Human Osteoblastic Activity

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HUMAN OSTEOBLASTIC ACTIVITY

INTRODUCTION

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It has long been known that in order to measure that portion of turnover of the human skeleton due to constant resorption of old bone and laying down of new bone, methods were needed to permit dealing with resorption and formation on a separate basis. In practice this proved difficult to achieve, the reasons being complex, largely technical, and more than enough to account for previous difficulty.

Since the conclusion of World War II numerous attempts have been made to measure bone formation by using various radioactive isotopes. Many of these attempts merit being called achievements instead of attempts, since the values they derived are significant and are the correct order of magnitude. Turnover measurements as done by men such as G. C. H. Bauer in Sweden, and C. Nordin in Britain, are in the writer's mind as these comments are made.

The basic difficulty with such measurements is well appreciated by the men who are making them. Radioactive calcium, strontium, and other isotopes are not only deposited in the skeleton in places where new bone is being added, but are also deposited in some parts of the large mineral mass in the so-called resting skeleton. Accumulating evidence suggests that this mineral mass may be divided into at least two and perhaps three compartments simply on the basis of the rates with which exchange between the bone mineral and blood may occur. As a result it is difficult to say how much of a retained isotope is deposited in new bone and how much is deposited in already existing bone. This is the nubbin of the problem of measuring bone accretion with radioactive isotopes.

This problem may be restated as follows: tracer methods need a quantitative frame of reference.

In the following series of four papers, and in some of the preceding work which will be referred to in these papers, we present measurements of osteoblastic activity in a sizeable series of human beings. It has been possible to state the amount of new bone formed per unit volume of bone per unit time. The methods serve as cross checks on each other and as a result the writer is confident of the validity of the measurements, the methods, and inferences or conclusions drawn from them. There is one drawback in the methods, in that the measurements are limited to a few bones in the skeleton. Most of the measurements in fact have been done on ribs, clavicles, and femurs. Cancellous bone and the bones in the axial skeleton apart from rib have largely been ignored.

To achieve a quantitative statement of new bone formation in the entire skeleton, these deficiencies must obviously be dealt with in some manner.

The other side of the turnover equation, that of bone resorption, will be dealt with by a separate and subsequent series of publications.
INTRODUCTION

Measurement of human osteoblastic activity has long been the dream of skeletal physiologists. The dream has escaped conversion to reality because of the existence of formidable technical problems and lack of basic physiological knowledge. Recent developments in this and other laboratories now make such measurements possible. This paper is the culmination of work done to provide these measurements. Some results are presented which, in conjunction with companion papers, cover measurements made on bones from over 150 patients.

Figure 1

Cross section, clavicle, human, undecalcified, basic fuchsin. An Haversian system lies in the center of the field. The central Haversian canal is outlined at its wall by the inner array of India ink marks. Between the inner and outer array of India ink marks lies an osteoid seam, elaborated by osteoblasts during the formation of this Haversian system. Accordingly this is a new Haversian system, formed as a part of the body's normal remodelling skeletal activity.

The center of the Haversian system is occupied by somewhat shrunken vascular and connective tissue elements. The Haversian system is about three-fourths completed. Formation proceeds in centripetal rather than in centrifugal, fashion.

There are numerous reasons for wanting measurements of human osteoblastic activity, apart from the obvious ones of curiosity and the wish to measure the previously unmeasurable. Three of these reasons will be briefly discussed in the following paragraphs.
Human Osteoblastic Activity

A) Osteoporosis

In general, it is known that bone is formed only through the activity of cells known as osteoblasts, and destroyed only through the activity of cells known as osteoclasts. If osteoporosis is defined as bone with thinner cortices and trabeculae, and fewer trabeculae than normal, and if it is assumed that the osteoporotic skeleton was once normal, then there must have been more osteoclastic activity than osteoblastic activity to produce this situation. There was a negative skeletal balance, in other words. A change in skeletal balance can occur in one of five generically possible ways. These are (1) unequal increase in accretion and osteoclasia, (2) unequal decrease in accretion and osteoclasia, (3) complete cessation of accretion or, (4) of osteoclasis, (5) increase of one and decrease in the other mode of activity.

