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AN EQUATION FOR THE MESENCHYMAL CELL ACTIVATION FUNCTION IN THE LAMELLAR BONE REMODELLING EQUATIONS*

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INTRODUCTION

STUDENTS OF human physiology in its broad sense may find parts of this text interesting and provocative. This is because it deals with remodelling of a tissue. Remodelling is a widespread physiological activity about which little has been known until recently, and most of what is known happens to concern bone. From the standpoint of its physiological dynamics, bone is not as unique as its morphological peculiarities would suggest. Bone may function as a model system or window through which physiological activities of general importance may be studied. This function is significantly potentiated by the fact that bone contains a record of past physiological dynamics.[1] This record is written in a unique symbology, it is semipermanent, and it contains material useful in understanding endocrinological control mechanisms, diabetes mellitus, aging, tissue repair and the operation of the nuclear code of cells, to name some of the areas already enlightened by study of bone.

A search for the understanding of bone disease initiated by this laboratory (Orthopedic Research Laboratory) 9 years ago inevitably led to the study of the absolute and relative rates of dynamic processes, both of chemical and cytological origin. The inevitability arose because disease is usually an abnormality in rates of chemical and cytological processes. In time these disturbances lead to the obvious physical manifestations of disease. The causal relationship between rates and disease tends to be concealed from us by medical education systems which concentrate (necessarily!) on definition of state, morphology, recognition and classification.

In the major number of bone diseases the normal and abnormal dynamics referred to were largely unknown, so as a first step in understanding bone disease it became necessary to obtain, study and characterize normal bone. This study required the solution of many sampling and methodological problems and the revision or

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destruction of many older concepts, some of them time-honored and so vigorously defended.

Because bone does not flaunt its dynamics by means of standard alphabets, its dynamics are difficult to study directly in vivo or in vitro. Dynamic events occurring in bone often do leave evidence behind in passing of their existence, nature and magnitude, as well as of the time when the events occurred. This evidence is imprinted in a special symbology, creating a book containing histological, physical and chemical information begging to be read by curious eyes and translated by curious minds. Some of this record can be read and the nature of some dynamic events that occur in it or, more important, that affect it, can be detected and observed in action.[1,4,6,11] This record has the major advantage that it is written in vivo, unaffected by the technique of or act of observation and measurement, since technique and act focus on the bone after the event, not during it.

One of the offshoots of the work done with bone has been the development of the kernel of a mathematical model of lamellar bone remodelling.[1] In the following text only lamellar bone physiology is discussed, and little that will be said or reported is applicable to fibrous bone physiology.[5]

The mathematical model has three elements or parameters. These parameters are accurately measurable in fresh, undecalcified bone sections. A considerable amount of bone physiology and pathophysiology may be described partly or wholly in terms of changes in these three parameters with age or in disease (i.e., normal remodelling; osteoporoses; osteomalacias; osteogenesis imperfecta; osteopetrosis; aging). The model accounts for and partly explains an observed stratification of regulation of metabolism which had been undetected prior to its discovery in bone.

The basic use and purpose of the model is investigative. Each of the parameters of the remodelling system varies in characteristic ways during life, indicating the existence of time-dependent changes in regulation or response to regulation on the part of the cells whose activities are under study. Once reliable curves of the normal changes in these parameters with age have been empirically established, equations may then be devised whose time-dependent solutions accurately follow the empirical curves. Such equations may be called mathematically valid models of the physiological behavior. These equations must then be tested for biological validity by comparing the behavior of the various elements of the equations to the various elements of the biological system. A biologically valid equation will contain elements which correspond in number and in time-dependent behavior to those in the biological system. The more complete is one's knowledge of the biological system, the easier it should be to devise biologically valid equations which model the behavior of the biological system. When an equation proves biologically invalid, the concepts on which the derivation of the equation was based are wrong, incomplete or both.

There is a glimpse in the above paragraph of a powerful investigational approach which is still relatively unused in the orthopedic basic sciences, although the

approach has achieved wide success in many other fields, including various branches of engineering, biometry, and the physical sciences. One purpose of this report is to acquaint potentially interested orthopedic basic scientists with this approach.

A second purpose is to present a mathematically valid model of the mesenchymal cell activation function. This function is one of the three basic parameters of the lamellar bone remodelling equations. The activation function model is tentative for three reasons: a) its biological validity is not established, b) probably not enough is yet known about the cytodynamic events involved in remodelling to permit de novo construction of biologically valid equations, c) and it is possible that a different form of mathematics will prove more applicable to the present problem.

Clarity will be served best if some facts about bone remodelling are reviewed briefly and if the basis of the basic remodelling equations is outlined. The mesenchymal cell activation function will then be derived as an equation, numerical values for its constants and initial conditions given and a comparison of the calculated with the observed function made. The nonmathematical reader who needs to use the equation will find it presented in usable forms in equations (6.0) which is the derivative form yielding a numerical value at any instant, and (7.2) which is the integral form yielding cumulative values over any time span.

LAMELLAR BONE REMODELLING

A) *Bone remodelling is the combination of bone resorption and bone formation which occurs throughout a normal life.* Resorption is done by cells called osteoclasts, formation by osteoblasts. It follows that resorption, formation, and their sum, remodelling, are cell-mediated activities and so are cell regulated.[6,11]

B) There are at least two types of bone remodelling, a distinction based on observed differences in response to regulation throughout life. *Internal remodelling* occurs inside cortex and trabeculae and does not involve remodelling of any major bone surface. *Surface remodelling* occurs only at the periosteal and endosteal surfaces. See figure 1. The main subject of this text is internal remodelling because adequate studies of it are available. Surface remodelling exhibits many of the same basic properties as internal remodelling, even though its dynamic response appears to be different from that of internal remodelling. The curves shown in figure 3 are obtained from studies of internal remodelling.

