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**ELECTRON MICROSCOPIC OBSERVATIONS OF  
THE EQUINE PARATHYROID GLANDS  
WITH PARTICULAR REFERENCE TO THOSE OF EQUINE  
OSTEODYSTROPHIA FIBROSA**

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INTRODUCTION

For many years, YAMAGIWA and his co-workers had investigated the histopathology of the parathyroid glands<sup>6,21,22</sup>, especially in horses, with parallel studies on pathology of the bone.

YAMAGIWA et al. (1958~59) examined the histopathological features of the parathyroid glands in one hundred autopsied cases of equine osteodystrophia fibrosa and emphasized that enlargement of the parathyroid glands and formation of the patchy appearance in their histological sections have reactive and hyperplastic significance. Later, INUBUSHI (1961) investigated the parathyroid glands not only in cases of equine osteodystrophia fibrosa, but also in normal horses which were selected with respect to comparable age and season of the year. He also recognized reactive and hyperplastic features even in normal horses with immature bone tissues. From these previous studies with light microscopy, the authors (1961)<sup>19</sup> came to the conclusion that the changes of the parathyroid glands in equine osteodystrophia fibrosa and in normal horses with immature bone tissues, developed after the advanced bone tissue changes. Recently KROOK & LOWE (1964) published an interesting report on nutritional secondary hyperparathyroidism in the horse with a description of the normal equine parathyroid gland.

In recent years, studies on the fine structure of the parathyroid glands in animals and humans have been reported by LEVER (1957, 1958), TRIER (1958), PORTE & PETROVIC (1961), LANGE (1961), ROTH (1962), ROTH & MUNGER (1962), MUNGER

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& ROTH (1963), ROTH & RAISZ (1964), SHELDON (1964) and CAPEN et al. (1965)<sup>2,3</sup>), but electron microscopic studies on equine parathyroid glands, especially in cases of equine osteodystrophia fibrosa have not been reported in the available literature.

This study was undertaken to examine the ultrastructure of the equine parathyroid glands with particular reference to those in cases of equine osteodystrophia fibrosa in order to confirm light microscopic findings<sup>22</sup>). Most of the findings in this report had been presented at the 52nd Meeting of the Japanese Veterinary Science in 1961 and published as an abstract<sup>19</sup>).

#### MATERIALS AND METHODS

All of the tissue specimens were collected from horses killed at the Sapporo and Asahigawa slaughter houses. Portions of the submaxillary bones and the upper pair of the parathyroid gland were collected from each horse. The materials were collected from August 25, 1958 to March 31, 1961. The animals were from one to twenty years old. Materials were obtained from 12 normal horses, less than 2 years old, with immature bone tissues, 31 normal horses, more than 4 years old, with mature bone tissues and 10 horses with osteodystrophia fibrosa (tab.)

TABLE *Investigated materials*

NO.	CASE NO. (UM NO.)	SEX	AGE	DATE OF COLLECTION	CLASSIFICATION
1	129	♀	1	25/VIII '58	Normal horses with immature bone tissue
2	151	"	"	9/IX '58	
3	158	"	"	25/IX '58	
4	188	"	"	7/XI '58	
5	190	♀	"	8/XI '58	
6	202	♂	"	15/XI '58	
7	203	"	"	17/XI '58	
8	214	"	"	22/XI '58	
9	215	♀	"	24/XI '58	
10	216	"	"	"	
11	217	♂	"	"	
12	218	"	"	"	
13	197	♀	5	12/XI '58	Normal horse with mature bone tissue
14	212	♂	"	19/XI '58	
15	196	♀	6	12/XI '58	
16	147	"	7	8/IX '58	
17	193	"	"	11/XI '58	
18	201	"	"	15/XI '58	

TABLE (continued) *Investigated materials*

NO.	CASE NO. (UM NO.)	SEX	AGE	DATE OF COLLECTION	CLASSIFICATION
19	213	♂	7	19/XI '58	Normal horses with mature bone tissue
20	221	♀	"	25/XI '58	
21	132	"	8	26/VIII '58	
22	133	"	"	"	
23	134	"	"	29/VIII '58	
24	192	"	"	8/XI '58	
25	209	"	"	19/XI '58	
26	210	"	"	"	
27	211	"	"	"	
28	170	♂	9	13/X '58	
29	200	"	"	15/XI '58	
30	205	♀	"	17/XI '58	
31	135	"	10	2/IX '58	
32	171	"	11	14/X '58	
33	153	"	13	10/IX '58	
34	154	"	14	"	
35	199	"	15	12/XI '58	
36	207	♂	"	17/XI '58	
37	189	♀	"	8/XI '58	
38	156	"	17	25/IX '58	
39	157	"	18	"	
40	198	"	"	12/XI '58	
41	206	"	"	17/XI '58	
42	208	"	"	18/XI '58	
43	152	"	19	10/IX '58	
44	316	"	2	31/III '61	Osteodystrophia fibrosa cases
45	317	"	4	"	
46	195	♂	7	11/XI '58	
47	313	"	"	23/III '61	
48	314	"	"	24/III '61	
49	194	♀	8	11/XI '58	
50	312	♂	"	20/III '61	
51	191	"	9	8/XI '58	
52	315	♂	12	31/III '61	
53	220	♂	20	24/XI '58	



The diagnosis of equine osteodystrophia fibrosa was based on the histological changes of the submaxillary bones, except 6 cases (UM Nos. 312-317) all of which showed clinical and macroscopical features of equine osteodystrophia fibrosa. The histological changes of osteodystrophia fibrosa cases and the bone tissues of apparently normal horses with immature or mature bone tissues had already been reported by the authors<sup>19,20,23</sup>. The authors classified the lesions of the bone tissues in equine osteodystrophia fibrosa as mild, severe and stand-still lesions.

After electrolytic decalcification of the bone tissues, they were embedded in celloidin and sections were stained with hematoxylin and eosin.

The parathyroid glands were collected as soon as possible after slaughter and the glands were divided into 3 parts. One part was fixed in 10% formalin solution. A second part was fixed with Carnoy fixing solution for light microscopy, and the rest was fixed in one percent osmium tetroxide buffered at pH 7.4 with acetate-veronal (PALADE, 1952) for electron microscopy. The paraffin sections were stained with hematoxylin and eosin, and methylgreen pyronine. Histochemistry for PAS and alkaline phosphatase reactions was also conducted. Frozen sections were stained with Sudan III. The specimens for electron microscopy were dehydrated in an ethanol series and were embedded in a mixture of butyl and methyl methacrylates. Sections were cut on a JUM-4 ultramicrotome with glass knives and subsequently examined in a JEM-4 CHD electron microscope. One to two micron sections of the osmium-fixed, methacrylate-embedded tissues were stained with hematoxylin and eosin, and the PAS reaction was done with diastase-digestion after removing the plastic.

## OBSERVATIONS

### I) Light microscopic observations

#### a) Histological features of the equine parathyroid glands

The equine parathyroid glands were composed of groups of closely arranged parenchymal cells with a net work of capillaries and small blood vessels. The parathyroid glands were frequently accompanied by KÜRSTEINER's canals or cysts of variable size and occasionally accompanied with thymic rudiments and parts of the thyroids. Parenchymal cells were recognized as chief cells and oxyphil cells. The chief cells represented most of the parenchymal cells of the equine parathyroid glands and are the most important. Chief cells were divided into light and dark types and oxyphil cells were divided into pale and dark types. Occasionally water clear cells were found.

i) Chief cells Compact cords of chief cells were surrounded by loose connective tissue. Connective tissue septa gave the glands a lobulated appearance. Light and dark areas were intermingled in stained sections. Light and dark staining cells could be noted as well as transitional form between both types of cell. Except for tinctorial properties there were no remarkable differences between the two. The light chief cells had vesicular nuclei eccentrically placed. The cytoplasm was usually clear, but sometimes it contained a small amount of basophilic dustlike material. The material was rough in appearance and sometimes appeared as granular or doughnut like structures. Usually, chief cells were smaller than the water clear cells. The cytoplasm of the dark chief cells contained cloudy, floating material and took a somewhat basophilic color. In general, the cytoplasm of the chief cells was

rich in glycogen and sometimes contained fine granular or droplet like lipid. The cytoplasm of the dark chief cells was rich in RNA in comparison with the light chief cells. Some of the light chief cells had a small mass of RNA (the juxta nuclear bodies or the basophilic bodies<sup>18</sup>) near the periphery of the cell. The parenchymal cells were negative for alkaline phosphatase in equine parathyroid glands and only a slight positive reaction was obtained in the basement membrane and the endothelial cells of the blood vessels.

ii) Oxyphil cells Oxyphil cells were very infrequent compared with the more common chief cells. Oxyphil cells were observed in horses of all ages. The oxyphil cells were large in size. Their cytoplasm contained fine granules and often appeared swollen and stained with the acid dyes. The oxyphil cells could be classified as the dark or pale cells whose nuclei were small and occasionally degenerative. The oxyphil cells usually had no glycogen and occasionally contained a small amount of fat droplets. Sometimes swollen cells were observed which might be an intermediary cell between the light chief cells and pale oxyphil cells. These swollen cells contained a small amount of fat droplets. The swollen cells comprised a small group and showed negative results in PAS reaction.

iii) Water clear cells Water clear cells may be considered transition from chief cells. Water clear cells appeared in the parathyroid glands of horses with equine osteodystrophia fibrosa and in normal horses with immature bone tissues. Frequently in the severe cases of osteodystrophia fibrosa water clear cells appeared as focal areas of acinar or lobular groups. The water clear cells were large in size. Their nuclei were eccentrically placed and often showed degenerative changes. The cytoplasm was vesicular with hematoxylin and eosin stain. Large or small sized stainable substances were clearly shown by PAS reaction. The cytoplasm of water clear cells contained a large amount of glycogen.

#### b) The parathyroid glands in normal horses with immature bone tissue

The size of the parathyroid glands in normal horses with immature bone tissue was smaller than that of the adult horse and in general, the lobular pattern was definite in the stained sections. The parenchymal cells in the parathyroid glands of horses with immature bone tissue were mainly the light staining chief cells. Group of vacuolated chief cells was found to appear as an island in the glands. In addition, even in horses with dark stained glands, there were many groups of light stained chief cells appearing as an island. It is worthy of note that there were many horses with foci of water clear cells. In addition, PAS reaction showed massive amounts of stainable substances in the cytoplasm of chief cells of a majority of the normal horses with immature bone tissue.

#### c) The parathyroid glands in normal horses with mature bone tissue

It should be pointed out that glands which had a clear lobular appearance and dark stained tissues were many in number compared with glands which had compact and light stained tissues. In the latter case, vacuolization of the cytoplasm was scarcely noted and the foci of water clear cells were difficult to find. On the other hand, only a small number of foci of light stained cells were found in most of the glands with a clear lobular appearance and dark stained tissues. In some parts of the glands, there were groups of the swollen cells which were considered transitional forms of oxyphil cells. These cells showed a negative PAS reaction.

d) The parathyroid glands in equine osteodystrophia fibrosa

Under low magnification, a beautiful patchy appearance was found in the stained sections of glands from many severe cases of equine osteodystrophia fibrosa. In mild cases, the patchy appearance was not so remarkable and large or small sized areas with varying staining properties could be found in lobular, acinar and small cell groups. That is, sometimes single or groups of vacuolated cells were found in the groups of pale cells and sometimes the pale cells which looked like an island were found in groups of dark cells. In severe cases, the foci of water clear cells were frequently observed, but in mild cases, the existence of such foci was rare. Histologically dominating cells in equine osteodystrophia fibrosa were mainly light chief cells.

