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# Histomorphometric analysis of osteoclastic bone resorption in metastatic bone disease from various primary malignomas*

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**Summary.** The present study deals with qualitative und quantitative analysis of osteoclastic bone resorption in metastatic bone disease. 267 cases were examined histomorphologically and divided into three developmental stages. In the first 'phase of early appearance' no bone resorption takes place. The stimulation of osteoclastic resorption in the surroundings of tumour tissue is typical in the second 'phase of interaction'. Pressure atrophy, aseptic necrosis and osteolysis by the tumour cells themselves are other mechanisms of bone destruction in the last 'phase of carcinomatosis'. Because osteoclasts are exclusively responsible for the loss of bone tissue in the 'phase of interaction', this stage is suited for precise quantitative analysis of osteoclastic resorption. 24 pure osteolytic secondary bone tumours of various primary lesions were examined histomorphometrically. The numerical values were compared with each other and with standard values of healthy individuals. In contrast with normal bone tissue the fractional resorption surfaces und osteoclast indices increase in metastases. Activated osteoclasts are larger and have more nuclei. The numbers of osteoclast index and nuclei per osteoclast are significantly higher in renal than in breast carcinoma. Osteoclasts can be activated in distances of more than 500 μm from tumour tissue. The mean stimulation distance in metastasis from squamous cell carcinoma is markedly higher than in secondary bone tumours of breast carcinoma. Several osteoclast activating substances and divers mechanisms of stimulation might be responsible for different numerical values of morphometric parameters in metastases from various primary malignancies.

**Key words:** Bone metastases – Osteoclasts – Bone resorption – Histomorphometry – Undecalcified preparation

* Dedicated to Prof. Dr. G. Seifert on the occasion of his 65th birthday

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## Introduction

Metastases are the most common malignant tumours of the skeleton. Osteoblastic, osteolytic and mixed forms can be distinguished. Bone destruction is typical in osteolytic metastatic bone disease. Pathological fractures often follow as clinical complications. Different pathogenetic mechanisms of bone destruction make it possible to divide metastatic bone disease into three developmental stages (Kulenkampff and Delling 1984). Before bone resorption begins, small groups of tumour cells appear in the marrow spaces ('phase of early appearance'). During the following 'interaction phase' (Kulenkampff und Delling 1984) osteoclastic bone resorption is activated in the surroundings of tumour tissue. Release of osteoclast stimulating factors by secondary bone tumours has been discussed in the literature. Parathyroid hormone (Raisz et al. 1979; Omenn et al. 1969; Trump 1983; Scherwood et al. 1967) PTH-like factors (Mundy et al. 1984), prostaglandins (Voekel et al. 1975; Seyberth et al. 1975; Tashjian 1978) an osteoclast-activating factor OAF (Mundy et al. 1974; Mundy et al. 1974; Raisz et al. 1975) and some growth factors (Mundy et al. 1984; Tashjian and Levine 1978; Todaro et al. 1980) have all been considered to be osteoclast activation in malignancy.

In the next stage tumour tissue occupies the whole marrow space. At that time ('phase of carcinomatosis') other mechanisms of bone destruction, for example pressure atrophy (Milch and Changus 1956) ischaemic necrosis (Cramer et al. 1981) and osteolysis by the tumour cells themselves (Cramer et al. 1981; Mundy and Raisz 1977; Koeffler et al. 1978; Galasko 1981; Galasko and Bennet 1976; Eilon and Mundy 1979) occur.

Cytostatic agents and irradiation are possible treatments in advanced stages. Inhibition of osteoclastic bone resorption through medication can be attempted in the 'phase of interaction'. Prostaglandin antagonists (Voekel et al. 1975; Galasko 1981; Caro et al. 1979; Powles et al. 1976; Jung 1983) diphosphonates (Fleisch and Felix 1979; Galasko et al. 1980; Elomaa et al. 1984; Hasling et al. 1984; Jung et al. 1981) mithramycin (Perlia et al. 1970; Stamp et al. 1975) and calcitonin (Binstock et Mundy 1980; De Maria et al. 1979) are used, each with a different mode of action. Such treatment requires a knowledge of the quantitative extent of osteoclastic bone destruction. This problem was examined by an histomorphometric analysis of osteoclastic bone resorption activities on trabecular bone surfaces in osteolytic metastatic bone disease from various primary malignancies.