C) Definite amounts of bone are resorbed and formed during remodelling activity. The index of an amount of bone used throughout this text is *absolute bone volume.* This is the volume of bone left after all of its porosities and spaces have been subtracted. The porosities and spaces are lacunae, canaliculae, Haversian canals, Volkmann's canals, primary longitudinal canals and marrow spaces. The value of this index of an amount of bone is that it does not change significantly with age, disease

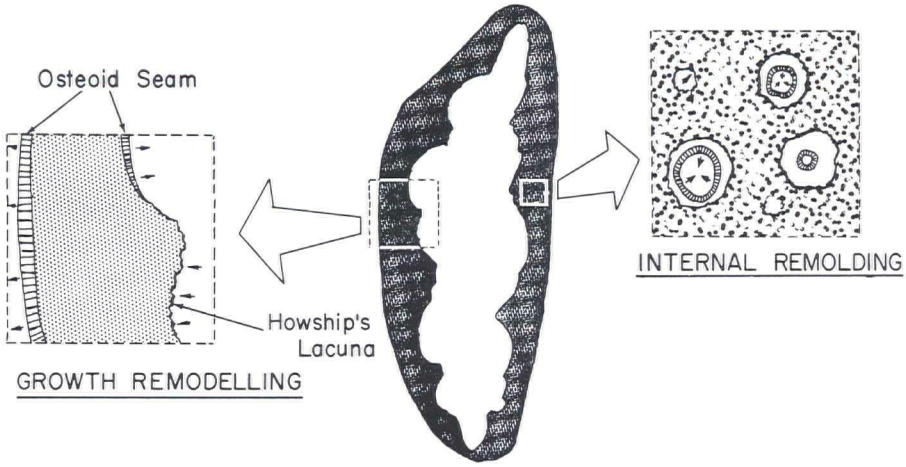

Figure 1

The difference between surface and internal remodelling of lamellar bone is shown. At the center of the figure is a diagram of a human rib in cross section. The perimeter of this rib is *periosteal* surface. The surface lining the central cavity is *endosteal* surface.
In the left box, new bone formation (left side) and bone resorption (right side) are shown in an insert enlarged from the central figure. Both of these processes occur at the surface, and are thus termed surface remodelling.
In the right box, in a similar insert, three foci of new bone formation and two of resorption are shown. This is internal remodelling because it occurs within the space encompassed between the periosteal and endosteal envelopes.
The reason for making the distinction between surface and internal remodelling is that their age-dependent changes are different, indicating a difference in regulation even though the same two types of cells actually do the work involved in removing or making bone[2].

or degree of mineralization. It is the most accurate index of an amount of bone, and of the amount of cellular activity in making it, that is available. See figure 2.

D) Remodelling activity possesses certain basic physical properties. These are: a) both types of remodelling occur in physically discrete and measureable units termed *foci,* b) there is a mean surface area of the foci of resorption and another of the foci of formation which is also measureable, c) there is a mean and measureable rate at which new bone matrix is added to the surface of formation foci, or destroyed at the surface of resorption foci, and d) only one type of remodelling activity (i.e., either resorption or formation) occurs at any moment at any one place in bone.

Note that in the above paragraph is the nucleus of three distinct means of controlling the amounts of bone remodelled in unit time: by changing the numbers of places where remodelling occurs, by changing the mean size of individual foci, or by changing the mean rate of evolution of individual foci.

E) There are certain basic dynamic properties of bone remodelling apart from the obvious possibilities pointed out in the preceding paragraph. There resides near the blood vessels which permeate the major porosities in bone a type of cell termed the *mesenchymal cell.* This cell is pluripotent, being able upon proper command to pro-

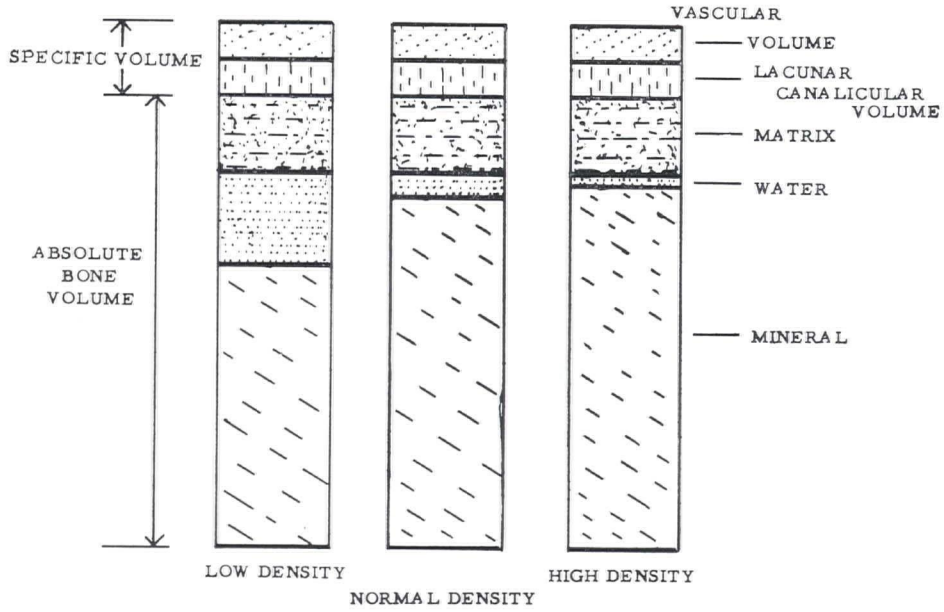

Figure 2

The three bars represent three bones from different people. They differ in mineral density, and therefore in mass. However, the amount of space occupied by the material lying within the confines indicated by the arrow designated absolute bone volume (ABV) does not change. Note that the amount of matrix is the same in all three; note that the volumes of lacunae and canaliculae is also nearly the same in all three.
The change in amount of mineral in terms of *volume* is permitted by equal but opposite changes in the *volume* of water present. Since an increment in volume of mineral is balanced by an equal loss in volume of water there is no overall change in absolute bone volume, although there is a change in the mass and density. The latter occurs because the mineral is more than twice as heavy as the water it displaces. This is the basic reason why it is best to discuss amounts of bone in terms of volume.

