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HUMAN OSTEOCLASTIC ACTIVITY: QUALITATIVE HISTOLOGICAL MEASUREMENT

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INTRODUCTION

IT HAS BECOME apparent that in a real sense osteoblasts and osteoclasts are the final common pathways for the evolution of most skeletal disease. Understanding the physiology and pathology of a myriad of bone disturbances reduces to understanding the pathological physiology of osteblasts and osteoclasts. The bone diseases themselves are the result of disturbances in the two cell-types named. These disturbances in turn are partly the result of inherent cellular aberrations (osteopetrosis; fragilitas osseum) and in part the result of aberrant control mechanisms acting on osteoblasts and osteoclasts (osteoporosis).

To investigate the inherent or control aberrations it is necessary to have some way of observing and measuring alterations in rates of activity. Some previous publications from the Henry Ford Hospital Orthopaedic Research Laboratory have dealt with various aspects of the physiology and measurement of osteoblastic activity in man.[4,6,7]

In this paper some measurements of human osteoclastic activity are presented. The method of measurement is based on the observation that an osteoclast leaves behind, as evidence of completed activity, a shallow, scalloped hollow termed Howship's lacuna[12] (Figure 1). Howslip's lacunae are normally found on the surfaces of the marrow, vascular and periosteal bone spaces. If osteoclastic activity, and thus bone resorption, were active, there should be more Howslip's lacunae than if osteoclastic activity were inactive.

Accordingly we have measured osteoclastic activity in terms of the area per unit volume of bone covered by Howslip's lacuna. This is termed the Howslip's specific surface after the accepted terminology used in the physical chemistry and physics of porous media. This is a purely qualitative measurement which does not describe the quantity of bone resorbed in a unit of time in a unit reference volume of the syeleton. There are certain other disadvantages of this method which will be considered later.

The idea of measuring the Howslip's specific surface in bone was, to our knowl-

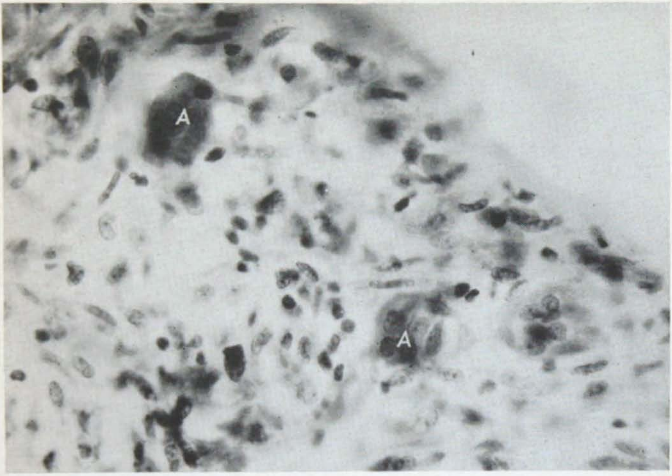

Figure 1

Tibia of an 11 year old boy with secondary hyperparathyroidism. The letters identify osteoclasts. (Material donated courtesy of R. Haliburton, M.D., Windsor, Canada)

edge, first proposed by Sissons.[10] Our method is based upon his ideas but has certain additional refinements that are essential and which will be referred to.

Forty-two bones from 37 patients have been examined. These bones were obtained either from the operating room as perfectly fresh material or from postmortem. The histories of all patients contributing material are known in detail and are on record in the hospital chart. Of the 37 patients, 19 were considered normal skeletally (Table (I). Most of the patients had thoracotomy for ligation of patent ductus, correction of hiatus hernia, correction of cardiospasm, lobectomy or penumonectomy for lesions found on routine photofluorographic survey, or amputation for fresh, soft tissue sarcomas of the extremitites. There is no patient in Table I with a chronic illness, weight loss, fever, or prior local operation. Some of the patients died suddenly of unexpected vascular incidents.

In Table II are listed cases in whom some skeletal abnormality was either known to exist or in whom some illness might have affected the results of measurement. Brief comment on the clinical aspects of these cases is included.

Methods

Fresh, undecalcified, unembedded, complete cross sections were made of the bones listed in the tables and stained with basic fuchsin by technique on record.[2,3]

A Zeiss integrating eyepiece II was used to measure the mm^2 of Howship's specific surface and the mm^2 of vascular specific surface per mm^3 of cortex. The mathematical reasoning behind the use of this eyepiece is discussed by Chalkley, Cornwall, and Park[1] by Hennig,[9] and by Uspensky.[11] The method of sampling the cortex is outlined in previous references.[6] The integrating eyepiece measurements involve the use of optical sectioning to obtain a nearly infinitely thin plane cut through the sections. This technique is also outlined in detail in other references and is too lengthy to reproduce here.[5] Since there is always some real depth of focus with real objectives, the ratio of the depth of focus to the vertical depth of the structure being measured determines roughly the amount of error introduced into the measurements. This error is correctable when both factors are known. The values in Tables I and II have been corrected for this error.

