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Quantitative Relations between Osteoblasts and Osteoid in Primary Hyperparathyroidism, Intestinal Malabsorption ad Renal Osteodystrophy

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Summary. In cancellous bone of iliac crest of healthy individuals a good correlation between the surface density of osteoid-osteoblast interface and the volume density of osteoid has been found. Using the same quantitative histological methods, the relation between these values was examined in primary hyperparathyroidism (40 cases), intestinal malabsorption (24 cases) and renal osteodystrophy (29 cases).

In all these conditions a significant increase of both surface density of osteoid-osteoblast interface and volume density of osteoid was observed. In primary hyperparathyroidism, the proportion between these values remains unchanged in spite of a considerable increase. A correlation exists also in intestinal malabsorption but the ratio shifted towards the osteoid. For renal osteodystrophy, however, no relationship between the mentioned parameters could be found.

Zusammenfassung. In der Beckenkammspongiosa gesunder Individuen konnte eine gute Korrelation zwischen der Oberflächendichte der mit Osteoblasten belegten Knochentrabekel und der Volumendichte des Osteoids nachgewiesen werden. Mit Hilfe der gleichen quantitativ-histologischen Methode wurde die Beziehung zwischen diesen beiden Parametern im primären Hyperparathyreoidismus (40 Fälle), intestinaler Malabsorption (24 Fälle) und bei der renalen Osteodystrophie (29 Fälle) untersucht.

In allen drei Krankheitsgruppen wurde eine signifikante Zunahme der Oberflächendichte der mit Osteoblasten belegten Knochentrabekel und der Volumendichte des Osteoids beobachtet. Trotz der erhöhten Werte bleibt das quantitative Verhältnis zwischen der mit Osteoblasten belegten Oberfläche und dem Osteoidvolumen beim primären Hyperparathyreoidismus normal. Eine Korrelation ist auch bei der intestinalen Malabsorption vorhanden, die Relation ist jedoch zugunsten des Osteoids verschoben. In der renalen Osteodystrophie konnte dagegen keine quantitative Beziehung zwischen den beiden Parametern nachgewiesen werden.

Introduction

Formation of new bone tissue is the result of the activity of osteoblasts. These cells deposit an originally non-mineralized organic matrix, which normally undergoes mineralization after a maturation period of about 8–10 days. Under physiological conditions, there is a direct proportionality between the number of matrix-producing osteoblasts and the amount of osteoid present. An increase or a decrease of the number of osteoblasts is paralleled by a corresponding increase or decrease in the osteoid volume (Merz and Schenk, 1970b). Quantitative evaluation of bone has shown that certain skeletal affections are associated with a change in the quantitative relation between the number of osteoblasts and the amount of osteoid (Schenk, unpublished data). This indicates a dissociation

of osteoid production by osteoblasts and its subsequent mineralization. We therefore examined the ratio osteoblasts:osteoid in three selected groups of bone disorders, where changes in osteoblastic activity and/or osteoid volume are known to occur.

Material and Methods

197 histological specimens of human iliac crests were evaluated quantitatively. 104 sections from autopsies of normal healthy individuals were used to define the normal values of bone turnover (Merz and Schenk, 1970). Of the remaining 93 bioptic specimens 40 come from patients with primary hyperparathyroidism confirmed by surgical removal of the parathyroid gland in 21 whereas 19 subjects have not yet operated up. 24 cases suffered from intestinal malabsorption and 29 from renal osteodystrophy.

The bone biopsies were embedded in methylmethacrylate without previous decalcification, and 5 μ thick microtome sections were stained according to Goldner (1938). Goldner's original method was modified mainly by using Weigert's iron haematoxylin instead of Hansen's and by increasing the staining time (Schenk, Merz and Müller, 1969). By this method mineralized intercellular substance is stained green-blue, in contrast to the unmineralized osteoid, which appears deeply red. Osteoblasts can also be identified and distinguished from inactive endosteal bone lining cells. Osteoblasts usually form a continuous layer upon newly deposited osteoid and are characterized by their ovoid shape, the excentrically located nucleus with distinct nucleoli and their orange-pink coloured cytoplasm. It is suggested from earlier investigations that the fraction of trabecular surface covered by this type of cells reflects the actual intensity of bone formation (Merz and Schenk, 1970a).