## II) Electron microscopic observations

a) Chief cells

**Light chief cells** Individual light chief cells were polygonal. The plasma membranes were parallel and moderately straight with irregular interdigitation of adjacent plasma membranes in some places. Sometimes numerous (micropinocytotic) vesicles of the smooth surfaced endoplasmic reticulum were noted, which was considered to be an infolding of the cell surfaces (fig. 3). The adjacent plasma membranes of the chief cells were characterized by occasional desmosomes (fig. 5), one of the characteristics of epithelial cells, but tonofilaments were not observed. Rarely, there were small filamentous structures which were considered to be cilia at the contact areas of two chief cells (fig. 4). Multivesicular bodies were occasionally present in the Golgi area and near the periphery of the cell (fig. 4). The nuclei of the light chief cells were large, spherical or oval, and located centrally and occasionally eccentrically. The outer and inner nuclear membranes were parallel, moderately straight and only occasionally had an irregular appearance. Sometimes the outer membranes projected into the cytoplasm like a sinus. In the nuclei, there were one or two nucleoli with high electron density. In general, the nucleoli of the light chief cells had granules of moderate electron density which were distributed diffusely and homogeneously. There were numerous various-shaped mitochondria (short or long rod-shape, guitar shape, oval and spherical shapes, etc.). Occasionally, the mitochondria accumulated in the vascular pole of the cell. The arrangement of Cristae mitochondriales was irregular and the mitochondria had granules of moderate electron density. The Golgi area was situated around the nucleus. A Golgi apparatus considered of an accumulation of numerous small vesicles, granules and lamellar sacs. The vacuoles in the Golgi area often showed somewhat increased electron density and sometimes contained small granules. There was a small number of vesicles of the smooth surfaced endoplasmic reticulum and parallel arrays of flat sacs of the rough surfaced endoplasmic reticulum in the cytoplasm. Sometimes localized lamellar (figs. 7 & 13) or whorl like bodies of the endoplasmic reticulum were found in the cytoplasm (figs. 1 & 2). These bodies were thought to coincide with the basophilic bodies or dust like stainable substances under light microscopy, because they were rich in ribosomes. In the center of these bodies, occasional groups of the Golgi vesicles or elongated sack structures were found. In the canalicular system of the endoplasmic reticulum, there were frequently

irregularly shaped lipid bodies of high electron density or various sized spherical or oval granules of high electron density. The size of these granules was occasionally larger than that of the mitochondria of the same cell. Though these granules occasionally had limiting membranes which were not always found, and may have been obscured by many granules. These granules presumably represent secretory granules (figs. 6, 8~10 & 12). These large granules were transitional to the small granules which were found in the Golgi region. These small granules presumably represent pro-secretory granules. These secretory granules were relatively numerous in normal horses with immature bone tissue. The cytoplasm also contained vacuolar inclusion bodies of various sizes (figs. 4, 8, 9, 13 & 20). The largest sized inclusion bodies were about the half size of the nucleus. These bodies were relatively numerous in normal horses with mature bone tissue. In aged horses especially, there was tendency to have the large and numerous inclusion bodies. The margin of these inclusion bodies was of high electron density and the ground substance was of moderate or low grade electron density although at times, there was a granular material of a somewhat high electron density. Occasionally filamentous structures were found in the inclusions. These vacuolar inclusions were thought to represent the granular or doughnut like bodies under light microscopy (This was identified by staining with hematoxylin and eosin for light microscopy after removing the plastic in the section prepared for electron microscopy). There were some areas in the cytoplasm of moderate electron density and relatively few organelles. These areas were identified as glycogen rich areas by PAS reaction with diastase-digestion after removing the plastic in the section used for electron microscopy.

**Dark chief cells** There was no difference between the dark chief cells and the light chief cells except the dark chief cells were rich in free ribosomes. Therefore, the cytoplasm of dark chief cells showed a higher electron density than the light chief cells and they were relatively poor in cytoplasmic glycogen (fig. 19).

#### b) Oxyphil cells

Dark and pale oxyphil cells were identified under the electron microscope. In addition, transitional oxyphil cells were recognized by electron microscopy (figs. 10 & 11).

**Dark oxyphil cells** The dark oxyphil cells were large in size. These cells were characterized by a large number of large mitochondria in the cytoplasm (figs. 14 & 16). In addition, a small number of secretory granules, lipid bodies and vacuolar inclusions existed in the cytoplasm of these cells. The small nucleus was located eccentrically and was occasionally degenerative. The nuclear membranes were double and occasionally the outer membrane projected into the cytoplasm. The interior of the mitochondria exhibited a somewhat high electron density and the arrangement of Cristae mitochondriales was irregular. Though the shapes of the mitochondria varied, due to the plane of section, most of them were short and rod shaped. Rarely, the mitochondria were extremely long. The extremities of the mitochondria showed swelling and a vacuolar appearance (fig. 15). Accumulation of lamellar and onion like bodies of the long mitochondria was rarely observed. In the cytoplasm, there was a small number of vesicles with a smooth surfaced endoplasmic reticulum. A small quantity of glycogen was recognized in the cytoplasm. The Golgi apparatus was not well developed and interdigitations of plasma membranes were rather few and flat (fig. 16).

**Pale oxyphil cells** The number of mitochondria was relatively few compared with that of the dark oxyphil cells (figs. 12 & 13). There were a few free ribosomes in the cytoplasm, which were light in color. Vesicles of the smooth surfaced endoplasmic reticulum were prominent and in some parts multivesicular bodies were found (fig. 13). The mitochondria showed variations in size and shapes. Vacuolar inclusions, lipid bodies and occasionally secretory granules were always found in the cytoplasm (fig. 13). The Golgi apparatus was small in size (figs. 12 & 13). In other respects, the pale oxyphil cells were similar to those of the dark oxyphil cells.

**Transitional oxyphil cells** These cells were large in size. The nuclei were oval and occasionally indented with an irregular shape (figs. 10 & 11). The inner and outer nuclear membranes were parallel. There were accumulations of parallel arrays of the rough surfaced endoplasmic reticulum near the nucleus (fig. 10). The Golgi apparatus was relatively well developed and the Golgi vesicles often had electron opaque granules. Numerous spherical or elliptical microbodies of moderate electron density and occasionally a filamentous internal structure were distributed in the cytoplasm (figs. 10 & 11). Among these numerous microbodies in the cytoplasm, there were small spherical or short-rod shaped mitochondria. These numerous microbodies were considered as precursory structures of the mitochondria. In some of these cells, the transition from the light chief cells might be considered from the degree of distribution of the mitochondria (fig. 10).

c) Blood vessels, subendothelial spaces and intercellular spaces

The wall of the blood capillaries was lined with a monolayer of endothelial cells and the outer side was surrounded by a monolayered thin basement membrane under light microscopy. Under electron microscopy, there were three layers between the endothelial cells of the blood capillaries and parenchymal cells (fig. 17). Adjacent to the parenchymal cells and endothelial cells, respectively, was a layer of rather high electron density. The intermediate layer of low electron density showed an amorphous structure. The endothelial cells lined the wall of the blood vessels and in some places, the cytoplasm of the endothelial cells thinned to a flattened sheet. Though the endothelial cells were connected closely with an adjacent basal lamina and the cytoplasm lined the entire capillary lumen, there were fenestrations of this cytoplasm so that in cross sections of the capillary, the endothelial cells appeared discontinuous (fig. 17). At the junctional portion of the endothelial cells, there was a terminal bar. The nucleus of the endothelial cells was usually elongated and irregular in shape. The inner and outer nuclear membranes were straight and parallel. The cytoplasm contained a small number of mitochondria and a rough surfaced endoplasmic reticulum. Large and small vesicles in the smooth surfaced endoplasmic reticulum were distributed in the cytoplasm. It was especially characteristic that the small vesicles stood in a line connecting with the basal lamina. The Golgi apparatus showed a medium grade of development. In some of the small blood vessels (fig. 18), the lumens were lined with endothelial cells and were completely surrounded by a basal lamina. On the outer side of the basal lamina, there were smooth muscle cells. Furthermore, on the outer side of the smooth muscle cell layer, there was a basal lamina. This membrane was immediately adjacent to the parenchymal cells of the parathyroid glands. The basal lamina between the endothelial cell layer and the smooth muscle cell layer was constricted in some parts, as if

both cell layers were adjacent to each other and manifested metabolic changes through the membranes between both cell layers. The cytoplasm of the smooth muscle cells showed well developed vesicles of the smooth surfaced endoplasmic reticulum, and many small (micropinocytotic) vesicles were adjacent to the outer basal lamina. In some of these vesicles (fig. 18) there were distinct flask like invaginations of the membrane lying in the peripheral cell. The Golgi apparatus of the cells was clearly seen and contained some electron opaque granules. The mitochondria of these cells were few in number.

**Subendothelial space** An intermediate layer of the basal lamina with a somewhat low electron density occasionally increased its width and contained collagen fibers, mesenchymal cellular elements (fibroblasts) and sometimes unmyelinated nerve fibers. These subendothelial spaces were continuous with the intercellular spaces between the parenchymal cells.

d) **The parathyroid glands in equine osteodystrophia fibrosa**

In equine osteodystrophia fibrosa, vacuolated chief cells, transitional water clear cells and water clear cells were strikingly evident in focal accumulations of lobular, acinar and occasionally small cell groups each with a different affinity for hematoxylin and eosin stain. The other parts of the glands were mainly occupied by light chief cells.

**Vacuolated chief cells** The irregularity of the inner and outer nuclear membranes was remarkable (figs. 19, 20 & 23) and the outer nuclear membrane was expanded in some parts. The nucleus was spherical or oval and occasionally had a somewhat irregular shape. There were one or two nucleoli. It was obvious that the vacuolated chief cells may have originated from the chief cells in view of the distribution of the organelles and morphology of the cytoplasm. The vacuolated light or dark chief cells were differentiated by the amount of free ribosome in the cytoplasm. Glycogen in the cytoplasm of vacuolated light chief cells was much richer than in the vacuolated dark chief cells. The vacuolated chief cells were characterized by the presence of the markedly dilated endoplasmic reticulum (figs. 19~23) The mitochondria were widely distributed in the cytoplasm and Cristae mitochondriales had characteristic arrangement, but destruction of the internal structure of the mitochondria was not found. The mitochondria had a medium grade of electron opaque granules. The Golgi apparatus showed, occasionally, cystic appearances. In the cytoplasm, there were small numbers of pro-secretory granules, lipid bodies and vacuolar inclusions.

**Transitional water clear cells** The transitional water clear cells were large in size and poor in cytoplasmic organelles (fig. 23). These cells were electron transparent. An increase of glycogen in the cytoplasm had a tendency to push the organelles to the periphery of the cell. In some parts of the cytoplasm, there were a few secretory granules, lipid bodies and vacuolar inclusions. The Golgi apparatus was small, but numerous vesicles, vacuoles and sacs had accumulated. The rough surfaced endoplasmic reticulum was flattened and situated around the nucleus in the periphery of the cell. There were one or two nucleoli. The inner and outer nuclear membranes were irregular. The plasma membranes were connected by interdigitation of the adjacent cell membranes. It was apparent that the transitional water clear cells might have come from the vacuolated light chief cells.

**Water clear cells** This cell (fig. 25) was extremely large. The nucleus was situated eccentrically and relatively small in size. The organelles were pushed to the periphery of the cell. The cytoplasm contained a large amount of glycogen. The existence of glycogen

was easily confirmed by the comparison of the PAS reaction used for electron microscopy. At the periphery of the cell, there were only a few small mitochondria, string like rough surfaced endoplasmic reticulum, vesicles of the smooth surfaced endoplasmic reticulum and lipid bodies.

Intercellular spaces, subendothelial spaces and blood vessels In cases of equine osteodystrophia fibrosa, intercellular spaces were dilated and occasionally contained substances with high electron density. Especially in the vacuolated chief cells, the cystic dilated endoplasmic reticulum was connected directly with intercellular spaces, there were spaces which made way for the release of secretory products from the endoplasmic reticulum to the blood vessels in the fashion of a canalicular system.

#### DISCUSSION

##### 1) Classification of parenchymal cells of the equine parathyroid glands

The available literature contains only a brief mention of a few reports (BOBEAU and LITTY) on the histology of the equine parathyroid glands. The reference to those reports gave only a brief comment and no details. Recently KROOK & LOWE (1964) reported the nutritional secondary hyperparathyroidism in the horse and showed various types of parenchymal cells of the equine parathyroid glands: light chief cells, dark chief cells, water clear cells and pale oxyphil cells. There are no known published reports on electron microscopy of equine parathyroid glands. In this study, by electron microscopy all of the cells which have been described by light microscopy<sup>21,22)</sup> could be recognized and in addition, transitional oxyphil cells, transitional water clear cells, and vacuolated chief cells could be recognized.

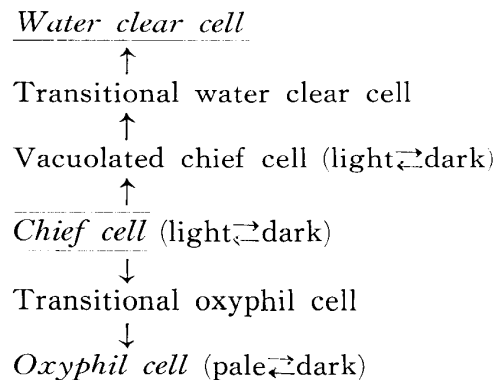
Although electron microscopic studies of the parathyroid glands in human and animals are comparatively few, LEVER<sup>9)</sup> has described light and dark chief cells. TRIER studies the parathyroid glands of monkeys. MUNGER & ROTH studied the glands of humans and of Virginia deer. ROTH & MUNGER investigated two cases of human parathyroid adenoma and two of primary chief cell hyperplasia, and demonstrated chief cells and oxyphil cells. LANGE classified the cells in 4 human cases of chief cell adenoma as dark oxyphil cells, small water clear cells, large water clear cells, and adenoma cells. PORTE & PETROVIC demonstrated only chief cells in parathyroid glands of hamsters. CAPEN et al.<sup>2,3)</sup> investigated bovine parathyroid glands and demonstrated light, dark, intermediate and atrophic chief cells in pregnant and nonpregnant cows fed without or with a high level of vitamin D. Specifically, there has been no report on electron micrographs of transitional oxyphil cells, transitional water clear cells, and vacuolated chief cells, which the authors have pointed out as the transitional cells.

From the results of this investigation, chief cells can be regarded as the stem cell of the parenchymal cells of the equine parathyroid glands. The various types

of cells of the parathyroid glands are thought to have derived from the chief cell. The chief cells are differentiated as two types, light and dark by electron microscopy. The cytoplasm of the light chief cells is rich in glycogen, but the cytoplasm of the dark chief cells is poor in glycogen and rich in free ribosomes. These characteristics are thought to represent some functional differences between the types of cell.

