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The Role of Lysosomes in Resorption of Bone Tissue[1]

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Tissue destruction in the body is known to be mediated by acid hydrolases (lysosomes) released from discrete cytoplasmic organelles (the lysosomes) ([1, 8]). Although a wealth of information has accumulated about the lysosomal activity of various organs, little is known about the lysosome levels of hard tissues. Resorption of bone and cartilage has been reported to occur in organ culture where excess vitamin A or parathyroid hormone was added to the media, and this process was shown to be caused by lysosomes ([2, 7]).

In human leprosy, resorption involving the small bones of denervated hands and feet is common; the phenomenon is similar to that encountered in other diseases manifesting peripheral denervation. However, the specific physiologic process which produces bone absorption in leprosy or in other diseases in still not understood. Large, ameboid, phagocytic cells (the osteoclasts) are believed to be involved in bone resorption ([4]). Because phagocytic cells are rich in lysosomes ([1]), it is likely that destruction of bone tissue is caused by the acid hydrolases released from the osteoclasts. Any factor which labilizes the lysosomal membranes results in liberation of the acid hydrolases ([1]). In tissues where active lysis takes place, high levels of lysosomes can be detected, while the enzyme activity is minimal in resting tissues ([1, 8]). In the present study, lysosome levels were assayed in bone and soft tissues removed at surgery from extremities of patients with leprosy, to see if there was any correlation between lysosome activity and bone destruction.

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MATERIALS AND METHODS

Bone and adjacent soft tissues were removed at surgery from feet and hands of leprosy patients, and were submitted for lysosome assay. Inactive (bacteriologically negative) cases of leprosy, which did not possess infected wounds associated with bone changes, served as controls, as opposed to all other cases in which localized infected wounds possessing inflammatory exudate were associated with bone changes, since such values are not available from normal human beings. A total of 31 soft tissues and 30 bone specimens were obtained from 20 patients. Medullary bone with marrow and adjacent soft tissue were collected in separate tubes kept at 0°C. The specimens were usually processed immediately, save for those cases in which processing was deferred over the weekend, during which time they were kept frozen at−20°C.

The tissues were minced with scissors, ground in a chilled agate mortar, and extracted in small amounts of cold distilled water. Acid phosphatase is considered a marker enzyme for lysosomal activity. Therefore, the acid phosphatase level in the extracts was assayed, using a modification of the method of Lowry *et al.* ([6]). In the determinations, p-nitrophenyl phosphate was used as substrate (Sigma Chemical Co., St. Louis, Mo.). The product formed from the substrate by the enzyme gives a yellow color upon the addition of alkali. The intensity of color, which is proportional to the amount of enzyme present, was measured in a Beckman Model CU spectrophotometer at 410 nm. The enzyme activity determined is expressed as absorbance units per milligram of protein obtained, which was estimated by the biuret method ([3]). Other enzymes could not be studied because of the limited amounts of human material available.

RESULTS

The data were arranged into three groups on the basis of the patients' disease classification. Group I consisted of patients with inactive leprosy who were without wounds and infections related to bone changes. Group II comprised patients with inactive leprosy who possessed infected wounds associated with bone changes, and Group III patients with active leprosy having infected wounds associated with bone changes. The following tables (1, 2, 3) show the site from which each specimen was obtained, the nature of the localized bone pathologic alteration, and the acid phosphatase values determined in the soft tissues and the bone specimens. The results indicate that bone tissue is rich in lysosomes and that elevated lysosome activity is associated with bone absorption.

The data were statistically analyzed. The method used to test the significance of the difference between groups was the independent samples t-test. Hypotheses were tested at the 5 per cent level of confidence. Acid phosphatase levels obtained from the soft tissue determinations revealed the following:

(a) The mean of Group III (1.24) is significantly larger than the mean of Group II (0.41).

(b) The mean of Groups II and III combined (1.04) is significantly larger than the mean of Group I (0.17).

Acid phosphatase levels obtained from the bone tissue revealed the following:

(a) Group II mean (1.41) does not differ significantly from Group III mean (1.92).

(b) The mean of Groups II and III combined (1.68) is significantly larger than the mean of Group I (0.23).