duce chondroblasts, fibroblasts, both types of osteoblasts and perhaps histiocytes. The mesenchymal cell is capable of mitotic replication of itself, in addition to being capable of producing the specialized cell types mentioned. Much of the following we owe to the work of R. Young.[13-15]

a) The first event in starting a focus of remodelling is one termed *activation* of the mesenchymal cell population at the site where the remodelling focus will evolve. This activation involves transmission of a specific command from the microenvironment to the mesenchymal cell nucleus, as a result of which the succeeding steps occur as though predetermined by the use of precoded information or predetermined activity inherent but previously masked in the mesenchymal cell nucleus.

b) The mesenchymal cell population produces waves of osteoclasts which in turn produce a resorption space in internal remodelling or a resorption front in surface remodelling. Since osteoclasts are not able to reproduce themselves by mitotic or

amitotic reproduction their number is determined by how many the mesenchymal cell population produces.

c) A resting phase ensues, lasting about 1 month, during which no histologically visible activity occurs.

d) Now the mesenchymal cell population produces waves of lamellar osteoblasts which form new bone matrix, covering up the resorption surface or filling in the resorption space. Since osteoblasts are also unable to reproduce themselves, their numbers are also determined by the mesenchymal cell population.

e) When the new bone formation phase is done, histological quiescence returns to the site. The total evolution of a remodelling focus seems to take about 5 months in man, and the events just listed appear to be well stereotyped, one bit of evidence that the evolution of a remodelling focus is the result of some precoded series of operations in the nucleus of the mesenchymal cell.

THE LAMELLAR BONE REMODELLING EQUATIONS

Designate an amount of bone by the symbol (V). Designate the numbers of places in this bone where remodelling activity occurs by (A), the mean surface area of each remodelling focus by (S) and the mean rate of destruction or growth of this surface by (M). Use the subscript ($_f$) to indicate formation and ($_r$) for resorption. Let (k) indicate the amount of bone originally present. Define units of length, area and volume in millimeters, time in years. Then we may write

$$V_f \Big]_N^{N+1} = k A_f S_f M_f \Big]_N^{N+1} \qquad (1.0)$$

meaning the amount of bone formed in a year is the product of the original amount in mm^3, times the mean number of formation foci per mm^3, times the mean surface area of these foci in mm^2, times the mean rate of growth of this surface in mm/year. The answer is in mm^3.

The meaning of this equation may be clarified by a simple analogy. Cut both ends out of a soup can so that only the cylinder of metal comprising the wall is left, the ends being open. This corresponds to an (A) unit. The interior of the cylindrical wall of the can has a surface area which could be measured in a number of ways. Assume that it is measured. This is the (S) parameter noted above. Now assume that layer after layer is added to the inner wall of this cylinder so that the diameter of the hole inside the can decreases steadily; this corresponds to (M), in this case the (M_f) parameter. If, instead of making the hole smaller, it were progressively enlarged, it would then become an (M_r) unit, since bone resorption is centrifugal, bone formation centripetal in direction.

Clearly if mean values for these parameters were known for any age or any short span of years, the amounts of bone resorbed and formed in that time could be

calculated easily and accurately, the latter depending on the accuracy of the individual measurements. Unfortunately this simple algebraic form cannot supply an accurate solution for any large period of the life span, centered on any moment in the life span. The reason is illustrated in figures 3 and 4. Here some of the empirically determined curves of the remodelling parameters are reproduced from studies of normal human ribs. It may be seen that they vary in characteristic ways throughout life, and they vary differently from each other.

Achieving a mathematical form equal to the problem of obtaining a solution for any length of the life span centered over any moment in the life span requires considerable complication of the mathematics. The increased effort appears to be justified by increased potentiality of using the equations so derived as research tools (for studying regulation) rather than as descriptive vehicles.

Inspection of equation (1.0) indicates that if any of the right hand elements of the equation are changed by a given increment, the solution (left hand side) will be similarly changed. In symbols this may be said:

$$\Delta V_f = k \Delta A_f \ \Delta S_f \ \Delta M_f \qquad (1.1)$$

It may be seen that the right hand side of equations (1.0, 1.1) are rates and have the dimensions of mm^3/time. Accordingly, resorting to calculus, we may write:

$$V_f \Big]_{to}^{t} = k \int_{to}^{t} f(A_f)_t \ f(S_f)_t \ f(M)_t \, dt \qquad (2.0)$$

In words the absolute volume of new bone formed (V_f), in an original amount of bone (k), is the sum of the instantaneous products of the instantaneous values of all three parameters or functions at all infinitesimally small intervals of time between (t_o) the beginning of the span of observation, and (t) the end of the span of observation.

The forms $f(A_f)$, $f(S_f)$, $f(M_f)$, indicate functions of the respective parameters. These are functions of time (age), and are intended to represent in symbolic form mathematically valid equations that, when solved for any moment during life, yield the observed numerical value of that parameter at that age.

As the subscripts show, only the formation equations have been dealt with so far. The resorption equations are very similar and all of them may be obtained by the simple substitution of the proper subscript. This parallelism is another bit of evidence for the common mesenchymal cell origin of the cells responsible for resorption and formation, and for their dependence on the same precoded sequence of events in the mesenchymal cell nucleus.

Thus one may write

$$V_r \Big]_{N}^{N+1} = k A_r S_r M_r \Big]_{N}^{N+1} \qquad (3.0)$$

meaning that the amount of bone resorbed in a year is the original amount times the mean values during the year of the three parameters. Since these parameters deal with resorption foci, their numbers, size and rates of evolution will be independently determined, and may not be deduced from the formation parameters.