## Material and methods

From 1971 to 1983 nearly 18,000 osteological cases were examined and registered in the department of bone pathology of the University of Hamburg. 267 of these patients were suffering from metastatic bone disease. Biopsy material was usually examined; a smaller number were necropsy cases. The last were radiographed to give a better impression of osteoblastic and osteolytic changes. Osseous tissue was fixed in Carnoy's or buffered formaldehyde solution. Specimens of $2 \times 2$ cm size were then embedded in methylmethacrylate without decalcification. 5 µm thick sections were cut (Delling 1972; Delling 1975) and stained by the Goldner method, a Kossa's modification and the toluidine blue reaction.

Only 10% of all 267 metastatic cases appeared purely osteolytic and demonstrated all

**Table 1.** Primary malignomas of quantitatively examined bone metastases

| Primary malignoma | Cases |
|---|---|
| Breast carcinoma | 5 |
| Renal carcinoma | 4 |
| Bronchial carcinoma | 3 |
| Melanoma | 2 |
| Thyroid carcinoma | 1 |
| Prostatic carcinoma | 1 |
| Cervical carcinoma | 1 |
| Lingual carcinoma | 1 |
| Hypopharynx carcinoma | 1 |
| Synovial sarcoma | 1 |
| Histiocytoma of thyroid gland | 1 |
| Unknown primary malignoma | 3 |
| Total | 24 |

the cellular phenomena of the 'interaction phase'. In these cases it was possible to perform quantitative analyses of osteoclastic bone resorption. The various primary malignomas of the 24 metastases examined are listed in Table 1. For determining the morphometric parameters a light microscope (magnification × 240) was used. Only bone surfaces in the region surrounding the tumour tissue were analysed. Perimeter intersections of trabecular edges on the lines of a parallel woven eyepiece grid were measured and the following fractions of endosteal surface were calculated (Merz 1967; Delling 1979; Frost 1977):

S fract RT (%): Percentage of endosteal surface with resorption cavities in the surroundings of tumour tissue (fractional resorption surface).

S fract $R_{Ocl}$ (%): Fractional lacunar surface (S fract RT) covered by morphological recognizable osteoclasts. This parameter quantitatively reflects the cellular resorbing activity at time of biopsy.

Osteoclast index (OI, N/cm): Number of osteoclasts per centimeter endosteal trabecular bone surface.

$D_{Ocl}$ (µm): Mean value of minimal distances between osteoclast-bone contact size (middle) and nearest tumour tissue in the surrounding marrow space.

S fract OS (%): Percentage of surface covered by osteoid seams in the surroundings of metastatic tumour tissue (fractional formation surface).

$K_{Ocl}$: Average number of nuclei per osteoclast.

The magnification for measuring minimal distances between osteoclasts and tumour tissue was × 125. An average number of 170 distances were measured in each case. Because there was no normal distribution of numerical values, the universal function:

$$f(r) = ar\, e^{-br}$$

was taken as basis for distribution. The variables a and b, as well as medium distances ($D_{Ocl}$, µm) and their errors ($De_{Ocl}$, $\mu m^{-2}$) were calculated by computer. The single function curves were plotted.

All histomorphometric parameters of metastatic bone disease were compared with a control group of healthy individuals (Delling 1975). The reproducibility of the method is 5% (Delling et al. 1981). Normal distribution and *t*-test are used for statistical analysis. The histomorphometric parameters, their symbols and units are listed in Table 2.

## Results

### *Qualitative morphological findings*

Extensive osteoclastic bone resorption surrounding metastatic tumour tissue is characteristic of secondary bone tumours in the 'phase of interaction'.

