

Review Article

Pathogenesis of Osteopetrosis: A Comparison of Human and Animal Spectra

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Introduction

The term osteopetrosis was introduced to describe a human condition characterized by generalized sclerosis of the skeleton with multiple fractures [1, 40]. Subsequently, several isolated reports have described the clinical spectrum of the human disease, genetic variants, and histological correlates in bone. The occurrence in several animal species of diseases involving increased bone density has suggested that satisfactory models of the human disease might exist which would permit evaluation of pathogenic mechanisms under a variety of experimental conditions. This review compares the human forms of the disease with those animal syndromes which have been termed osteopetrosis on the basis of gross and radiological observations.

Human Osteopetrosis

Two genetic variants of osteopetrosis in humans have been described; autosomal recessive and malignant; dominant and benign. Discrimination between groups is based upon analyses of family history and clinical features.

Malignant Recessive Osteopetrosis

Clinical features. The clinical features of 50 cases of the malignant, recessively inherited variety of osteopetrosis (MRO) were recently reviewed [37]. The prominent manifestations include optic atrophy, splenomegaly, hepatomegaly, poor growth, frontal bossing, fractures, loss of hearing, mental retardation, large head, osteomyelitis, tooth enamel hypoplasia, facial palsy, and genu valgum. The disease may be manifest at birth and has been diagnosed radiologically *in utero*

[34]. The calcium, phosphorus, and alkaline phosphatase values in serum are usually within normal limits, but decreased calcium levels in early infancy have been documented [3, 43, 75]. Radiological examination reveals increase in density of all the bones with narrowing of medullary spaces and minimal trabecular pattern [30]. The metaphyses of the long bones are clubbed or flask-shaped. Fine striations are found in the cortices, and transverse and longitudinal lines of relatively increased density are seen in the metaphyses. All the long bones, the pelvis, the spine, and the ribs are symmetrically and markedly involved.

The malignant form is complicated by hydrocephalus and by replacement of the bone marrow by unresorbed bone [17]. The latter feature leads to anemia and thrombocytopenia, and these abnormalities in conjunction with increased hemolysis from hypersplenism [46, 68] usually cause death. It is generally agreed that MRO arises from a rare recessive gene [53].

Bone morphology. The affected bones are characterized by retardation of endosteal resorption leading to formation of a thick cortex and replacement of the marrow cavity with spongiosa [15, 83]. The columns of calcified cartilage are not replaced by well organized bone but persist along with increased lamellar bone, resulting in a disorganization of the Haversian systems. The number of osteoblasts may be decreased or normal. Osteoclasts are usually decreased but occasionally may appear to be normal in number [17]. These findings are usually interpreted to mean a failure of normal resorption rather than increased bone formation but are insufficient to explain abnormal osteoclast function.

Metabolic studies. Pathogenic abnormalities in the dynamics of calcium metabolism have been sought. Ex-

tant hypotheses include: (a) increased absorption of dietary calcium; (b) increased formation of bone or deposition of bone calcium; (c) decreased bone resorption either due to end-organ hyporesponsiveness, an humoral inhibitory factor, or a defective humoral resorptive substance. Many of these hypotheses are incompletely supported, but a variety of evidence may be cited favoring one or the other.

The observation that there are similarities in the radiographic appearance of bone in idiopathic hypercalcemia of infancy [16] and in osteopetrosis has led to the proposal that an oversensitivity to vitamin D may be present in osteopetrosis [11]. The appearance of hypercalcemia, soft tissue calcification, and excessive increase in the calcium level in serum following administration of small amounts of vitamin D as seen in idiopathic hypercalcemia [41] are not, however, observed in osteopetrosis [19]. Calcium balance studies invariably reveal marked calcium retention despite low calcium intake [11, 48]. Only the addition of cellulose phosphate to a very low calcium diet may reverse the positive balance [11], and even oral intake of phytate may be ineffective in reversing the excessive absorption [41]. A strict calcium-depleting regime may reduce the density of new bone [11].

