Henry Ford Hospital Medical Journal

Volume 13 | Number 2 Article 5

6-1965

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Harold M. Frost

Henry Ford Hosp. Med. Bull.
Vol. 13, June, 1965

A SYNCHRONOUS GROUP OF MAMMALLIAN CELLS WHOSE IN VIVO BEHAVIOR CAN BE STUDIED*

H. M. Frost**

Introduction

1.) In a synchronous cell culture, all its individual cells undergo division at nearly the same time; i.e., they are "in step" with each other. These cultures have been particularly productive of information about those aspects of cell physiology that are related most directly to DNA (deoxyribo nucleic acid) and RNA (ribonucleic acid). In microorganisms it has been shown with this technique that switch-like and step-like mechanisms, and ordered sequences of events, characterize the control of some kinds of cell metabolic behavior (see Pardee and Wilson 22; Shailberger et al, 25; Sorokin, 27; Halvorson, 14). These kinds of behavior seem to arise largely from the nature of the chemical dynamics and control of (*i*) DNA replication, (*ii*) transcription of encoded information from DNA to messenger RNA, (*iii*) and translation of this information into specific cellular proteins.[13,24,25,27] These kinds of cell behavior will be designated *discrete modes* of behavior in the following text. They differ from that of chemical reactions in solution, and from chemical processes which attain equilibrium, which are the kinds of chemical behavior with which physicians and biologists are most familiar.

While these discrete modes of behavior are known to exist in microorganisms, it has been proposed (for example, by Pardee and Wilson[22]) that in higher animals including man, metabolism of differentiated cells in the adult is mostly or all controlled by altering enzyme activity. While this is a reasonable proposal in view of the evidence that is available, its truth is hard to assess in the absence of *in vivo* studies of synchronous mammalian adult cells. While many kinds of differentiated cells from adult animals will survive in cell or tissue culture, discrete modes of metabolic behavior in such preparations have not been reported to my knowledge, although they have been seen in embryological material, for example by Grobstein[13] and by Fugita et al.[11]

*Work supported by the following grants-in aid: 293, Henry Ford Hospital; AM-04186, N.I.H. A publication of the Orthopaedic Research Laboratory.

**Associate Orthopaedic Surgeon.

2): But there are serious difficulties in trying to draw analogies between the behavior of a mammalian cell in tissue culture, and its behavior *in vivo* in its natural state. These difficulties are in part: (*i*): By definition, cultures of differentiated mammalian cells or tissues do not duplicate the natural state of the cells. Therefore the relevance to the intact organism of observations made in such cultures is suspect until comparisons with cell behavior *in vivo* establish such relevance. (*ii*): Cells in these cultures tend to be asynchronous at the start of their culture, and (*iii*) they tend subsequently to dedifferentiate and resume active, repeated and asynchronous cell division.[1,23] These factors make it unlikely that discrete modes of metabolic behavior of individual cells would be seen in such preparations even if they did exist *in vivo*. In this respect I believe it is significant that the discrete modes of behavior in uncellular organisms tend to occur *between*, rather than during, cell divisions, to such a degree that the time of cell division is usually taken as time zero in such studies.[13,14,25] (*iv*): As noted by Weiss among others, in animals the non chemical aspects of the cell environment (in terms of neighboring cells, physical structures and properties, electrical fields, and in terms of changes in and orientation of these things) are an essential part of the cell milieu to a degree that is seldom approached by unicellular organisms or duplicated in culture.[23,34,35] Consequently even if its natural chemical environment could be *exactly* duplicated, the behavior of an adult animal cell in a culture would probably still not reproduce that which occurs *in vivo*.

3): If a system existed in which the natural behavior of a synchronous group of cells of higher animals or/and man could be studied *or deduced*, and this with *assurance* that the act of study would not affect the cell behavior being studied, this would be of great interest to many scientists. This article calls attention to such a system: the secondary osteon (i.e., Haversian system), as defined by Enlow,[5] in compact bone. And interestingly, there is already a body of evidence which shows that cells in this system in man do have discrete modes of behavior. To see which cells in this system are synchronous, and why and how, the dynamic anatomy of an actively forming osteon must be described; then we must deduce how its cells arise. Following this some pertinent differences between synchronous groups of micro-organisms and of animal cells will be pointed out, and some advantages of bone as a tool in studying mammalian cell synchrony will be described.