With regard to the formation of the oxyphil cells, it might be suggested that oxyphil cells are formed from chief cells by way of the transitional oxyphil cell. The transitional oxyphil cell is characterized by numerous microbodies with a medium grade of electron density, which are thought to represent the precursory structures of the mitochondria, in the cytoplasm. The dark oxyphil cell has large and numerous mitochondria in the cytoplasm, while the pale oxyphil cell has fewer mitochondria and therefore has a paler cytoplasm.

It has been demonstrated by electron microscopy that the parenchymal cells of the equine parathyroid glands have various transitional types of cells between the oxyphil cells and the water clear cells. These relationships can be summarized in the following schema.



## 2) Interpretation of electron microscopic findings on the parathyroid glands of equine osteodystrophia fibrosa

In previous reports<sup>5,19,21</sup>, it was presumed that the morphological changes of the parathyroid glands as seen with light microscopy followed the advanced bone tissue changes in equine osteodystrophia fibrosa (secondary hyperparathyroidism), and these morphological changes were considered as reactive and hyperplastic. The important morphological features in horses with severe changes of bone tissues, were enlargement of the parathyroid gland and a patchy appearance in stained sections, though these changes did not always occur.

Electron microscopically, the light chief cells comprised most of the parenchymal cells as found by light microscopy. In horses with severe changes of bone tissue, one of the characteristic features was the intermingling of the light



and the dark chief cells and most of these cells were a type of the vacuolated chief cell. In these cell groups, the endoplasmic reticulum showed a vacuolar or cystic appearance. Lipid bodies and secretory granules were found in some. The Golgi apparatus usually had vacuolar cystic appearance. These findings are considered to be an abnormal state produced by secretory hyperfunctions. Slight dilatation of the intercellular spaces was noted in glands from some horses with immature bone tissue, but not in horses with normal mature bone tissue. In equine osteodystrophia fibrosa, both vacuolization of the cytoplasm of the parenchymal cells and conspicuous cystic and saccular dilation of the intercellular spaces occurred. Outflow of electron opaque substances was frequently observed in the intercellular spaces (fig. 21). The intercellular spaces from a meandering maze were connected with the subendothelial spaces around the blood capillaries (fig. 22). The blood capillaries of parathyroid glands were lined with endothelial cells which did not completely surround the lumen of the capillaries and were, in some places, flattened with numerous fenestrations of their cytoplasm (fig. 17). Since the basal lamina is then directly exposed to the lumen of the blood vessels through these fenestrations, it is possible to release secretory products directly into the blood circulation.

MUNGER & ROTH found secretory granules in capillary endothelial cells of both the normal human and the Virginia deer parathyroid, numerous granules were also found in parathyroid adenomas and primary chief cell hyperplasia in humans. Furthermore a rare granule has been found in the extracellular perivascular space. From these findings, they suggested the following secretory process: the intact secretory granules leave the parathyroid chief cell, traverse the epithelial basal lamina, the perivascular space, and the capillary basal lamina to enter the capillary endothelial cell. Granules then appear to accumulate in the endothelial cells and then released into the blood stream.

Even though the light chief cells were relatively rich in glycogen and large in size, MUNGER & ROTH considered them as inactive because they had almost no secretory granules and a small Golgi apparatus even though the dark chief cells were small in size and relatively poor in glycogen. The concept of the dark chief cell as an active cell should be considered in connection with changes of the bone tissue. Since the dark chief cell is thought to play a main role in normal horses with mature bone tissue, some authors regard this cell as a functionally mature cell. The dark chief cell may have some high potency of activity. In our studies, many of the light chief cells had a relatively well developed Golgi apparatus and many secretory granules. Therefore the light chief cells were considered to be functioning cells. The vacuolated chief cell displayed a dilatation of the Golgi vesicles and vacuolization of the endoplasmic reticulum. These cell changes were

therefore regarded as an abnormal state produced by hyperfunction. For these reasons, the light chief cells and the vacuolated chief cells have some relationship in hyperfunction. Glycogen in the cytoplasm of the light chief cells was much richer than in the dark chief cells. The water clear cells represent a transition from vacuolated light chief cells and have a marked content of glycogen in their cytoplasm. The water clear cells are regarded as abnormal inactive cells produced by hyperfunction.

### 3) The mechanism of formation of secretory granules in the equine parathyroid glands

It is a well known fact that parathyroid hormone is produced from parathyroid glands and regulates the bone metabolism. The parathyroid hormone is composed of a polypeptide. In our study, three components, secretory granules, lipid bodies and vacuolar inclusions, were encountered mainly in the cytoplasm of chief cells. Similar components, though fewer, were noted in other kind of cells. Although the character of the vacuolar inclusions is not fully understood, they might have some relation to cellular function. Some relationship seems to exist between lipid bodies and secretory granules (pro-secretory granules). LEVER<sup>9)</sup> and TRIER suggested that lipid aggregates, lipid bodies, and vesicles were substances produced in the process of secretion, though they did not recognize secretory granules by electron microscopy. DAVIS & ENDERS found small bodies of high electron density in the Golgi region of the parathyroid glands of rats, and they considered them to be small secretory granules. They showed these granules to have some relationship to the multivesicular bodies. MUNGER & ROTH demonstrated bodies with high electron density, similar to those observed by DAVIS & ENDERS, in normal parathyroid glands of humans and the Virginia deer. They regarded these bodies as secretory granules, however they could not demonstrate a relationship between these granules and the multivesicular bodies. They reported that these granules vary in size, are produced from the Golgi apparatus, and coincide with hematoxylin positive bodies and argyrophilic bodies under light microscopy. CAPEN et al.<sup>2,3)</sup> also recognized a similar secretory mechanism in the bovine parathyroid gland.

The Golgi apparatus may be considered to be a differentiated part of the vacuolar membranous system. Lipid synthesis and metabolism tend to be active in the smooth surfaced endoplasmic reticulum. Recently, it has become known that the smooth surfaced endoplasmic reticulum plays an important role in carbohydrate metabolism, while the rough surfaced endoplasmic reticulum is active in protein metabolism. It is therefore reasonable to think that the secretory granules are formed in the vacuolar membranous system. There are high electron opaque bodies in the endoplasmic reticulum and in the Golgi vacuoles. These

bodies are thought to represent secretory granules which were accumulated and concentrated as secretory products in the Golgi vacuoles. It is a well known fact that the Golgi apparatus plays an important role in the secretion of materials synthesized by the cell. Therefore it may safely be said that secretory or pro-secretory granules may be produced in the Golgi apparatus.

#### SUMMARY

An electron microscopic study was made of equine parathyroid glands from 53 horses (12 normal horses with immature bone tissue, 31 normal horses with mature bone tissue, and 10 cases of equine osteodystrophia fibrosa), and compared with light microscopic findings.

1) By electron microscopy, the authors could identify not only chief cells (light and dark), oxyphil cells (pale and dark) and water clear cells, which were previously noted by light microscopy, but also vacuolated chief cells, transitional water clear cells and transitional oxyphil cells.

2) The chief cell is the stem cell and plays an important role in equine parathyroid glands. From the electron micrographs, the evidence may suggest that the various types of cells have been derived from the chief cell in the equine parathyroid glands.

3) The light chief cells were impressive in cases of equine osteodystrophia fibrosa. Especially in severe cases, many of the light chief cells took the form of vacuolated light chief cells (only a few vacuolated dark chief cells were seen), and in some parts of the parathyroid gland, foci of water clear cells were frequently observed.

4) The vacuolated light chief cell was regarded as an abnormal cell produced by hyperfunction, because of its richness in glycogen, marked vacuolization of the endoplasmic reticulum, vacuolization and dilatation of the Golgi vesicles and existence of lipid bodies. The water clear cell was also regarded as an abnormal inactive cell produced by hyperfunction, because of the extremely rich glycogen in the cytoplasm. The light chief cell was regarded as a functioning cell, because of the secretory granules, and dilatation of the Golgi vesicles.

5) It is suggested that secretory products may accumulate and concentrate in the vacuolar or vesicular system of the Golgi apparatus. Pro-secretory granules are formed and these may develop into secretory granules in the endoplasmic reticulum system. It is further suggested that there is some relationship between secretory granules and lipid bodies.

6) The intercellular spaces were not remarkable except in equine osteodystrophia fibrosa when they were markedly dilated or cystic and an outflow of high

electron opaque substances was frequently observed in the intercellular spaces.

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## EXPLANATION OF PLATES

All the scales printed in the plates show 1  $\mu$ .

### PLATE I

- Fig. 1 Case No. 7 (UM 203) 1 year old  $\times 18,600$   
A whorl like body (arrow) in the cytoplasm of a light chief cell  
Normal horse with immature bone tissue
- Fig. 2 Case No. 26 (UM 210) 8 years old  $\times 18,600$   
In the center of a whorl like body (arrow) of the cytoplasm of a light chief cell, there are some vesicular components and an elongated sack structure.  
Normal horse with mature bone tissue
- Fig. 3 Case No. 7 (UM 203) 1 year old  $\times 16,500$   
Numerous micropinocytotic vesicles of the smooth surfaced endoplasmic reticulum (arrow), which are considered to be the infolding of the cell surfaces, are noted.  
Normal horse with immature bone tissue
- Fig. 4 Case No. 36 (UM 207) 15 years old  $\times 18,600$   
Cilia like structure (arrow), multivesicular body (Mv) and a vacuolar inclusion (V) are seen in the light chief cell.  
Normal horse with mature bone tissue
- Fig. 5 Case No. 50 (UM 312) 8 years old  $\times 17,500$   
Desmosomes (arrow) in the adjacent connecting plasma membranes of vacuolated light chief cells. The cytoplasm of these cells are rich in micropinocytotic vesicles.  
Case of equine osteodystrophia fibrosa

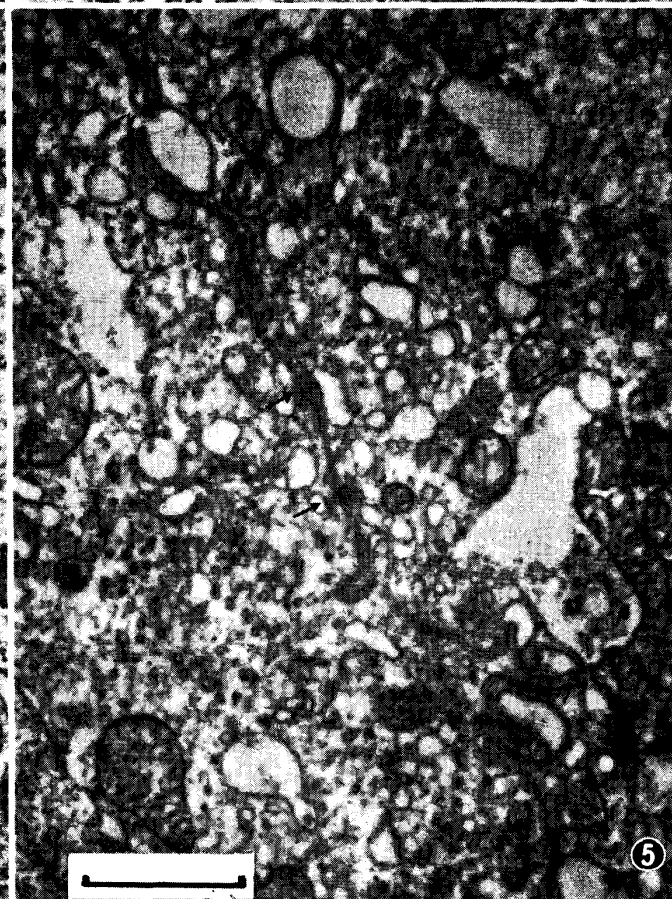
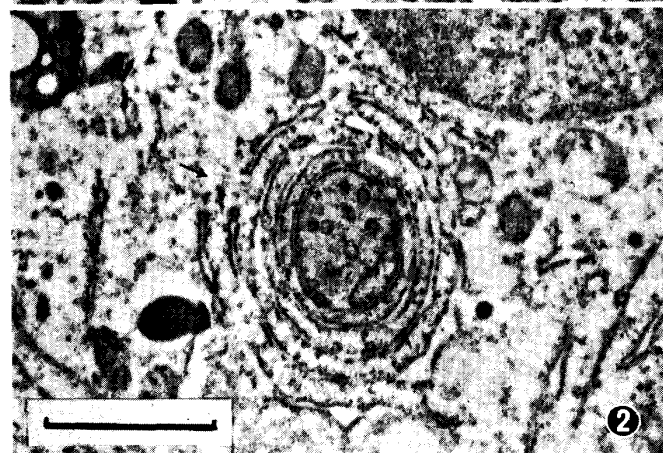
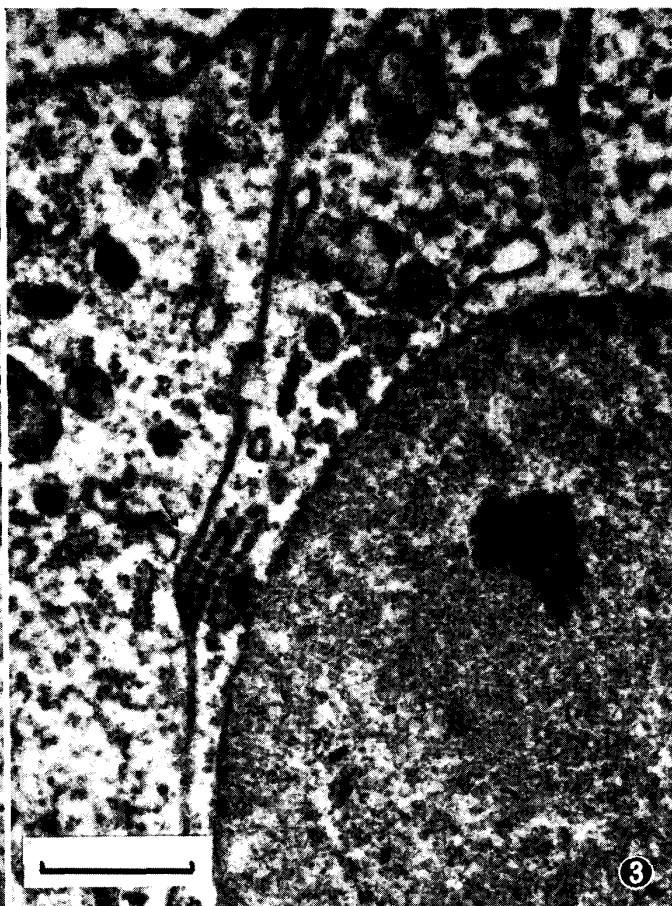
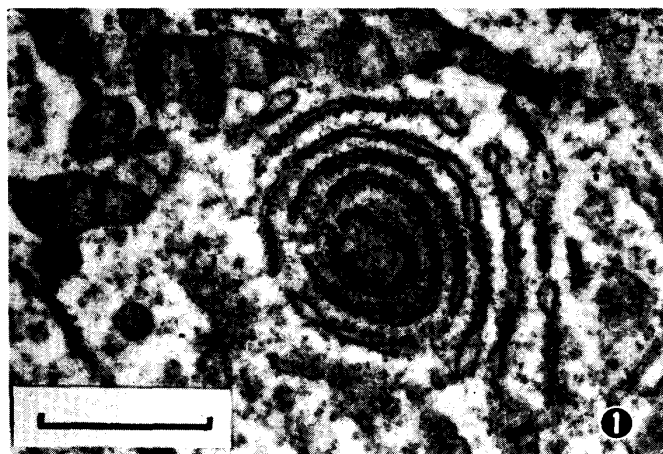