A number of attempts have been made to evaluate the responses exhibited by children with osteopetrosis to a variety of vitamin and hormone preparations. An extensive study of one patient treated with large doses of dihydrotachysterol, parathyroid extract, and vitamin A while receiving both normal and low calcium diets revealed no effect on bone resorption. An increase in intestinal absorption of calcium following administration of parathyroid hormone, however, was observed [48]. Another report of an affected child documents the persistence of hypocalcemia during severe calcium restriction despite the injection of large amounts of parathyroid extract or vitamin D [19]. Parathyroid hormone was noted to cause phosphaturia in this case, which indicates normal responsiveness of the renal tubules to the action of parathyroid hormone. In two osteopetrotic siblings, ingestion of aluminum hydroxide has been reported to restore normal parathyroid extract-induced phosphaturia and to increase hydroxyproline excretion in urine [27]. Aluminum hydroxide treatment also diminished the positive calcium balance. It has been suggested that this response may result from an unmasking of the effects of parathyroid hormone on bone [59]. It is worthwhile noting that a high phosphate diet or the simultaneous injection of phosphate enhances the hypocalcemic response to calcitonin in the rat bioassay system [49].

Since calcitonin acts by inhibiting bone resorption [21], it is important to establish the possible influence of calcitonin in human osteopetrosis. Two children with MRO showed no increase in parafollicular light cells of the thyroid [48, 69], which are a primary source of calcitonin [10]. Another patient had a thyroid concentration of calcitonin 200 times that found in control infants [27]. In the latter case and before treatment, calcitonin was undetectable in serum using a bioassay. An inhibitor of bone resorption as tested in a bone culture system *in vitro*, however, was slightly increased after calcium infusion. After thyroidectomy, the serum inhibitory factor was abnormally great. It is premature to incriminate calcitonin in the pathogenesis of osteopetrosis based on these limited experiences.

Whereas the mode of inheritance, clinical pattern, pathological findings, and the prognosis of MRO appear predictable, the experimental data on the pathogenesis are fragmentary and inconclusive. They confirm the presence of an abnormality in the mechanism of bone resorption with little precise definition.

Benign Dominant Osteopetrosis

A recent review [37] of the more benign, dominant form of osteopetrosis (BDO) includes an analysis of the mode of genetic transmission of the disease in 19 families reported in the literature as well as of 2 families evaluated by the authors.

Clinical features. Although a large number of patients with BDO may be asymptomatic, there are three major complications: fracture, osteomyelitis, and cranial nerve palsy [37]. In contrast with RMO, these patients develop neither the manifestations of extramedullary hematopoiesis nor anemia and can be expected to survive to old age. Calcium, phosphorus, and alkaline phosphatase values in serum are usually normal although there is one report of a patient with a normal calcium level at 8:00 AM and marked hypocalcemia at 12:00 PM [81]. In addition, one brother of the latter patient had hypocalcemia [1]. Acid phosphatase values in serum are reported to be high in a majority of cases [37]. Acid phosphatase activity in bone cells is found primarily in the lysosomal fraction [74, 82] and release of acid phosphatase into the incubation media of calvarium explants is enhanced by parathyroid hormone and correlates with active bone resorption in some studies [74]. There are no differences in roentgenographic findings of the dominant form (BDO) compared with the recessive disease (RMO) although the former may have less involvement [31].

Bone morphology. The histological appearance of the bones of adults is similar to that described in chil-

dren. Disorganized matrix, hyaline bars, and absence of a defined medullary cavity are described in the adult form [37]. There is no uniformity of opinion as to whether this appearance represents failure of remodeling or continued overproduction of a poorly formed bone structure, since examples of each have been described [37].

