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A QUANTITATIVE HISTOLOGICAL STUDY OF RIB FROM 18 PATIENTS TREATED WITH ADRENAL CORTICAL STEROIDS[1]

By

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INTRODUCTION

Many investigators have followed the lead of *Bauer, Carlsson & Lindquist* (1) in developing and applying *in vivo* tracer methods for the study of bone physiology and disease. These methods have proven powerful and valuable, and within their province yield information not available by other existing methods.

One of the early hopes in using these methods was that they could yield direct information about the kinetics of the cells ultimately responsible for most features of bone physiology and pathophysiology. This hope has not yet materialized. It became clear, at least to some, that interpretation of skeletal tracer based studies in terms of cell kinetics must await better knowledge of these cells obtained with other techniques, and subsequent correlation of this knowledge with tracer studies.

This impasse was broken by two major developments on the cell front.

One was the discovery and use of *in vivo* tetracycline labelling of newly forming bone as a research tool, initiated by the reports of *Milch, Rall & Tobie* in 1957 and 1958 (13, 14). This provided bone histologists with a marker in time and a reliable label of foci of new bone formation. The second was the use of autoradiography and tritium labelled thymidine to study the physiology of new cell generation in a wide range of

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tissues and organisms including bone. This was started by the reports of *Belanger & LeBlond* (2), and has been followed up by many, among them *Quastler* (16), *Kember* (12), *Young* (22, 23) and *Owen* (15).

These developments afforded histologists an unparalleled chance to study dynamic bone cell phenomena. A considerable body of knowledge provided by work based on these methods now exists, and it is time to begin studying disease in the light of this knowledge.

In this report studies are reported of bone obtained from 18 patients treated with adrenal cortical hormones or their synthetic homologs.

The system we have studied is internal remodelling of lamellar bone. The methods used are quantitative histological.

A summary of some relevant cell dynamics in bone remodelling will help to understand our data.

RELEVANT SUMMARY OF BONE REMODELLING

Bone is made by cells called osteoblasts, and destroyed or resorbed by others called osteoclasts (*Ham & Leeson* (10)). Neither osteoclast nor osteoblast can reproduce itself. Both are generated by cell division from a primitive cell called the osteoprogenitor cell by *Young* and the *mesenchymal cell* by us. (*Young* (22) 1962, (23) 1963, and *Frost* (6) 1963). Mesenchymal cells apparently cannot resorb or make bone themselves and must somehow be instructed from their environment to begin making daughter cells. Once so instructed, they are said to be *activated,* and a remodelling *focus* then occurs in the region where the activation happened.

Counts of such foci are thus a way of studying the location and frequency of activation. Each remodelling focus involves a finite amount of bone resorbed and made, each with some mean size and speed of evolution. A measure of size is surface area, because in resorption surface is being shaved off at so many microns per day, while in formation it is being added to.

A measure of evolution speed is the depth that is removed from, or added to, the surface per time unit.

The sizes and evolution speeds of remodelling foci are determined directly by osteoclasts and osteoblasts, and indirectly by agents and situations that regulate them.

It follows that changes in sizes and evolution speeds indicate changes in the activity of osteoclasts and osteoblasts, but do not have meaning in terms of the presence or lack of activation of mesenchymal cells.

Therefore, one can measure separately indices of both mesenchymal cell activation and of the activities of osteoclasts and osteoblasts.

THE GEOMETRY OF INTERNAL REMODELLING OF LAMELLAR BONE

Three geometric properties of lamellar bone remodelling processes are very useful in relating anatomy to cell dynamics (6). These are:

There is a mean yearly depth at which bone is shaved off or added to the surfaces of active remodelling foci. Express this in millimeters per year and use the symbols (M_r, M_f) to designate it, the subscripts identifying resorption or formation. These have dimensions of length and time. This can be measured in tetracycline labelled bone.

There is a mean size of the resorption and formation foci. Express this as the surface area to which active resorption or formation is limited. It is given in square millimeters and designated by (S_r, S_f). These have dimensions of length squared. This can be directly measured in mineralized sections.

There is some mean number of foci during the year at which remodelling activity occurs, one for resorption, another for formation. Designate these by (A_r, A_f) and express them in numbers per cubic millimeter. These counts are easily done on mineralized sections.