PLATE II

- Fig. 6 Case No. 5 (UM 190) 1 year old  $\times 12,300$   
Many large secretory granules (S) (arrow) are noted in the light chief cells.  
Normal horse with immature bone tissue
- Fig. 7 Case No. 36 (UM 207) 15 years old  $\times 13,200$   
Lipid bodies (L) (arrow), flattened rough surfaced endoplasmic reticulum (ER) and a Golgi apparatus (G) are seen in the light chief cells.  
Normal horse with mature bone tissue



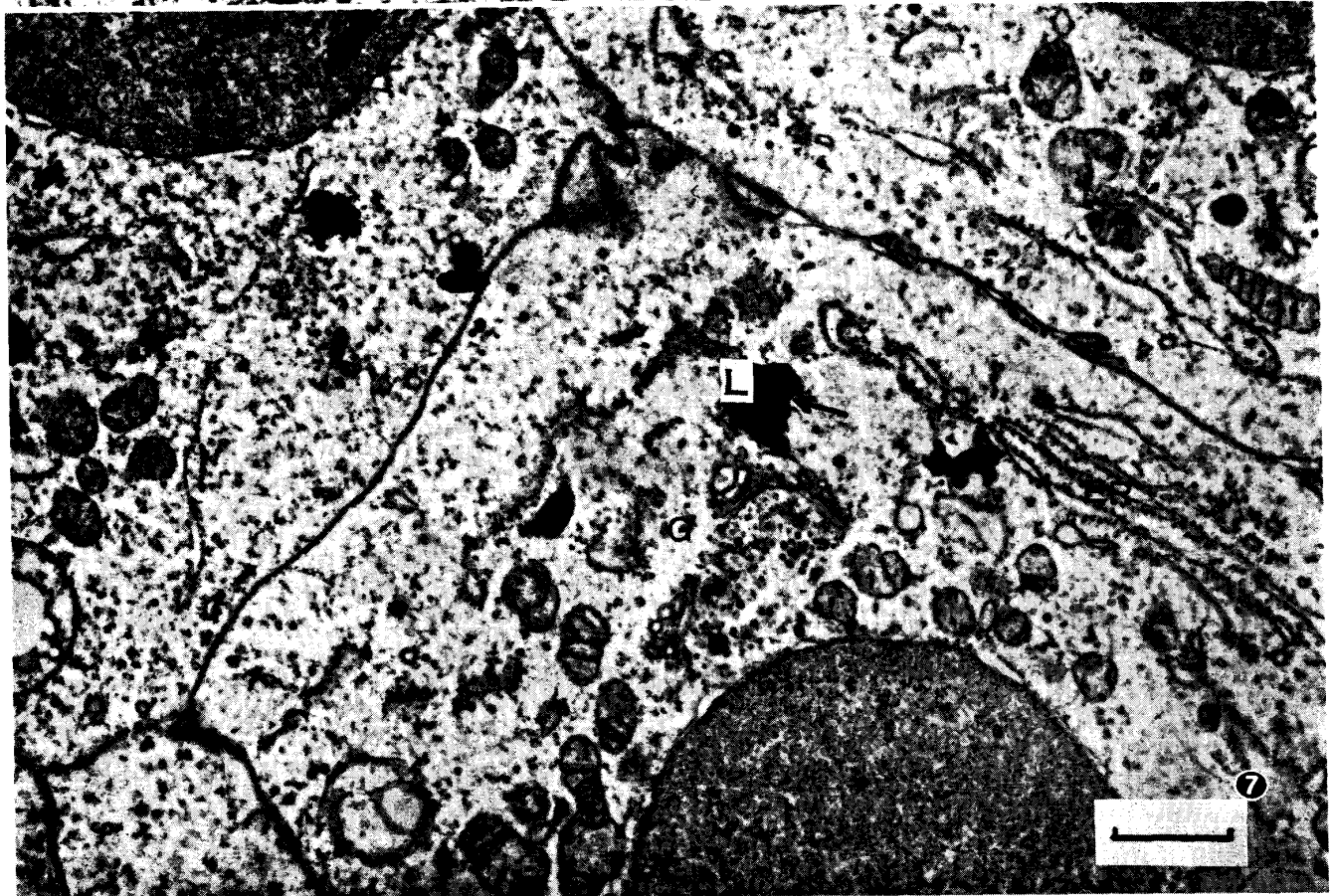
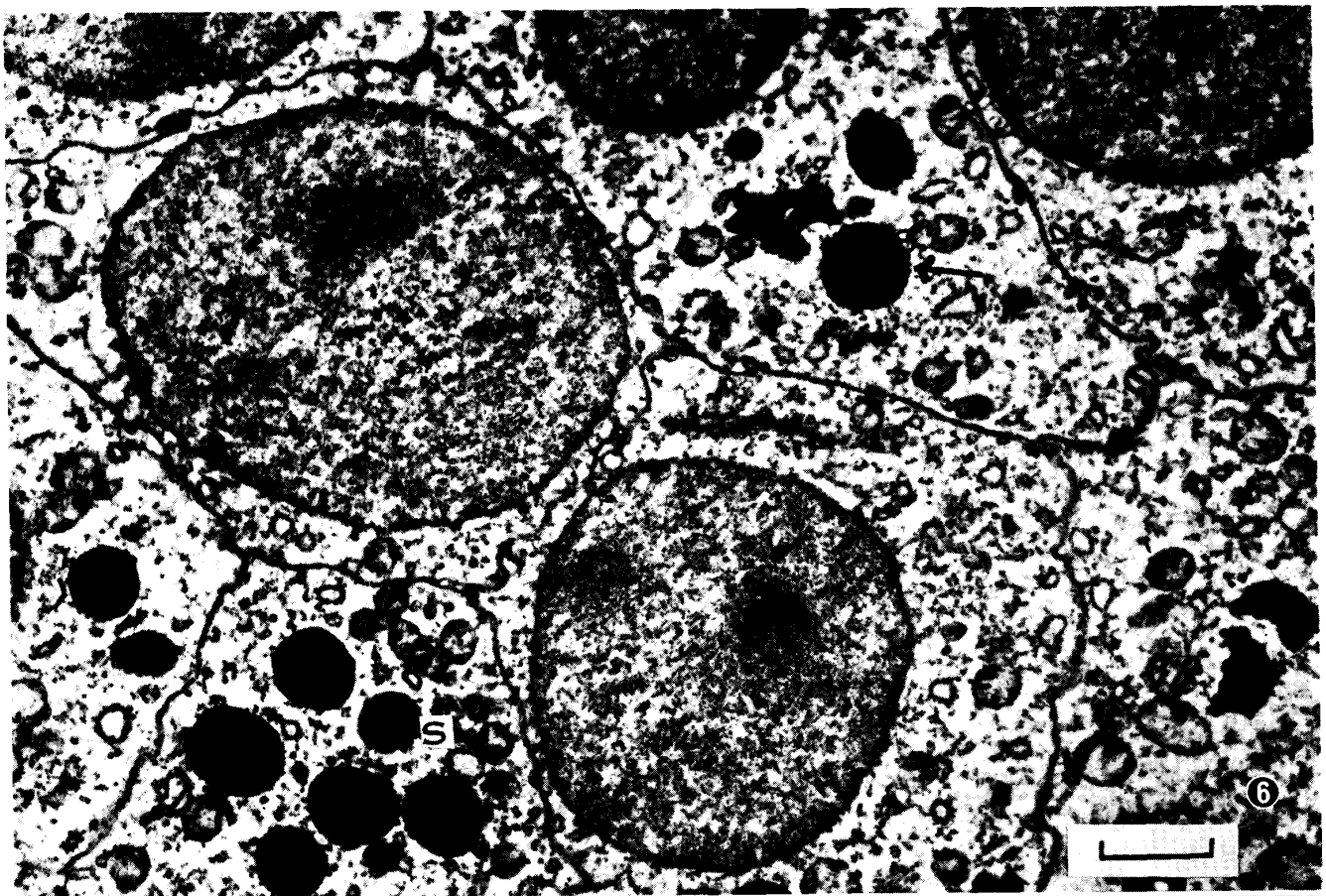




PLATE III

Fig. 8 Case No. 20 (UM 221) 7 years old  $\times 10,500$

Vacuolar inclusion bodies (V) (arrow), lipid bodies (L) and secretory granules (arrow) are seen in the light chief cells. The blood capillary is seen in the right corner of the micrograph. B: basal lamina  
Normal horse with mature bone tissue