Metabolic studies. Data on investigative approaches to the physiology and pathogenesis of BDO are fragmentary. Abnormalities in calcium balance have not been detected in the few patients studied [37]. Administration of parathyroid extract may elicit normal bone resorption as evidenced by increased excretion of hydroxyproline and phosphorus in urine [37]. Osteopetrotic adults, however, may not respond normally to exogenous parathyroid extract while calcium infusions may fail to suppress parathyroid hormone action and may result in paradoxical hypocalcemia [1]. Recently, normal levels of calcitonin in plasma in subjects with osteopetrosis have been reported [26, 70]. In one instance, the disease occurred in four children of a woman with medullary carcinoma of the thyroid [70]. In neither report was the form of the disease stated. Bone calcium accretion rates have been reported to be high [37] or low [45] whereas bone resorption was low in one instance [21]. Plasma from an osteopetrotic adult was said to have increased diurnal variations in rat serum calcium-lowering activity as compared with controls [81].

In summary, RMO and BDO can be differentiated on the basis of the time of onset, the family history, and the eventual course of the disease. Roentgenographic and morphological criteria cannot distinguish between the two varieties. Although the metabolic data obtained from patients with either form are inconclusive, evidence is accumulating which implicates an abnormality in bone resorption. The specific roles of calcitonin and parathyroid hormone in this marked human state are yet to be defined.

Animal Models of Osteopetrosis

Hereditary Osteopetrosis of the Rabbit

Pearce [54-57] described a unique condition arising spontaneously in a strain of purebred Dutch rabbits which has remarkable similarities to human osteopetrosis. She studied the clinical, genetic, hematological, biochemical, and pathological features of this condition in 300 animals. These studies remain the only published reports of the disease in rabbits.

At birth the first clue to the diagnosis of osteopetrosis

was the absence or hypoplasia of one or more of the incisor teeth. Tooth growth was retarded or suppressed and premature shedding occurred. Within the first 2 weeks of life, the rate of body weight gain decreased followed by loss of weight with progression to cachexia, weakness, and death by 4-5 weeks of age. Other clinical signs included hydrocephalus, exophthalmos, tremors of the head and body, and nystagmus. The disease occurred in 28.9% of the F₁ generation and 25.2% of the F₂ matings. This was compatible with a simple recessive condition which was not sex-linked.

Roentgenographic abnormalities were present at birth and consisted of loss of structural detail with a dense homogenous appearance of the entire skeleton, delayed epiphyseal development, and deficient calcification of the calvarium. With increasing age, delayed skeletal growth occurred with obliteration of marrow spaces without predilection for any particular bones. The base of the skull and the maxilla showed deficient calcification.

The rabbits exhibited macrocytic anemia, thrombocytopenia, and a moderate myeloid leukocytosis. The calcium concentrations in the serum of the osteopetrotic rabbits were consistently lower than those of normal litter mates. Younger osteopetrotic rabbits had much lower phosphorus levels in serum than controls, which gradually became normal by 3 weeks and were higher than normal thereafter. Affected rabbits had consistently higher alkaline phosphatase levels.

The affected bones were described as hard and brittle with no marrow cavity. The diaphysis was filled with an opaque, white, bony, fibrous mass resembling spongy bone which was continuous with the metaphysis. There were irregularities of the cartilage columns. The calvarium was irregularly calcified and very brittle. There was striking persistence of spongy bone in the metaphysis and diaphysis. The cortical bone was disorganized with poor development of Haversian systems. Irregular and small osteocytes and persistence of fibrous tissue with large numbers of osteoblasts and fibroblasts were found. Osteoclasts were present but were less numerous than osteoblasts.

Several additional interesting features of this disease deserve emphasis. Histochemical studies revealed intense phosphatase staining in several organs and tissues other than bone, and increased calcium content occurred in similar distribution. These observations suggest that the disturbance of calcium metabolism involved several tissues other than bone and may indicate either a generalized hormonal influence or an abnormality of calcium metabolism which affected sev-

eral tissues and organs. The thyroid glands of the affected rabbits were described as normal in size although pale and containing a predominantly acidophilic staining colloid rather than the usual basophilic reaction. The parathyroid glands were enlarged and frequently contained accessory nodules. The descriptions of the cells of the thyroid were inadequate to permit judgement on the state of the parafollicular cells.