Equation (3.0) has the same limitation as equation (1.0), so it cannot provide a continuous solution for any segment of the life span centered on moment during life. An equation comparable to that for formation may be written

$$V_r\Big]_{to}^{t} = k \int_{to}^{t} f(A_r)_t f(S_r)_t \, f(M_r)_t \, dt \qquad (3.1)$$

Equations (2.0, 3.1) may be differentiated in the usual way, noting that the three (A,S,M,) parameters are all functions of time, and using equation (3.1) as the model:

$$\frac{dV_f}{dt} = k\left[A_f S_f \frac{dM_f}{dt} + S_f M_f \frac{dA_f}{dt} + S_f M_f \frac{dA_f}{dt} \right] \qquad (3.2)$$

and:

$$\frac{dV_r}{dt} = k\left[A_r S_r \frac{dM_r}{dt} + A_r M_r \frac{dS_r}{dt} + S_r M_r \frac{dA_r}{dt} \right] \qquad (3.3)$$

An expression for the skeletal balance at any moment during life would then become, where B_{sk} indicates the balance,

$$B_{sk}\Big]_{N}^{N+1} = \int_{o}^{t} dV_f \, dt - \int_{o}^{t} dV_r \, dt \qquad (4.0)$$

meaning that the balance is the difference between the derivatives. This might also be written in the simpler algebraic form, using equations (1.0, 3.0):

$$B_{sk}\Big]_{N}^{N+1} = V_f\Big]_{N}^{N+1} - V_r\Big]_{N}^{N+1} \qquad (4.1)$$

meaning the balance over a one year period. In both cases normal balance is zero, meaning that there has been no change in the amount of bone present during the year because as much was formed as was resorbed.

The amount of bone in the whole skeleton, or in one bone such as a rib, is the cumulative balance between formation and resorption throughout previous life. This might be written

$$V\Big]_{o}^{t} = V_f\Big]_{o}^{t} - V_r\Big]_{o}^{t}, \qquad (4.2)$$

or it might also be written

$$V\Big]_{o}^{t} = \int_{o}^{t} f \, (B_{sk})_t \, dt \qquad (4.3)$$

EQUATION FOR THE MESENCHYMAL CELL ACTIVATION

The remainder of the text will deal with the derivation of an equation suitable mathematically for the $f(A_f)$ element of equation (2.0). A similar form of equation would then be suitable also for the $f(A_r)$ function of equation (3.1), although an additional term would be needed in this equation to model accurately a resorption feature that need not be amplified further here.

MATHEMATICAL NOTATION

A: A focus of remodelling activity

A_f, A_r: foci of formation and resorption respectively, in numbers/mm^3

ABV: Absolute bone volume

β: Beta, the negative skew term

e: The base of Naperian logarithms

f: as a subscript: pertaining to a formation focus.

$f(A_f)_t$, $f(M_r)_t$, etc.: read "function of A_f of t, function of M_r of t"

$f, {}^{I}f, {}^{II}f, {}^{III}f$: First, second and third derivatives respectively

k: A constant, or the original amount of bone present in mm^3

M_f: Linear rate of addition of new bone matrix to the surface of an osteoid seam per year in millimeters

M_r: Linear rate of destruction of bone at a resorption site in mm/year

r: As a subscript: pertaining to a resorption focus

R: Amplitude of oscillation

R_o: Amplitude at the beginning of observation

S: The mean surface area of remodelling foci in mm^2

t: Time in years

t_o: Time at the beginning of observation, years

t_1, t_2, etc.: First time, second time, etc.

V: A volume of bone in ABV terms

V_f, V_r: Volumes of bone formed and resorbed respectively

ω: Omega: radians per unit time.

$\int$: The symbol for integration

dt: The symbol meaning with respect to time

$]^{t}_{to}$: The time limits between which the mathematical operation is performed

cos, sin: Cosine and sine (trigonometric) respectively

$\triangle$: An increment of change in a value

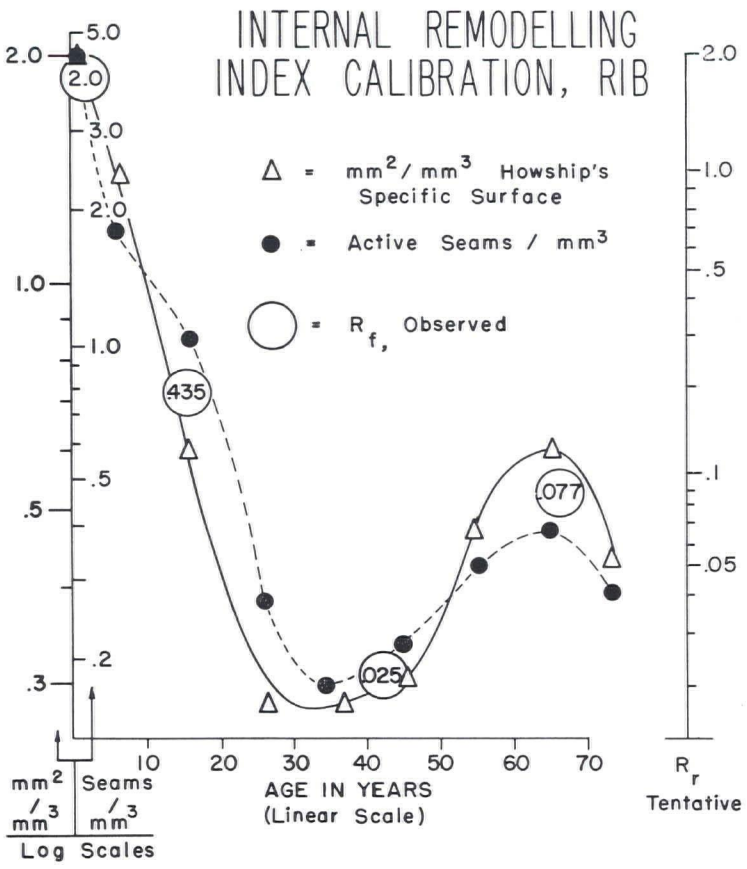

Figure 3

This is a graph of the changes with age of an index of bone formation and an index of bone resorption in 139 normal human ribs. The study is being separately reported by Sedlin and coworkers[9] and Villanueva and coworkers[10].

