been made only exceptionally. MAREK et al. studied the change of bone minerals in rickets of piglets and colts. NAKAJIMA et al.¹³⁾ studied the change of bone minerals in so-called *osteomalacia* in horses, and HORI and NIWA reported the change of amino-acids component in the bone affected by this disease.

The present author has already reported in the first part²²⁾ of this study that in bone analysis the composition should be evaluated by per unit-volume. In this second part, a report is made of both histopathological examinations and chemical analyses using this method of evaluation, which were carried out on many bone materials gathered indiscriminately from various slaughterhouses. From the results of these examinations, the author wishes to describe some findings regarding the composition of the bone accompanied by histopathological changes on *osteodystrophia fibrosa*.

MATERIALS AND METHODS

Source of materials, parts of skeleton used for this study, and the analytical and evaluating methods were already reported in the first part.²²⁾ Chemical analyses and histological examinations were made from the samples of both *os nasale* and *metacarpus*. However, in this part, only the changes of *os nasale* in the two samples collected from the same horse have been examined, because *os nasale* distinctly manifests the change of this disease in comparison with *metacarpus* (this fact is expected to be discussed in the third part).

Duplicate analyses were carried out on the following points: total water in wet bones; the specific gravity of dried bones; the ash, phosphorus, calcium, magnesium, and total nitrogen per unit-volume of bones. According to the description by BAKER et al., the measure of the degree of calcification of the bone matrix might be given by the calcium/nitrogen ratio. This ratio was also calculated in the present report. In addition, calcium/phosphorus ratio which may indicate the principal structure of bone salt was also calculated.

The selection of the samples used for this study was made from a total of 178 cases as described in part I.²²⁾ Namely, 124 cases of *os nasale* of the horses between the ages of 4~14 years in order to be free from the influences of age factor were first selected. From these cases, 112 were selected from animals kept in rice-paddy areas; these were classified into five stages according to the process of pathological changes in the bone tissue revealed by the microscope. Finally, 13 cases from each of five stages have been selected in order easily to compare the chemical results by statistical analysis. Mean values of the bone compositions in each group were compared.

RESULTS

Microscopical findings of bone tissue observed in *osteodystrophia fibrosa* were fully

discussed by YAMAGIWA and SATOH. A summary of the main histological changes is presented again in order to assist the understanding of compositional changes.

In the early stage, small "focal loosening" lesions can be detected in the bone tissue connected with canal system or in other parts, and the focus is in the habit of taking irregular shape. Appearance of "pale-nuclear cells" in small focal loosening portions is successively observed. These cells seem to be connected with the bone destruction in the earliest stage. In the next stage, multinuclear giant cells are recognized in the interior of the focus accompanied by pale-nuclear cells, and the peripheral portion of foci becomes demarcated. The holes are somewhat enlarged, but still they are of microscopically-small size ("progressive" small-sized hole formation) (Figs. 3 & 4).

Accompanied by enlargement of the holes fine connective tissue fibers proliferate and form reticular structure in these holes and blood vessels penetrate into the enlarged focus. It is evident in these "progressive" cases that many active giant cells and pale-nuclear cells are found in the large-sized holes and that the walls of these are lined with osteoblastic layer, even though appositions of osteoid tissue are rarely observed. Such a proliferation of connective tissue fibers and the holes found in the bone tissue become so distinct as to be visible macroscopically in this stage ("progressive" large-sized hole formation) (Figs. 5 & 6).

However, it is frequently observed that most of the once proliferated connective tissues in the various-sized holes do not show progression of reactive changes, but on the contrary even a retrogression of reticular structure is detected. In such holes, there are only few cells and few multinuclear giant cells which remained closely to the wall of the hole and these giant cells have the protoplasm emphasized with eosin-color and the pycnotic nuclei. These pathological conditions are designated as "silence".

The histological findings of the cases classified as in the stage of "silent" small-sized hole formation (Figs. 1 & 2) have shown that the pathological conditions are in "silence" after only slight progression of the change. The materials in this stage are used as control cases, because normal cases could not be found in materials collected for the present study.

There is another stage in which though many large-sized holes are observed in the diseased bones, pathological changes of the foci show them to be in the "silent" condition. The stage showing such a condition is classified as "silent" large-sized hole formation (Figs. 8 & 9).

Among many advanced cases, the periosteal or endosteal proliferation of new bone tissue takes place frequently, and this reinforces the fragile bones. These findings are observed mainly in occasions where many large-sized holes are found in the bone tissue and histological changes generally show severe destruction. Such a condition is classified as large-sized hole formation accompanied by hyperplasia of new bone tissue (Fig. 7).