The Histodynamics and Cell Dynamics of Osteon Formation

A) Morphological Dynamics

Figure 1 shows the histological dynamics of a newly forming osteon, as seen in longitudinal section in human compact bone. Five subprocesses are involved in its manufacture,[5,10] which move in space as shown by the arrows. If one were to watch these processes from some stationary point, such as the planes of section, (A), in the inserts in the figure, he would see, passing from right to left in correct temporal order:* (A_1) bone resorption done by *nondividing*[15,30,36,37] motile, multinucleated, differentiated cells called osteoclasts, which in effect "drill" a hole called a resorption

*The subscripts refer to the order in time of successive moments of observation.

space through the cortex; (A_2) followed by a group of spindle shaped, undifferentiated cells among which mitotic figures can be found; (A_3) followed by a capillary blood vessel; (A_4) followed by the appearance of *nondividing,*[23,21,36,37] nonmotile, uninucleate, differentiated cells called osteoblasts which condense in a ring-shaped monolayer on the wall of the resorption space (whereupon the space is renamed the *Haversian canal);* (A_5) followed by filling the canal up, centripetally, with new bone made by the osteoblasts. After the canal is filled up the osteoblasts disappear, some having been trapped inside the new bone to become osteocytes but most having died and autolysed*.

These five processes move through bone as a coherent group, with approximately constant longitudinal and temporal spacing. Their end product is a 200 micron diameter tube whose 70 micron thick walls are made of lamellar bone.[5,9,16] According to L. C. Johnson, in man the resorption front moves longitudinally through the bone *about* 100 microns in 20 days,[16] while I have calculated that the centripetal closure of the resulting hole takes *about* 80 days.[8,9] The distance between the spindle cells and the ring-like zone where the osteoblasts appear is about 350 microns, and between the resorption front and the ring of osteoblasts about 400 microns. Thus from the time that resorptive activity first appears at some transverse level such as (A_1), to the time of the formation of the first new bone at the same level, now (A_4), about 80 days elapse. While there is some uncertainty about these numbers (which must be removed in the future), this does not alter the validity of the *relationships* which are described.

B) Cell Dynamics

To be considered synchronous, the cells in this system (henceforth restricted to the spindle cells, osteoclasts and osteoblasts) must be shown, (a) to have an ancestral origin* that can be determined in both time and space, (b) to be an ancestrally homogenous group, unmixed with cells of different origin and age, and (c) to be identifiable as a group by some practical *selection criterion* which will allow the group, *or its behavior,* to be distinguished and isolated for study purposes from neighboring extraneous cells.

a): *Concerning origin,* there are three possibilities (*i*) the cells are brought to the region by the blood, having been made by progenitor cell division at indeterminate times and places elsewhere in the body; (*ii*) or they are temporarily specialized versions of local cells that were present before the osteon started to form, but whose ages are indeterminate with respect to the cell divisions of which they are daughters; (*iii*) or they derive from progenitor cell division which occurs in the locale and at determinate times before these cells appear and begin to function. The evidence bearing on these hypotheses is, *one:* workers with tritiated thymidine have uniformly

*About 3300 osteoblasts start making the bone in a one mm. length of a new osteon; 700 of them subsequently become osteocytes, and 2600 just "disappear".

*The origin of a cell here means the location in time and space of the progenitor cell division of which it was a daughter, using progenitor cell division in the general sense of the mitotic division which made the cell, regardless of the nature or location of the parent cell.