The dashed line shows the variations in numbers of sites of new bone formation with age in these ribs. This is the (A_f) function considered in the text. The study reports only remodelling foci involved in internal remodelling. Note the minimum at age 35 and the secondary maximum at age 65, followed by a further decline.

The solid line represents an index of resorption which incorporates both the (A_r) function in the text and the (S_r) function. Since the (S_r) function exhibits a nearly horizontal value with age, the more marked changes in the (A_f) function "show through".

The circles represent numerical values of the bone formation rate in these ribs, determined with the aid of suitably tetracycline labelled specimens.[4] The bone formation rate is the decimal fraction of the original amount that is produced as new bone in one year. Note a slight excess of formation in the younger ages, and of resorption in the older. The log scale at the right is a reasonably good fit with the observed bone formation rate determined, (R_f), and may be used to interpolate.

Work by Kulp and Schulert[7] indicates that from the standpoint of remodelling ribs are about 1.4 times more active than the mean for the whole skeleton, and as much as 30 times more active than normal human tibia. The value of this curve lies more in that it reveals the time of life when changes in slope of the curve occur, and less in that it gives numerical values for one bone although this has been useful.

DERIVATION OF AN EQUATION FOR THE MESENCHYMAL CELL ACTIVATION FUNCTION

This text focuses on (A_f), or the numbers of foci of bone forming activity in an arbitrary amount of bone, because this is the most accurately known of the remodelling parameters in normal bone.

The curves in figures 3 and 4 suggest (a) simple harmonic motion (b) which is damped in amplitude with increasing time and (c) superimposed on a progressive

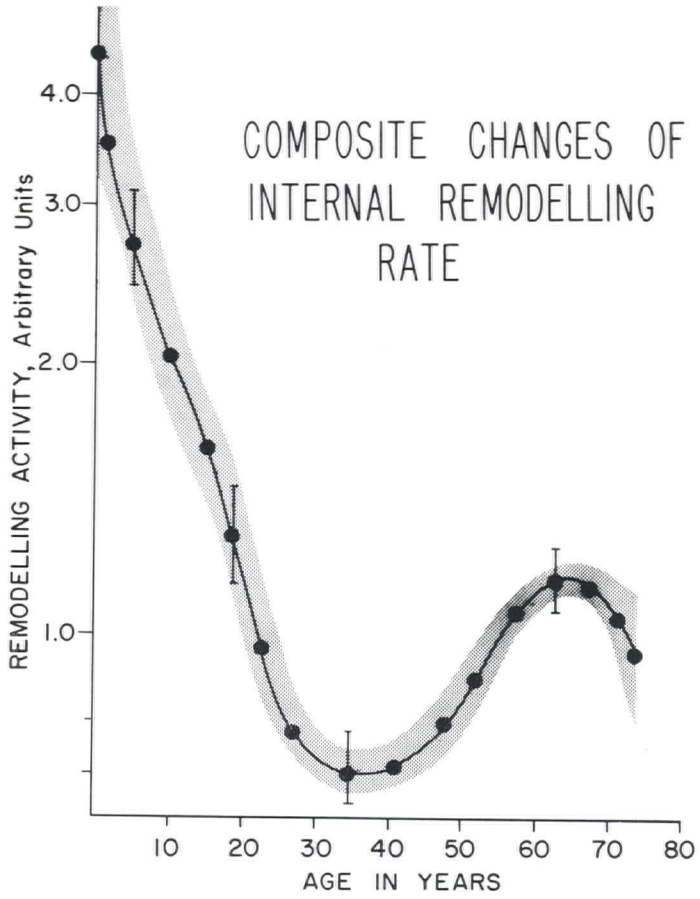

Figure 4

In this curve five separate indices of internal remodelling activity in the 139 ribs of figure 3 are combined into a composite. Justification for combining formation and resorption indices is this: in internal remodelling in healthy people, resorption nearly exactly balances formation. The same cannot be said for surface remodelling.
The dots are means of the five different indices after suitable scale transformation had been done to make the ordinates and abcissas comparable.
This curve exhibits a striking sine-wave characteristic. The short vertical bars indicate standard errors of the combined indices. Note that the ordinate is logarithmic while the abcissa is linear. Internal bone remodelling may be analogous to cellular turnover in soft tissues, epithelium and remodelling of tendon and fascia.

negative skew on the abcissa (negative Y axis shear). There may also be an increase in the frequency with time. Read $f(A_f)$ as "function of A_f"; this function is an equation. When solved for a specific value of time, its solution is a locus in Cartesian coordinates (i.e., X-Y) on a graph.

A) The equation for simple harmonic motion (i.e., oscillation), assuming that the origin of the oscillation is at the maximum height on the Y axis, is

$$Y = \cos \omega t \tag{5.0}$$

where omega represents radians per unit of time and (Y) is the varying Y axis component of the oscillation. The curve in figure 4 suggests that the period of the oscillation in $f(A_f)$ with age is 65 years. It might be longer, but with limited knowledge of the shape of the curve prior to birth and after age 75 this cannot be determined with certainty at present.

A period of 65 years means that omega varies from 0° to 360° in 65 years. Using pi to indicate pi radians or 180°, equation (5.0) may now be written

$$Y = \cos 2\pi \frac{t}{65} \tag{5.1}$$

Solutions of equation (5.1) plotted against time yield the cosine wave illustrated in figure 5A.

B) Designate by (R) the amplitude of the harmonic motion on the Y axis. Figures 3 and 4 indicate that the amplitude of $f(A_f)$ decreases with time or is damped. The amount of damping is concealed to some extent in figure 3 by the logarithmic y-axis scale. The degree of damping does not appear to be linear, but appears to decrease at an exponentially decaying rate. Using (R_o) to designate the amplitude at time zero we write

$$f(R) = R_o e^{-kt} \tag{5.2}$$

where (k) is an arbitrary constant and (t) is time in years. Damped harmonic motion is diagrammed in figures 5B and 5C.