The low, high, and average values for all groups are given in Table 4.

The following clinical observations were also made. In two cases of active lepromatous leprosy in which disintegrated bone only was encountered, one was due to leprous osteitis and the other was nonspecific in origin on a noninfectious basis. In these cases the acid phosphatase levels were elevated, with those of bone being considerably higher than in the soft tissues. Also, in two cases of infected olecranon bursitis possessing open wounds, the lysosome activities in both the soft tissue and the bone were considerably elevated.

TABLE 1. *Acid phosphatase of inactive leprosy cases with quiescent deformity but without infection.*

Number	Site	Pathology	Acid phosphatase absorbance 410 nm/mg. tissue	
			Soft tissue	Bone
1441 (a)	Prox. phalanx R. great toe	Clawed	0.060	0.246
(b)	Prox. phalanx L. second toe	Clawed	No tissue	0.366
1894 (a)	Prox. phalanx L. index finger	Flexion crease crack	0.126	0.143
(b)	Second metacarpal L	As above	0.128	0.200
2848 (a)	Prox. phalanx L. great toe	Clawed	0.087	0.126
(b)	Prox. phalanx L. fourth toe	Clawed	0.138	0.213
2885 (a)	Distal phalanx R. great toe	Clawed	0.276	0.387
(b)	Distal phalanx L. great toe	Clawed	0.250	0.260
(c)	Prox. phalanx L. second toe	Clawed	0.268	0.142

TABLE 2. *Acid phosphatase of inactive leprosy cases with deformity associated with active infection.*

Number	Site	Pathology	Acid phosphatase absorbance 410/nm mg tissue	
			Soft tissue	Bone
2692	First L. metatarsal head	Drop foot and plan tar ulcer	0.360	0.439
2848	First L. metatarsal head	Absorbed MP joints and plantar ulcer	0.221	0.484
1514	First L. metatarsal head	Absorbed MP joints and plantar ulcer	0.669	1.177
2848 (a)	Distal phalanx L. great toe	Gangrenous clawed toe	0.319	1.689
(b)	First L. metatarsal head	Same as above	1.224	1.927
2885	Prox. phalanx R. third toe	Clawed toe with plantar ulcer	1.350	1.187
2921	Os calcis R.	Plantar ulcer	0.810	No tissue
823	Distal phalanx L. long finger	Sec. osteo. with sequestra	1.352	1.293
271	Olecranon bursae L.	Infected ulcer	1.500	2.235
1441	First R. metatarsal head	Clawed with plantar ulcer	0.319	2.264

DISCUSSION

Although "normal" values for bone lysosomes are not known, the levels of lysosome activity in our series without active infection were relatively low. In contrast, lysosome levels were significantly elevated in those cases with active specific disease or secondary infection involving bone. This association suggests that the high level of acid hydrolases may be a major factor in the resorption of bone which is associated with active infection. Elevated levels of lysosome activity have also been shown in other diseases in which there is tissue destruction and inflammation (8).

The question whether bone resorption is "neurotrophic or infectious" has been asked by Hodgson *et al.* (5). Their report on a series of 61 cases of different origins which possessed bone resorption showed that only one factor, namely, infection, existed in every case and therefore was thought to be of clinical significance. This finding correlates with our observations regarding similar bone changes in cases of leprosy with infected lesions involving bone. A direct study made on the effect of adding lysosome preparation from guinea pig liver and egg white lysosome to culture media in which mouse calvaria were maintained showed that the enzymes cause bone dissolation[3]. It has also been demonstrated that

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TABLE 3. *Acid phosphatase of active leprosy cases with deformity associated with active infection.*