found in mice, rats and rabbits (excepting osteon formation, which has not yet been adequately studied in any animal with this technique) that osteoclasts and osteoblasts are made by progenitor cell division* which occurs in the locale and at determinate times before, these cells appear and begin to function.[20,21,30,31,36,37] Man and dog are probably similar. *Two:* but, since no studies with tritiated thymidine exist in man or dog that bear on this problem, 7th ribs from 100 metabolically normal adult people and 17 two year old Beagle dogs* were longitudinally sectioned, suitably stained*,[6,7,32] and the actively forming osteons in them searched for mitotic figures with the light microscope. These figures should occur if the progenitor cell-osteoclast-osteoblast relationship in man and dog is similar to that in the rodents already mentioned. Of the mitotic figures that were found, 94% were confined to the region shown by the plus signs in figure 1. Thus, at least some of the cells in this system in man and dog are made by local progenitor cell division. *Three:* the number of cell nuclei in an actively forming osteon is larger (by a factor exceeding 20) than the number present in the same volume of bone before active formation of the new osteon began. This means that at the minimum more than 95% of the cells in the actively forming osteon are new, and cannot be temporary specializations of cells that were previously present in the area. It follows that hypotheses (*i*) and (*ii*) are untenable, and in both man and dog, as in rodents, osteoclasts and osteoblasts

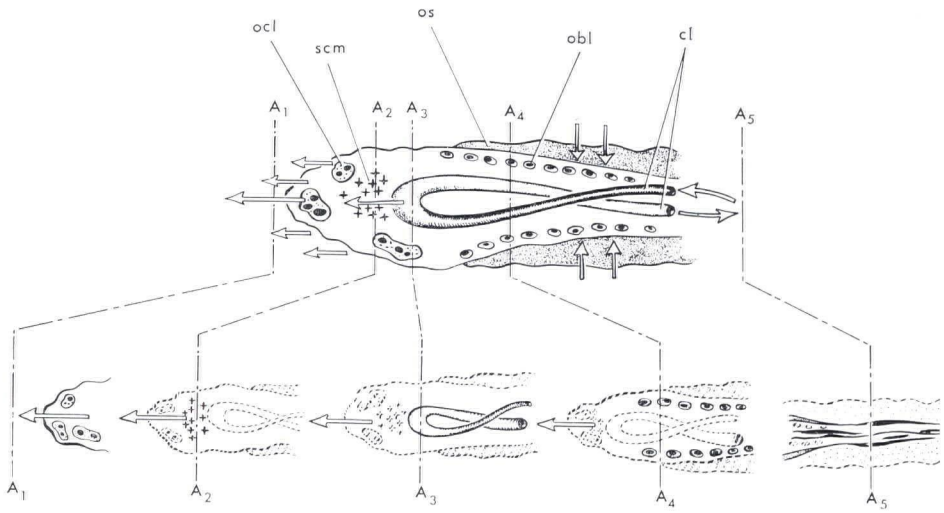

Figure 1

Upper part: Diagram of longitudinal section through an actively developing new osteon. The processes move from right to left as a coherent group with approximately constant longitudinal and temporal spacing. The planes of section (A) represent successive moments of observation at some fixed joint, and are ordered in time according to the subscripts. The distinct processes are: A_1: the resorption front; A_2: the spindle cells with mitoses (plusses); A_3: the capillary loop; A_4: appearance of a ring-shaped layer of osteoblasts on the wall of the hole; A_5: closure of the hole with new bone.

ocl: osteoclast. scm: spindle cell mass. os: new bone osteoid or matrix.
obl: osteoblast. cl: capillary.

*The dogs were supplied courtesy of Dr. R. Johnston, and of the Upjohn Co., Kalamazoo, Mich.

are made by progenitor cell division in the region were, and at a determinate time before, they appear and begin to function.

b): Concerning ancestral homogeneity, the following two-part argument is made:

1.) *Osteoclasts* are nondividing cells which have been found without exception in tritiated thymidine studies (but in animals other than man) to originate from progenitor cell divisions in the locale where they appear and begin to function.[20,21,30,31,36,37] The plus marks in figure 1 show where the vast majority of the mitotic figures were found in the bones examined in this laboratory. They are very close to the osteoclasts, so this is almost certainly where the progenitor cell divisions occur which make osteoclast nuclei.

