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A. R. Villanueva

M. Klein

Harold M. Frost

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EFFECT OF ADRENALCORTICOID HORMONES ON HUMAN LAMELLAR BONE: QUANTITATIVE HISTOLOGICAL MEASUREMENTS OF RESORPTION AND FORMATION INDICES*

A. R. Villanueva, B.S., M. Klein, M.D., and H. M. Frost, M.D.

Introduction

In recent years considerable interest has arisen concerning the mechanism of the effects of the hormones of the adrenal cortex on bone. The motivation behind this interest has been partly the need to understand the genesis of some of the undesirable effects of these hormones (such as osteoporosis, spontaneous rib and metatarsal fractures, aseptic necrosis of the humeral and femoral heads), and partly the hope that understanding the mechanism of action of these agents will provide knowledge which would significantly improve ability to treat metabolic bone disease.

Lamellar bone may serve as a biological record in which clues to past physiological dynamics are recorded. These clues are recorded in a special symbology which can be partly read by several means including quantitative histology. Some of the clues in the record provide information about the absolute rates of bone formation and resorption. When a standard of normal is available for these clues, then bone from diseased skeletons may be compared to the norm and the nature of the absolute rate changes often observed directly or deduced.[11]

This is a report of comparative measurements in which some normal indices of bone formation and resorption are compared to the same indices in 18 patients who had received pharmacodynamic doses of an adrenalcortical hormone or synthetic homolog for a known period of time. We believe we have shown clearly the effect of these drugs on formation and resorption. The measurements are quantative-histological.

Outline of Bone Remodelling

There are two basically different bone physiologies: *lamellar bone* and *fibrous bone*. These differences were known and appreciated by Baker and Cretin and have

From the Orthopaedic Research Laboratory.
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been re-emphasized recently.[1,4,11,12] Each has a distinctive end product. The bulk of the human skeleton is composed of lamellar bone and it is lamellar bone whose physiology is disturbed in metabolic bone diseases including Cushing's syndrome. Henceforth this text limits its remarks to lamellar bone and lamellar bone physiology. Little that will be said can be applied without modification to fibrous bone physiology.[11]

Bone remodelling is the sum of two physiological activities called resorption and formation. The former is caused by specific cells termed osteoclasts, the latter by osteoblasts. Resorption of bone involves the removal of mineralized bone matrix. Formation involves the production of new bone matrix and of a new supply of bone cells called osteocytes. Since resorption and formation and their sum, remodelling, are cell mediated, they are cell regulated and understanding their physiologies reduces mostly to understanding the physiologies of osteoclasts and osteoblasts.[16,23]

Neither osteoclasts nor osteoblasts normally undergo cell division, knowledge we owe to work with tritiated thymidine by authors such as Kember and Young.[18,24,25] The total number of these cells depends on the number produced by a primordial or progenitor cell population. This is called here the *mesenchymal cell population* and members of it are usually found near the blood vessels that permeate the porosities normally present in bone.

There are two types of bone remodelling. In *internal remodelling* the remodelling activity occurs in the interior of the cortex and of large trabeculae. In *surface remodelling* the activities occur only on the periosteal and endosteal surfaces. This text is addressed primarly to internal remodelling. The changes that will be described are nearly the same for both types insofar as bone dynamics in Cushing's syndrome are concerned.*

Internal remodelling involves a stereotyped sequence of events which probably has a large part of its origin in the operation of the information processing devices in the nucleus of the mesenchymal cell.[11] Whatever its origin, the first observed event is the appearance of waves of osteoclasts which produce a resorption space. Completion of the space in human adults takes two to four months. Then the second observed event occurs: waves of osteoblasts appear and deposit new bone matrix. When the matrix mineralizes, which it begins to do about 14 days after its formation, it becomes bone. When formation of new bone matrix is finished, histological quiescence returns again to the region.[8,11]

A site where remodelling activity occurs is termed a *focus of remodelling* and will be either a resorption or formation focus. One way of controlling the amount of bone being remodelled is to control the number of places or foci where this activity occurs. It is important to understand that all (or almost all) individual in-

*In principle, cortical bone is under attack by remodelling processes from only one side, while a trabeculum is under attack from four sides. Thus trabeculae reflect changes in bone balance four times faster than cortex. Rigorously, the relationship is the ratio of endosteal surface area per unit amount of bone.

ternal remodelling foci (and probably surface foci, too) evolve through the same sequence of events, so that resorption foci will later exhibit formation, while formation foci, at some time prior to the moment of observation, exhibited resorption.[11] While this sequence of events is established in normals, it remains to be shown that it also prevails in bone in Cushing's syndrome.