By combining equations (5.0, 5.1, 5.2) we obtain

$$Y = f(R) \cos 2\pi \; t/65 \tag{5.3}$$

An increase in frequency could be modelled by including in (t) a multiplier of the form (e^{kt}) or $(e^{\frac{t}{k}})$. Because we are not certain that this frequency change exists we omit this feature from the remainder of the text.

C) The negative Y axis shear shown in the curves in figures 3 and 4 may be modelled by adding an additional, term to the final equation. This will be termed beta, and may be written so:

$$= e^{\alpha - t/k} \tag{5.4}$$

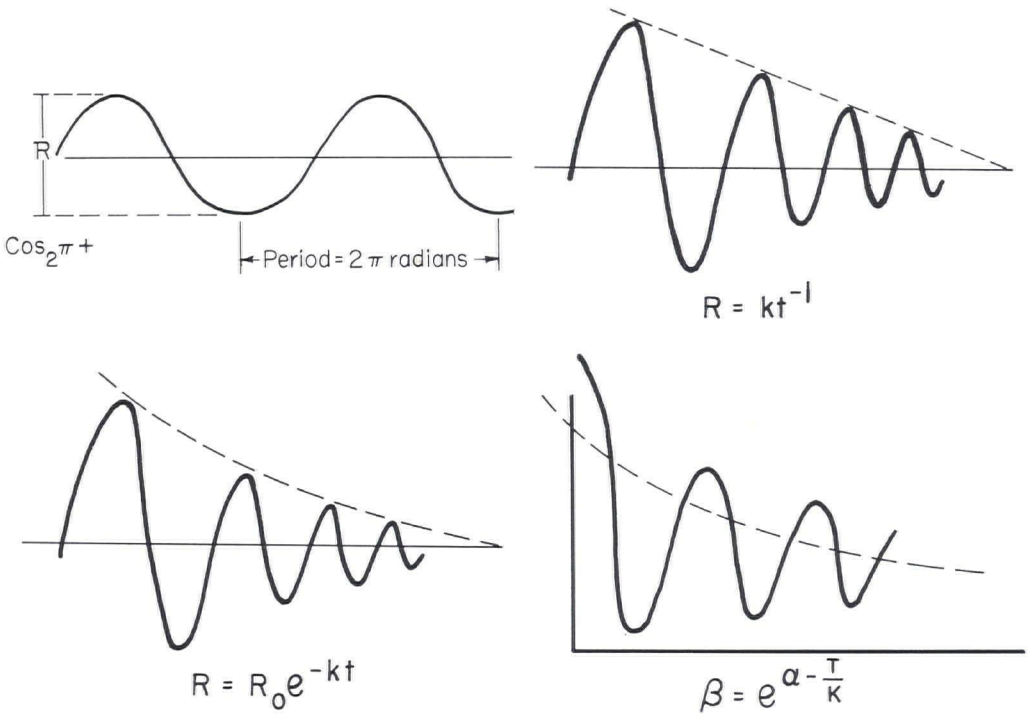

Figure 5a

Upper left. A sine wave (or cosine wave) is illustrated. The interval noted is the *period* of oscillation. The height of the oscillatory motion above the central axis is the *amplitude*. The *frequency* would be that portion of a complete cycle occurring in one time unit, say in one year. The frequency of measured (A_f) is about 0.015 cycles per year. The period is about 65 years.

Figure 5b

Upper right. A sine wave with linear damping of the amplitude with respect to time is shown.

Figure 5c

Lower right. An exponential negative skew of damped harmonic motion is diagrammed. Note that

Figure 5d

Lower right. An exponential, ngative skew of damped harmonic motion is diagrammed. Note that this curve has a strong resemblance to the curve in figure 4.

In figure 5D an exponential skew of damped harmonic motion is shown.

D) By combining equations (5.3, 5.4) we obtain this equation:

$$f(A_f) = R_0\, e^{-kt} \cos 2\pi\, t/65 - e^{\alpha - t/k} \qquad (6.0)$$

or, adopting some of the simplifying symbols already used,

$$f(A\ ) = f(R)\ \ t - \beta \qquad (6.1)$$

where omega stands for ($2\cos \pi/65$). The various (k) constants are separate and would ordinarily be so designated by being termed k_1, k_2, k_3, etc.

E) With appropriate initial condition values and values of the constants, solutions of equations (6.0, 6.1) for actual values of time will yield actual values of (A_f) as observed at that age plotted in Cartesian coordinates. Therefore $f(A_f)$ in equations

(6.0, 6.1) is the first derivative of the function with respect to time. This is equivalent to saying that when equations (6.0, 6.1) are integrated, the result is the cumulative value of $f(A_f)$ over the span of time of the integral. In solving equation (3.1) just such an integration is required. Accordingly a method of achieving this integration will now be outlined which permits numerical solution of the function. We write, from equation (6.0), without proof or derivation (this is being reported separately):

$$f(A_f)_t^{Av} = \frac{1}{t-t_o} \int_o^t R_o e^{-k_1 t} \cos \frac{2\pi}{65} t \, dt - \frac{1}{t-t_o} \int_o^t e^{\alpha - t/k_2} \, dt$$

$$= \frac{1}{t-t_o} \left[e^{-k_1 t} \left(A \cos \frac{2\pi}{65} + B \sin \frac{2\pi}{65} t \right) \right] - \frac{1}{t-t_o} \int_o^t e^{\alpha - t/k_2} \, dt \tag{7.0}$$

where: A = 12.93

B = 31.25

Since the integral of the function $e^{\alpha - t/k_2}$ is improper, it is necessary to make a numerical approximation of the integral of $e^{\alpha - t/k_2}$ utilizing a graph of the function and a planimeter or an algebraic approximationo, utilizing for instance Simpson's rule. When solved for any age span equation (7.0) yields the integral of the cumulative values of the constants and initial conditions are inserted.