In order to obtain a quantitative estimation of the bone remodelling and mineralization processes, the sections were morphometrically evaluated according to our method of point counting and linear intersectioning (Merz, 1967). This method permits quantitative determination of the structure of cancellous bone as well as the measurement of the surface extent of bone formation and resorption areas along the trabeculae. As criteria for the estimation of matrix deposition and osteoid mineralization, the following parameters were chosen for the present study:

Surface Density of Osteoid-Osteoblast Interface (S_{Vob})[1]. This value represents the area of the interface between osteoid and osteoblasts, or in a more general way, the surface area of the trabeculae lined by osteoblasts. It is expressed as mm^2 surface per cm^3 total volume of cancellous bone (bone + bone marrow). Surface density is defined as the surface area of the component per unit containing volume (Weibel, 1972).

Volume Density of Osteoid (V_{Vos})[1]. It indicates the volume of osteoid present per unit volume of cancellous bone and will be calculated as mm^3 osteoid tissue per cm^3 total bone.

Detailed instructions for the determination of these parameters are given in previous papers by Merz and Schenk (1970a, b). In each group of subjects mean values and standard errors were calculated for both parameters. In order to define the quantitative relationship between the surface density of osteoid-osteoblast interface and volume density of osteoid the results of each group were submitted to a correlation test.

Results

The results of the morphometric measurements are given in Table 1. The surface density of osteoid-osteoblast interface is abnormally large in all three bone disease groups, average values exceeding the normal range by a factor of 2–3. The changes of the volume density of osteoid are even more pronounced. In primary hyperparathyroidism the volume density of osteoid was markedly

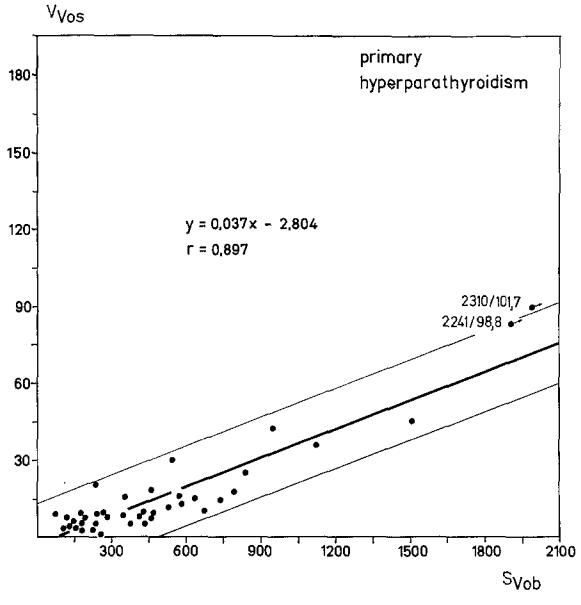
1 In the further description the following short forms may be used: 'osteoblast surface density' instead of surface density of osteoid-osteoblast interface (S_{Vob}); and 'osteoid volume' for volume density of osteoid (V_{Vos}).

Table 1

	Normal individuals	Primary hyperparathyroidism	Intestinal malabsorption	Renal osteodystrophy
Number of cases	104	40	24	29
Surface density of osteoid-osteoblast interface ($mm^2/cm^3 \pm SE$)	154.96 ± 12.58	441.35 ± 65.06	498.48 ± 93.04	504.00 ± 110.56
Volume density of osteoid ($mm^3/cm^3 \pm SE$)	4.92 ± 0.33	13.75 ± 2.72	48.20 ± 8.13	53.59 ± 10.60
Correlation coefficient (r)	0.948	0.897	0.793	0.302
Significance of correlation coefficient ($P <$)	0.001	0.001	0.001	n.s.

increased, however not as much as in the case of intestinal malabsorption and renal osteodystrophy where the values raised 10–12 times above the normal. The increase of osteoblast surface density as well as osteoid volume are highly significant in all three groups ($P < 0.001$). A difference between the average values of the osteoblast surface density cannot be verified statistically in these three groups. On the other hand, the mean values for the osteoid volume are significantly higher for malabsorption and renal osteodystrophy than for primary hyperparathyroidism ($P < 0.05$, resp. 0.02).