Osteopetrosis in rabbits has several features in common with the human disease, particularly RMO, and seems to have a similar course and prognosis. It, undoubtedly, would make a fine experimental model in which to study bone metabolism and calcium control mechanisms in terms of comparisons with human osteopetrosis.

Osteopetrosis in Grey-Lethal Mice

An autosomal recessive condition in the house mouse which results in suppression of the yellow color of the hair and abnormalities of the skeleton was described by Grüneberg [23-25]. The disease is characterized by abnormalities in fur pigmentation, delayed dentition and skeletal development, delayed growth, starvation, and early death. Affected mice are frequently smaller than their normal litter mates at birth and gain poorly thereafter; starvation leads to early death. Teeth fail to erupt and the long bones are shorter than normal and are abnormally shaped.

The genetic data are compatible with an autosomal recessive inheritance in this condition although its occurrence in families with large litters is less than 25% [25]. Metaphyseal areas of the long bones, the facial bones, and the base of the skull show uniformly increased density [25]. Calcification of the head of the femur and several bones in the feet, the vertebrae, and the tail is delayed. Once calcification has started, these bones also become more dense than normal.

Although the bone marrow space is diminished, there is no anemia [25]. Both calcium and phosphorus levels in serum of mutant mice are lower than normal [50, 77], with the latter decrease being most marked [77, 78]. Alkaline phosphatase levels in serum are increased in mutant mice whereas acid phosphatase levels are normal [50].

Histologically, the bones of grey-lethal mice are characterized by persistence of the spongiosa whereas in normal bone development most of the spicules primarily formed are later destroyed [23]. Calcification of bone is incomplete, with layers of uncalcified bone covering many of the spongiosa spicules. The anomalous development of the teeth has been attributed to

abnormalities of the surrounding socket rather than to the teeth *per se* since teeth which develop in normal sockets have normal size and shape [24]. Quantitative studies show a marked decrease in the numbers of osteoclasts [6, 32]. The bone histology has been interpreted to indicate a defect in the process of resorption rather than an abnormal increase in new bone formation [25, 32, 50, 77, 78].

The first attempt to define the nature of the presumed resorptive defect in osteopetrosis was that of Barnicot [4]. He transplanted abnormal ribs from grey-lethal mice to subcutaneous sites in normal hosts. This frequently resulted in return to normal structure. In contrast, in transplants of grey-lethal to grey-lethal, abnormal grey-lethal structure persisted. Conversely, normal bones transplanted to grey-lethal animals occasionally attained an appearance similar to that of the dense grey-lethal bone. He concluded that the failure of bone resorption in grey-lethal animals might be due to a humoral factor. Barnicot [5] also noted that the grey-lethal mutants were relatively resistant to doses of parathyroid extract which were frequently lethal to normal animals. In normal animals following the administration of parathyroid extract, a period of excessive bone deposition preceded the phase of bone resorption. At doses of parathyroid extract which caused resorption in normal animals, excessive bone formation was observed in the mutants. It was noted, however, that with massive doses of parathyroid extract, marked dissolution of the excessive bone occurred. Furthermore, soft tissue calcification involving the kidney, adrenal, heart, and bronchi which was associated with the induction of bone resorption in normal animals was not detected in osteopetrotic mice who were undergoing similar bone resorption under the influence of high doses of parathyroid extract. This is of interest with regard to the demonstration that calcitonin inhibits metastatic calcification induced by either bilateral nephrectomy or by overdosage with parathyroid extract [22].