Fig. 9 Case No. 20 (UM 221) 7 years old  $\times 10,500$

The same findings as fig. 8

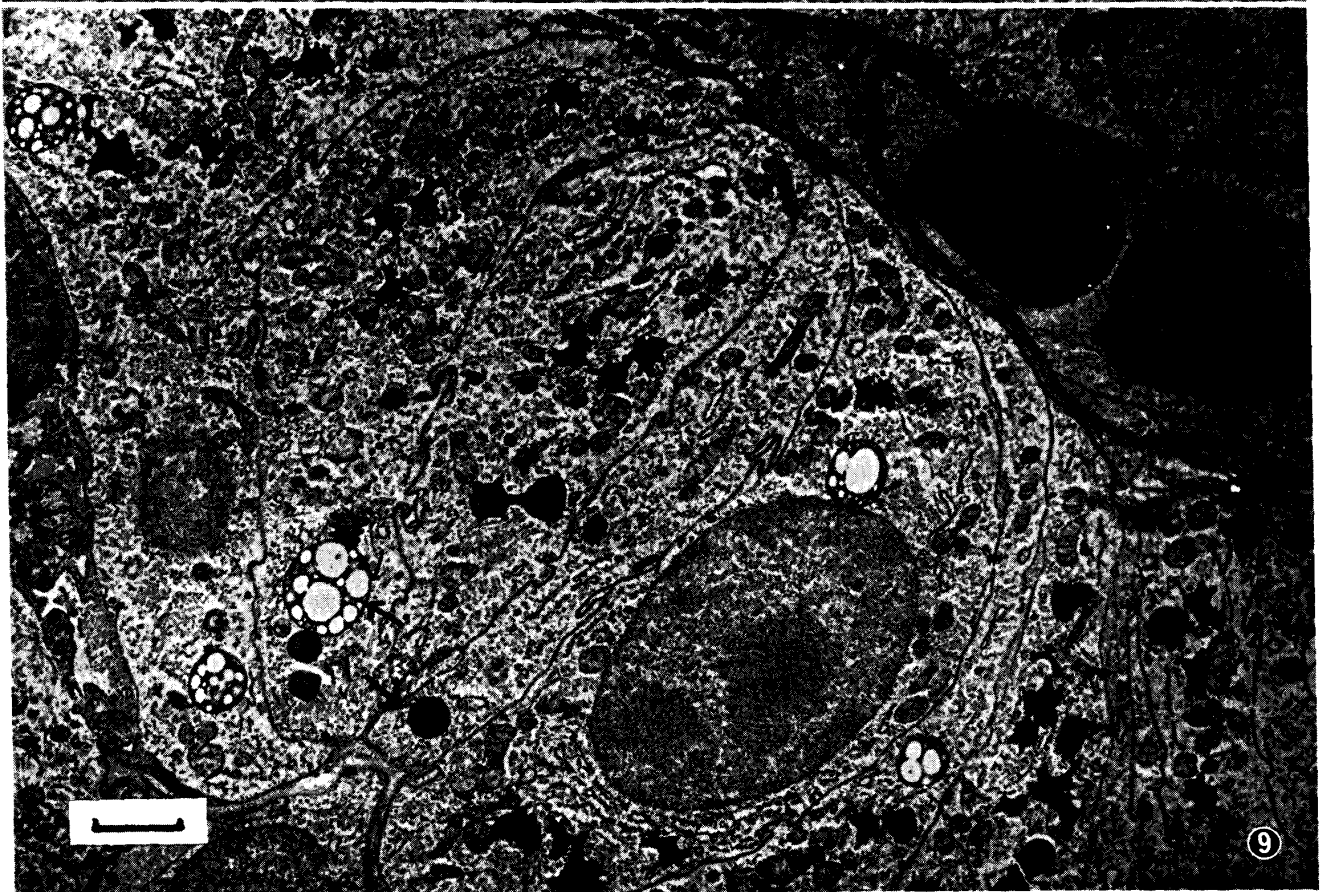
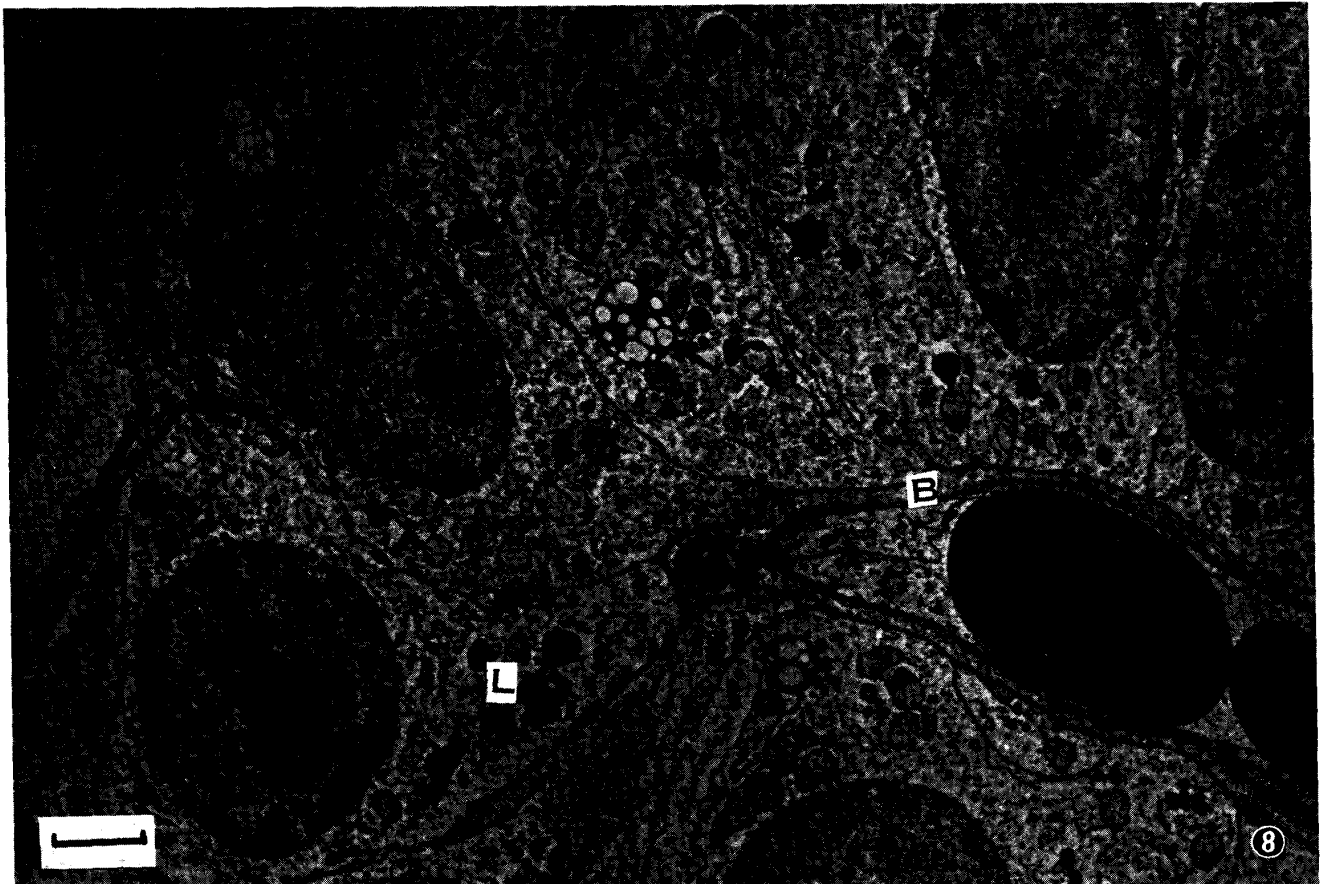


PLATE IV

- Fig. 10 Case No. 36 (UM 207) 15 years old  $\times 10,200$   
Microbodies (m) are scattered in the cytoplasm of a transitional oxyphil cell and Golgi vesicles (G) still have pro-secretory granules. Rough surfaced endoplasmic reticulum (ER) is accumulated on one side of the nucleus. M: mitochondria  
Normal horse with mature bone tissue
- Fig. 11 Case No. 36 (UM 207) 15 years old  $\times 9,600$   
Numerous microbodies (m) are noted in the cytoplasm of a transitional oxyphil cell. M: mitochondria, G: Golgi area  
Normal horse with mature bone tissue

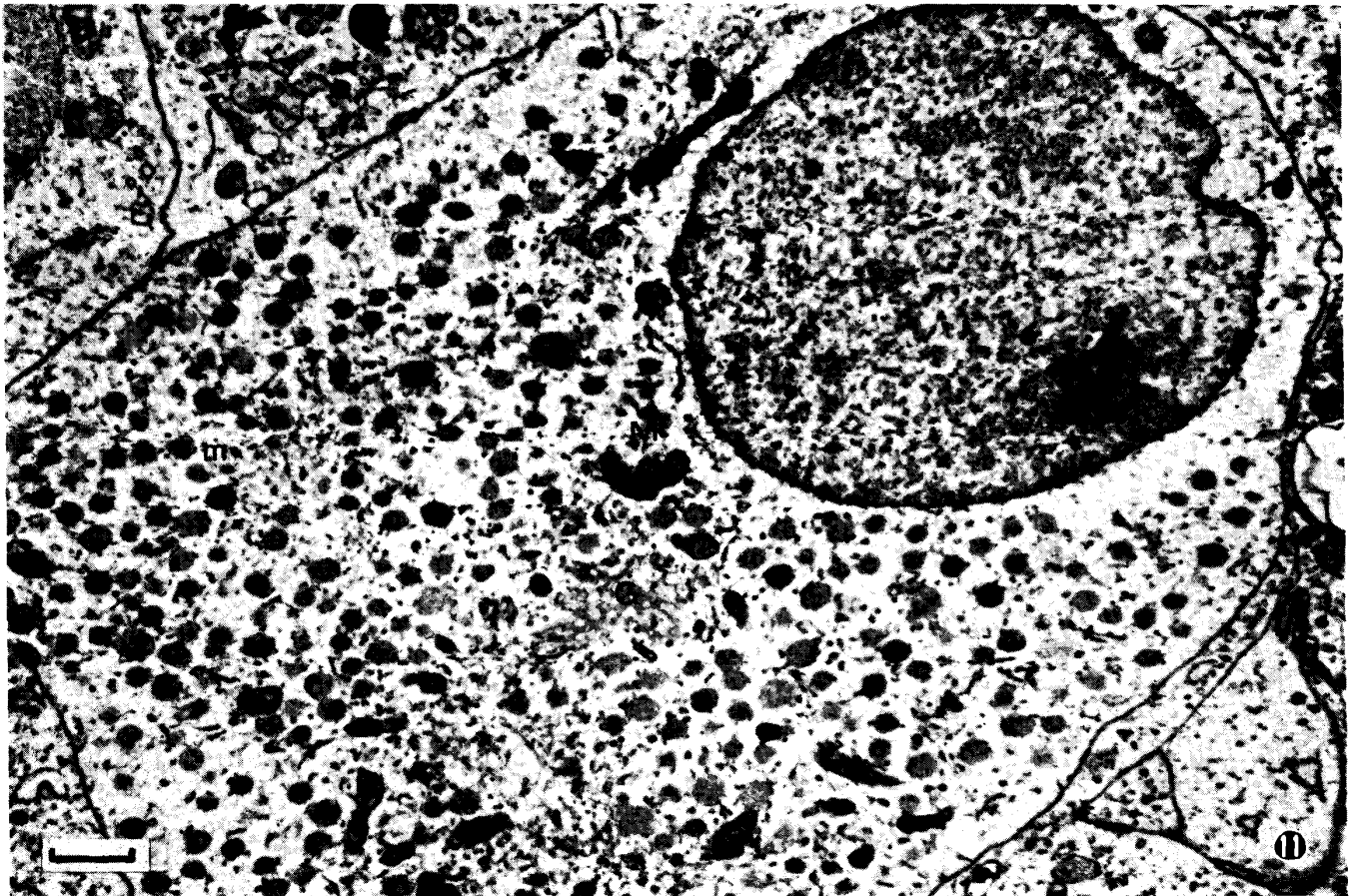
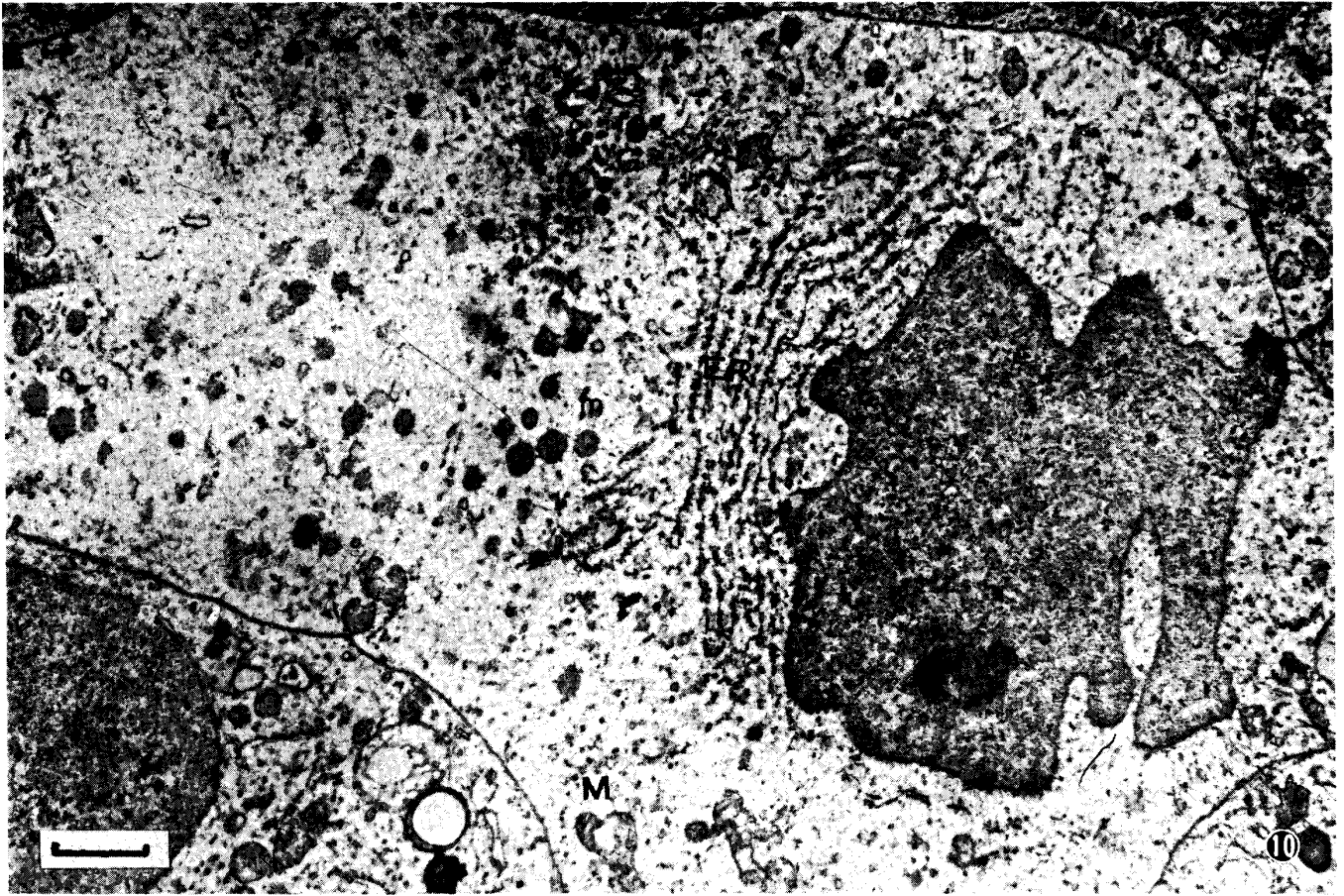