**Table 2.** Parameters of histomorphometric analysis

| Parameters | Symbols | Units |
|---|---|---|
| – Fractional lacunar surface (=fractional resorption surface) | S fract RT | % |
| – Fractional lacunar surface covered by osteoclasts | S fragt $R_{Ocl}$ | % |
| – Fractional surface covered by osteoid seams | S fract OS | % |
| – Oseoclast index | OI | n/cm |
| – Average number of nuclei per osteoclast | $K_{Ocl}$ | n/Ocl |
| – Minimal distances between osteoclasts and tumour tissue (Medium value) | $D_{Ocl}$ | μm |
| – Error of minimal distance (=$D_{Ocl}$) | $De_{Ocl}$ | $\mu m^{-2}$ |

Increased numbers and size of the multinucleated cells are seen. The number of nuclei per osteoclast increases a little. Closely packed ranks of bone resorbing cells cover the endosteal trabecular surface in some of the cases examined. Enlarged Howship's lacunae are frequently found. Narrow trabeculae and loss of bone mass are also typical microscopic findings in osteolytic metastatic bone disease, Osteoclasts with more than 20 nuclei are observed sporadically (Fig. 1). Osteoblasts and osteoid seams are scarce on the surrounding trabecular surfaces of osteolytic metastases. Small fibrous layers often develop between tumour and bone tissue, while direct contact with both rarely occurs in the 'phase of interaction'.