2): Consider the *osteoblasts* lining the Haversion canal in any thin section cut transverse to the longitudinal axis of the canal. There are two possibilities: (*i*) they are continuously replenished during the time it takes to make the osteon, and so must be heterogeneous; (*ii*) or the first ones to appear on the wall of the canal (as a ring shaped monolayer at the plane of section A_4 in figure 1) are solely responsible for the subsequent closure of the canal with new bone at that level, and so are homogeneous. The relevant facts are: *one:* if (*i*) were true, and since osteoblasts are nonmotile and with rarest exceptions nondividing, the mitoses accompanying a postulated continuous replenishment should occur in a layer near and parallel to the layer of osteoblasts. *Two:* since osteoblasts outnumber osteoclast nuclei at any representative moment by more than five to one in the average actively forming osteon, a commensurately larger number of mitoses should be found associated with osteoblast than with osteoclast production. *Three:* But the plus signs in figure 1 show where 94 percent of the mitoses were found in 600 longitudinal sections of ribs from 100 adult humans, and in 83 sections of ribs from 17 adult Beagle dogs. This means that the continuous supply hypothesis is unreasonable, and that osteoblasts are probably also daughters of cell divisions that occur in the spindle cell region. This means too that in any plane transverse to the longitudinal axis of an actively forming osteon, the monolayer of osteoblasts therein all appeared at about the same time at all points around the circumference of the Haversian canal. Thus osteoblasts are definitely synchronous in the limited sense that those in a plane transverse to the osteon appeared as functionally competent osteoblasts at about the same time.

d): The Relation between Osteoclast and Osteoblast: The facts adduced so far indicate that both osteoclasts and osteoblasts derive from mitotic cell divisions in the same progenitor cell mass, which lies in the region (A_2) shown by the plusses in figure I. This mass is determinate in space, and its mitotic activity is determinate in time (see also below). It remains to solve the relationship between osteoclasts and osteoblasts. There are three possibilities: (*i*) the progenitor cell mass makes both but separately: (*ii*) or the osteoblast is made first and then changes into an osteoclast; (*iii*) or the osteoclast is made first and then changes into an osteoblast. The facts that seem relevant to me are: *One:* resorptive activity precedes formative

as shown by Takahashi et al,[29] whenever space-and-time-contiguous pairing of the two processes occurs. *Two:* time-space-continuous pairing of resorption and formation processes is the rule in *adult* man and dog at all periosteal, endosteal and Haversian canal surfaces of bone.[29] These facts imply that osteoclasts transform to osteoblasts, i.e., (*iii*). *Three:* the reverse order, i.e., formation before resorption, does not seem to occur,[29] while the older osteoblasts, which would seem more likely to transform to some other state than young ones, are the farthest removed physically from the resorption front,[5] meaning that osteoblasts do not transform to osteoclasts. *Four:* the effect of cortisone on this system is (in the steady state[9]) to decrease the number of osteoclast nuclei[29] but to nearly arrest the appearance of osteoblasts.[8] *Five:* the spindle cells lie *much* closer in space to the osteoclasts than to the osteoblasts, so that were osteoblasts made directly by progenitor cell division, they would have to travel an unreasonable distance through a dense mass of tissue to materialize on the wall of the Haversian canal, where they begin to make new bone. *Six:* carbon particles injected into rabbit limb arteries subsequently appear in osteoclasts but not in the osteoblasts. These facts mean that hypothesis (*i*) is unreasonable, while (*ii*) seems excluded, to me at least, leaving unchallenged the admittedly unorthodox concept that the osteoclast nucleus is made first and later transforms, without any cell division, into an osteoblast. This point requires direct confirmation by other techniques. See figure 2.