The resistance of the grey-lethal mutant to parathyroid extract was confirmed recently by Walker. He reported that the concentration of calcium in serum of mutants did not rise normally when the parathyroid extract was administered for several days [77]. In addition, a large number of parafollicular light cells were found in the thyroid glands of the grey-lethal mice stimulated with parathyroid extract [78]. The significance of this finding is not clear since comparisons with the response of normal mouse parafollicular cells to parathyroid extract administration were not stated. Indeed, hypercalcemia has been shown to induce cyto-

chemical changes in normal parafollicular cells [18, 47]. Chronic administration of parathyroid extract may lead to parafollicular cell hyperplasia in both normal and congenitally osteopetrotic mice [47]. This treatment of normal mice results in marked increase of metaphyseal spongy bone and is prevented by prior thyroidectomy. We have noted that the calcitonin content of the ultimobranchial body of the normal chicken increases after parathyroid extract administration [12]. Other studies have shown that the ultimobranchial body in the frog undergoes rapid hyperplasia when stimulated by hypercalcemia [62]. A recent report stated that the calcitonin content of thyroid glands of grey-lethal osteopetrotic mice was the same as in normal litter mates. Nevertheless, administration of parathyroid extract caused a 50% increase in calcitonin content in grey-lethal mutants whereas in the glands of control mice the calcitonin content decreased by 20–50% [71].

It has been shown by the use of an *in vitro* bone fragment explant system upon which were placed parathyroid glands that parathyroid glands from grey-lethal mice can induce the formation of osteoclasts in grey-lethal bone [32]. These observations effectively exclude a primary defect in the parathyroid-osteoclast interaction in murine osteopetrosis.

The ability of grey-lethal bone to maintain a steady state distribution of calcium and phosphorus in an incubation medium *in vitro* was normal although heat-inactivated mutant bone released more calcium into the medium than did the normal bone [24]. In this system, the concentration of calcium maintained in the incubation fluid by heat-inactivated samples of bone can be considered to be representative of the solubility of the bone mineral phase, while any increment in concentration above this value maintained by living samples can be considered to be due to active cellular metabolism in the bone [77]. Thus, it was concluded that the amount of calcium in the incubation medium which was due to active cellular metabolism was greater in normal than in mutant bone [24]. Such an increase in the passive solubility of bone mineral would be expected under conditions of parathyroid hormone influence [22].

Several chemical events in bone resorptive processes have been examined using the grey-lethal mouse. The release of a collagenolytic substance into incubation media from the bone of parathyroid extract-treated grey-lethal mutants was greater than from similarly treated normal mice [78, 79]. The osteoclasts of mutant mice possessed abnormally large vacuoles after parathyroid extract administration [78]. Collagenase activ-

ity in bone cells has been localized to the particulate fraction which has many of the sedimentation, physical, and biochemical characteristics which are attributable to lysosomes [80]. These studies in grey-lethal mice indicate a normal reaction of the cellular component but suggest only a partial resorptive capacity.

Examination of the possibility that resorptive processes are linked to the production of organic acid by bone cells has led to the finding that both the serum and bone content of citric acid were elevated in the mutant mice [51]. When a sufficient amount of coenzyme (reduced nicotinamide adenine dinucleotide phosphate, NADPH) was added to the extraction medium, the activity of isocitric dehydrogenase (EC. 1.1.1.42) was normal. In contrast, absence of the coenzyme was associated with diminished activity of isocitric dehydrogenase [51]. The decrease in activity of isocitric dehydrogenase induced by parathyroid extract has been attributed to increased binding of a fraction of isocitric dehydrogenase to some insoluble cellular material. The bound enzyme is presumably released only when nicotinamide adenine dinucleotide phosphate (NADP), or NADPH, or both, are incorporated into the extraction medium [28]. Parathyroid hormone can cause a decrease in the amounts of the coenzyme in calcified tissue [29, 76]. The breakdown of NADP in bone by dephosphorylation has been attributed to alkaline phosphatase activity [76]. Hence, parathyroid extract may block the activity of isocitric dehydrogenase by inhibiting the cofactor, NADP or NADPH, leading to elevated levels of citric acid. Histochemical estimations of diaphorase and isocitric dehydrogenase activities of osteoclasts after parathyroid extract administration *in vivo* were the same in mutant and normal mice [78]. Oxygen uptake by bone from mutant animals is greater than from normal animals [51], which suggests increased cellular activity but does not indicate which cells are involved.