PLATE V

- Fig. 12 Case No. 36 (UM 207) 15 years old  $\times 10,200$   
Pale oxyphil cell have, relatively, many mitochondria (M) in the cytoplasm. S: secretory granule, G: Golgi apparatus  
Normal horse with mature bone tissue
- Fig. 13 Case No. 29 (UM 200) 9 years old  $\times 13,600$   
A pale oxyphil cell is seen at the two-thirds of the micrograph and have vacuolar inclusions (V). Upper right corner of the micrograph shows a light chief cell with lamellar structure of endoplasmic reticulum (ER). G: Golgi apparatus, Mv: Multivesicular body, M: Mitochondria  
Normal horse with mature bone tissue
- Fig. 14 Case No. 48 (UM 314) 7 years old  $\times 15,000$   
Dark oxyphil cell have numerous mitochondria (M) in the cytoplasm.  
Case of equine osteodystrophia fibrosa
- Fig. 15 Case No. 18 (UM 201) 7 years old  $\times 10,500$   
Numerous, extremely long, mitochondria are noted in the cytoplasm of an oxyphil cell and extremities of the mitochondria (M) show swelling and vacuolar appearances. V: vacuolar inclusion body  
Normal horse with mature bone tissue



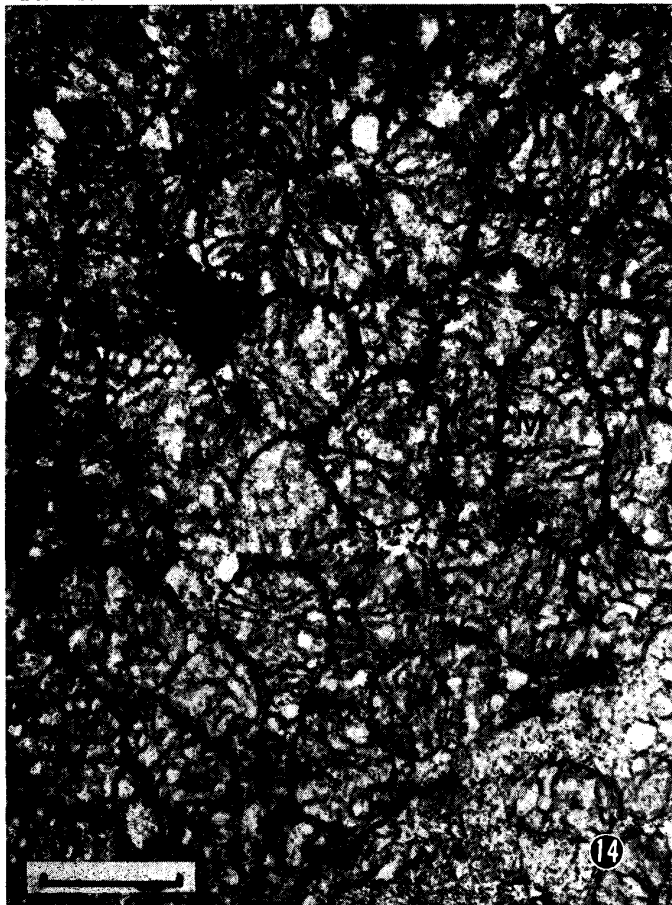
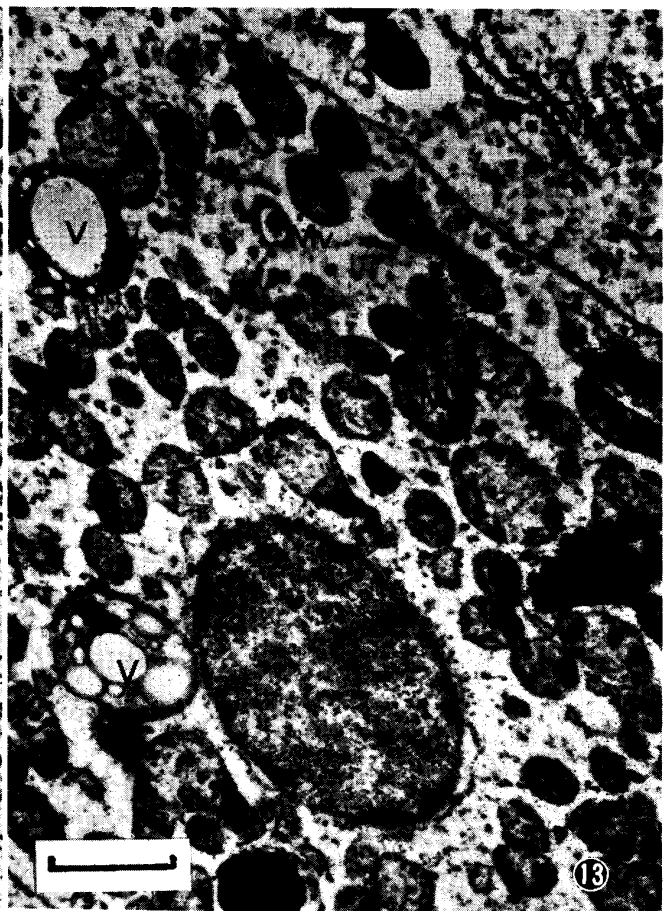
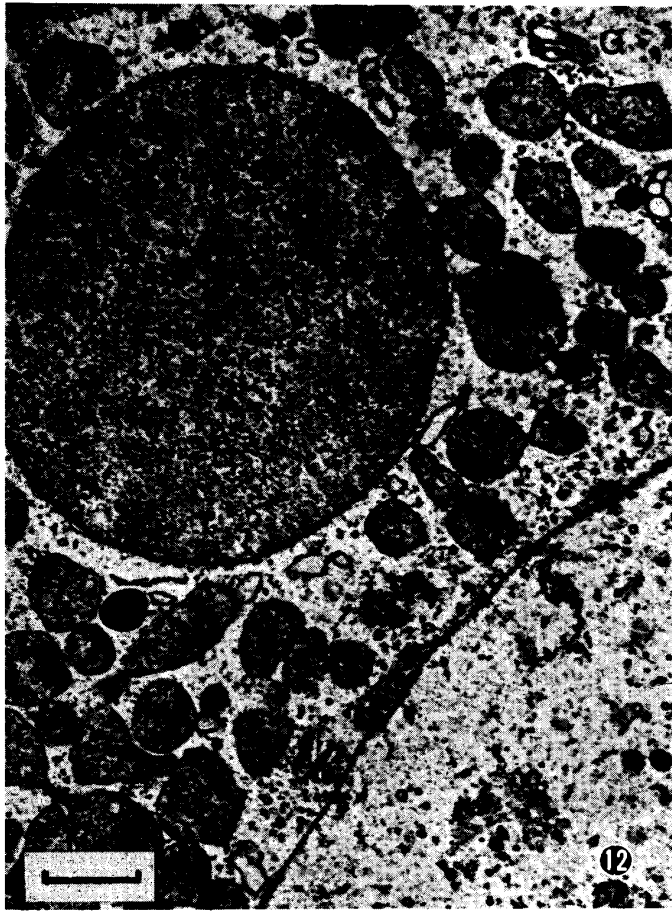


PLATE VI

- Fig. 16 Case No. 26 (UM 210) 8 years old  $\times 12,300$   
Two dark oxyphil cells (upper part of the micrograph) and a pale oxyphil cell (lower part of the micrograph) are seen.  
Normal horse with mature bone tissue
- Fig. 17 Case No. 36 (UM 207) 15 years old  $\times 20,700$   
Wall of a capillary: The cytoplasm of the endothelial cells becomes thinned forming a flattened sheet. Arrows show fenestrations of the cytoplasm of the endothelial cells (En). B: basal lamina, T: terminal bar  
Normal horse with mature bone tissue

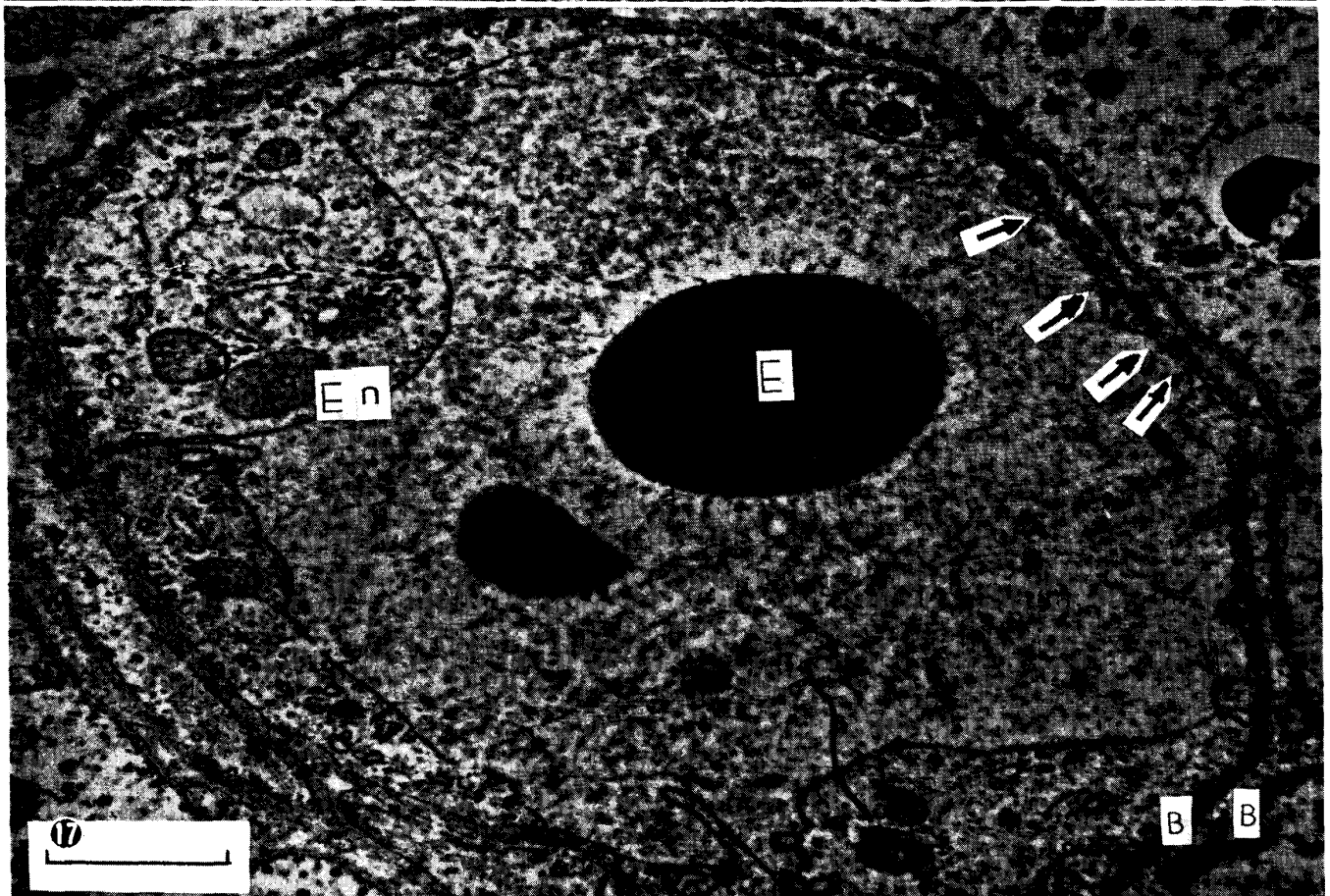
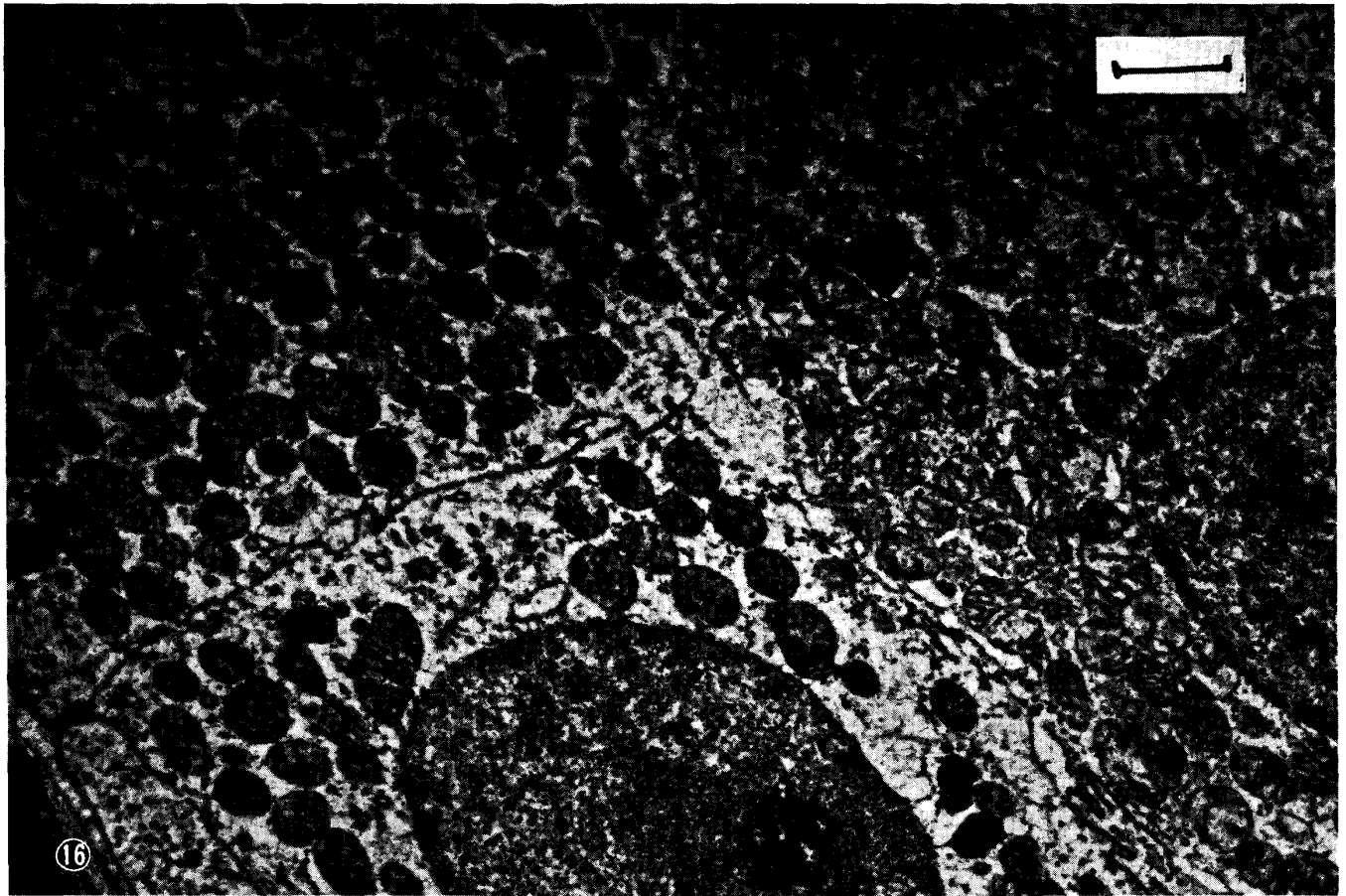