F) Another way of obtaining this integral is now outlined which has the potential value that the equation elements may be studied in different form. For this derivation equation (6.1) is used.

$$\int A_f \Big]_{to}^{t} = \int_{to}^{t} f(R) \cos \omega t - \int_{to}^{t} d\beta \, dt \tag{8.0}$$

The first term of the right hand side is in standard form

$$\int U \cos a x \, dx \tag{8.1}$$

so that by making appropriate substitutions the integral for equation (8.0) becomes

$$\int f(A_f) \Big]_{to}^{t} = \frac{\sin \omega to}{\omega} \left(R - \frac{\ddot{f}(R)}{\omega 2} + \frac{\ddot{\ddot{f}}(R)}{\omega 4} \cdots \right) + \frac{\cos \omega t}{\omega} \left(\frac{\dot{f}(R)}{\omega} - \frac{\dddot{f}(R)}{\omega 3} + \cdots \right) \Big]_{to}^{t} \tag{8.2}$$

The $t - t_o$ bracket at the right of the right hand term means that to solve for any span of life which does not begin at birth one should do the following:

$$\int f(A_f) \Big]_{to}^{t} = \int^{t} f(A_f) - \int^{to} f(A_f) \tag{8.3}$$

meaning that the integral from birth to t_o should be subtracted from the integral from birth to t, the end of the period of observation. The higher derivatives in (8.2) are meaningless so that the series of derivatives cause less computational difficulty than they would appear to at first glance.

Beta was omitted from (8.2). The complete equation for $f(A_f)$ should be written as follows:

$$f(A_f)\Big]_{t_1}^{t_2} = \left[\frac{\sin \omega t}{\omega}\left(R - \frac{\ddot{f}(R)}{\omega^2}\right) + \frac{\cos \omega t}{\omega}\left(\frac{\dot{f}(R)}{\omega}\right) - \beta\right]\Bigg|_{t_1}^{t_2} \qquad (9.0)$$

G) In figure 6 a graph is shown of the empirically observed curve of $f(A_f)$ with age. With it is plotted a calculated $f(A_f)$ curve, using equation (6.0). The numerical values obtained are listed in Table I.

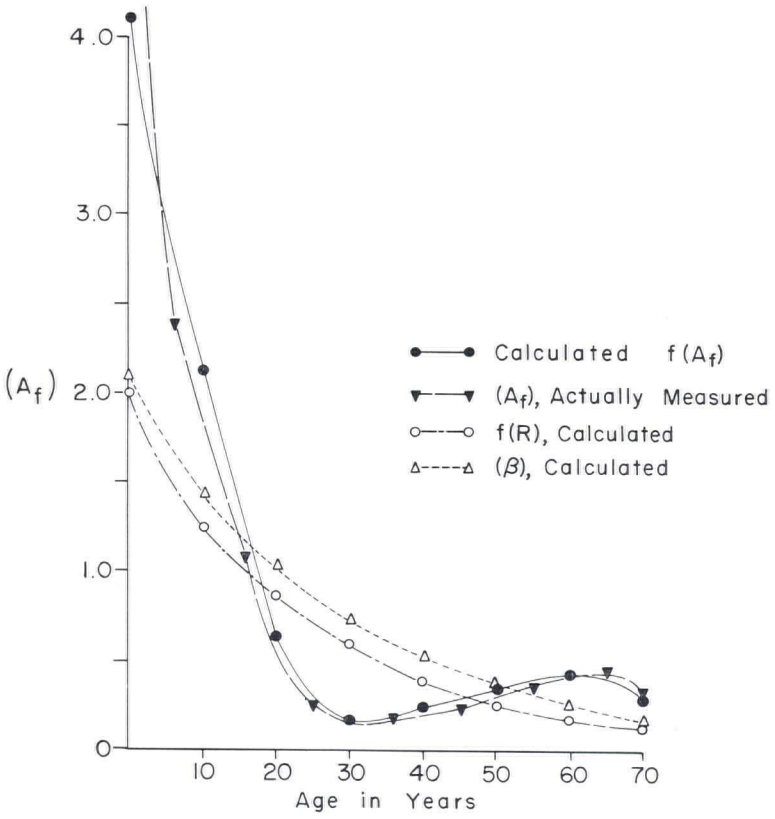

Figure 6

The heavy, continuous line with circular plotting points is the plot of calculated values of (A_f) with age using equation (4.1). The ordinate is linear rather than logarithmic.
The light line with square plotting points is the curve of empirically determined values of (A_f) in human ribs, with the log axis of figure 3 transformed into a linear one. The fit between the calculated and empirical curves is impressive. The fit could be materially improved by incorporating a slight frequency change in the equation for $f(A_f)$.
The curve of short dashes represents the change in value of the beta function with age, while that of long dashes shows the change in f(R) or amplitude of oscillation with age.
The choice of initial values is such that the oscillatory motion never crosses the x-axis and therefore never assumes negative values. This is important bcause the real system, of which this is a model, *cannot* assume negative values for (A_f).

An initial amplitude, (R_o), of 2 scale units was selected, and a value of 0.04 for the associated (k) of (e) was chosen. The damping thus obtained is separately plotted in figure 6.

In fitting beta it was assumed that the neutral axis of the harmonic motion intercepted the Y axis at 2.1 scale units. Numerical values of 0.7 for alpha and of 30.0 for the (k) were chosen for the associated (e). The resulting Y axis shear is separately plotted also in figure 6.

In Table I the numerical values of $f(A_f)$ for various ages obtained with the above constants and initial conditions are listed. The Y axis in figure 6 is linear rather than logarithmic. An excellent fit is obtained and could be bettered provided the reliability of the measurements of (A_f) warranted the extra trouble.

Discussion

While there is ample evidence of cyclic behavior in biology, introducing this concept in bone physiology in the manner outlined is new. The major reason for this introduction is the repeated observation in this laboratory that lamellar bone remodelling exhibits such a cyclic change in rate with age, according to a variety of indices. These variations escaped previous detection because they are rather small in absolute value compared to the sensitivity of methods available for detecting such change, and because no previous systematic study of normal bone along the lines developed in this laboratory were available.