The presence of a bone resorption-inhibitory factor in grey-lethal mice has been further suggested by studies of the effects of parathyroid extract upon bone matrix formation [51]. Whereas a marked reduction of ^3H -proline incorporation into calvaria after acute parathyroid extract administration was seen in normal animals, lesser reductions in ^3H -proline incorporation were seen in calvaria from grey-lethal mice. Chronic administration of parathyroid extract resulted in increased ^3H -proline incorporation into bone of both normal and osteopetrotic mice.

These data must be interpreted in terms of the comparative effects of parathyroid hormone and calcitonin upon collagen and mineral metabolism in other exper-

imental models. Calvaria from parathyroid extract-treated animals synthesize increased amounts of hexosamine and decreased amounts of hydroxyproline [36]. A component of extract from calvaria of parathyroid extract-treated animals when added to normal bone minces reproduced the decreased labeling of matrix hydroxyproline [36]. Thyrocalcitonin, however, also can cause decreased bone hydroxyproline synthesis associated with decreased glucose uptake, decreased lactate production, and decreased release of calcium from bone [21, 36]. These experiments have been interpreted to indicate that parathyroid extract causes decreased collagen synthesis with increased lysis of matrix followed by rebound increased rate of synthesis whereas calcitonin inhibits bone lysis and subsequently inhibits synthesis [35]. This hypothesis has been partially substantiated by the demonstration that calcitonin inhibits resorption as well as bone and matrix formation with the resorption-inhibitory effect being greater [7]. The simultaneous administration of parathyroid extract and calcitonin will obliterate the acute effects of either alone upon the rates of calcium release from bone *in vitro* [20, 36]. Additionally, calcitonin inhibits parathyroid hormone-induced increases in levels of hydroxyproline in extracellular water [42] and in urine [58]. There is considerable evidence that calcitonin acts at sites or steps which are different from those affected by parathyroid hormone [20, 58, 61].

It is obvious that the few measurements of proline uptake by grey-lethal mutant bone after parathyroid extract administration are not adequate to justify a conclusion as to the nature of the inhibitor in the mutants. However, the evidence currently available is compatible with a state of excessive calcitonin influence. In the intact animal the state of hypercalcitoninism would be expected to result in secondary stimulation of parathyroid hormone release which in turn would influence the cellular resorptive processes in osteoclasts. Completion of the resorptive process, however, may be continually overridden by the inhibitor. Furthermore, the interpretation of all metabolic variables must take into account the probability that feedback mechanisms in bone and calcium homeostasis make it extremely difficult to separate the contributions of various components until more information is available as to their interactions.

In summary, in the grey-lethal mutant mouse is found an example of spontaneous osteopetrosis which shares many features in common with RMO of humans including the course of the disease and many roentgenographic and histological characteristics. The

evolving information on the pathogenesis of osteopetrosis in grey-lethal mutant mice is compatible with a primary disorder in the mechanisms of bone resorptive processes.

Osteopetrosis in the Bull

Tumors of the ultimobranchial-derived component of the thyroid gland are present in approximately 30% of bulls raised upon an artificial high calcium diet although no such tumors have ever been described in the cow [38]. In addition to those with neoplasms, another 16% of the bulls have parafollicular hyperplasia. These findings correspond with the observation that bulls have an incidence of vertebral osteophytosis approaching 100% at 7 years of age, although the incidence in the cow is insignificant [58]. Furthermore, cortical bones of the bull gradually become thicker and denser with age whereas the cortex of long bones is considerably thinner in cows even when considered in relation to their smaller body size [73].