Four of the five possible types of alteration of skeletal balance listed could cause osteoporosis. There are no existing quantitative measurements which point out which of the four possibilities actually is responsible, for example, for senile osteoporosis. The current theory is that osteoblastic activity is largely controlled by the protein anabolic hormones, that the decrease in output of these hormones with advancing age is accompanied by decrease in osteoblastic activity without alteration of osteoclastic activity, and that this is the cause of the osteoporosis. In spite of this theory, patients seldom correct the skeletal defect on anabolic hormone treatment, and some even get worse. This indicates a lack in the theory of the genesis of osteoporosis of old age. Measurements of osteoblastic activity are needed to inject a core of fact into this problem and to suggest which of the possible mechanisms mentioned is the cause of the osteoporosis.

B) Interpretation of in vivo Balance Studies

Many methods of measuring calcium balance are known and in use. Broadly speaking their purpose is to determine if more bone is being formed than destroyed during the epoch of the study. It is usually assumed, in the absence of evidence to the contrary, that a positive calcium balance occurs as the result of an excess of bone accretion over osteoclasia, and conversely. It can now be stated with some assurance that this need not be so.

For example, assume a steady-state skeleton - i.e.: one in which the total amount of bone matrix is not changing, and in which formation equals destruction. Theoretically a positive calcium balance in such a situation may occur as the result of halo volume accretion, progressive mineralization of feathered bone, progressive mineralization of relatively young but incompletely mineralized bone, and crystallite adsorption or exchange. On the other hand, a negative balance could occur theoretically from halo volume and crystallite elution. These factors, and others perhaps unknown, plus non-skeletal changes not considered here, could operate simultaneously.

So many unmeasured variables (including osteoblastic and osteoclastic rates) makes interpretation of balance studies in which these variables are ignored a very

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*A former controversy over this point should be laid to rest because conclusive evidence documenting this statement now exists, supplied by several independent laboratories.

Not to be confused with Calcium balance.
unreliable process. Measurement of osteoblastic activity would enable us to place a quantitative estimate on at least one of these variables. From this would be learned the relative importance of the remaining variables. They might be minute or overwhelming.

C) Radioisotope Studies

When radiocalcium, radiosodium, or other skeletally depositing isotopes are given to animals or man, and the skeletally retained moiety subsequently determined, several variables can theoretically combine to explain an observed effect. These factors have been mentioned: feathering, micropetrosis, halo volume, mineralizing new matrix, osteoclasia, and at more fundamental levels, factors governing diffusion impedance between crystallite and the blood supply, and factors governing the solubility and activity coefficient of the bone crystallites. Almost no attempt has been made to quantitate the first 7 factors named, partly because most of them are new knowledge, and partly because there were no feasible methods available. The number of variables eloquently portrays how essential quantitation of one or more of them is in order to assign quantitative significance to the remainder.

THEORY OF MEASUREMENTS OF SEAMS/ MM³

A brief review of some material published by the Orthopedic Research Laboratory will orient the reader of this paper. Details will be found in the references listed.

1) Properly stained fresh, undecalcified, cross sections of the diaphyses of long bones were utilized in this work. The sections average about 35 microns thick, comprise the entire cross sections of the bone and are cut accurately perpendicular to the longitudinal axis of the bone.

2) Osteoid seams may be quantitatively stained and quantitatively recognized in sections prepared as recommended above.

3) Longitudinal homogeneity, transverse inhomogeneity, has been found to characterize the skeletal distribution of a number of features, among them osteoid seams, feathering, micropetrosis and tetracycline labelled new bone formation. This orientation has obvious effects on the type of sampling and sections that are needed.

4) Only measurements on diaphyses are reported. Measurements of cancellous bone can be made but correction factors must be applied due to lack of uniform orientation. These correction factors are laborious and subject to large errors.

The restriction of material to diaphyses means that the values of osteoblastic activity cannot be considered values for the entire skeleton or entire bones, since cancellous bone might have different activities than cortical bone. It has already been noted that significant differences occur in activities between the axial and appendicular moieties of the skeleton.