In a control group of autopsy cases, a close relation between osteoblast surface density and osteoid volume has been reported ($r = 0.948$, Merz and Schenk, 1970 b). This correlation reflects the physiological synchronization between matrix production by osteoblasts and matrix mineralization. The statistical analysis of our values has demonstrated that the proportionality found in normal cases is also valid for primary hyperparathyroidism ($r = 0.897$, Fig. 1). In intestinal malabsorption a linear function is also present (Fig. 2), though with a considerable variation of the individual values ($r = 0.739$). The correlation coefficient in both groups is statistically significant ($P < 0.001$). In contrast to these results, in renal osteodystrophy (Fig. 3) no correlation between osteoblast surface density and osteoid volume could be statistically verified. In Fig. 4, the regression lines for primary hyperparathyroidism and intestinal malabsorption are compared with the normal range. In primary hyperparathyroidism it is defined by $y = 0.037\,x - 2.804$, which is very close to the normals. It falls into the 95% confidence limit of the normal curve, but the majority of individual values is increased thus extending the normal curve into the high bone formation range. Contrary to this finding the regression line for intestinal malabsorption deviates on the ordinate, pointing to an accumulation of unmineralized matrix.



Figs. 1—3. Surface density of osteoid-osteoblast interface (S_{Vob}) and volume density of osteoid (V_{Vos}) in primary hyperparathyroidism (Fig. 1), intestinal malabsorption (Fig. 2) and renal osteodystrophy (Fig. 3). Regression line and 95% confidence limits are indicated in Fig. 1 and 2, whereas in renal osteodystrophy (Fig. 3) no correlation has been found

Discussion

Bone formation is a two-phase-process:

1. formation of osteoid and
2. mineral deposition into this newly formed organic matrix.

Normally both processes are linked, occuring synchronically. Mineralization of osteoid starts with an initial rapid phase which seems to be controlled by the osteoblasts (Harris and Heaney, 1969; Merz and Schenk, 1970b; Sherman and Sobel, 1965; Wilson *et al.*, 1966). The second, slow phase appears to be governed by a physico-chemical process and therefore takes place in the absence of osteoblasts, too. Physiologically the amount of osteoid present corresponds to the number of osteoblasts. Therefore a quantitative change in the osteoblast-covered surface is necessarily connected with an equivalent change of osteoid volume. Measurements in 104 normal individuals showed a positive linear correlation between the two values ($r = 0.948$), thus verifying this assumption (Merz and Schenk, 1970b).

In various skeletal diseases an increase of the osteoid volume can be observed. This may be the result of an increased matrix production, or a disturbed mineralization, or combination of both (Frost, 1966; Morgan and Fourman, 1969; Paterson *et al.*, 1968). In cases of primary hyperparathyroidism the increase of osteoid volume is due to a proportionate increase of osteoblast surface density as demonstrated by our morphometric data. In other words, primary hyperparathyroidism

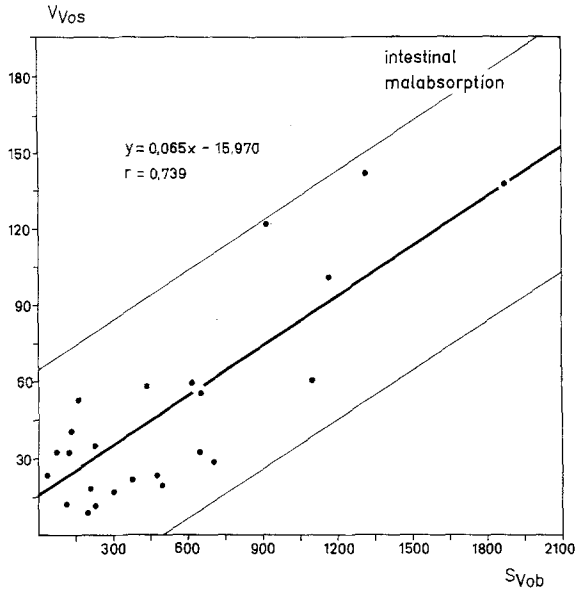


Fig. 2

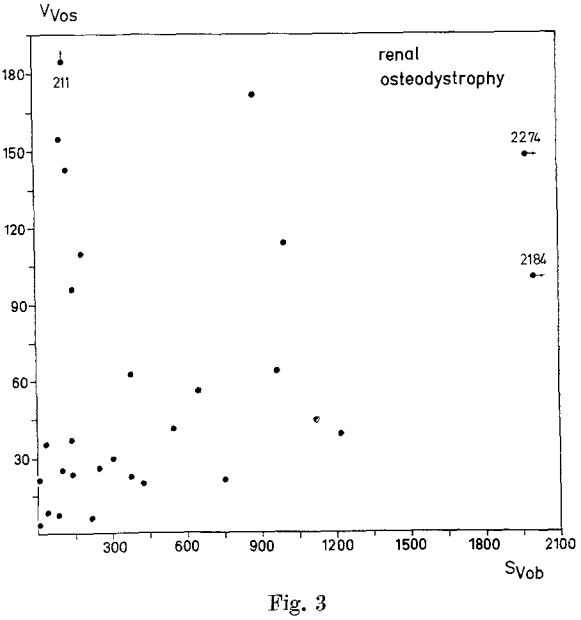


Fig. 3

accelerates new bone formation leaving mineralization of osteoid tissue undisturbed. This finding confirms earlier reports of various authors (Frost, 1966; Harris and Heaney, 1969; Jowsey, 1967; Schenk, unpublished data; Wilson *et al.*, 1966) who suggested that in most cases of primary hyperparathyroidism the synchronization of matrix formation and mineralization remains normal.