In any given bone, there is some mean number of resorption and formation foci per unit amount of bone. Each type of focus will exhibit a mean surface area analogous to the inner surface of an open-ended soup can, and a mean rate of destruction of this surface in the case of resorption, or addition of new material to the surface in the case of formation. These remarks may be expressed symbolically in the following simple equations:

$$V_f = k\ A_f\ S_f\ M_f \qquad (I)$$

$$V_r = k\ A_r\ S_r\ M_r \qquad (II)$$

Equation I says that the amount of new bone formed (V_f), is equal to the product of the amount of bone originally present (k), times the number of places where new bone formation occurs per unit amount of bone (A_f), times the mean surface area of each formation focus (S_f), times the mean rate at which new osteoid is added to this surface (M_f).

Equation II says much the same about resorption: the amount of bone resorbed is the product of the original amount, times the number of resorption foci per unit amount, times the mean surface area of these foci, times the mean rate of destruction of this surface.

In these equations customarily (V) is in mm^3, (k) likewise, (A) is numbers per mm^3, (S) is mm^2 per focus, and (M) is mm per year. If the time period is arbitarily limited to one year, then equations I and II are true rates and their solutions are the amount of bone formed or resorbed per year.[11] The volume of bone is in terms of absolute bone volume, meaning that all the spaces have been subtracted from the whole. The spaces are lacunae, canaliculae, Haversian and Volkmann's cannals and marrow spaces.[11]

These equations and the discussion surrounding them may be clarified by use of an analogy.

Given: a large hole in the ground that is to be filled with earth by dump trucks. Then the speed with which the hole will be filled is a function of three things: the number of trucks hired (analogous to A in the equations above); the average size of the trucks (analogous to S); and how fast the trucks are driven between source of fill and the hole (analogous to M).

Materials

There are two groups of materials in this study: the normal, and the adrenalcorticoid treated.

(1) The normal group comprises the fifth-seventh ribs of 33 persons who did not have any known chronic illness, metabolic bone disease, diabetes, metastatic malignancy, congestive heart failure, cirrhosis or treatment with any hormone including the adrenal corticoids. There were no patients who received x-ray therapy or cytotoxins. Three-quarters of the bones in this group were obtained at thoracotomy for indications such as hiatus hernia, cardiospasm, biopsy of the lung, repair of patent ductus, resection of aortic coarctation or repair of acute thoracic trauma. The remaining quarter of the ribs were obtained at autopsy for causes such as homicide, suicide, car accidents and sudden, fatal vascular incidents.

(2) The corticoid group comprises the same numbered ribs from 18 patients who came to autopsy. These patients were ill to varying degree for various reasons which are listed in Table I. All had received pharmacodynamic doses of one of the adrenalcorticoid hormones or one of the synthetic homologs for a period exceeding two months before death. Except for the common denominators of severe illness and corticoid treatment, there is little homogeneity of the various variables among the patients in this group*

Methods

Fresh, undecalcified, undehydrated cross sections cut accurately perpendicular to the longitudinal axis of the ribs were made and stained with basic fuchsin by special methods.[9,10] An average of over three sections per case was available for study in both groups of patients.

The cortical area of a bone as seen on cross section is defined as the area enveloped by the periosteum minus the area of the marrow space. These latter two areas were measured, using a modification of Chalkley's method, to an accuracy of 5 per cent.[2] The cortical area was obtained by subtracting the marrow area from the total and checked by direct measurement of the cortical area using the same method.

The total number of active osteoid seams per case was counted,[8] and the mean number of osteoid seams per mm^2 of cross section calculated by dividing the number of seams by the cortical area. Because of the geometry of rib cross sections, and of the lamellar bone and Haversian systems in these sections, and because of the design of the measuring method, the number of seams per mm^2 is equivalent to the number of seams per mm^3 as seen in sections exactly one millimeter thick.