It is possible that in the future a normalization factor can be determined which will enable measurements of one bone to be normalized to the entire skeleton. This would remove the present, bothersome restriction on interpretation of the present data.
5) Osteoblastic activity sounds fine, but how to measure it? Should one measure oxygen consumption per osteoblast, new matrix formed per unit volume or the number of osteoblasts per osteoid seam? We have elected to measure the number of osteoid seams per unit volume of cortex. Such a measurement does not reveal the rate of new bone formation, and does have inherent in it a number of possible defects which will be discussed later. Seams per unit volume are easy to measure, are reproducible, and when averaged in sufficient number provide a smooth curve of variation with age.

6) In a previous publication it has been noted that osteoid seams are normally present in human adults as well as in children. In the present 500 cases in the laboratory's files there are no cases in which seams are not found except in some cases of severe cardiac failure, some cases of severe hepatic cirrhosis or in most patients receiving large doses of cortisone or ACTH. Included in the case material are 90 healthy, normal people dying of violence, embolism, sudden hemorrhage, or coming to surgery for the correction of anatomic defect rather than systemic disease. These cases exhibit measurements lying in the median range of the curves in Graph I.

7) The actual method of doing the measurements is detailed in reference (13) which was published specifically to detail this part of this paper. The measured parameter is the number of seams/mm³.

8) It should be noted that only osteoid seams in the cortex are measured in this work. Endosteal and periosteal seams are not measured. It should also be noted that the normal, lamellar bone remodelling processes that occur in childhood and adulthood are always accompanied by the presence of seams at sites of new bone formation. A peculiar, resting state of osteoid seams induced by abnormal factors has been eliminated as a variable in this work by excluding resting seams from counts.

**MATERIALS**

Sixty-two ribs and 52 clavicles from 93 patients were measured. Males comprise 62% of the cases. Thirty of the ribs were obtained fresh from the operating room from thoracotomies. The remainder of the material was obtained at postmortem.

Hindsight has been exercised in determining which cases to include and which to exclude from this study. The following have been excluded: any patient on cortisone or related compound or ACTH for more than 2 weeks prior to skeletal sampling; any patient on androgens or estrogens; any patient with severe hepatic cirrhosis; any patient with severe congestive heart failure; any patient with known local disease of the bone obtained; any patient with systemic bone disease, including multiple myeloma and skeletal metastases; any patient with disturbed serum calcium, phosphorus or alkaline phosphatase; any patient with symptomatic osteoporosis.

**METHODS**

1) Fresh, undecalcified, cross sections were made and stained as recommended by Frost. Section thickness averaged about 35 microns and should not exceed 50 microns.
2) The number of osteoid seams per unit volume was measured as described in reference (13) and expressed as seams per mm³.

3) The measurements were then tabulated in groups according to the age of the patient, the divisions in age being made every 5 years. Totally separate tabulations were made for the ribs and the clavicles.

4) The average values for a given age — say age 60 — were made by averaging the measurements from all cases 5 years younger, and all cases 5 years older, than the given age. The average figure for age 60 then is the mean of the measurements of all cases aged 55 to 65 in the series. This method of obtaining values for a curve has the advantage that the curve is a smooth one and thus easy to grasp. It has the disadvantage that possible actual short time fluctuations in values are averaged out. Until the number of “normals” is larger by a factor of about 5, it will be impossible to be sure whether short-term fluctuations were real or due to chance.

5) The values for rib and calvicle are plotted separately, using a linear scale for age and a logarithmic scale for seams/mm³.

6) A discussion of errors is necessary to place the values in perspective. Empirically it has been determined that the measurement of channel/mm² varies plus or minus 0.005 and the seam counts plus or minus 0.01. With the optical constants specified in reference (13) there is an additional constant, proportional error in seam counts because about 3% of them are too thin to be recognized at the magnification and numerical aperture used. Shrinkage of undecalcified cross sections is about 2% in the radial and tangential axes (longitudinal shrinkage can be completely ignored in the present measurements).¹³

There is no error due to failure to demonstrate an osteoid seam, and there is no error due to failure to recognize an osteoid seam, except for the proportional error referred to previously.

All the above errors combined are so small in comparison to the actual fluctuations in seams/mm³ in different cases that correction for them is superfluous. In other words, the sensitivity of the method exceeds the needs of the task.