PLATE VII

Fig. 18 Case No. 20 (UM 221) 7 years old  $\times 10,600$

Wall of a small blood vessel: The basal lamina (B) are noted between the endothelial cell layer (En) and the parenchymal cells through a smooth muscle cell layer (Sm). The basal lamina between the endothelial cell layer and the smooth muscle cell layer is constricted in some parts (arrows), as if both cell layers were adjacent and manifested metabolic changes through the membranes between both cell layers. (E): erythrocyte

Normal horse with mature bone tissue

Fig. 19 Case No. 50 (UM 312) 8 years old  $\times 26,000$

A dark chief cell (lower part of the micrograph) and a vacuolated light chief cell. Intercellular spaces (I) show dilatation.

Case of equine osteodystrophia fibrosa

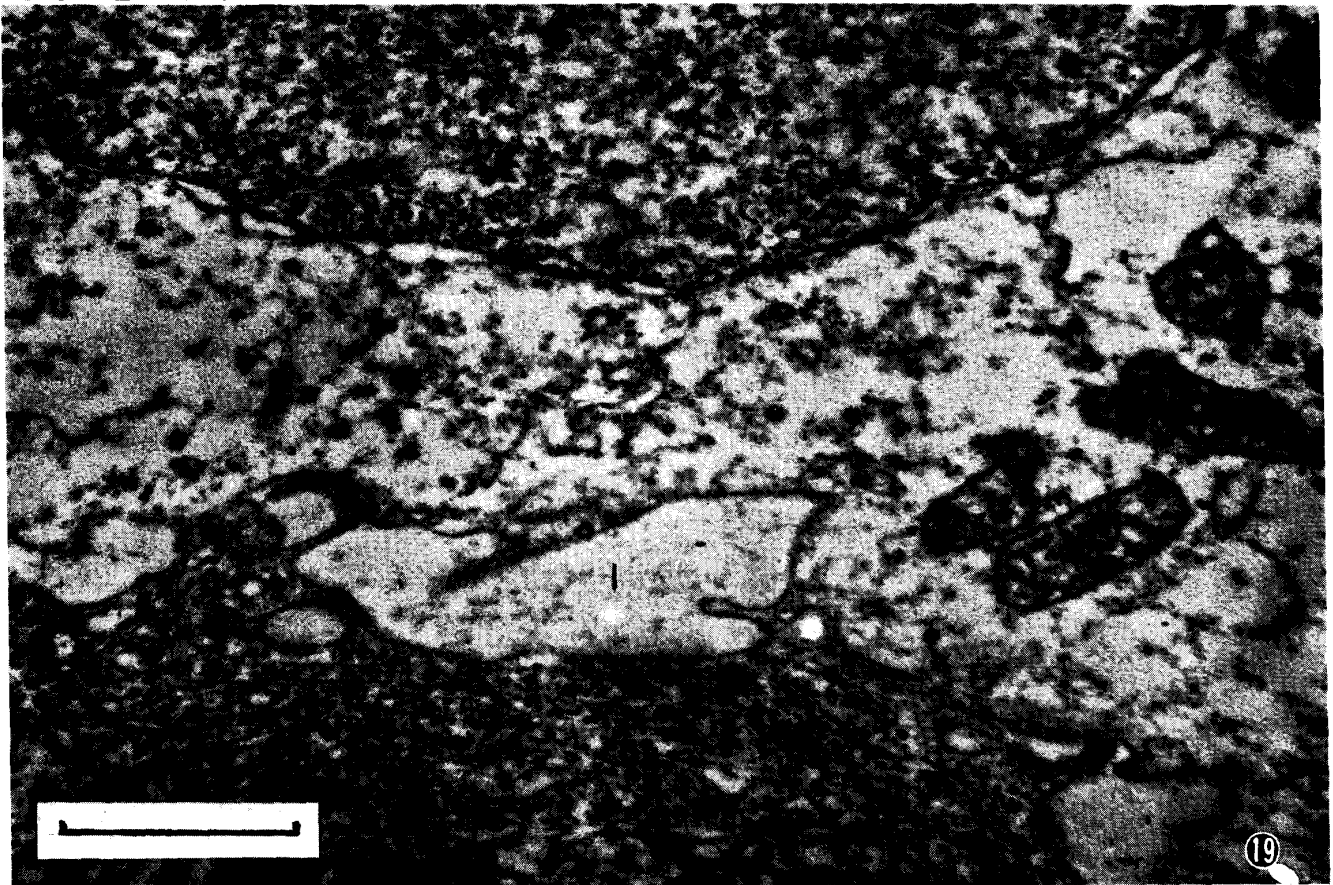
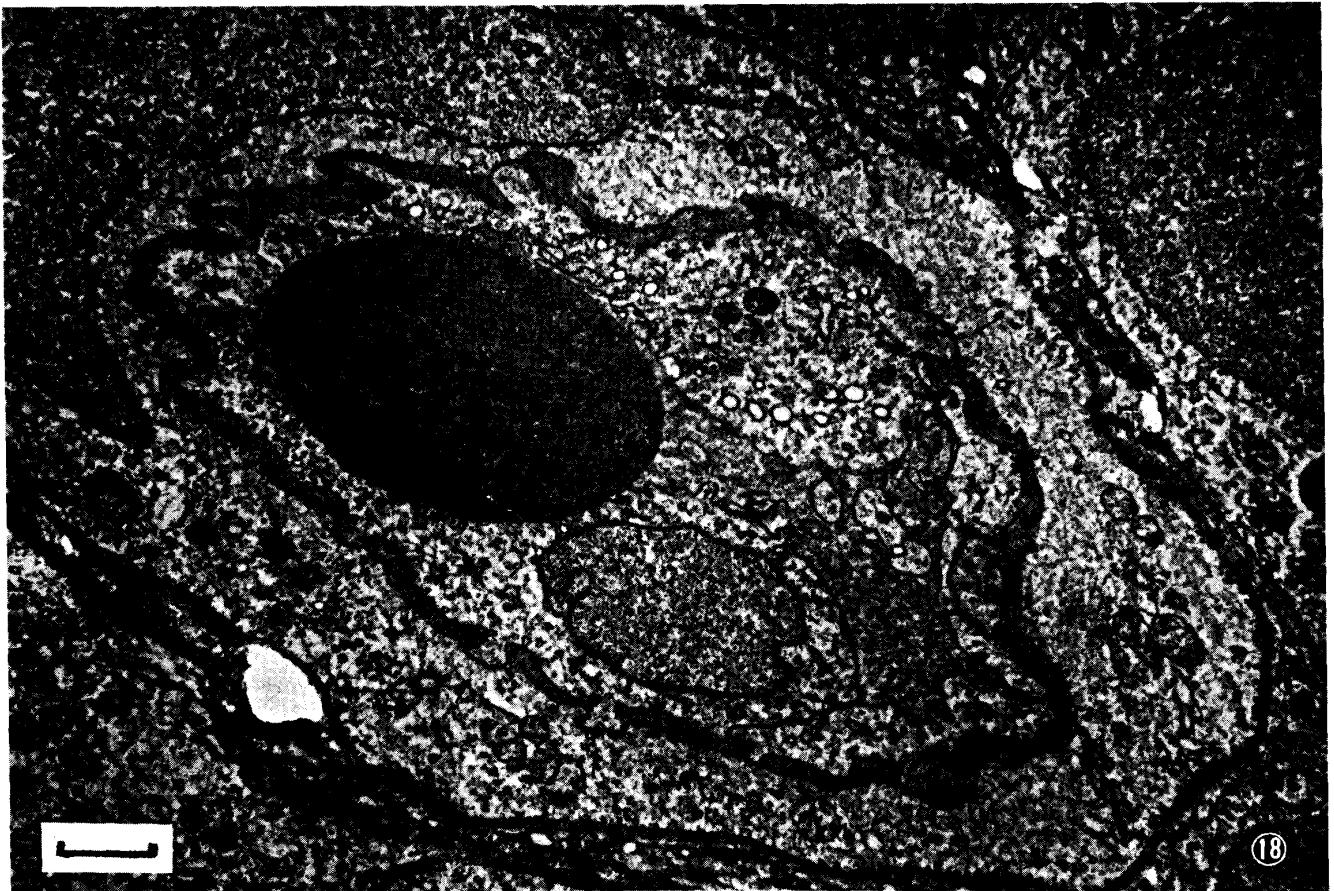


PLATE VIII

Fig. 20 Case No. 50 (UM 312) 8 years old  $\times 21,000$   
Vacuolated light chief cells  
Case of equine osteodystrophia fibrosa

Fig. 21 Case No. 50 (UM 312) 8 years old  $\times 21,000$   
Vacuolated light chief cells: In the intercellular spaces (I), there  
are some electron opaque materials.  
Case of equine osteodystrophia fibrosa