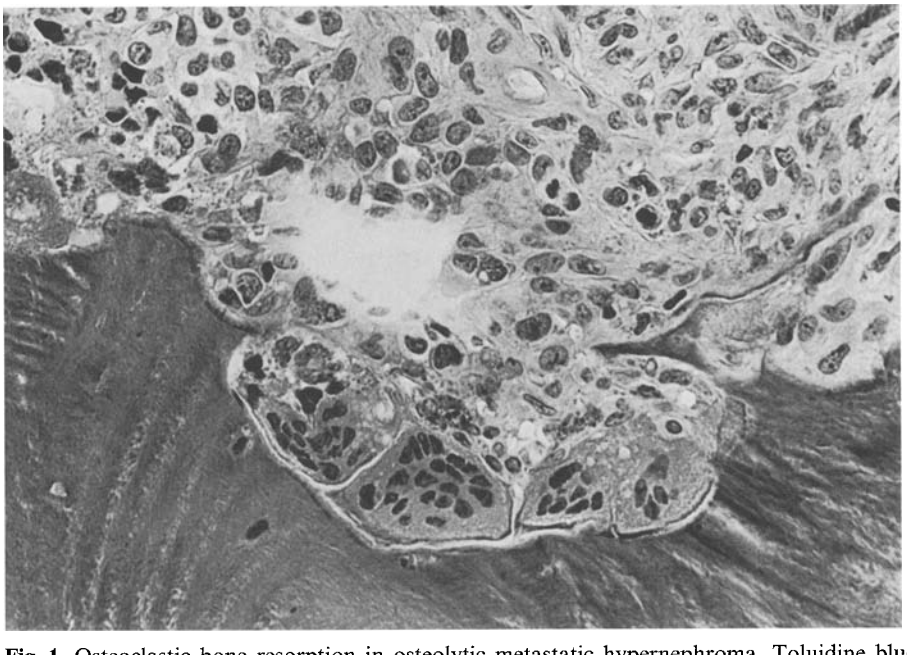

**Fig. 1.** Osteoclastic bone resorption in osteolytic metastatic hypernephroma. Toluidine blue, undecalcified, ×450

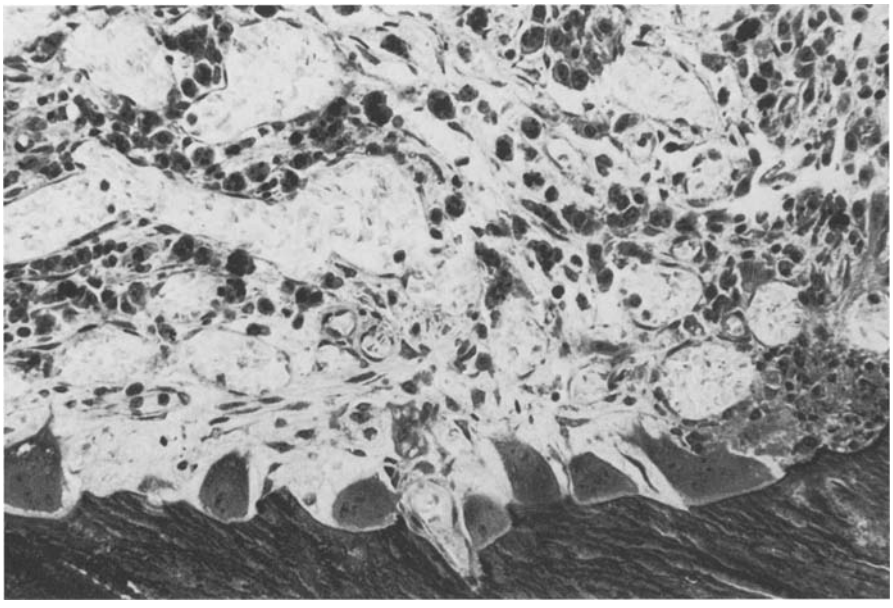

**Fig. 2.** Osteoclastic bone resorption in osteolytic metastatic breast carcinoma. Toluidine blue, undecalcified, × 270

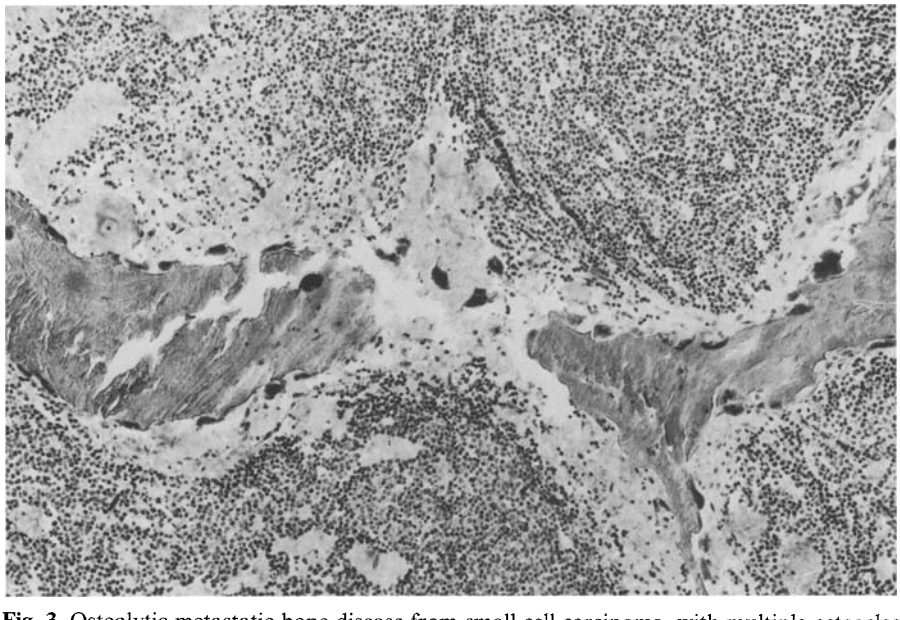

**Fig. 3.** Osteolytic metastatic bone disease from small cell carcinoma, with multiple osteoclasts. Goldner's stain, undecalcified, × 180

No correlation was detectable between the range of myelofibrosis and the extent of osteoclastic resorbing activity. Apparently metastatic bone disease in renal carcinoma exceeds other secondary bone tumours in the stimulation of osteoclasts (see Figs. 1 and 2).

*Quantitative Results*

*1. Parameters of trabecular bone surface.* All surface parameters studied in osteolytic metastatic bone disease from the 'phase of interaction' and those of healthy individuals are listed in Table 3.