The total number of resorption foci was similarly counted, and the mean number per mm^2 similarly calculated.[15]

The mean surface area of resorption foci was then measured, using a modification of Chalkley's and Cornfield's method, and expressed as the mm^2 of resorption surface (Howship's lacunae) per focus of resorption in cross sections exactly one millimeter thick.[3]

The standard bone approach[11] is the basis for the selection of samples in this study. Ribs were selected because they are the only normal human bone available in quantity, so a reliable study of the normal values of various indices of physiological activities could be done first and most completely in this bone.

Observations

The data for individual cases is listed in Table I along with pertinent information about the cases.

In Table II, a statistical summary of the mean values, standard deviations and per cent changes is listed, comparing the corticoid to normals. The adults and children in both corticoid group and normals are listed in separate columns.

In adults, with mean age 57 years, the mean number of foci of new bone formation per cubic millimeter, (A_f), is 0.06 in corticoids and 0.32 in normals, a de-

*The changes that will be described have been seen in about 40 other bones from 22 other patients treated with adrenalcorticoid hormones. The changes are uniform, and the only common denominator in all these people was the hormones. This is why we think enough of measurements of only 18 people to publish them. While the pure drug-induced state would be desireable, it is not easily found in people!

Table I

CLINICAL DATA ON CORTICOID GROUP

Case	Hospital Number	Age Yrs.	Sex	Diagnosis	Month's Treatment
1	910259	5	M	Acute lymphatic leukemia	12
2	539327	11	M	Acute lymphatic leukemia	18
3	984023	15	M	Regional enteritis, peritonitis, thrombocytopenia	2
4	855929	15	F	Rheumatoid arthritis, pulmonary fibrosis	24
5	1025051	15	F	Metastatic sarcoma	4
6	947773	34	M	Metastatic Ca. of lung	4
7	988512	46	M	Metastatic Ca. of lung	2
8	1029086	50	M	Uremia	4
9	965668	54	F	Leiomyosarcoma of pancreas	4
10	808741	56	F	Metastatic Ca. of colon	13
11	947250	57	M	Duodenal ulcer, emphysema, pulmonary fibrosis, post gastrectomy	5
12	982666	58	M	Ca. Lung, rheumatoid arthritis, osteoporosis	48
13	829554	60	F	Metastatic Ca. of rectum, CHF	3
14	063298	60	M	Chronic lymphatic leukemia	5
15	562544	66	F	Subdural hematoma, leukemia, Ca. of tonsil	12
16	622474	68	F	Metastatic Ca. of breast	18
17	762092	68	F	Cirrhosis of liver, diabetes mellitus	4
18	959760	68	F	Rheumatoid arthritis, metastatic Ca.	5

crease in the corticoids of 84 per cent from normal. The number of resorption foci/ mm^2, (A_r), is 0.76 in corticoids and 0.53 in normals, an increase of 43 per cent over normal. In children similar suppression is observed in the number of foci of new bone formation in corticoids, a decrease of 63 per cent from normal. The number of resorption foci in children is essentially the same in both groups. The mean surface area of resorption foci in both groups of patients (corticoid and normal), (S_r), is also essentially the same.

The mean area of cortex per cross section in the adult corticoid ribs is 14.8 mm^2 while in the normal group it is 18.5 mm^2. The adults' total cross section area is 52.7 mm^2 for the corticoids and 52.0 mm^2 for the normals, while in children is is 43.5 mm^2 for the corticoids and 46.0 for the normals. This is a significant decrease in cortical area compared to the normal, without any significant difference in the total area between the two groups, indicating that the source of the difference in cortical areas lies in difference in the size of the marrow areas in the two groups. This has been confirmed by direct measurement.

Table II

A COMPARISON OF NORMAL AND CORTICOID INDIVIDUALS

Histologic Index	Mean Values		Per Cent Change	Mean Values		Per Cent Change
	Normal Adults N=28	Corticoid Adults N=13		Normal Children N=5	Corticoid Children N=5	
Mean Area per Cross Section in mm^2	18.58 ± 4.46	14.80 ± 3.90	−20	22.80	21.20	−7
No. Resorption Foci per mm^2 (A_r)	0.53 ± 0.24	0.76 ± 0.43	+43	0.95	0.85	−10
Specific Surface per Resorption Focus in mm^2/mm^3 (S_r)	0.47 ± 0.13	0.49 ± 0.18	+4	0.64	0.75	+17
No. Formation Foci per mm^3 (A_f)	0.32 ± 0.13	0.06 ± 0.09	−84	1.30	0.48	−63
Specific Surface per Formation Focus in mm^2/mm^3 (S_f)	0.29 ± 0.08	0.17 ± 0.20	−41	0.29	0.43	+48