The major source of possible error is in the number of sections measured in each case.¹³ A total of 191 rib sections and 98 clavicle sections were measured, an average of about 3 rib sections and two clavicle sections per bone examined. The variations found in single bones will be reported separately.¹³ Suffice it to say that measurement of one rib section carries a probable error in the neighborhood of 100%; of 3 rib sections about 20%; of two clavicles about 10%. Since in some cases only one section was measured, and in others 7 or 8, the reliability of the individual measurements varies widely. Since the scatter in values has repeatedly proved to be real rather than a sampling defect, we have resorted to the device of lumping cases together to minimize the scatter and to permit graphing the changes in osteoblastic activity with age.

**OBSERVATIONS**

The mean values of seams/mm³ for both rib and clavicle will be found in
**Human Osteoblastic Activity**

Graph I. The characteristic curve should be noted, and the fact that over the majority of their courses the curves of the two bones parallel each other. Note the sharp decrease in osteoblastic activity near age 30 and the fact that it gradually increases thereafter. A decrease after age 30 is manifested only in the clavicle and only after age 65. Note that, with the exception of some variation in the 30-40 age range, the curves obtained are smooth, indicating that the number of cases measured and sampled is sufficient for the present purpose. If the number were too small, there would be sizable sinusoidal variations along the majority of the curve.

![Graph I](image)

The curves are drawn around the mean values plotted by 5-year age groups. The curve of the ribs is to the right, that of clavicles to the left. There is no curve for clavicles below age 40 because local undertakers object to the deformity resulting when a part of this bone is removed in younger clients.

Age is the abscissa on a linear scale. Seams/mm\(^3\) of diaphyseal cortex is the ordinate on a logarithmic scale. The curve as depicted is artificially smoothed.

In Table I the mean values of the various rib age groups plotted in Graph I are listed along with the number of cases in each group. Since there is considerable overlap in these group mean determinations (see Methods) they do not total out to the total number of cases available.
Frost and Villanueva

**RIBS**

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<th>Mean Age</th>
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<th>Number Cases Averaged</th>
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**Table I**

**CLAVICLES**

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<tr>
<td>75</td>
<td>.092</td>
<td>8</td>
</tr>
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</table>

**Table II**

All of the cases in this study have been placed in 5-year age groups and tabulated in these columns. Ribs and clavicles are tabulated separately. The age of the group is on the left, the mean value of the group in the middle, and the number of patients in the group in the right columns.

In Table II the mean values of the various clavicle age groups are similarly listed.

In Table III some individual age groups are listed, each case in the group and its sex being noted. The purpose of this is to permit the reader to see for himself the amount of scatter to be expected in these measurements.

The values of seams/mm² in young children is probably deceptively low, the reason being that endosteal wall osteoid seams were not counted in the measurements.
Human Osteoblastic Activity

A considerable number of such seams are to be found in the bones of rapidly growing children.

Table III

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Sex</th>
<th>Value Seams /mm³</th>
<th>Age Group</th>
<th>Sex</th>
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</table>

Table III 

AGE GROUPS FROM THE SERIES OF RIBS, AND TWO FROM THE SERIES OF CLAVICLES, are presented here in detail. Some idea of the scatter may be obtained from inspection of the individual measurements. There is no clear-cut difference between the sexes.

DISCUSSION

1) As was intimated earlier, saying that osteoblastic activity will be measured is fine, but when the task materializes the investigator must decide exactly how osteoblastic activity will be measured. In general one might measure some aspect of metabolic activity, or the total amount of matrix formed in unit time, or the total amount of mineral deposited in unit time, or the total volume or number of osteoid seams formed per unit time. There are other possibilities but the point is made — the task seems complex.

2) This laboratory has been very fortunate in having available (a) simple methods for preparation of fresh, undecalified sections which contain less artifact than bone sections made by any other existing method; (b) possessing the largest collection of such sections in the world with complete clinical data on hand on every case; (c) in possessing material from a large number of cases to whom tetracyclines were administered at varying times and durations prior to skeletal sampling; (d) in possessing superiors with a true desire and understanding of the need for basic research.

With these advantages a study such as the present one is possible. The most likely phenomenon that could be used to measure osteoblastic activity at first appeared to be the osteoid seam, which had already been observed to be normal, rather than abnormal, accompaniment of skeletal maturity and senescence.
3) Measuring osteoblastic activity in terms of seams per unit volume possesses certain possible faults which had to be checked. These faults, and the manner in which they were dealt with, are briefly:

A) The possibility that new matrix is mineralized so quickly that it is not present in sufficient thickness to be detectable optically. This was checked using tetracycline-labelled material in which the tetracycline had been administered within two weeks of skeletal sampling. The tetracycline-labelled new bone always corresponded to the presence of an osteoid seam. No tetracycline-labelled new bone existed without an accompanying seam in this material.