All of the bone resorption and formation that occours in a bone or skeleton can be calculated from the above measurements if they can be obtained. This calculation involves the solution of two simple algebraic equations, *viz.:*

$$V_r = k\, A_r\, S_r\, M_r \quad (1)$$

$$V_f = k\, A_f\, S_f\, M_f \quad (2)$$

In these equations (V) is the volume of bone resorbed or formed per year, according to the subscript, and (k) is the number of mm^3 originally present. For a one-year span of time with (k) equal to unity, their solutions are the yearly bone resorption and formation rates respectively. They are rates because they have dimensions of time, and length cubed.

MATERIALS

The materials consist of the 5th, 6th or 7th ribs of two groups of patients.

1) The first group of 31 people had no known metabolic bone disease, hormonal therapy, cytotoxins, metastatic malignancy, chronic illness, congestive heart failure, chronic infection, diabetes mellitus or cirrhosis. Three-quarters of these ribs were

obtained at thoracotomy for indications such as hiatus hernia, cardiospasm, biopsy of undiagnosed solid parenchymal lesions, repair of patent ductus or aortic coarctation, or repair of acute thoracic trauma. The rest were obtained at autopsy, causes of death including sudden vascular incidents, homicide, suicide and trauma. It is believed that this group of ribs provides a representative sample of normal human bone. Ages of these patients were from 51 to 60 years, with a mean age of 57 for the group.

2) The second group of 18 patients received an adrenal corticoid hormone or synthetic homolog in pharmacodynamic doses for more than two months before skeletal sampling. Seven received these agents for a year or more.

There were a variety of reasons for giving the corticoids, ranging from lymphatic leukemia, rheumatoid arthritis, and pulmonary fibrosis to metastatic malignancy and thrombocytopenia. Pertinent clinical data on these patients is given in Table 1. All of these skeletons were sampled at autopsy. None of these patients received any other hormone during the time of corticoid therapy. Only one (case 12) was clinically Cushingoid at the time of death.

3) In addition to the above material, there are in the laboratory's files over 200 normal bones other than ribs, and over 50 bones other than ribs from corticoid treated patients. These have been examined by the same techniques as those referred to next. The changes in the corticoid bones are qualitatively similar to those in the ribs and add considerable weight to the significance of the 18 cases reported in detail. This study is restricted to ribs because only this bone is present in normals in sufficient quantity to provide a valid norm for age, based on measurement.

METHODS

1) Fresh, hydrated mineralized cross sections of the ribs were made and stained with basic fuchsin by special methods (4, 5). Completed sections were about 50 microns thick. There were an average of more than three cross sections per case. The sections were oriented within 5 degrees of perpendicularity to the longitudinal axis.

2) The total cross section area is that enveloped by the periosteum as seen on cross section. The cortical area is the total area minus the marrow area. All three of these areas were measured, using a modification of *Chalkley*'s method (3, 18). Accuracy was 1.0 mm^2, precision 0.3 mm^2. The cortical area was tabulated both as a total for the three sections, and as the mean per section, for later calculations.

3) The total numbers of active osteoid seams (21) and of resorption foci (18) were then counted in the sections as described by *Sedlin et al.* and *Villanueva et al.* The means per mm^2 of cortex were obtained by dividing the total numbers of foci by the cortical area of all sections of a case. Because of the geometric properties of the sections, and of the bones examined, and of lamellar bone studied in cross section, and because of the design of the measuring method, active seams/mm^2 is equivalent to actice seams/mm^3 (18, 21). In establishing this equivalence it is assumed that one always refers to cross sections exactly one millimeter thick. All numerical data in this work has this dimensional basis.

4) The circumferences of the average active osteoid seam and resorption space were measured, using a modification of *Chalkley, Cornfield* and *Park*'s method (3, 18), Zeiss integrating eyepieces and methods of calculation outlined elsewhere.

TABLE 1

Corticoid Patients.

The 18 cases in this study are listed with their age, sex and major medical diagnosis at the time of post-mortem. Only one case was obtained at surgery (for lung biopsy: #12).