63,3 ± 11,2% of trabecular endosteal surface around tumour tissue consists of Howship's lacunae (= S fract RT; 24 cases). There is no significant difference between groups of various primary lesions. In comparison with normal bone tissue (S fract RT = 6,5 ± 2,2%) a ten fold increase is seen.

29,3 ± 10,3% of lacunar surface is covered by osteoclasts. This is 2,0 ± 1,1% in normal bone. There is no statistical difference between secondary bone tumours of the various primary malignancies.

The number of osteoclasts per unit surface (OI; Ocl/cm) also increases. The combined osteoclast count from all cases examined was 57,6 ± 24,3 Ocl/

**Table 3.** Histomorphometric parameters of osteoclastic bone resorption in metastatic bone disease and normal trabecular bone

| Parameters | Units | Bone metastases (24 cases) | Normale Bone (14 cases) |
|---|---|---|---|
| S fract RT | % | 63.8 ± 11.3* | 6.5 ± 2.2* |
| S fract $R_{Ocl}$ | % | 29.9 ± 10.3* | 2.0 ± 1.1* |
| OI | n/cm | 57.6 ± 24.3* | 7.5 ± 5.2* |
| $K_{Ocl}$ | n/Ocl | 3.3 ± 0.6* | 2.1 ± 0.5* |
| $D_{Ocl}$ | µm | 101.7 | – |
| DeOcl | $\mu m^{-2}$ | 17.9 | – |
| S fract OS | % | 8.5 ± 5.6* | 23.2 ± 4.6* |

* $p < 0.05$

**Table 4.** Histomorphometric parameters of osteoclastic bone resorption in metastatic bone disease of various primary malignomas

| Parameters (symbols) | Units | Squamous cell carcinoma | Breast carcinoma | Renal carcinoma | Other malignomas |
|---|---|---|---|---|---|
| S fract RT | % | 71.1 ± 6.6 | 60.3 ± 9.2 | 62.7 ± 13.0 | 63.0 ± 12.9 |
| S fract ROcl | % | 30.4 ± 13.6 | 27.2 ± 5.4 | 37.0 ± 8.0 | 28.4 ± 11.5 |
| OI | n/cm | 65.0 ± 32.8 | 46.0 ± 6.9* | 71.9 ± 24.9* | 55.0 ± 25.5 |
| KOcl | n/Ocl | 3.3 ± 0.6 | 3.0 ± 0.3* | 3.8 ± 0.6* | 3.2 ± 0.6 |
| DOcl | µm | 158.4 | 89.6 | 107.0 | – |
| DeOcl | $\mu m^{-2}$ | 28.3 | 41.6 | 67.75 | – |

* $p < 0.05$

cm. Compared with normal bone tissue (OI = 7,5 ± 5,2 Ocl/cm) a 7-fold increase is detectable. In metastases from renal carcinoma there are statistically higher indices than in breast carcinomas (Table 4).

The average number of osteoclasts nuclei is raised due to tumour cell stimulation from 2,1 ± 0,5 ($K_{Ocl}$ in healthy individuals) to 3,3 ± 0,7 ($K_{Ocl}$ in metastatic bone disease). In some osteoclasts more than 20 nuclei are found (Fig. 1). In contrast to metastatic carcinoma ($K_{Ocl} = 3,0 \pm 0,3$) the average number of nuclei in renal carcinoma is significantly higher ($K_{Ocl} = 3,8 \pm 0,6$).

Bone formation is rarely seen on the trabecular endosteal surfaces in the surroundings of osteolytic metastases. Resorbing surfaces are nearly 8 fold more prevalent (S fract RT = 63,3 ± 11,2%) than osteoid covered surfaces (S fract OS = 8,5 ± 5,6%). The fractional formation surface in healthy individuals is 23,2 ± 4,6%.