B) The possibility that the rate of production and mineralization of new osteoid varies with age, as well as the measured variation in number per unit volume. In other words, there is a possibility that a decreased measured value of seams/mm² might correspond to increased osteoblastic activity because the few seams present are being formed and mineralized much more rapidly than would be normal at another age. While variations in rate of formation do occur, increases of rate are accompanied by increases in seams/mm². If the resting state of osteoid seams is compensated during the counts, then it is also true that retardation in rate of osteoid formation is accompanied by fewer seams/mm². Finally, no systematic variation in rate, other than the high activity expected in children, has yet been detectable in tetracycline-labelled material. The predicted slowing up of osteoblastic activity in the years past age 40 were not only not observed; there is a slight increase in rate of formation of new osteoid with advancing age.

C) The possibility that osteoid seams are abnormal. This matter has been dealt with elsewhere but, in brief, this is a false notion. Seams are a normal finding in all ages. Absence of seams at any age seems to be evidence of severe disturbance in the body's physiology.

D) The possibility that cortical remodelling may show variations in age totally different from the variations in cancellous bone. At present there are no quantitative measurements which dispose of this point, only qualitative observations in a few tetracycline-labelled skeletons. While there are differences in rate between cancellous and cortical bone, their variations seem to vary in the same manner in the small amount of material observed to test this matter.

If this possibility should prove true — which is not likely — knowledge of such a skeletal characteristic should prove most valuable.

E) The possibility that clavicles and ribs are peculiar bones not representative of the skeleton as a whole. The laboratory possesses over 200 femora, tibias, fibulas, radii, ulnae, humerii, phalanges, metacarpals, metatarsals, vertebrae and iliac crest specimens. There is no essential difference between these bones and those reported

*Checked with tetracycline labelled cases.
Human Osteoblastic Activity

... on in this paper. Clavicles and ribs were selected for this study solely because enough of them are available and have been measured to derive significant mean values according to age.

4) It hardly needs to be pointed out that the nature of fluctuation of the curve of osteoblastic activity in Graph I is quite the opposite from that which would be expected from current theories about factors governing osteoblastic activity. It is widely felt that the protein anabolic hormones exert a considerable effect on osteoblastic activity, acting as an accelerator. It is further felt that with increasing age, after the 30 year decade, a progressive fall in the endogenous supply of protein anabolic hormones, plus a disproportionately smaller fall in the endogenous supply of protein catabolic hormones, leads to “defective matrix formation”. The latter term is usually interpreted to mean that a decreased amount of matrix is formed but that the quality of the matrix which is formed is normal.

The curves in Graph I do not agree at all with the above thesis. There are three possible reasons for this disagreement:

(a) The measurements in this paper are in error (they have been checked most carefully).

(b) Some quirk of osteoblastic physiology exists which has not been detected and which, when compensated for, will make the measurements presented in this paper compatible with the above-mentioned theory.

(c) The theory is wrong.**

These three possibilities are stimulating, and it is hoped that skeletal physiologists find them of interest.

SUMMARY

Measurement of human osteoblastic activity, expressed as osteoid seams per unit volume, has been done. Measurements on human ribs and clavicles are graphed according to age. The curves indicate that at birth osteoblastic activity is quite high, that it decreases rapidly until age 25-30 where it levels off, only to climb slowly but steadily until age 65. Beyond this age the significance of the curves is impaired by lack of numbers. It appears at present that ribs continue to reveal a relatively high osteoblastic activity beyond this age, but that clavicles show a dropping off in activity after this age.

The present methods and measurements are qualitative because the problem of Volume of bone formed per unit volume of bone per unit time has not been solved. Until such a solution is at hand the present measurements, while instructive and indicative of the quantitative nature of the variation of osteoblastic activity, cannot be interpreted in terms of so many milligrams of calcium per day retained, so much matrix nitrogen per day retained, so much turnover per day and so on.

**This is our present opinion.
REFERENCES