Case	HFH no.	Sex	Age Years	Months corti-coid the-rapy	Pertinent diagnoses
1.	91 02 59	M	5	12	Acute lympathic leukemia
2.	53 93 27	M	11	18	Acute lymphatic leukemia
3.	98 40 23	M	15	2	Regional enteritis; peritonitis; thrombocytopenia
4.	85 59 29	F	15	24	Rheumatoid arthritis; pulmonary fibrosis
5.	102 50 51	F	15	4	Metastatic sarcoma, obscure bleeding diathesis
6.	94 77 73	M	34	4	Metastatic Ca – lung
7.	98 95 12	M	46	2	Metastatic Ca – lung
8.	102 90 86	M	50	4	Uremia
9.	96 56 68	F	54	4	Leiomyosarcoma Pancreas
10.	80 87 41	F	56	13	Metastatic Ca – colon
11.	94 72 50	M	57	5	Duodenal Ulcer; pulmonary fibrosis; emphysema; gastrectomy
12.	98 26 66	M	58	48	Ca – lung; rheumatoid arthritis; osteoporosis
13.	82 95 54	F	60	3	Metastatic Ca Rectum; congestive heart failure
14.	06 32 98	M	60	5	Chronic lymphatic leukemia
15.	56 25 44	F	66	12	Leukemia; subdural hematoma; Ca – tonsil
16.	62 24 74	F	68	18	Metastatic Ca – breast
17.	76 20 92	F	68	4	Cirrhosis liver; diabetes mellitus
18.	96 97 60	F	68	5	Rheumatoid arthritis; Metastatic Ca

Mean Age Cases 6—18: 57 years.

These measurements are reported as mm^2 per formation or resorption focus and are calculated on the basis that the value obtains in cross sections one millimeter thick.

5) The rate at which new osteoid is added to the surface of an active osteoid seam may be measured by observing the rate at which the zone of demarcation converts osteoid to bone as it sweeps over new, previously unmineralized bone matrix. The motion of the zone of demarcation is labelled by the tetracycline anti-

TABLE 2

The Measurements of Each Steroid Treated Case are Listed.

Column 1. Case	2. A_f #/mm²	3. A_r #/mm²	4. S_f mm²/mm²	5. S_r mm²/mm²	6. M_f mm/year	7. A_r/A_f	8. Total cortical cross section area mm²	9. Ratio cortical to total area	10. Sections	11. Cortical area mm² per section
1.	0	1.38	...	.603	(0.20a)	>100	13.0	.45	1	13
2.	0	.50	...	.979	(0.20a)	>100	32.5	.40	2	16.2
3.	1.80	.83	.51	.639	(0.20a)	.54	22.7	.49	1	22.7
4.	.32	.38	.60	.725	(0.20a)	1.0	110.3	.51	3	36.6
5.	0	.81	...	.799	(0.20a)	>100	39.4	.56	2	19.7
6.	.075	1.10	.38	.464	(0.20a)	14.7	39.9	.36	2	19.95
7.	.34	1.65	.40	.492	(0.20a)	3.7	64.0	.23	4	16
8.	0	.664	...	.554	(0.20a)	>100	55.7	.24	3	18.5
9.	0	.90	...	.444	(0.20a)	>100	30.5	.28	2	15.25
10.	0	1.14	...	.538	(0.20a)	>100	22.7	.23	2	11.35
11.	.061	.23	.53	.343	(0.20a)	3.5	146.8	.24	7	20.97
12.	.027	.29	.306	.699	(0.20a)	15.2	36.7	.29	2	18.35
13.	.17	.13	.35	.212	(0.20a)	1.0	29.8	.34	3	9.93
14.	0	.58	...	.479	(0.20a)	>100	18.7	.33	2	9.35
15.	0	1.05	...	.499	(0.20a)	>100	36.0	.28	2.7	13.1
16.	.068	.94	.244	.90	(0.20a)	18.8	14.8	.41	1	14.00
17.	0	.46	...	.241	(0.20a)	>100	30.5	.23	3	10.17
18.	0	.66	...	.527	(0.20a)	>100	43.8	.33	3	14.6

TABLE 3

The X, σ and σ X are Compared for the Normal and Steroid Treated Patients.