C) Summation

The evidence can reasonably be taken to mean that (*i*) the progenitor cell divisions which make osteoclasts and osteoblasts occur in the spindle cells that lie between the osteoclasts and the capillary loop, and so are determinate in both time and space, (*ii*) and that in any transverse plane through an osteon the osteoclasts and/or osteoblasts there are synchronous with respect to their age after the time of both (*i*) their *appearance* as specialized cells and (*ii*) the *cell divisions* which made them. The selection criterion mentioned earlier emerges as the group of cells in any thin cross section through an osteon. This criterion applies to osteons at any stage of formation, and of any age after completion, noting that for osteocytes (i.e., inactive bone cells) there is an additional qualification. This is that the osteocytes lying in a common lamella in a thin cross section cut through an osteon are synchronous (within $\pm$ 10 days). With respect to determinability in time, note that at different transverse planes cut through the same osteon, the ages of the cells in such planes are related to each other as is their separation in microns along the longitudinal axis divided by the number of microns per day at which the resorption front moves through the bone. Using the numerical values quoted earlier, calculation gives the rate of this motion as 5 microns per day, a number which must be defined more accurately than it has been. This refinement should not alter the validity of the relationships just described. These relationships mean that the 400 microns between the resorption front (A_1), and the ring of osteoblasts (A_4), covers *about* 80 days, and cells in each successive cut 400 microns farther away from the resorption front are 80 days older than those in its immediate predecessor and 80 days younger than in its successor.

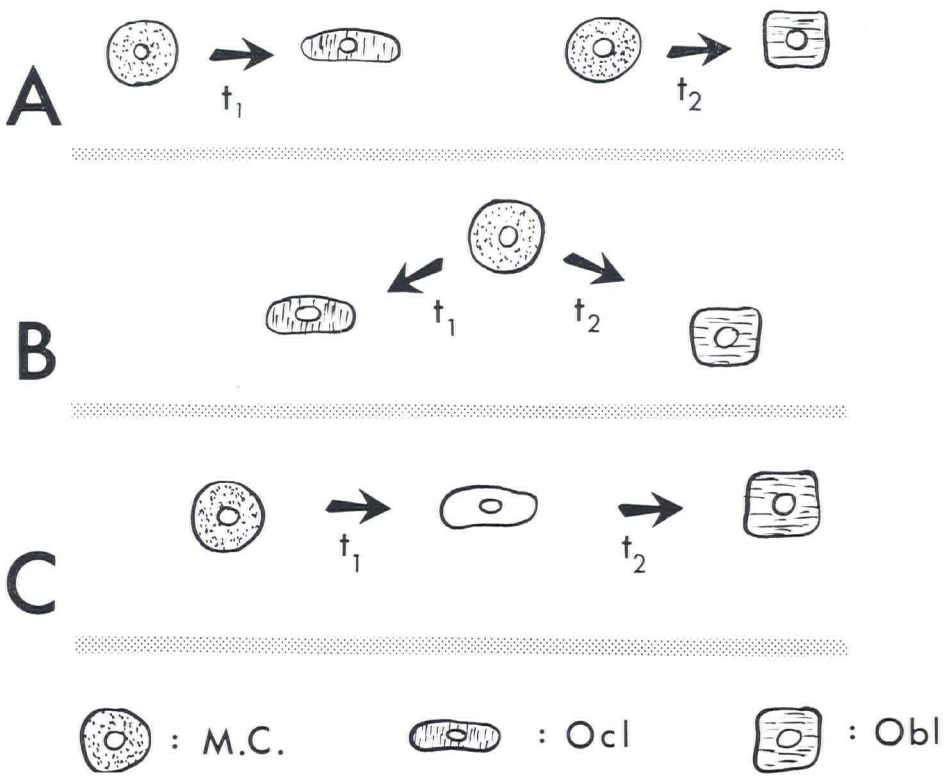

Figure 2

The possible, simplest-case relationships between progenitor (i.e., mesenchymal) cell, osteoclast and osteoblast are shown diagrammatically. A: osteoclasts and osteoblasts are generated independently. B: The same progenitor cell generates the osteoclast first, the osteoblast second, by separate divisions. C: The progenitor cell makes an osteoclast, which later transforms to an osteoblast. MC: The mesenchymal (i.e., progenitor) cell. ocl: osteoclast. obl: osteoblast. While the facts presently available are compatible with C but not with A or B, these facts are not sufficient unto themselves to *prove* C.

Discussion

I: SOME DIFFERENCES in the MEANING of SYNCHRONY in MICRO-ORGANISMS and in HIGHER ANIMALS.

Several comparisons must be made of the cell system just described with synchronous cultures of unicellular organisms, because there are some unrecognized but *very* important and pertinent differences in cell behavior at these two widely different levels of biological organization. These differences will affect the manner of study and interpretation of the behavior of synchronous *animal* cells.