Table I

Age, years	0	10	20	30	40	50	60	70
f(R)	2.0	1.24	0.898	0.602	0.400	0.268	0.182	0.122
$\text{cosw} = \cos 2\pi \frac{t}{65}$	1.0	0.568	-0.454	-0.970	-0.743	-0.104	0.883	0.891
	2.1	1.44	1.04	0.740	0.531	0.381	0.272	0.188
A_f	4.1	2.14	0.634	0.156	0.232	0.353	0.433	0.297

$f(R) = 2e^{-.04t}$

$\beta = e^{.7 - \frac{t}{30}}$

$\text{cosw} = \cos 2\pi \frac{t}{65}$

Table I

The numerical values on which the curves in figure 6 are based are shown. The top row of figures is the age of the patient in years. The succeeding rows are identified using the symbology in the text. The bottom row shows the numerical values for $f(A_f)$ at the ages indicated. The sign changes in the third row are important in making the calculations.

EQUATION FOR THE MESENCHYMAL CELL ACTIVATION

The oscillatory motion of the mesenchymal cell activation function is an example of regulation of the metabolism of an organ by regulating the number of places in it where a metabolic activity occurs. In the case of bone this involves bone resorption and formation with their associated fluxes of inorganic ions and organic molecules between bone and blood. There is reason to believe that this particular function depends upon an operation or command on the nuclear code of mesenchymal cells. If so, an appropriate mathematics for this operation would appear to be a Boolean algebra because codic operations probably involve the transmission of finite amounts of information and are probably of an all or none nature as far as individual cells are concerned. Only the existence of millions of these cells makes a mathematical approach using continuous functions appropriate.

It may be that the best mathematical form to use in describing the mesenchymal cell activation function would be differential equations. Certainly the curves in figures 3 and 4 strongly suggest a two-actuator biological system with negative feedback along a closed loop. This indicates that analysis of the curves by means of servomechanism math might be fruitful, and we plan such a study when the biological part of the present investigation has been completed.

The curves of the change in f(R) and in beta in figure 6 reveal a monotonic decrease with time. This might be the result of an increasing inhibition with time, along the lines suggested by Weiss and Kavanau in their model of growth and differentiation of new tissue.[12] It will be interesting to try to devise a test which will permit one to decide whether these decreases represent an increase in inhibitory activity or a decrease in stimulatory activity.

Finally, it should be noted that the oscillatory motion depicted in figures 3 and 6 is well hidden from the casual observer by a simple means: the total size of an organ or organism, or the total metabolic output of an organ or organ system, is the result of the surplus of anabolic activity over catabolic activity. In other words anabolism and catabolism occur simultaneously (although, in the case of bone, not at the same loci in physical space), and there is usually a pretty good balance between them. The surpluses or deficits which may arise from imbalance are small. The time dependent changes of imbalances as empirically observed do not tend to reflect the underlying oscillatory fluctuations in absolute values of the associated rates. The imbalances characteristically are rather uniform in direction and tend, themselves, to vary monotonically (i.e., in the same direction) with time.

These ideas have found concrete application in bone physiology. (1) It has been reported elsewhere that the remodelling rate of bone varies in characteristic manner during normal life. This manner is illustrated well in figure 3. On the other hand the skeletal balance also varies in a characteristic manner during life, but the curve of this change is totally different from that of remodelling rate changes. Both skeletal balance and remodelling rate are the product of the same two cell activities, and thus the same two metabolic activities: bone resorption and bone formation. Yet it is empirically observed that as far as their regulation is concerned they are independent of each other.

The mathematical model of Weiss and Kavanau (which is a superb combination of observation and thought) deals basically with growth, which in the terms we are using here means with the cumulative balance between anabolism and catabolism as they apply to growth.

The model we are attempting to develop deals with anabolism and catabolism separately, a feature that is the chance outcome of the material we study and the methods by which this is done. It may be that the cyclic variation in bone formation caused by the cyclic variations in the mesenchymal cell activation function will in the future serve as a base for a further development of the Weiss-Kavanau model. Certainly Weiss and Kavanau have modelled the integrated balance between growth and destruction (and death) of tissue; i.e., they have derived an equation whose solution is that of equations (4.2, 4.3) in this text. Certainly they had no reason we are aware or, at the time their model was formulated, to suspect that there was, hidden under their measurements of growth, a cyclic variation of major magnitude in the absolute values of the rates of growth and destruction. The differences in observed dynamic behaviour between growth and internal remodelling raise intriguing and basic questions about the nature of their regulation. The clues to these matters may lie in the area of cytodynamic events and regulation thereof, as opposed to the orthodox concept that regulation acts directly on the target cell and target metabolic activity.

Summary

Lamellar bone remodelling involves the elaboration of osteoclasts and osteoblasts by mesenchymal cells residing near the vessels that permeate the larger bone porosities. Upon command, the mesenchymal cells in a part of bone produce these cells in a stereotyped sequence. The specialized cells then produce resorption spaces and new bone.

Foci of remodelling (i.e., bone resorption or formation) occur in some mean number per unit amount of bone, and have both a mean size and a mean rate of evolution.

A normal curve for the variation in the numbers of foci of bone formation in a unit amount of bone is presented. This curve is termed the mesenchymal cell activation function. A mathematical model of bone remodelling activity is outlined, based on the properties already mentioned. This model contains three parameters in terms of which a great deal of bone physiology may be described. The mesenchymal cell activation function is one of these parameters.

Two equations for the mesenchymal cell activation function are then constructed. They have the property that when solved for any age they yield the observed numerical value of the mesenchymal cell activation function at that age.

It is then pointed out that bone remodelling is a hidden process because the new bone formed tends to be concealed by the resorption of a like amount, leaving

little or no change in the total amount of bone present. This concealment has obscured the existence of the magnitude and the nature of remodelling changes with age and has therefore prevented previous theoretical investigations of the nature of the growth process from including remodelling activity and from including realistic absolute values for anabolic and catabolic actiivty.

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