In this table, the arithmetic mean values of the corticoid group and normal group are compared. The mean age for both groups is 57 years. The symbols have the same meaning as in Equations I and II in the introduction of the text. (A_f) are foci of bone formation per mm^3; (A_r) are foci of bone resorption per mm^2; (S_r) is the mean area of foci of resorption assuming the foci are examined in cross sections one millimeter thick. Total area is the area enveloped by the periosteum on cross sections. Cortical area is total area is total area less the area of the marrow space. The symbol (N) in the table is the number of cases on which the values in that column is based.

Discussion

LAMELLAR BONE FORMATION*

There was a decrease in the number of foci where new bone was being formed (A_f) in the group treated with adrenocorticoids. The decrease is a major one, to a level only 16 per cent of normal when the means of adult normal and corticoid groups are compared. Unless there were an equally major compensatory increase in the mean size of these formation foci or/and in their mean rate of formation (S_f and M_f respectively in equation (I) in the Introduction), the meaning of the decrease in numbers of new bone forming foci per cubic millimeter is that there is a pronounced absolute decrease in the rate of bone formation in the corticoid ribs. This conclusion is in agreement with conclusions reached by Eisenberg and Gordan[5], Rich, Ensinck and Fellows[19], Heany and Whedon[17], Frost and Villanueva[14], Stanisavljevic and co-workers[21] and Sissons[20]. The work referred to embraces radioactive tracer and stable tracer methods as well as quantitative and qualitative histology.

We did not note any significant difference in size of the osteoid seams (S_f) during this study. The decrease in size noted in Table II is based on too few seams to draw valid conclusions. This observation is suggestive because in order to compensate for the tendency of the decrease in numbers of formation foci to decrease the bone formation rate, the mean size of each focus would have to be increased six-fold. Such a difference could not escape notice.

Finally, one of us has reported separately some tetracycline based measurements of the bone formation rate in normal and in corticoid human bones.[11] A definite depression in the bone formation rate in the adrenalcorticoid treated patients was observed, indicating that the mean rate of evolution of individual formation foci has not changed enough to compensate for the decrease in numbers apparently caused by these drugs.

LAMELLAR BONE RESORPTION

(A) Internal Remodelling

The nature of the resorption indices we have used is such that the status of bone formation must be known before qualitative interpretation of the resorption index can be made. The reasons are outlined in the references listed but basically are these: The total number of resorption foci observed is a function of two things: the rate at which they are introduced, and the rate at which they are removed (by being converted to foci of bone formation). The numerical value obtained in measuring this index is a measure of the *balance* between these two processes. It is noted elsewhere that changes in balance do not reveal the nature of the changes

*Obviously, this group of patients had major variables superimposed on their hormone treatment. We are not deluded into thinking we have outlined the definitive bone effects of the corticoids. But we have shown a definitive approach to this problem, which is the reason for this report; others may wish to adopt it.

in absolute rates responsible for them. Other information is needed before such absolute changes can be definitely characterized.[11,15]

In the present case, the major decrease in the amount of new bone formed is the clue to the nature of the change in the absolute resorption rate. This indicates (to us!) that resorption undergoes an absolute decrease in rate in the corticoid ribs, because the observed 50 per cent increase in the numbers of resorption foci (See Table II) is not enough to compensate for the decrease in formation foci (meaning there is a *relative* excess of resorption compared to formation, but an *absolute* decrease compared to normal). Note in Table I that there are seven cases who were on corticoids longer than one year. Had there been an absolute increase in resorption or even a normal resorption rate in this group of patients, their bones should have become extremely porous due to marked increase in number and in size of their resorption foci. Such porosity was not observed. Note that, with the profound depression of formation, a *relative* excess of resorption compared to formation will lead to *accumulation* of the numbers of resorption foci because they are not being converted to bone forming foci at normal rates or after normal intervals of time. The observed 50 per cent increase in the numbers of resorption foci in the corticoid group compared to normal can be accounted for almost wholly by the cumulative effect just mentioned.