This syndrome in bulls has been attributed to excessive calcium in the bull ration which is used at artificial insemination stations [44]. The bulls are fed the same diet as cows, which provides up to 5.9 times more calcium than is recommended by the National Research Council [52]. Thus, one can postulate that excessive calcium intake in the bull provides a stimulus for excessive activity of the calcitonin-producing cells of the thyroid, which in turn leads to excessive secretion of calcitonin and resultant increase in bone density.

Avian Osteopetrosis

A disorder of bones occurring spontaneously in domestic fowls was described by Pugh [60] and was called "sporadic diffuse osteoperiostitis." Transmissibility by suspensions of cells obtained from various tissues in florid cases despite prolonged storage was demonstrated in 1938, and the condition was named osteopetrosis gallinarum [39]. Avian osteopetrosis has been thought to be a virus-induced disease of the avian leukemia complex on the basis of studies with cell-free material obtained from affected chickens [33]. Recently Dougherty [13] has shown that the virus of avian osteopetrosis is probably distinct from the usual oncogenic viruses belonging to the group. However, chick embryos injected with blood from humans with chronic myeloid leukemia develop osteopetrosis [72] as did 1-day-old chicks injected with duck tumor cells derived from Rous sarcoma virus [14]. Viruslike parti-

cles have been found in the periosteum but not in osteoblasts [66].

The progression of the disorder and the pathological characteristics are best described after the disease is artificially induced [8, 67]. Chickens affected with osteopetrosis have a progressive debilitating disease which involves the periosteum at the middiaphysis. Radiographically, the osteopetrotic lesion indiscriminately appears as an area of increased density under the periosteum and on the surface of the original cortical bone.

Osteopetrotic birds are usually not anemic [8]. Both diffusible and nondiffusible calcium levels in serum may be normal [8] or elevated [33], although alkaline phosphatase levels are elevated and acid phosphatase levels are slightly greater in affected than in control birds [8].

Histologically, the disease is characterized by thickened periosteum with excessive formation of spongy bone, distortion of developing Haversian canals by a fibrillar matrix material, and by large lacunas containing several osteocytes. The primary change in the avian osteopetrotic lesion is an alteration of the periosteal osteoblasts since the medullary osteoblasts may or may not be affected [64, 67]. Osteoclasts are noticeably absent from the lesion although they are found around the trabeculas and endosteal surfaces of all normal bones [8, 64]. There are no typical lesions in other tissues of osteopetrotic chickens with the exception of extramedullary hematopoiesis in the liver and kidneys [8]. Parathyroid glands have been described as both enlarged [12, 33] and normal [8].

Because of a possible abnormality in calcium control mechanisms in avian osteopetrosis, a study of several variables of calcium metabolism was undertaken [12]. The calcitonin content per unit dry weight of ultimobranchial body of osteopetrotic chickens was consistently lower than in control chickens although the total weight of the glands was the same in each group. Injection of parathyroid extract resulted in both increased ultimobranchial body dry weight and increased ultimobranchial calcitonin content in each group although the calcitonin content from the osteopetrotic group remained lower than that from the control in the stimulated state. Also the calcium levels in serum of osteopetrotic chickens rose less than those of the controls after parathyroid extract administration (500 units/kg) and were lower than those of the controls 12 hr postinjection. The rate of recovery from hypercalcemia induced by calcium infusion was normal. To date, these studies have failed to implicate an abnormality of calcitonin secretion or function in the

pathogenesis of avian osteopetrosis. It may be possible to define the role of calcitonin to calcium metabolism in avian osteopetrosis by ultimobranchial extirpation studies [9] performed in conjunction with the experimental induction of the disease.

Avian osteopetrosis does not appear to be similar to human osteopetrosis or to the animal models of osteopetrosis previously discussed. It differs etiologically, genetically, and morphologically from typical osteopetrosis. There are some resemblances, however, to three human disorders of bone, Caffey's disease, Englemann's disease or progressive diaphyseal dysplasia, and Ribbing's disease [63].