*2. Minimal distances between osteoclasts and tumour tissue.* Nearly 4,000 single distances were determined in 24 cases of metastatic bone disease. $D_{Ocl}$ yields 101,7 µm ($De_{Ocl} = 17,9\ \mu m^{-2}$) for all examined cases (Table 3). The peak of the curve which represents the highest measured percentage occured at a distance of 65 µm. 1,3% of all distances were greater than 500 µm (Fig. 5). In two cases of squamous cell carcinoma, one of breast carcinoma and two of renal carcinoma more than two percent of all numerical values exceed 500 µm. In other secondary bone tumours (one synovial sarcoma, one bronchogenic carcinoma and one breast carcinoma) the maximal stimulating distance was shorter than 200 µm. In the range from 100 µm to 300 µm about 20% more activated osteoclasts can be found in squamous cell carcinoma than in renal or breast carcinoma (Fig. 5). Thus the mean distance in metastases of squamous cell carcinoma ($D_{Ocl} = 158,4$ µm;

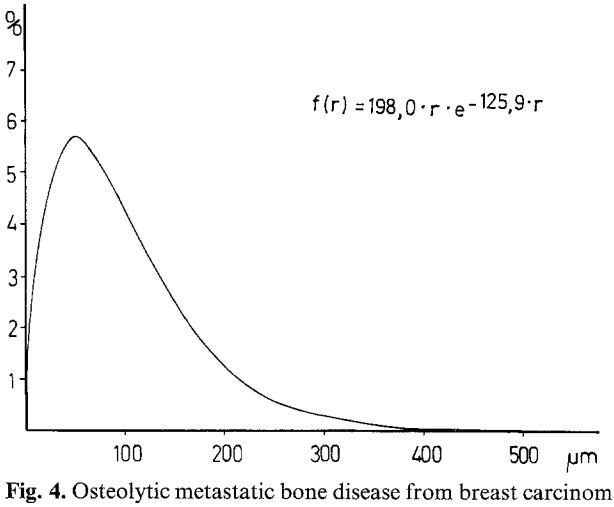


**Fig. 4.** Osteolytic metastatic bone disease from breast carcinoma with extremely long osteoclast-stimulating distances

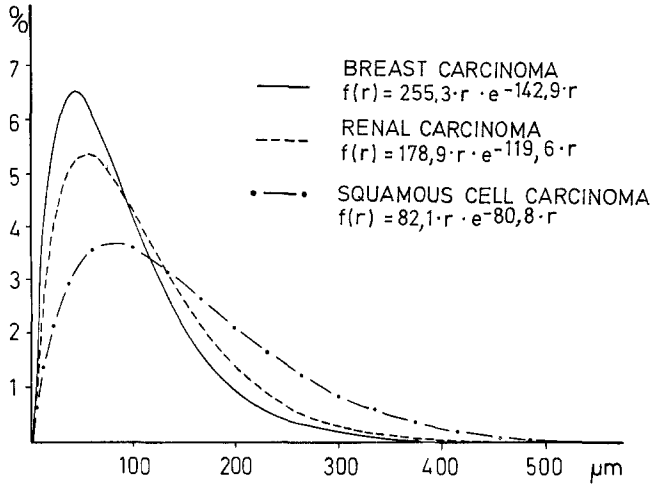


**Fig. 5.** Distribution of minimal distances between osteoclasts and tumour tissue in metastatic bone disease of breast-, renal- and squamous cell carcinoma. (13 cases)

$De_{Ocl} = 28{,}3\ \mu m^{-2}$) is markedly higher than in secondary bone tumours from breast carcinoma ($D_{Ocl} = 89{,}6\ \mu m$: $De_{Ocl} = 41{,}2\ \mu m^{-2}$). Because of the smaller osteoclasts in breast carcinoma this tumour seems to have produced a more pronounced increase in the distribution curve of minimal distances than the secondary tumours of kidney and squamous epithelium.