As supporting evidence that this effect is cumulative and not the result of an absolute increase in resorption, Sisson's observations, and his summary of the observations of others, to the effect that osteoclasts are unusually hard to find in bone from Cushingoid patients, may be referred to.[20]

As to the average size of the resorption foci, these are about normal (See Table II). This is supporting evidence for the deductions already made.

We freely admit the possibility of different interpretations of this data.

(B) Endosteal Remodelling

Our index of changes in the balance between resorption and formation at the endosteal surfaces of the ribs is the cortical area. The area enveloped by the periosteum in the corticoid ribs is normal except in the younger children. Yet the cortical area is diminished, and this is the result of enlargement of the marrow canal at the expense of cortical thickness. Such enlargement can occur only by an excess of endosteal resorption *relative to* endosteal formation. Note that the excess need not be the result of an absolute increase.

Refer again to the seven patients in Tables I and II who received corticoids for a year or more. Had there been an absolute increase in resorption there should have been little bone left in these ribs, yet the amount observed is not significantly different from the mean for all of the cases. In the face of profound decrease in bone formation, the other points mentioned indicate to us that there has been a significant decrease in the absolute value of the resorption rate in these bones.

We suspect that the large negative calcium balances observed both in animals and in man soon after institution of pharmacocynamic daily dose levels of an adrenalcorticosteroid represent a *transient* response of the physiological system, and misrepresents the steady state, which requires some months to become established.

REMAINING PROBLEMS

Eighteen patients do not comprise a large series, so that any conclusions based on such a series are tentative. In addition the patients we studied had major medical and surgical problems in addition to their iatrogenically induced hypercortisonism, so a pure drug effect was not studied. Balancing these facts are two things: (a) The observations quoted are qualitatively similar to our findings in a much larger stock (circa 3X) of Cushingoid bones other than ribs, in a much larger "library" of corticoid treated patients. We have retrospective reasons for excluding these cases from the present study. Their inclusion would have *accentuated* the findings we report here rather than minimize them. (b) To our knowledge this is the first quantitative histological study of reliable indices of the amounts of bone formed and resorbed in Cushingoid patients. There have been quantitative cellular studies in the past, but previous attempts to do quantitative studies of the amounts of bone matrix formed and resorbed have, in all instances known to us, been afflicted with methodological problems which made their interpretation difficult. We have noted elsewhere that much of the rationale of our own methods has been possible because of the ideas contributed by these authors, without which there would not yet be a true bone quantitative histology.[11]

Internal bone remodelling is probably analogous to the turnover of cells and extracellular protein that seems to occur to some degree in most of the body's soft tissues. An interesting question to pursue would be whether the depression of formation of new tissue observed in bone in corticoid treated patients, and its node of genesis, is also characteristic of some or many other tissues in this drug-induced state, for example, in the hematopoietic system and in epithelium.

Surface remodelling, particularly endosteal remodelling, is important in understanding the mechanism of the osteoporosis of Cushing's syndrome. For this reason, we plan to obtain more complete data on this type of remodelling to match that already obtained for internal remodelling. In this study, we have shown effects of the cumulative imbalance between resorption and formation at the endosteal surfaces, but have not presented any data which reveal the nature of the absolute change in rates here. Accordingly our inference that these rates are depressed remains to be substantiated by direct measurement of either the endosteal resorption or the endosteal formation rate.

CONCLUSION

We infer, as an hypothesis worthy of consideration and testing, that the adrenalcortical hormones exert a major effect on the proliferative activity of tissue progenitor cells, and that this effect occurs as the result of direct action on the nucleus of these

cells. We suspect the hormones alter the operation of the information processing apparatus in the nucleus, and that this is a quite general action of the drugs which exists in addition to and superimposed upon, direct actions on the biochemical machinery involved in metabolic activity.

SUMMARY

Selecting ribs as a standard bone, the numbers of places where bone resorption and bone formation occur in an arbitrary amount of bone have been measured, plus the average size of resorption areas. These measurements were done on 33 ribs from metabolically normal, and 18 ribs from corticoid treated patients. Comparison of the normal with corticoid groups indicates that bone formation is profoundly depressed in the corticoids, and resorption appears to be depressed also but to a lesser degree than formation. The major mode of depression appears to be by decreasing the total numbers of places where resorptive and formative activity occurs. This suggests that the corticoid effect is largely due to an action on the nuclear mitotic activity of the bone progenitor cells, and only minimally due to actions directly on the biochemical machinery of osteoclasts and osteoblasts.

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