Summary and Conclusions

Several features of human and animal osteopetrosis have been compared and are summarized (Table I). The recessively inherited, malignant clinical course of osteopetrosis in childhood is similar in several respects to the disease described in rabbits and in grey-lethal mutant mice. The time of onset, degree of bone involvement, histological characteristics of bone, several laboratory measurements, and the lethal course of the disease suggest that the pathogenic mechanisms may be similar. Indeed, both the human recessive form and the form seen in grey-lethal mutant mice demonstrate decreased bone resorption and resistance to resorptive influences. The osteopetrosis seen in the grey-lethal mutant disease probably can be attributed to a humoral factor which prevents the complete process of bone resorption although there is considerable evidence that several osteoclastic responses are adequate. The possibility that calcitonin may be present in excessive amounts must be confirmed by quantitative techniques. The data implicating a humoral factor or factors in human recessive osteopetrosis are still inconclusive.

The dominant, benign, adult onset form of osteopetrosis in humans cannot be easily differentiated from the recessive variety in terms of histological characteristics. There is no known animal model of osteopetrosis which has similar genetic and prognostic features. A factor lowering calcium levels in serum has been implicated in isolated cases, but the data are inconclusive.

Increased skeletal density or thickness may be a result of several presumably unrelated mechanisms. Diverse examples include the vertebral changes accompanying parafollicular cell tumors and hyperplasia in bulls induced by increased dietary calcium, chronic

Table I. Comparisons of osteopetrosis syndromes in man and animals¹

Species	Genetics	Age at onset	Course	Radiographic involvement	Levels in serum			Pathology of bone			Response to PTH ²		Pathogenesis thyroid-parafollicular cell, calcitonin measurement
					Calcium	Serum phosphorus	Alkaline phosphatase	Area involved	Osteoblasts	Osteoclasts	Bone	erum	
Human	Recessive	Birth, infancy	Lethal early childhood	increased density all bones	N,D*	N	N	Cortex thick, replacement marrow cavity by spongiosa, disorganized columns cartilage	N,D	D,N*	D (measured indirectly)	D	Parafollicular cells: N Thyroid calcitonin content: I
	Dominant	Adult	Benign	Increased density, variable involvement, metaphyses first affected	N,D*	N	N	Similar to recessive form	N	D	D,N (measured indirectly)	D	Factor lowering Ca levels in rat serum: I
Rabbit	Recessive	Birth, infancy	Lethal first 4 weeks	Increased density all bones, deficient calcification skull	D	D (early) I (late)	I	Cortex thick, replacement marrow cavity by spongiosa in metaphysis and diaphysis, increased fibrous tissue, disorganized cartilage columns	I (in fibrous tissue)	I (in fibrous tissue)	Not measured		Not measured
Grey-lethal mice	Recessive	Birth, infancy	Lethal first 4 weeks	Increased density all bones, may be associated with early delay calcification	D	D	I	Cortex thick, persistence of spongiosa mixed with layers of uncalcified bone	N	N,D	D	D	Parafollicular cells: I Probable humoral factor interferes with bone resorption
Bull		Increasing incidence up to 6 years	Benign	Vertebral osteophytosis, thick cortex all bones									Ultimobran- chial tumors and hyperplasia secondary to hypercalcemia or increased dietary calcium
Avian		Spontaneous: 6 weeks Virus-induced: 2 weeks	Diminished life span	Increased density midshaft long bones	N,I	N	I	Thickened periosteum, excessive spongy bone, trabeculae at right angles to cortex	I	N, in areas of normal base bone D, areas of involvement		D	Virus-induced Decreased ultimobran- chial gland calcitonin

*: infrequent; N: normal; D: decreased; I: increased. ² Parathyroid hormone.

administration of parathyroid extract in rats, and the viral-induced disorder of long bones in fowl. It is obvious that different etiological mechanisms evoke responses involving humoral as well as end-organ factors that can lead to increased density or thickness of bone.

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