	A_f all cases	A_f cases 6/18	A_r all cases	A_r cases 6/18	S_f all cases	S_f cases 6/18	M_f	S_r all cases	S_r cases 6/18	All cortical areas (C.A.)	Adult cortical areas	Ar/A_f
Corticoids												
Arithmetic Mean	.158	.058	.771	.758	.418		.20	.562	.489	16.7	14.8	100
Standard Deviation	.42	.09	.41	.43	.121			.20	.17	6.35	4.04	
Standard Error	.09	.03	.09	.01	.046			.054	.048	1.5	1.12	
N	18	13	18	13	8		6	18	13	18	13	18
Normals												
Arithmetic Mean	.380		.53		.53			.465			19.0	1.4
Standard Deviation	.26		.24		.056			.13			5.9	
Standard Error	.047		.045		.01			.02			1.06	
N	31		31		31		8	31			31	31

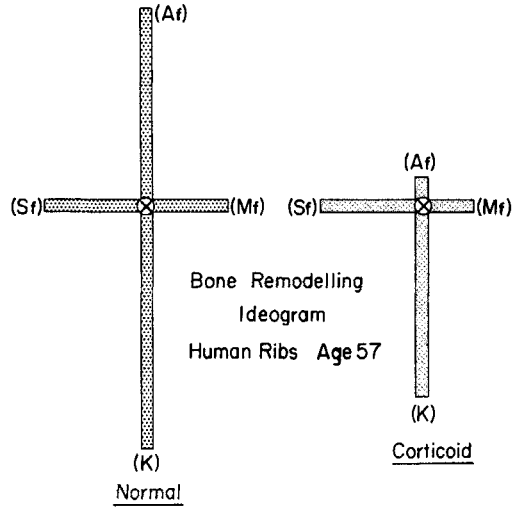

Fig. 1.

In this ideogram several of the measured parameters of bone remodelling are plotted. These are from the formation equation (2). Normal values for age 57 are on the left while the values for the corticoid group in the present report are on the right. The numerical values are taken from Table 3.—The bars on each side are homologous with each other and are identified in terms of the parameters in equation (1), text. Note the marked decrease in numbers of sites of formation. The bottom bars marked (k) represent the amount of bone in mm^3 that would be present on the average cross section. The mm^3 of bone is in absolute bone volume terms (5), meaning this is the amount of bone remaining after subtracting all of the normal porosities. The porosities are the lacunae, canaliculae, Haversian and Volkmann's canals, primary longitudinal canals and the marrow space.

biotics when these agents are given for infections (7). About 45 per cent of the general hospital population contains one or more tetracycline labels, according to *Sedlin.*

6) It has been observed that in a standard bone the ratio of the cortical area to the total area is a useful index of the degree of osteoporosis (19). Accordingly, this ratio was calculated for both groups and is shown in column 9, Table 2.

The normals for age 57, which is the mean age for the corticoid group, have been determined by the means referred to. While there are 31 patients in the normal group whose ages lie between 52 and 62 years, the weight assignable to this group is augmented by the values of the groups on either side of it. The likelihood that the numerical values of the normal group, based only on the 31 patients in that group, are in error due to chance is considerably less than 0.02 ($p < .02$).

The corticoid treated patients are listed in Table 1 with pertinent information about their age, sex, and major medical diagnoses. The number of months each received the corticoids is also noted. All received the cortcoids continuously during the interval indicated.

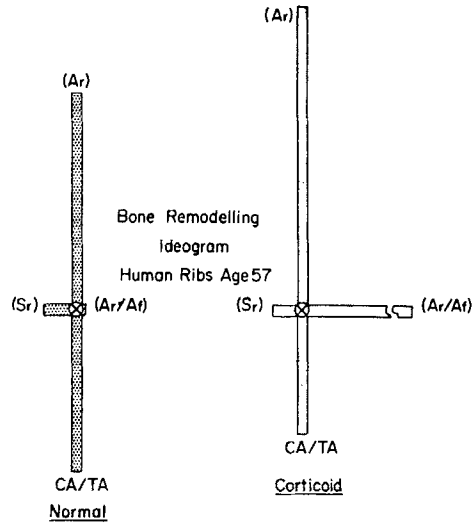

Fig. 2.