Materials used for the present study have been grouped according to the stages of the pathological process described above as follows:

Stage I.	"Silent" small-sized hole formation	47 cases
Stage II.	"Progressive" small-sized hole formation ...	21 cases
Stage III.	"Progressive" large-sized hole formation ...	16 cases

Stage IV. Large-sized hole formation accompanied by
hyperplasia of new bone tissue 13 cases

For purpose of observation, 15 cases of the stage of "silent" large-sized hole formation have been compared in addition to these four stages.

Thirteen cases from every one of the groups have been selected at random. Mean analytical results of these cases are set out in table 1. Statistical comparisons among these results are shown in table 2.

DISCUSSION AND CONCLUSION

The author has attached importance, in this report, to relative comparison of each chemical components between the four stages, rather than to changes of the absolute composition of the bone.

As shown in tables 1 and 2, distinct changes of composition were in the decrease of mineral substances and total nitrogen in dried bone as contrasted with increase of water in wet bone (Table 1).

Considerable increase of water was observed, i. e., 36.9 per cent for Stage IV (large-sized hole formation accompanied by hyperplasia of new bone tissue) while it was 19.5 per cent for Stage I ("silent" small-sized hole formation). The most significant difference in water content was observed in the stage where the various-sized holes are enlarged from microscopical to macroscopical size (Table 2).

In the results of analysis of mineral substance, the mean values of the ash content of Stage I, normally containing 94.5 g/100 cc, has decreased to 62.7 g/100 cc at Stage IV. In particular, No. 295 of the former has shown 107 g/100 cc, while No. 344 of the latter only 46 g/100 cc, showing an extreme decrease but less than below 50 per cent was to be noted. Similar figures have been occasionally seen regarding the contents of calcium as well as phosphorus of bone at both Stages I and IV. For instance, No. Ch. 73 has contained 43.5 g/100 cc of calcium at Stage I, compared with No. Ch. 55 containing 17.5 g/100 cc at Stage IV; No. Ch. 73 has been found to contain 18.8 g/100 cc of phosphorus at Stage I, while No. Ch. 50 contained 7.5 g/100 cc at Stage IV.

Such decreases as found in bone salt content seem to have caused the decreases of the specific gravity of dried bones. For examples, No. 356 of Stage I showed a specific gravity of 1.83, while both No. Ch. 55 and Ch. 50 of Stage IV showed specific gravity of 0.91 (the dried bones may occasionally float in water when highly porous). From these analytical results, it may be reasonable to consider that the severe reductions in mineral substances seem to be closely connected with the enlargement of various-sized holes histologically observed, because of the fact that mineral composition are significantly reduced in contrast to the increase of water, when the change of the bone tissue is advancing from small-sized holes (Stage II) to large-sized holes (Stage III) (cf. Table 2).

TABLE 1. *The Relation between Histological Findings and Chemical Composition of Os nasale Affected by Osteodystrophia fibrosa*

PROCESS OF THE CHANGE	HISTOLOGICAL FINDINGS		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	\bar{x}	19.50	94.50	16.20	38.50	2.20	8.00	4.83	2.40	1.62
		σ	3.39	7.41	1.38	3.60	0.09	0.45	0.25	0.06	0.10
Stage II	"Progressive" small-sized hole formation	\bar{x}	21.20	88.00	15.20	35.30	0.20	7.70	4.66	2.35	1.48
		σ	4.41	3.48	0.77	1.88	0.04	0.38	0.25	0.09	0.06
Stage III	"Progressive" large-sized hole formation	\bar{x}	28.90	76.40	13.10	30.60	0.21	7.25	4.22	2.34	1.35
		σ	6.91	8.82	1.63	3.59	0.04	0.50	0.39	0.09	0.12
Stage IV	Large-sized hole formation accompanied by hyperplasia of new bone tissue	\bar{x}	36.90	62.70	10.50	24.20	0.29	7.06	3.40	2.32	1.20
		σ	10.15	13.5	2.46	5.51	0.08	1.16	0.51	0.11	0.21
	"Silent" large-sized hole formation	\bar{x}	23.50	80.50	14.40	32.80	0.21	7.45	4.38	2.34	1.43
		σ	4.04	6.51	0.99	2.72	0.05	0.43	0.32	0.08	0.09

\bar{x} : Mean results of 13 cases

σ : Standard deviation

S. G.: Specific gravity of dried bone (mass)

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TABLE 2. Summary of Statistical Comparison among the Mean Results Given in Table 1