We measured Howship's lucanar area only on vascular space walls. The walls of the marrow spaces, and the periosteal surface, have been ignored in the present study.

The best measure of the accuracy of measurements such as the present ones is their reproducibility. The reason is that there are too many separate steps and too many variables involved to make a theoretical analysis valid. In four cases repeated measurement of the same sections was inadvertently done. The difference between the first and second determinations was 0.1 mm^3/mm^2 in 3 cases and 0.2 mm^2/mm^3 in the 4th case. These differences were not related to the absolute value of Howship's lucanar area, which was larger than 0.7 in two instances and smaller than 0.3 in the other two instances.

This difference is uncomfortably large, particularly in comparison to the rather small difference of less than 5 percent relative in measurements of osteoid seams $/mm^3$ (7) and of formation half-lives from teracycline labelled material.[4] As a result the value of the present measurements lies in the determination of the mean from groups of bones from a group of similar cases, whether these groups be normal or abnormal. The sigficance of a measurement of a single case must be considered dubious for the present. Since we are interested in what happens and why it happens in general, rather than in a specific case, this limitation of the value of the present method is less serious than it would be otherwise.

RESULTS

Referring to Tables I and II, the cases, ages, sex, and bones examined are listed in the first five columns. In the 6th column are listed the mm^2/mm^3 of

Table I

Case	Hospital Number	Age	Sex	Bone	mm^2 Howship's Lacunae per mm^3 Cortex	mm^2 Vascular Channel Surface per mm^3 Cortex	Howship's Lacunae as % Total Vascular Surface
1.	102 45 96	9	F	Rib	.61	2.4	
2.	102 05 45	11	M	Femur	.69	5.3	
3.	95 58 82	12	M	Rib	1.2	3.3	
4.	102 66 18	17	M	Humerus	.01	3.9	
5.	31 62 01	21	M	Rib	.29	3.1	
6.	97 64 09	31	F	Rib	.29	3.5	
7.	99 98 68	32	M	Rib	.14	1.2	
8.	99 48 65	45	M	Rib	.03	2.3	
9.	95 37 28	52	M	Rib	.57	2.9	
10.	101 39 83	53	M	Clavicle	.1	3.1	
11.	11 73 56	54	F	Rib	.23	3.5	
12.	56 07 68	57	M	Rib	.2	4.0	
13.	97 92 57	57	F	Rib	.3	3.5	
14.	99 50 41	59	F	Rib	.2	4.5	
15.	85 99 71	63	M	Rib	.5	4.8	
16.	07 77 38	63	F	Rib	.5	4.2	
17.	99 86 18	69	M	Rib	.6	4.4	
18.	01 30 96	75	M	Clavicle	.34	3.8	
19.	87 87 62	75	M	Rib	.41	4.8	
Mean, Cases 1 — 4		12.2			.86	3.4	25.3
Mean, Cases 5 — 11		41.1			.23	2.8	8.2
Mean, Cases 12 — 19		64.7			.38	4.2	9.3

Data of 19 normal skeletons sampled and measured for Howship's specific surface. Mean values for mean age groups are given in the bottom 3 rows. The last column in these 3 rows lists Howship's specific surface as the % of the vascular specific surface.

Table II

Case	Hospital Number	Age	Sex	Bone	mm² Howship's Lacunae per mm³ Cortex	mm² Vascular Channel less Howship's per mm³ Cortex	Howship's Lacunae as % Total Vascular Surface	Remarks
20.	06 57 53	64	F	Clavicle	.21	3.0		Cortisone Osteoporosis Estrogen
21.	09 03 13	51	F	Rib	.14	2.4		Osteoporosis Uremia Estrogen
22.	60 34 51	74	M	Clavicle	.17	3.2		Osteoporosis Cortisone Cirrhosis
23.	70 91 33	66	F	Rib	.4	3.4		Osteoporosis Cortisone Carcinomatosis
24.	98 26 66	58	M	Rib	.3	4.4		Osteoporosis Metastases
25.	40 67 74	67	F	Clavicle	.3	3.7		Osteoporosis Uremia Diabetes
26.	38 33 16	73	M	Clavicle	.12	3.8		Osteoporosis Lues Uremia
27.	21 10 77	73	F	Rib	.3	2.8		Osteoporosis Uremia Diabetes
28.	60 16 81	71	F	Clavicle	.4	3.0		Osteoporosis Congestive Failure
29.	52 97 54	12	M	Rib	.57	3.7		Osteoporosis Rickets Atresia—Common duct Cirrhosis
30.	95 24 58	58	M	Rib	.54	3.5		SBE
"	"	"	"	Clavicle	.17	2.1		Rupture aortic Valve
31.	95 44 39	15	M	Rib	.40	2.7		Plastic Mitral Prosthesis Cardiac arrest
32.	95 43 43	13	F	Radius	.8	4.2		Severe burn; elbow contracture; subluxed radial head, resection 5 years later
33.	95 41 55	66	M	Clavicle	.64	4.8		Brain Tumor Gradual Decline
34.	94 30 14	57	M	Femur	.1	4.0		Aortic Aneurysm; By pass; Sepsis
35.	06 20 70	56	F	Clavicle	.3	3.3		Carcinomatosis Cachexia
36.	95 50 47	64	M	Clavicle	.2	3.8		Nodular Goiter
	"	"	"	Rib	.8	4.0		Multiple Adrenal
	"	"	"	Ilium	.6	4.5		Cortical Adenomata
37.	98 78 50	36	F	Rib	.05	2.3		Mitral Stenosis Commissurotomy
Mean, Cases 20 - 28		66.3			.26	3.3	7.9	