A) *Control of Cell Division:* In cultures of microorganisms cell division is made to occur simultaneously in all cells by some artificial device. But in osteon production the cell divisions that make osteoclasts and osteoblasts occur serially and uninterruptedly so that in the natural state the kind of synchrony produced in cultures of unicellular

organisms does not and cannot exist. But the valuable property of osteons is that daughter cells of the same age are physically located in the same plane transverse to the longitudinal axis of the osteon. Using this knowledge the cells and their behavior can be studied.

B) Time Scale: The approximate order of time between successive cell divisions in unicellular organisms is an hour, and in this "between time" synchronous metabolic activity of the cells in the culture is studied, and discrete modes of cell behavior are seen best. But the osteoclast-osteoblast nucleus lives (in its natural surroundings!) *about* 160 days (80 days as an osteoclast, and 80 as an osteoblast[9,10]).* Being characterized by nondivision, this period is comparable to the period between divisions in microorganisms. Thus the difference between microorganisms and animal bone cells, in the length of time during which the cells are doing metabolic "work" but not dividing, is on the order of one to 3800 respectively.

C) The Relation of Cell Division to Metabolic Work: In its natural environment, a unicellular organism continues to divide, metabolize and divide repeatedly and indefinitely, while in its natural state the osteoclast-osteoblast is a differentiated, *nondividing* cell whose natural fate is, irreversibly, either death (without further division) or incorporation into new bone as an osteocyte (again without further division). In other words, in microorganisms the two functions of doing metabolic work and of making new cells are combined in the same cell as a repeating cycle of events. *But in adult animals* (i.e., their secondary osteons, and by inference in other animal tissues too) *cells are of two functionally different kinds: (i)* those which are undifferentiated, and make new cells but do not provide the metabolic functions that are necessary to maintain the life of the whole organism, (*ii*) and those which are differentiated, each individual among them providing one "package" of the metabolic work needed by the whole organism, and then dying or otherwise disappearing.† Were these "lost" differentiated cells not regularly replaced with new ones, their metabolic functions would disappear and the whole animal would then become ill or die (examples: aplastic anemia; Addison's disease; pemphigus).

D) This division of responsibility may have evolved in multicellular organisms because of the cellular specializations (i.e., differentiations) that characterize them. Thus, in man, a liver cell can perform the metabolic "work" needed for its own survival whether it be in the body of the host or in a cell culture. But it is expected to do more than this when in the host's body; it must perform *other* metabolic tasks for the other tissues of the body, tasks of which these tissues have (seemingly) been relieved so that they can better make *their* specialized contributions to the health of the whole animal or plant. And it is just this kind of metabolic work that higher plants and animals seem to assign to *nondividing* cells. This division of responsibility is not razor sharp: it is blurred but nonetheless real.

*I refer only to the cells involved in *lamellar* bone remodeling.
†This "package" may last 2 weeks (bowel epithelium), 3 months (red cells of the blood) or the lifetime of man (neurons).

E) The Meaning of Relative Synchrony: In a culture of bacteria, synchrony usually means that most of the cells are within a few minutes of the mean phase of the entire culture. Clearly this order of synchrony does not exist in individual mammalian osteons. However, the ratio of the period between successive divisions in microorganisms, divided by the period between the creation (by progenitor cell division) and death of an osteoclast-osteoblast, is one measure of how much less synchronous the animal cell needs to be in absolute time to provide comparable relative synchrony. It is already said that this ratio is about 1/3800. This statement assumes that any discrete transitions in metabolic behavior in a cell in an osteon are of the same order of number as those in microorganisms, and clearly this statement needs to be evaluated by experiment.

II: EXISTING EVIDENCE of DISCRETE BEHAVIOR in OSTEONS

Actively forming osteons have some interesting properties which in abstraction resemble the discrete behavior of unicellular organisms referred to before, and which suggest that they derive from synchronous cells, but which do not fit the idea that the metabolic work contributed to the whole man by a differentiated, adult cell is all continuous over time (i.e., is not switched on or off), is all regulated by nearly infinitely adjustable mechanisms (such as changing enzyme activity or substrate concentration), and never involves irreversible sequences of different kinds of metabolic behavior.