## Discussion

By using undecalcified preparations in histological examination of bone, decalcification artefacts are avoided and small details of tumour cell structure, osteoblasts and osteoclasts, can be studied more carefully. In the 'phase of interaction' a separate disposition of tumour and bone is a characteristic finding. Direct contact of tumour cells with trabecular surfaces can be seen only in the last phase of metastatic development. While osteoclastic bone resorption is the only pathogenetic mechanism of bone destruction in the 'interaction phase', pressure atrophy (Milch and Changus 1956) ischaemic necrosis (Cramer et al. 1981) and osteolysis by the tumour cells themselves (Mundy and Raisz 1977; Koeffler et al. 1978; Galasko 1981; Galasko et al. 1976; Eilon and Mundy 1979; Cramer et al. 1981) are additional possibilities for bone loss in the late phase of carcinomatosis, when tumour cells completely occupy the whole of the marrow spaces and expand up to the trabecular surface. Precise histomorphometrical analysis is only possible in the 'phase of interaction'. Total occupation of medullary spaces, myelofibrosis and regressive developments prevent topographical lucidity.

The number of Howship's lacunae increases markedly in the trabecular endosteal bone surface of around the tumour. Rates of more than 60% have only been found exceptionally in cases of metabolic bone disease (Delling 1975). The number of Howship's lacunae has been established as an

**Table 5.** List of various osteoclast stimulating substances released from tumour tissue in metastatic bone disease

| Stimulating factors |
|---|
| Parathyroid hormone and PTH-fragments |
| PTH-like factors |
| Osteoclast-activating factor (OAF) |
| Prostaglandins (especially PGE2) |
| Growth factors |
| – tumour growth factor |
| – epidermal growth factor (EGF) |
| – transforming growth factors |
| Catecholamine |
| Bone resorbing factor of melanoma |
| Thyroid hormone, heparin and adrenocorticotropic hormone |
| Stigmasterol and sitosterol |

indirect indicator of resorption. The osteoclast index (OI) expresses the active resorption at time of biopsy much more accurately. Because an increase of this parameter was found we may assume that in metastatic bone disease either more osteoclasts are created unter influence of tumour cells or that the life span of multinucleated cells is exceeded by continuous activation from the malignancy (Galasko 1981; Bonucci 1981). Both possibilities also explain the higher portion of lacunar surface covered by osteoclasts (S fract $R_{Ocl}$). Osteoid seams are scanty on trabecular surfaces in the neighbourhood of tumour tissue. Therefore it is suggested that osteoclast – stimulating – substances produced by malignancy interrupt the normal cycle of basis metabolic units (BMU), described by Frost (Frost 1963). Another interpretation might be rapid expansion of tumour tissue so that the tumour exceeds the BMU time and occupies spaces in dissolved bone before bone formation can take place. Baron (1977) assumed that in pathological conditions coupling messages get lost if reversal phases are too long, and the remodeling starts again with a new resorption phase.

The results of this investigation suggest that resorption activity in the surrounding trabeculae of osteolytic metastases increases rapidly in the 'phase of interaction', but there are also differences between groups of various primary lesions. Osteoclast quantity, size and number of nuclei are greater in metastases from renal than from breast carcinoma. In the range from 100 µm to 300 µm, more activated osteoclasts can be found in squamous cell carcinoma than in renal or breast carcinoma. There is some evidence that the various osteoclast activating substances known have different influences on primary bone tissue cells (Table 5). In one of our cases parathyroid hormone was immunohistochemically demonstrable. Additional data are needed on the controversy about whether tumour cells release pure PTH or PTH-like-substances (Mundy et al. 1984; Simpson et al. 1983). Various pathogenetic mechanisms in stimulating osteoclasts by different substances are known. Chambers (1980) makes a distinction between direct and indirect activating forms. Osteoclast activating factor (OAF) stimulates osteoclasts directly. Because the multinucleated bone resorbing cells have

no specific receptors for PTH, PTH-like factors and prostaglandins, indirect mechanisms of stimulation are necessary (Rodan and Martin 1981). Reduced levels of prostacyclin released by lining cells might result in the activation of osteoclasts (Chambers and Dunn 1982). Growth factors stimulate synthesis of prostaglandins in bone tissue and thus osteoclasts are activated indirectly (Trump 1983). By using additional immunohistochemical methods the morphological effects of various osteoclast-activating substances will be studied. It will be possible to investigate the mechanims of disordered remodelling more closely.

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