17 Cases known or suspected to be skeletally abnormal are listed along with their measurements. The mean of cases 20 - 28 is given in the bottom row.

Howship's lacunae. In the 7th column are listed the mm^2/mm^3 of all vascular spaces not covered with Howslip's lacunae.

Table I: In the children a large value for Howship's area is noted. This value falls during young adulthood and then rises again after age 50. The large value in children might be expected since there is a great deal of growth remodelling occurring. The fall in value during young adulthood also might be expected simply because growth remodelling has ceased and only internal remodelling remains. The fall in young adulthood is not very significant because this group only contains four cases (5 - 8).

Over age 50 the value of Howship's lacunar area rises again, reaching the highest average values in the older cases.

There is a similar fluctuation in the non-Howship's vascular specific surface with age, but the values are surprisingly uniform.

Table II: Cases 20 - 29 had osteoporosis severe enough to have been noted on two or more occasions in the record. Four of these cases received cortisone or related drug for prolonged periods. The mean values of the normals and of the osteoporotics is listed in Table II. The osteoporotics have lower values than the norm, at least in the cases presented here.

In cases 3, 29, 30, and 36 two or more bones from the same person were measured. Evidently there may be wide variations among bones from the same skeleton at any on time. These same variations have been previously noted in osteoid seams,[7] and in tetracycline-based formation half-lives[4] and are probably the rule rather than the exception. This variation is the most potent argument for lumping measurements on a group of similar cases rather than focussing on single determinations.

Discussion

In the paper in which the present method of measurement is presented in some detail[8] it was pointed out that a Howship's lacuna, once formed, is a static phenomenon. It remains until covered over by new bone. The likelihood that a given Howship's lacuna will be observed upon subsequent sampling of the skeleton is directly dependent upon how soon after its formation it is covered over by new bone formation.

Assume for the moment two patients with identical rates of osteoclasia. In the first patient assume that new bone formation is very active; in the second assume that new bone formation is very indolent. It can be appreciated that after a time the Howship's specific surface in the first case will be rather small, but in the second case it will gradually increase with time and become large. The reason is that in the second case, due to failure to cover the Howship's lacunae with new bone, the lacunae accumulate. In the first case, due to active new bone formation, the lacunae are covered up soon after they are produced.

As a result, measurements of the Howship's specific surface cannot be interpreted unless there is also a measure of the new bone formation against which to judge the lacunar specific surface.

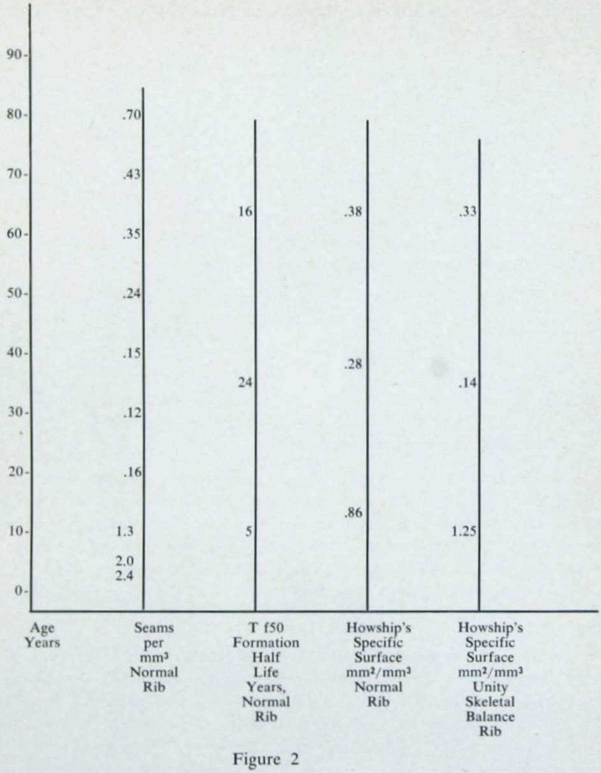

Figure 2

Age, first bar, plotted against osteoid seams/mm^3, second bar, (from reference[7]), T_{f50}, third bar, (tetracycline-based formation half life from reference[4]). Observed Howship's specific surface from this study, fourth bar, and the Howship's specific surface that would theoretically be expected with unity skeletal balance, fifth bar. The method of calculating the latter is not considered and the reader may accept it as tentative.