COMPARISON		WATER % in wet-bone	ASH	P	Ca	Mg	TOTAL N	Ca/N	Ca/P	S.G.
		g/100 cc								
Stage I : II	"Silent" small-sized hole formation : "Progressive" small-sized hole formation	non	non	non	non	non	non	non	non	non
Stage II : III	"Progressive" small-sized hole formation : "Progressive" large-sized hole formation	II < III (5 %)	non	II > III (1 %)	II > III (1 %)	non	non	II > III (1 %)	non	II > III (1 %)
Stage III : IV	"Progressive" large-sized hole formation : Large-sized hole formation accompanied by hyperplasia of new bone tissue	non	III > IV (5 %)	non	III > IV (1 %)	III < IV (5 %)	non	III > IV (5 %)	non	III > IV (5 %)
Stage I : II : III : IV	(Analysis of variance)	(1 %)	(1 %)	(1 %)	(1 %)	(1 %)	(1 %)	(1 %)	non	(1 %)

(1%) or (5%): Significant in 1% or 5% significance level
non: Non-significant difference

However, calcium/phosphorus ratios have been constantly found in the range of 2.3~2.4 throughout each stage of the progress of the disease; these values are similar to those which have been so far reported as the ratio of normal bones. From this fact, it may be said that these pathological changes have been made without causing any changes in chemical structure of bone salt, so far as calcium/phosphorus ratios are concerned.

The figures of magnesium content (0.20 g/100cc for Stage I and 0.29 g/100cc for Stage IV) showed only a slight change with the progress of the disease (Table 1), even though a small increase has been significantly shown in Stage IV (hyperplasia of new bone tissue). Only fragmentary data have been published for magnesium in bones¹⁴⁾, but it is generally accepted that the greater part of magnesium ion in bones exists on the crystal surfaces of bone salt. Accordingly, the fact that the content of magnesium was slightly increased in comparison with the striking reduction of bone salt, would suggest that the magnesium in the bone salt may conversely increase with the progress of the disease, and furthermore, this increase may be closely related with the proliferated new bone tissue (Tables 1 & 2).

The author would like to emphasize that special attention should be paid to the change of total nitrogen in the diseased bone. When decalcification takes place in the bone due to any cause it is generally expected that the content of total nitrogen relatively increases while the bone salt decreases. HORI and NIWA indicated, as was expected, that the content of total nitrogen in bone has a tendency to increase in the so-called *osteomalacia* in horses (5.6 per cent for the diseased bone in comparison with 4.1 per cent for normal bone in dry materials). However in the present study, the results of total nitrogen have shown a decrease in contrast with the finding of the above mentioned authors, viz., the content per unit-volume was significantly decreased according to the progress of the bone change (7.06 g/100 cc for Stage IV while it was 8.0 g/100 cc for Stage I, cf. Table 1). The reduction of the total nitrogen is also considered to take place, just as in the case of mineral substances when the holes in the diseased bone tissue grow from small to large size, though the statistical analysis does not indicate a significant difference. Even in these large-sized holes, one usually may observe the proliferation of both fibrous tissues and hyperemic blood vessels both which contain much protein. The fact that the content of total nitrogen decreases with the progress of the disease, may suggest the reduction of collagen as bone matrix which makes up as much as nearly 35 per cent of the dry, fat-free bone weight⁸⁾. The degree of the bone salt deposition in an organic matrix can be estimated by the calcium/nitrogen ratio. This ratio has been also found to decrease significantly with the progress of the bone change, particularly in Stage IV (large-sized

hole formation accompanied by hyperplasia of new bone tissue), in which the imperfection of calcification may occur in the new bone tissues.

The findings on the bone of *osteodystrophia fibrosa* in horses through the above investigation are summarized as follows.

Firstly, severe reduction of the bone minerals of diseased horses has been found. The main cause of this reduction seems to be closely connected with the enlargement of "the hole" in the bone tissue. Secondly, the total nitrogen content has been also found to decrease. The reduction in nitrogen may be suggested as the cause of the enlargement of "the hole" histologically found, because the decrease of the bone matrix is considered to induce the enlargement of the hole. So far as *osteodystrophia fibrosa* is concerned, it may be said that the reduction in bone minerals takes place simultaneously with the reduction of the bone matrix. Calcium/nitrogen ratios which may represent the degree of calcification, also decrease as in the case of bone minerals, and this reduction seems to be closely related with the proliferation of new bone tissues in which the imperfection of calcification may occur.

The chemical structure of bone salt which may be given by the calcium/phosphorus ratio seems not to change in *osteodystrophia fibrosa*.