Six discrete properties are: (*i*) the formation of a new osteon begins and ends absolutely, at definite points in time, so the cells making it take a definite amount of time to make one and the relevant chemical reactions are started and stopped by mechanisms which are functionally discrete.[9] (*ii*) Once made, the amount of bone in an osteon does not subsequently change through the activity of the same cells, even after many years, so that in effect bone resorption and formation by these cells is irreversibly "switched off" at the completion of each osteon;[9,10] (iii) the amount of bone in an osteon is a measure of the amount of metabolic "work" contributed by its cells to the man, and as Currey, Landeros and Takahashi and I found, tends to be constant on the average throughout life,[2,3,19,28] although the insolubility of bone in the body fluids means that chemical feedback of the product on the reaction mechanisms making it cannot cause this; (*iv*) either a complete group of cells and processes is engaged in making a new osteon, or none are; there is no gradual transition between these states, so that, functionally and dynamically, making new osteons is a binary valued as well as a discrete activity;[9] (*v*) As Takahashi et al found, resorption almost always precedes formation in this system,[29] and since the same cell nucleus provides both metabolic functions this is an irreversible sequence of forms of metabolic behavior in one cell; (*vi*) the shape of the kinetic curve of osteon formation is age invariant[18] although the rate at which the osteon is made is not;[8,19] which implies that the mechanisms determining the manufacture of new bone by an osteoblast produce a functional dependence of the amount of bone made on the dimensionless *period* of osteon formation, but an independence of this amount on absolute time.[19]

III: PRACTICAL ADVANTAGES of OSTEONS as CASES of SYNCHRONOUS CELL BEHAVIOR.

Osteons have practical and potent advantages which make them attractive as model systems of synchronous cell behavior in mammals. Among them are: *one:* the observing act need not disturb the cells being studied. This is because their behavior can be studied *after* it has occurred, a property which is based on the following facts. (a): In adult man, bone turns over slowly (once every 20-40 years);[8] (b): the microscopic architecture of mineralized bone is temporally stable for hundreds of years after the death of the individual; (c) the cells in bone are responsible for its microscopic architecture and for changes in it which are associated with disease, aging, growth and nutrition. (d): These changes are a form of intelligence concerning the behavior of the cells. *Two:* these facts mean that bone keeps a record of the behavior of its cells, whose intelligence may read *after* being transcribed therein.[8,9] *Three:* in the living animal this intelligence is transcribed by cells that function *in vivo*, under completely natural conditions. *Four:* simple methods have been developed for preparing mineralized sections of bone, measuring them, and interpreting the information obtained therefrom.[6,7,8,9,10] These methods make it possible to study the dynamics of several kinds of bone cells, both as individuals and as cell populations, in some depth and with considerable accurarcy. *Five:* This novel approach to the study of cell behavior has the asset that the composition and content of the cells are irrelevant to the objective, which is to define *what they did,* with respect to things such as quality, amount, rate and pattern of metabolic activity.[4,16,17,19,28,29,33]

These methods should work nearly as well in bones of dead or/and extinct animals as they do in bones of live ones, provided only that the subjects from which they came formed good secondary osteons, as these structures are defined by Enlow.[5]

SUMMARY

In a thin transverse section, an actively forming osteon in mammalian compact bone contains a group of cells which seem to be synchronous with respect to (*i*) time of appearance as differentiated cells, (*ii*) and age after the progenitor cell divisions which made it. This system offers an opportunity to study the behavior of both differentiated and undifferentiated, mammalian (including human) cells as this behavior occurs *in situ, in vivo,* under completely natural conditions, immune to disturbance by the observing act. Several features of this system imply the presence of control mechanisms which are functionally and temporally ordered, discontinuous and discrete. Dynamically and in abstraction this behavior resembles some of the DNA- and RNA- dependent discrete controls which occur in microorganisms, and suggests that (*i*) analogous phenomena occur in at least some other mammalian cells, and that (ii) these phenomena may serve as tools for studying the *in vivo* operation of DNA- and RNA- dependent controls in mammalian including human cells.

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