At present there are only two histological methods of measuring new bone formation which are quantitative and significant. These are the osteoid seam/mm3 [7] and the tetracycline-based formation half-life methods[4] published by us.

In Table III we list some of this data. Alongside the Howship's specific surface measurements given in Tables I and II are the average value of seams/mm^3 for these same patients. In interpreting these values it is necessary that we know the numerical relationship of the Howship's specific surface to the seams/mm^3 when the skeletal balance is unity. By unity skeletal balance we mean that as much new bone is being formed as old bone is being resorbed. In the theoretically normal human adult skeleton it is assumed that skeletal balance is unity and that as much bone is formed in unit time as is resorbed. Since these activities have not yet been compared in a conclusive way in humans this concept of normal skeletal balance of unity must remain theoretical and somewhat suspect. After all, what is normal but, in a statistical sense, that which is usually observed?

This problem is the subject of a separate study and publication by this laboratory but it is unfair to the present study to avoid relating the Howship's specific surface to a norm. This is done by, first, relating our previously published curve of seams/mm^3 plotted against age to a subsequent study in which the formation half-lives of 40 bones were measured with the aid of tetracycline labelling phenomenon. The formation half-life is a true statement of rate, defining the reference volume, the volume of new bone formed and the unit of time in which it was formed.

The graph, Figure 2, reveals the relationship between age of the patient, the normal mean values of seams/mm^3 and of tetracycline-based formation half-lives for a series of human bones, mostly ribs. Plotted alongside these values are the Howship's specific surface as given in the present paper, and the Howship's specific surface that would be theoretically expected if the skeletal balance were unity throughout life. The differences between the observed value (4th scale) and the theoretical value (5th scale) represent the existence of observed and real positive skeletal balance during growth and negative skeletal balance in the older age groups.

When cases 20 - 28 in Table 3 are evaluated in the light of the graph, Figure 2, it is apparent that this group of osteoporotics is in negative skeletal balance because of a disproportionate decrease in osteoblastic activity compared to resorption, as measured by seams/mm^3 and Howslip's specific surface respectively.

The deductions made in the preceding paragraphs must be considered tentative. The reason is that there are insufficient cases in either the normal or abnormal

Table III

Case Group	Mean Age	Mean Howship's Area	Mean Vascular Space Area	Howship's % Vascular Space Area	Seams /mm^3
1 — 4	12.2	.86	3.4	25.3	1.1
5 — 10	39.	.28	3.2	8.7	0.3
13 — 19	65.8	.41	4.3	9.5	.71
20 — 28	66.3	.26	3.3	7.9	.27

The mean values of cases listed in Table I and Table II are presented. The last column is the mean value of osteoid seams/mm^3 for the cases in each group. The mean of cases 5 — 10, with mean age of 39, is considered to be very close to the point of unity skeletal balance. In the older age groups there is a seemingly disproportionate increase in seams. Actually in older patients it takes more seams to do the same amount of manufacturing and some of this increase is the result of compensation for the loss in efficiency.

Because cases 20 — 28 are a mixed group no deductions should be made about the underlying pathophysiology of osteoporoses in general from this material.

groups to consider the figures conclusive. We estimate that about three times as many cases as we present here will be needed before this reliability can be achieved. In spite of this it is encouraging to note that the variation in osteoid seams and in tetracycline-based formation half-lives previously noted by us are in agreement with the variations in respective activity presented here.

We feel that the measuring technique and the measurements derived therefrom are so dependent upon the determination of a group mean value for significance that at present the technique is primarily a research tool. There is too much normal variation in time, and too much variation between bones to make single case measurements useful in themselves. In other words with this technique it is possible to study general processes in groups but it is not feasible to study individuals. Other and better ways will have to be developed to do this.

Summary

Measurement of bone resorptive activity has been presented. The measurements were made on complete, undcalcified cross sections of human long bones and are expressed as the mm^2/mm^3 of the internal cortical vascular spaces covered with Howship's lacunae. This is termed the Howship's specific surface in line with the terminology used by physicists in discussing porous media.

Typical mean values for corresponding mean ages are:

0.86 at age 12
0.23 at age 41
0.38 at age 65

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