Number	Site	Pathology	Acid phosphatase absorbance 410 nm / mg. tissue	
			Soft tissue	Bone
2692	Second L. metatarsal head	Leprous osteitis with comminuted fracture	1.007	3.068
2555 (a)	Medial cuneiform R.	Nonspecific disintegration	0.300	3.023
(b)	Medial cuneiform R.	Same as above	0.450	2.183
2055	Third R. metatarsal head	Plantar ulcer	1.456	3.008
2317	Proximal phalanx R. thumb	Ulcer dorsum IP joint	0.600	1.390
2788	Distal phalanx R. index finger	Sec. osteomyelitis with ulcerations	0.538	0.919
1825	Middle phalanx R. long finger	Ulcer dorsum PIP joint	2.065	1.170
2867	Distal phalanx R. great toe	Plantar ulcer	0.325	0.825
1727	Prox. phalanx L. fourth toe	Plantar ulcer	3.859	No tissue
2033	Olecranon bursae L.	Ulcerated, infected	1.823	1.380
2629	Distal phalanx R. 5th finger	Sec. osteomyelitis with ulceration	1.400	1.189

TABLE 4. *The low, high & average acid phosphatase values in soft tissues and in bone (Absorbance 410/milligram tissue).*

Group	Soft tissue			Bone		
	Low	High	Average	Low	High	Average
I	0.060	0.276	0.166	0.112	0.387	0.228
I	0.221	1.500	0.412	0.439	2.264	1.410
III	0.300	3.859	1.240	0.825	3.068	1.915

Group I—Patients with inactive leprosy and without wounds or infection.
Group II—Patients with inactive leprosy but with wounds and infection associated with bone changes.
Group III—Patients with active leprosy and wounds and infection associated with bone changes.

the administration of certain drugs and hormones which stabilize the lysosomes, such as chloroquine, chlorpromazine, acetylsalicylic acid, antihistamines, cholesterol, and corticosteroids alleviates inflammation and tissue destruction in certain diseases and also prevents bone absorption in organ culture ([1, 8]). The results reported here indicate that acid hydrolases may be involved in bone dissolution, as is the case in other tissues.

SUMMARY

Lysosome activity, in terms of acid phosphatase, was estimated in bone and soft tissues removed at surgery from extremities of patients with leprosy. Since normal values are not known, inactive cases without active disease of bone or adjacent soft tissue served as controls for active and inactive lepromatous cases which possessed either primary or secondary infection. Acid phosphatase activity was relatively low in cases without active infection. In active lepromatous cases with primary leprous osteitis and a majority with secondary infections, acid phosphatase levels were elevated, more so in bone than in soft tissue. These studies suggest that the high level of acid hydrolases in the tissues associated with active infection may be a significant factor in the resorption of bone.

RESUMEN

Se estimó la actividad lisosomial, en términos de fosfatasa ácida, en huesos y tejidos blandos extirpados quirúrgicamente de las extremidades de pacientes con lepra. Ya que los valores normales no se conocen, se utilizaron como control casos inactivos sin enfermedad activa de huesos o de tejido blando adyacente, los cuales se compararon con casos lepromatosos ac ivos e inactivos que tuvieran infecciones primarias o secundarias. La actividad de fosfatasa ácida fué relativamente baja en casos sin infección activa. En casos lepromatosos activos con osteitis leprosa primaria, la mayor a de los cuales tenían infecciones secundarias, los niveles de fosfatasa ácida estaban elevados, más aún en el hueso que en los tejidos blandos. Estos estudios sugieren que el alto nivel de hidrolasas ácidas en los tejidos asociados con infecciones activas puede ser un factor significatico en la reabsorción del hueso.

RÉSUMÉ

Dans des tissus osseux et dans des tissus mous prélevés lors d'interbentions chirurgicales au niveau des extrémités chez des malades atteints de lèpre, on a estimé l'activité des lysosomes, en termes de phosphatase acide. Du fait que les valeurs normales ne sont pas connues, on a utilisé comme témoins des cas inactifs sans atteinte active des os ou du tissu mou adjacent, et on a comparé ces t´moins à des cas de lèpre lépromateuse active ou inactive qui souffraient d'infections primaires ou secondaires. L'activité en phosphatase acide était relativement basse dans les cas sans infection active. Dans les cas de lèpre lépromateuse active, présentant une ostéite lépreuse primaire, et dans la majorité des cas avec infection secondaire, les niveaux de phosphatase acide étaient élevés. Ils étaient plus élevés dans les os que dans les tissus mous. Cette étude suggère que le taux élevé des hydrolases acides dan les tissus, associé à une infection active, pourrait représenter un facteur significatif dans la résorption des os.

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