As for magnesium in bone tissue, only fragmentary evidence was available from reports on the subject, and the present study has no significant addition to make.

Through the results of analyses described above, it is evident that all the *osteomalacia* cases, generally so-called in Japan, are different from true *osteomalacia*. Previous studies on the bone diseases of horses have been chiefly limited to the problems concerning calcium and phosphorus, but the present investigation strongly suggests that special attention should also be paid to the other complicated metabolic disturbances.

SUMMARY

The present study is concerned with the pathochemical examination of the bone diseases of horses which is popularly called *osteomalacia* in Japan.

Chemical analyses of 65 cases, which were selected from 178 cases of *osteomalacia* collected from three slaughterhouses in Hokkaido, were carried out and the following results have been obtained, as shown in table 1, 2 and plates I, II.

Severe reductions of bone minerals (particularly bone ash, calcium and phosphorus) have been found in the diseased bone. In such diseased bones a simultaneous reduction of bone matrix, which is represented by total nitrogen content, was observed. The change of magnesium content is not so evident as to add any findings, even through a slight increase has been found in the

advanced stage with the proliferation of new bone tissue.

YAMAGIWA and SATOH were the first to prove that the disease so-called *osteomalacia* in horses should be diagnosed as *osteodystrophia fibrosa* according to their histopathological investigations. The present results from the chemical analyses also indicate that this disease in horses is different from true *osteomalacia*.

It is further noted that the metabolic disturbances are not limited to calcium and phosphorus, but that other complicated elements should be taken into consideration.

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EXPLANATION OF PLATES

PLATE I.

Figs. 1, 3 and 5. $\times 7.5$. *Os nasale*; Transverse-sectioned preparation photographed by transmitted light.

Figs. 2, 4 and 6. $\times 50$. Microscopical magnification of Figs. 1, 3 and 5.

Fig. 1. At first glance it is observed as nearly normal.

No. Ch. 24, ♀, 13 yrs.

Bone composition: water: 20.5%; ash: 102.5 g/100 cc; P: 17.2 g/100 cc;
Ca: 40.3 g/100 cc; Mg: 0.18 g/100 cc; total N: 7.95 g/100 cc.

Fig. 2. Microscopically "silent" small-sized holes can be seen, i. e., few cells and sparse network in the hole; atrophic giant cells are observed on the walls.

Fig. 3. Macroscopically pathological conditions can not be observed.

No. Ch. 25, ♂, 8 yrs.

Bone composition: water: 20.85%; ash: 82.8 g/100 cc; P: 14.9 g/100 cc;
Ca: 33.8 g/100 cc; Mg: 0.27 g/100 cc; total N: 7.6 g/100 cc.

Fig. 4. Microscopically "progressive" small-sized hole formation; many cells and ample network, hyperemic blood vessels in the hole, osteoblastic layer and giant cells can be observed.

Fig. 5. Macroscopically many holes, hyperemia and proliferation of fibrous tissue can be observed.

No. Ch. 40, ♂, 8 yrs.

Bone composition: water: 30.2%; ash: 69.0 g/100 cc; P: 12.5 g/100 cc;
Ca: 27.7 g/100 cc; Mg: 0.19 g/100 cc; total N: 7.05 g/100 cc.

Fig. 6. "Progressive" large-sized hole formation; hyperemic blood vessels, proliferation of reticular structure of connective tissue fibers, osteoblastic layers can be observed.

PLATE II.

Figs. 7 and 8. $\times 4$ and $\times 7.5$. *Os nasale*: Transverse-sectioned preparation photographed by transmitted light.

Fig. 9. $\times 50$. Microscopical magnification of Fig. 8.

Fig. 7. Severe destruction of the bone, periosteal proliferation of new bone tissue and thickened periosteum.

No. Ch. 50, ♀, 11 yrs.

Bone composition: water: 50.3%; ash: 46.4 g/100 cc; P: 7.5 g/100 cc;
Ca: 17.6 g/100 cc; Mg: 0.24 g/100 cc; total N: 6.5 g/100 cc.

Fig. 8. Many large holes can be distinctly observed, but hyperemia and the proliferation of fibrous tissues are scarcely observed.

No. Ch. 101, ♂, 11 yrs.

Bone composition: water: 18.4%; ash: 92.3 g/100 cc; P: 15.7 g/100 cc;
Ca: 33.0 g/100 cc; Mg: 0.24 g/100 cc; total N: 7.85 g/100 cc.

Fig. 9. "Silent" large-sized hole formation; few cells, flattened osteoblastic layers in the hole; reticular structure of connective tissue fiber has become fat-tissue-like by retrogression.