In this ideogram the parameters of the resorption equation (1) are diagrammed. Note the significant increase in the numbers of sites of resorption (A_r); we feel this is due to accumulation which in turn is due to failure to initiate the formation of new bone in resorption spaces at a normal rate.—Note the increase in the ratio of (A_r/A_f) in the corticoids. This indicates that resorption spaces exist in corticoid treated bones much longer than normal before being filled in by new bone formation. Since the bone formation process is probably also slowed down (see M_f, Table 3), the persistence of resorption spaces is more accentuated than initially apparent.—The bars at the bottom indicate that there is less cortex present in the corticoids than in the normals in terms of the ratio of the amount of bone present on cross section to the volume of space enveloped by the periosteum. CA designates cortical area, TA total cross section area. This ratio is a sensitive index of growth or osteoporosis.

In Table 2, the individual measurements of the corticoid group are listed. In Table 3 the means, standard deviations, and standard errors of the corticoid patients are listed and compared to the norm as found in the group of 31 normal ribs referred to. In Figs. 1 and 2, the normal and corticoid group are compared to each other graphically.

RESULTS

The symbols used elsewhere for the features that were measured are included (6).

1) (A_f) :The numbers of active osteoid seams/mm^2 are 0.28 in normal and 0.15 in corticoid groups of ribs. If only the adult corticoid treated patients (cases 6–18) are averaged their value is 0.058, a reduction to 15 per cent of normal.

2) (A_r): The numbers of resorption foci per mm^2 are 0.52 in the normal and 0.77 in the corticoid treated groups, an increase of 48 per cent from normal.

3) (M): The linear rate of mineralization of new lamellar matrix in mm/year at the average focus of active new bone formation is 0.31 in normal and 0.20 in corticoid bones. These figures are from a udy reported elsewhere (8, 9) of measurements based on tetracycline labelled human bone. Although a 30 per cent decrease was observed in some corticoid treated patients, the number of patients in the study was small, so the figures are tentative.

4) (M_r): The linear rate of destruction of bone at a resorption front cannot be directly measured with our methods. A lower limit is given by the ratio of (A_r) to (A_f). The reason is that, if both types of foci existed for the same time, they would occur in equal numbers. If one focus existed twice as long as the other, twice as many would be found, because at some time in internal remodelling each type of focus has been or will evolve to the other type.

The ratio of A_r/A_f reveals how much longer a resorption focus exists than a formation focus. This assumes certain things which have not been established.

One is the assumption that enough time has elapsed since onset of treatment with the corticoids for a steady bone state to develop. Another is the assumption that the natural sequence of resorption followed by formation[1] is not altered by the corticoids.

The A_r/A_f ratio is listed in column 7, Table 2. It is much larger in the corticoid treated patients than in normals. The normal ratio is 1.4 and can be calculated from the data in Table 3.

5) (S_f): The mean specific surface of active osteoid seams in mm^2 is 0.33 in normal and 0.42 in corticoid ribs. The difference is below the borderline of significance ($p>.05$) because of the sparsity of active seams in the corticoid bones.

6) (S_r): The mean specific surface of resorption foci in mm^2 is 0.44 in normal and 0.49 in corticoid ribs. This is not significantly different and the two may be assumed to be the same. This means that the average resorption focus in these corticoid treated persons was normal in size.

7) (k): The cortical cross section area in mm^2 is 19 in normal and 14.8 in the corticoid ribs. This means that the corticoid ribs are a little

[1] Which is the case in internal remodelling.

osteoporotic compared to the norm, which may be taken as evidence of an excess of endosteal resorption relative to endosteal formation. The meaning of this measurement is that in one millimeter thick cross sections of ribs there are normally 19 mm^3 of bone, but in the corticoid treated group there were only 14.8 mm^3, a decrease of 22 per cent from normal.

DISCUSSION

The steroid treated group of patients is small and its individuals were afflicted with serious although varied medical and/or surgical problems. So the preceding data and the following attempts at interpretation are not conclusive. They are illustrative: They show one way to get definitive answers from human bone, and they may be correct. It should be pointed out that previous attempts to define the cell dynamics in Cushing's syndrome have been difficult to interpret because of methodological problems. The major uncertainties in this report are due to a limited sample, which nevertheless is one of the largest in a quantitative histological study of Cushingoid bone yet reported.