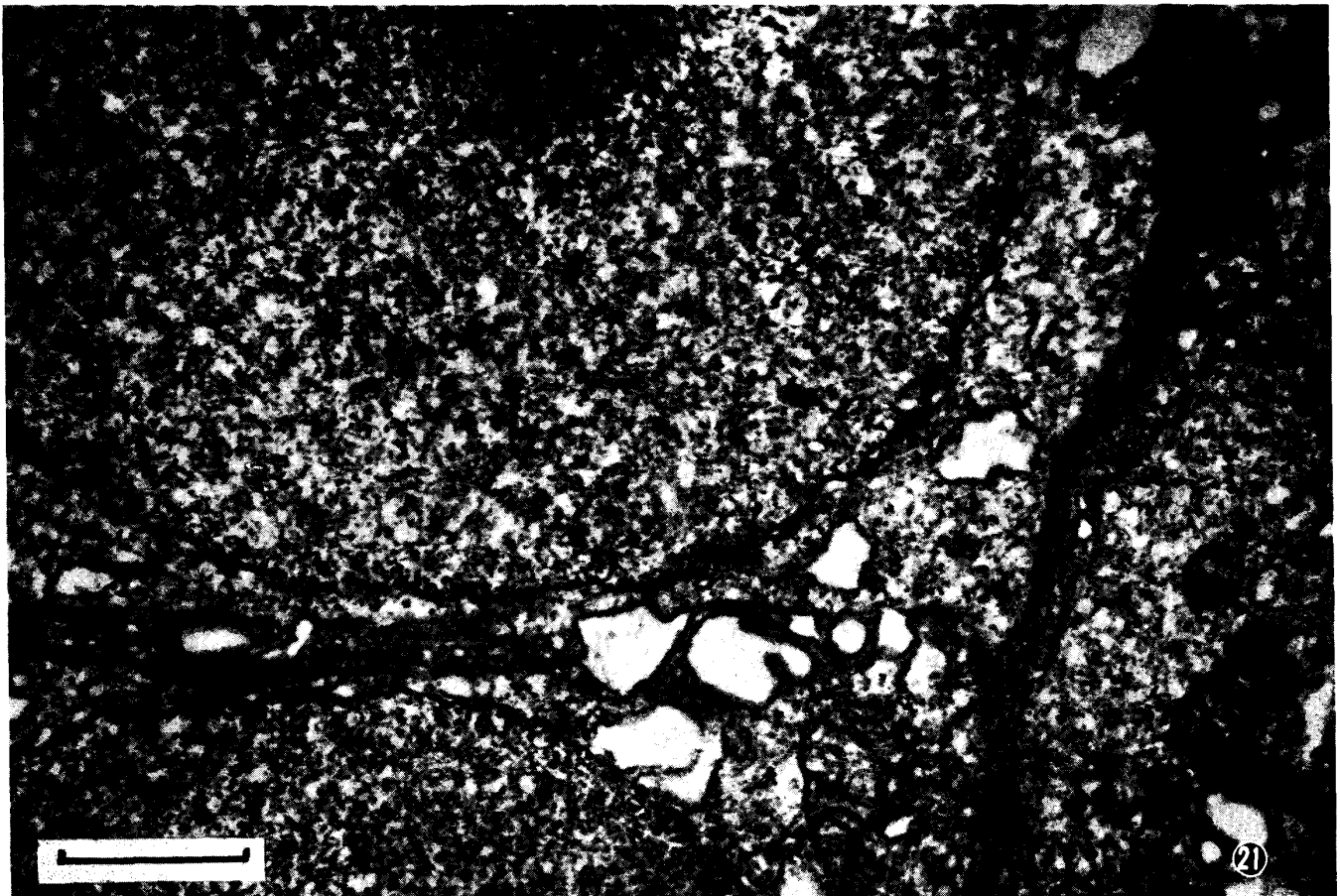
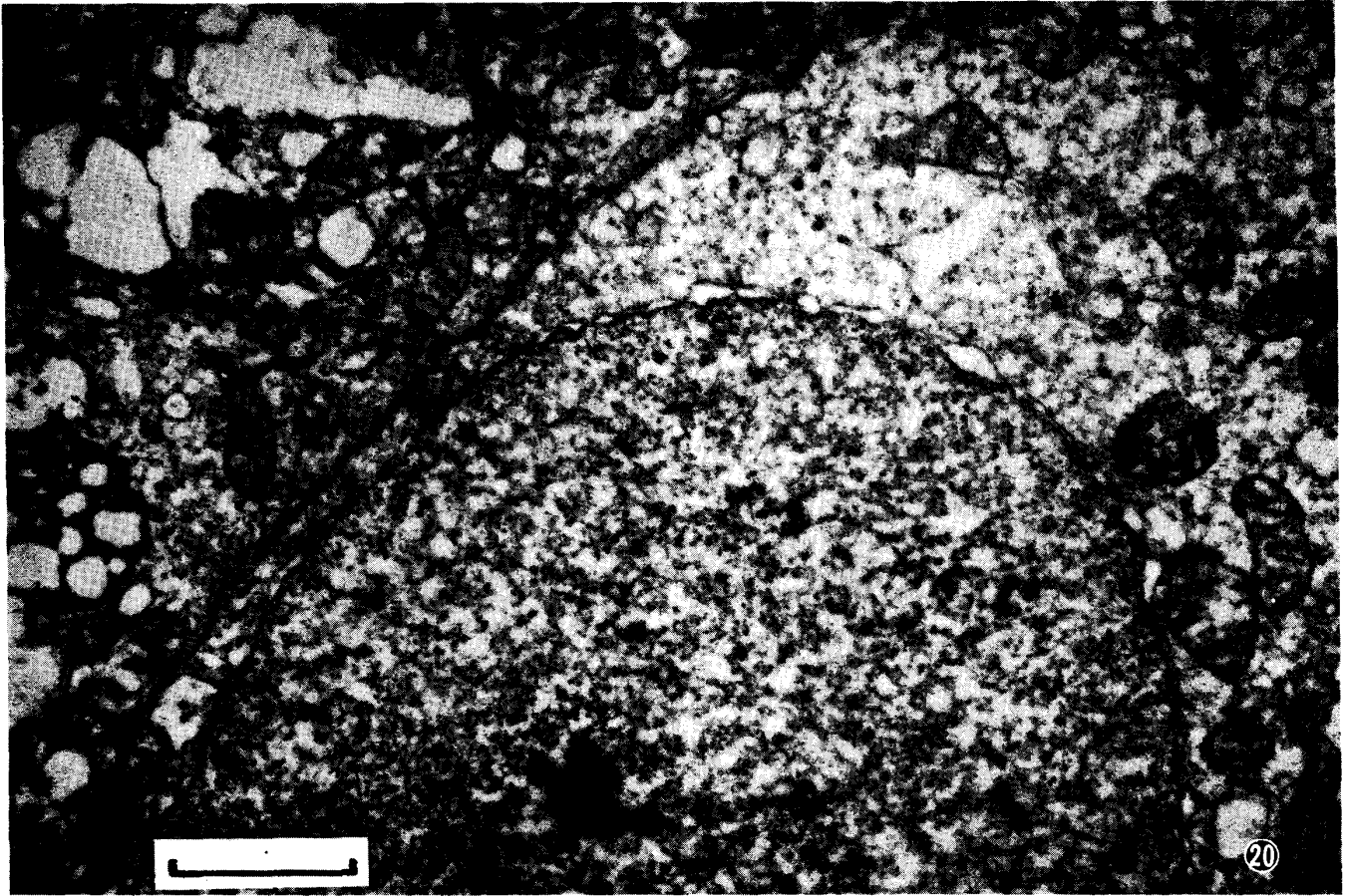


PLATE IX

- Fig. 22 Case No. 50 (UM 312) 8 years old  $\times 21,000$   
Dilatation of the endoplasmic reticulum and the intercellular spaces (I) are seen. The intercellular spaces are connected with (arrows) the subendothelial spaces. B: basal lamina, En: endothelial cell  
Case of equine osteodystrophia fibrosa
- Fig. 23 Case No. 44 (UM 316) 2 years old  $\times 26,000$   
A vacuolated light chief cell L: lipid body, ER: dilated endoplasmic reticulum  
Case of equine osteodystrophia fibrosa



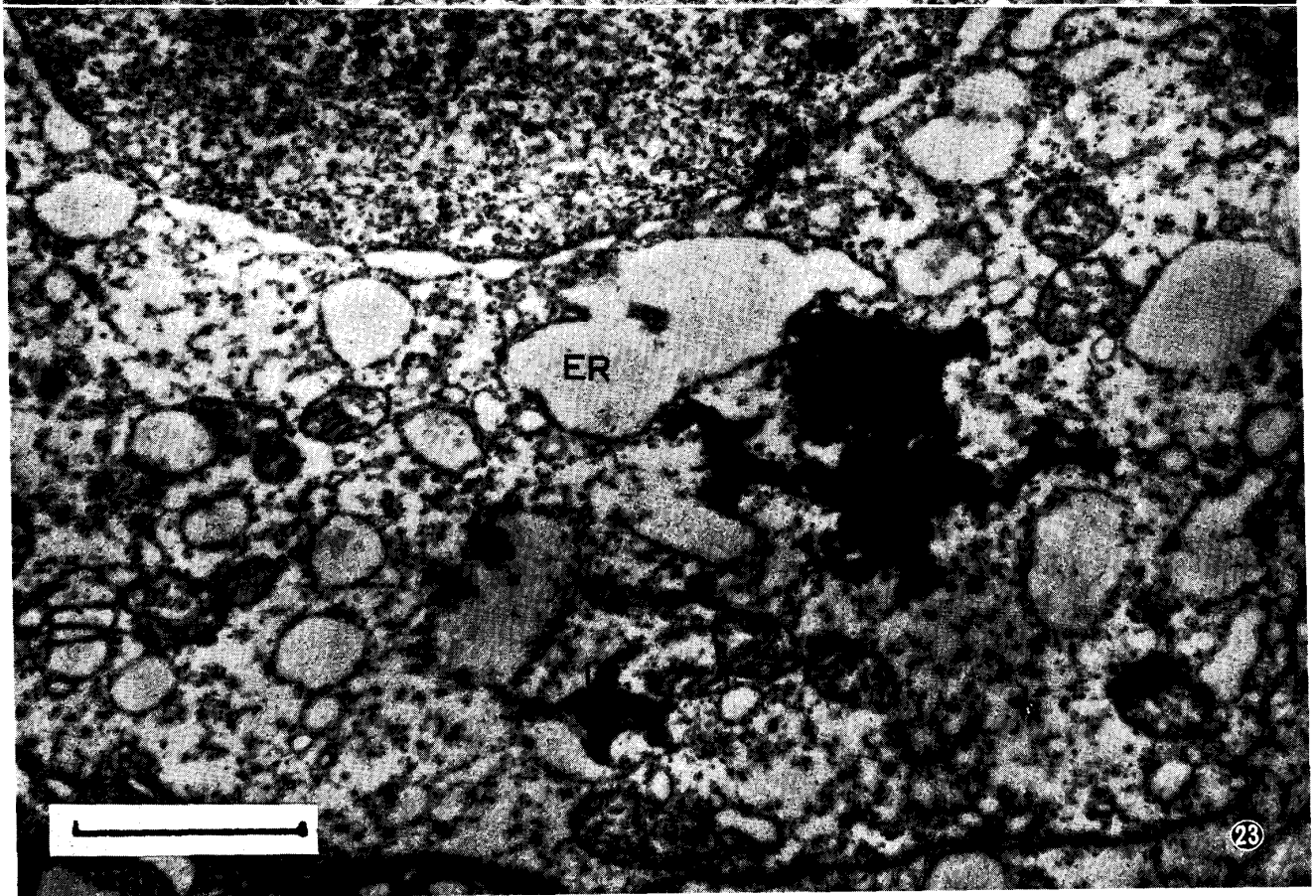
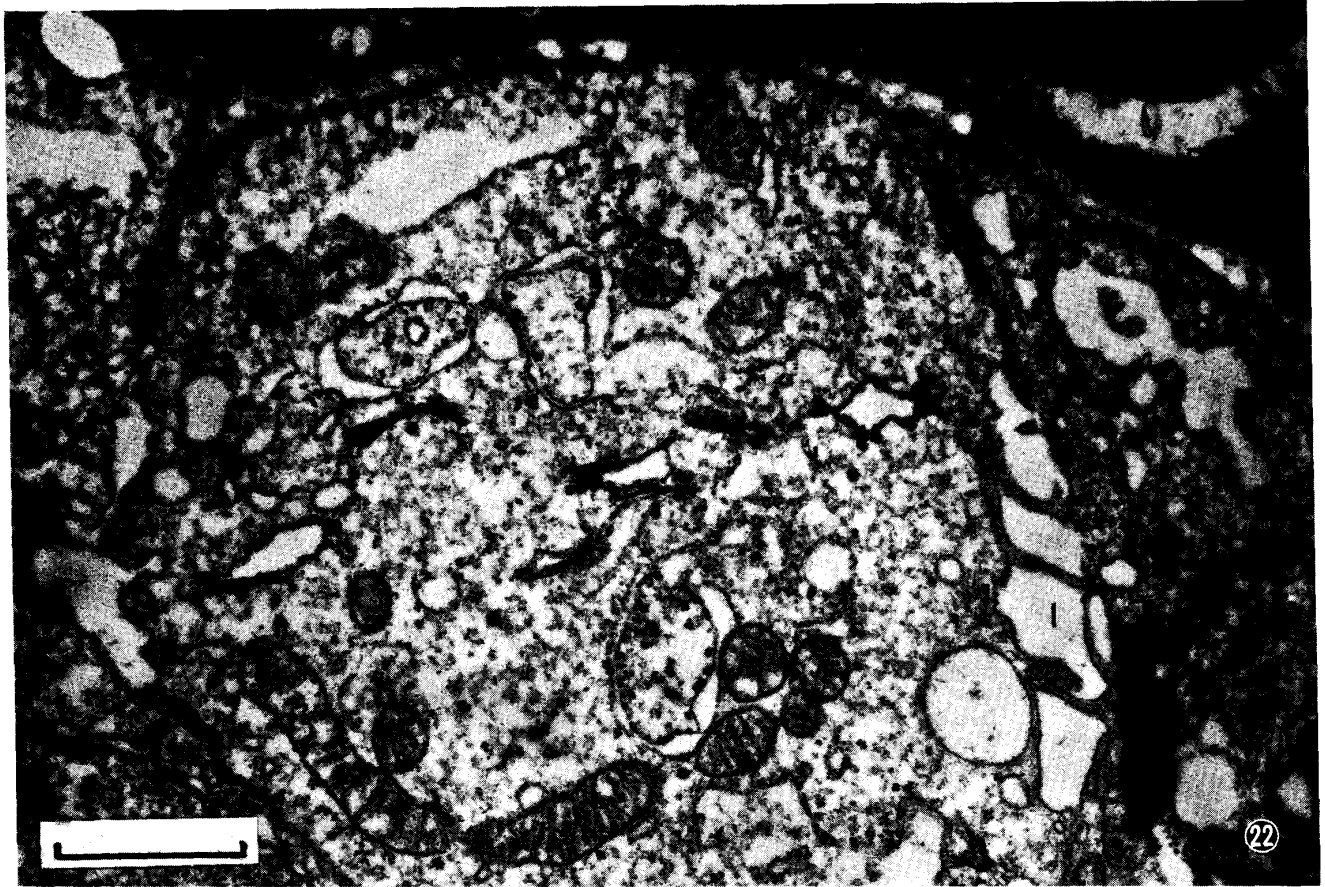


PLATE X

- Fig. 24 Case No. 47 (UM 313) 7 years old  $\times$  16,500  
Transitional water clear cells are seen. Cytoplasmic organelles are situated along the plasma membranes. Most of the cytoplasmic transparent area are occupied by glycogen.  
Case of equine osteodystrophia fibrosa
- Fig. 25 Case No. 52 (UM 315) 12 years old  $\times$  6,200  
Water clear cells  
Most of the cytoplasm are occupied by glycogen.  
Case of equine osteodystrophia fibrosa