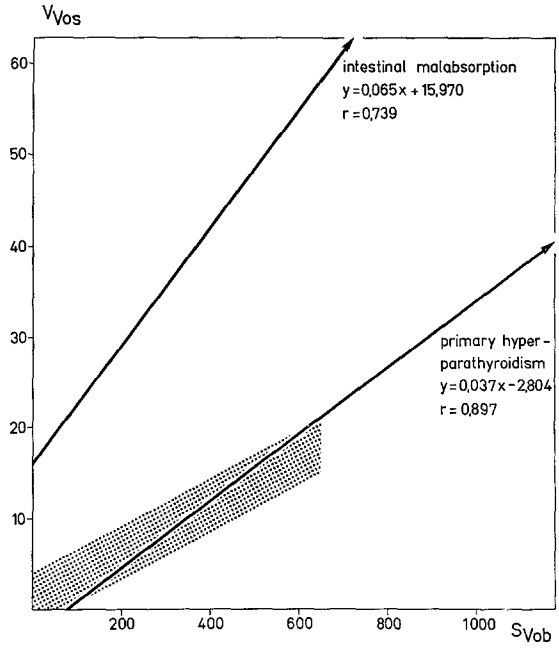


Fig. 4. Position of the regression line of the osteoblast:osteoid relation in primary hyperparathyroidism and intestinal malabsorption, compared to the normal range (dotted area)

In most cases of intestinal malabsorption the main histological feature is an accumulation of osteoid, termed osteomalacia (Fischer *et al.*, 1970; Garner and Ball, 1966; Morgan and Fourman, 1969; Paterson *et al.*, 1968; Wilson *et al.*, 1966; Woods *et al.*, 1968). Morgan and Fourman, as well as Paterson and coworkers measured the amount of osteoid and found that patients with osteomalacia due to gastrectomy showed "a large excess of osteoid". In accordance with these results, our examinations of malabsorption cases also yield a significant increase of the volume density of osteoid, which suggests an inhibition of mineralization. This has been postulated and discussed by other authors. Paterson supposed delayed mineralization to be due to qualitative alterations of the collagen or the ground substance, leading to a reduced affinity for calcium and phosphate. According to Morgan and Fourman primary skeletal changes are due to a delayed maturation of collagen. This leads to a prolonged half life of the immature and therefore noncalcifiable bone matrix, and thus to a delay of mineralization. In addition to the increased osteoid volume, we observe in our material an enlargement of the osteoblast surface density. This was true for the mean, whereas the individual results ranged from almost normal values to very high ones. But in osteoid volume also, there is a considerable scattering (Fig. 2). Despite this large variation and the small number of patients, a good correlation was detected ($r = 0.739$). This finding leads to the presumption that also in cases of intestinal malabsorption osteoblastic activity may influence mineralization. Thus the synchronization of matrix production and mineralization is still effective, but

the rate of mineralization is slowed down. The relation of osteoblasts to osteoid has, in comparison to normal values, distinctly shifted to the side of osteoid. This means that osteoid volume is not only absolutely increased but also in relation to the number of osteoblasts. This is the main difference to primary hyperparathyroidism.

Contrary to the results discussed so far, no correlation between osteoblasts and osteoid was observed in renal osteodystrophy. In this bone disease, pathological alterations of bone formation are caused by coincident, at least temporary disturbances of matrix synthesis (Jowsey *et al.*, 1969) and mineralization (Jesserer, 1965; Russell *et al.*, 1969; Sarnsethsiri *et al.*, 1969). In addition, there obviously is also a disconnection between deposition and mineralization of bone matrix. The irregular alteration of blocked and sporadically accelerated mineralization (Lalli and Lapides, 1965) results in a complex disturbance. It becomes even more complex by secondary hyperparathyroidism. In histological sections these periodic changes are superimposed and therefore make it impossible to prove any correlation between osteoid formation by osteoblasts and the mineralization processes.

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