Now our interpretation:

1) The corticoids cause (directly or indirectly) a dramatic decrease in the number of foci of new bone forming activity. This is seen consistently in all bones from corticoid treated patients. There is insufficient change in the size of these foci to offset the 85 per cent decrease in their number, and a decrease is reported elsewhere in their individual rates of evolution (9).

The next inference naturally is:

The steroids decrease the rate of bone formation. This is in qualitative agreement with tracer-based *in vivo* studies, among them being those of *Heany & Wheadon* (11), and *Rich, Ensinck & Fellows* (17). Since our study suggests that this is due mostly to decrease in the *numbers* of formation foci, it seems to us that the steroids primarily affected the mesenchymal cell population. The result of this effect is not only a decrease in the numbers of formation foci; it is probably also an absolute decrease in the numbers of osteoblasts (see *Sissons* (20)).

2) If the values of the corticoid and normal groups in Table 3 are substituted into equation (2), assuming a value of unity for (k), the resulting numerical values for (V_{fl}) are 0.0048 for the corticoid treated and 0.039 for the normal ribs. These numbers are the decimal part of the rib that would be duplicated as newly formed bone per year. This

is termed the bone formation rate (9) and indicates that there is an 88 per cent decline from the normal to the corticoid state.

3) Assembling the resorption terms, it seems to us that there was suppression of resorption in the corticoid treated group. There are 7 patients in Table 1 who received corticoids a year or longer. Were resorption accelerated, there should have been little bone left due to enlargement of the marrow cavity. Yet, there is little change in cortical cross section area in these 7, compared to the rest of the group, as column II, Table 2 reveals. Also had resorption been accelerated, the cortices should have become porous in these 7, due to enlargement of old resorption foci and the appearance of new ones. This was not observed. The increase in number to about 50 per cent over normal, we interpret as the result of *accumulation* of foci waiting for the next phase (*i.e.* bone formation) rather than an increase in resorption rate. This conclusion is also in line with *Sisson*'s report on the paucity of osteoclasts in this state (20). We freely grant the right of the reader to disagree with this interpretation.

SUMMARY

The number of places where bone remodelling activity occurs was found to be depressed in ribs from 18 patients treated longer than 2 months with adrenal cortical steroids. This depression seems to arise from some change in the activity of the mesenchymal cell population, which generates osteoclasts and osteoblasts.

The speed with which an individual formation focus evolves seems to have been depressed also in these ribs. This depression appears to arise from a change in the activity of the osteoblast population.

There was no evidence of increased bone resorption, and possibly a suggestion that resorption was decreased.

RESUME

Il a été constaté une dépression de l'activité de remodelage des os en un certain nombre d'endroits dans les côtes de 18 malades traités pendant plus de deux mois par stéroïdes de cortico-surrénale. Cette dépression semble découler de certaines modifications de l'activité de la population des cellules mésenchymales qui produisent les ostéoclastes et les ostéoblastes.

La rapidité avec laquelle se forme un foyer individual semble égale-

ment avoir diminué dans ces côtes. Cette diminution semble provenir d'un changement intervenu dans l'activité des ostéoblastes.

Il ne semblait pas y avoir une résorption osseuse accrue et on peut même supposer que la résorption avait diminué.

ZUSAMMENFASSUNG

Die Anzahl der Stellen in denen eine Knochenumbildungsaktivitet vor sich geht, wurde bei Rippen von 18 Patienten, die länger als 2 Monate mit adreno-cortikalen Steroiden behandelt worden waren, als herabgesetzt gefunden. Diese Herabsetzung scheint durch irgendeine Veränderung der Aktivitet der mesenchymalen Zellen, die Osteoklasten und Osteoblasten bilden, zu entstehen.

Die Schnelligkeit mit der ein individuelles Bildungszentrum entwickelt wird, scheint bei diesen Rippen ebenfalls herabgesetzt zu sein. Diese Herabsetzung scheint von einer Veränderung der Aktivitet der Osteoblasten hervorgerufen zu werden.

Keine augenscheinliche Zunahme von Knochenresorption war vorhanden, doch handelte es sich möglicherweise um eine Verminderung der Resorption.

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