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Quantitative Histological Data on Disuse Osteoporosis

Comparison with Biological Data

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The effect of immobilization on human bone was studied through a longitudinal, as well as cross-sectional, quantitative and dynamic histological analysis of 34 decalcified and undecalcified iliac crest biopsies. They were obtained at various times after the onset of immobilization in 28 patients of which 22 were suffering from post-traumatic spinal cord lesions. Trabecular bone volume, osteoid volume, trabecular osteoclastic resorption surfaces, size of the periosteocytic lacunae, thickness of iliac cortices and volume of the cell population of the marrow were measured. The histodynamic study was made by double tetracycline labeling in 19 patients. The histological data were compared with biological data from another group of 68 immobilized patients including 22 of the patients undergoing biopsy. Calcemia, phosphoremia, alkaline phosphatase, calciura, phosphaturia and hydroxyprolinuria were measured. The decrease of the trabecular bone volume averaged 33% over 25 weeks and then stabilized. Immobilization also caused an early increase in the trabecular osteoclastic resorption surfaces and later in the size of periosteocytic lacunae, an early depression of osteoblastic bone formation and a thinning of the cortices. Calciuria was high, as was hydroxyprolinuria which correlates with resorption surfaces. The histological and biochemical changes suggest an histodynamic hypothesis according to which the global lifespan of the BMU (Basic Multicellular Unit from Frost) would be increased. These changes reflect a *transient*, leading to a new *steady state:* rarefied bone with a low rate of subsequent turn-over. They emphasize the importance of mechanical factors in the development of bone cells.

Key words: Bone — Histology — Morphometry — Osteoporosis — Immobilization.

Introduction

While numerous radiodensitometric (Mack and Lachance, 1967; Bruce and Wiebers, 1969) and biological studies (Semb, 1966; Lutwak *et al.*, 1969; Sevastik and Mattson, 1971; Chantraine, 1971; Hardt, 1972) have been made of disuse osteoporosis, a scarcity of quantitative histological studies on this type of osteoporosis led us to study 34 iliac crest biopsies from 28 immobilized patients. The results of this quantitative histological survey were compared with biological data from another group of 68 immobilized patients. It was hope that such a comparison might further our understanding of the pathophysiology underlying the bone rarefaction occurring in immobilized patients. This study allowed a comparison of human histological and biological data, with experimental data obtained

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from previous studies in animals (Burckhart and Jowsey, 1967; Landry and Fleisch, 1964).

Methods and Materials

Histology

Methods

a) Biopsy and Histological Preparation. The samples were obtained by transfixing the iliac bone, at a point 2 cm behind the anterior superior iliac spine and 2 cm below the summit of the right iliac crest. Biopsy was performed under anesthesia, or, in case of cord lesions without it, using Bordier's trephine (inner diameter: 6 mm). The trocar provides a cylindrical bone sample limited by the two iliac cortices which protect the integrity of the spongy bone (Fig. 1). This integrity is an indispensable condition for valid morphometric analyses. In most cases (28 out of 34), two samples were taken, one for decalcification, the other for treatment without decalcification. The cylinders to be partially decalcified with nitric acid were fixed in Bouin's fluid. They were cut into 32 serial sections (5 μ thick) which were stained with hematoxylin-phloxin-safranin. The undecalcified samples were fixed in 80% alcohol, and embedded by successive Bioplastic baths. They were then cut into 8 serial sections (8 μ thick) using a Jung K microtome and then stained with solochrome cyanin and Villanueva's osteochrome. Four additional unstained sections (20 μ thick) were used for observing tetracycline labels under fluorescence.

b) Quantitative Reading Methods. Six bone and medullary tissue parameters were measured:

1. Trabecular bone volume (TBV). This parameter represents the percentage of a given volume of iliac bone occupied by trabeculae, excluding medullary and vascular spaces, but including calcified and osteoid tissues. The measurement was performed either manually with a Zeiss I integrating eyepiece exposing 25 points or with an image analyzing computer (Quantimet 720).

2. Relative osteoid volume of the cancellous iliac bone (R.O.V.). This is the percentage of bone tissue occupied by osteoid tissue and was measured with a Zeiss integrating eyepiece exposing 100 points. From the relative osteoid volume it is possible to then calculate the osteoid volume related to the specimen sample volume, by means of the following formula:

$$\text{Osteoid volume} = \frac{(\text{R.O.V.}) \times (\text{T.B.V.})}{100}$$

3. Trabecular osteoclastic resorption surfaces. The resorption sites notch the edges of the bone trabeculae. Their surface were measured with the Zeiss II integrating eyepiece. This parameter was expressed as the percentage of the total trabecular surface.

4. Mean size of the periosteocytic lacunae. Periosteocytic lacunae generally appear on decalcified preparations as ellipsoidal cavities with clearly outlined contours. According to an original method (Meunier *et al.*, 1973a) the larger and the lesser diameters of the ellipse were measured with a micrometric eyepiece. We consider the product of the two diameters as representing the surface of a rectangle within which the cross-section at cavity can be placed as an indication of the actual size of the lacuna. The measurement was performed on 50 periosteocytic lacunae selected at random in each bone sample. This was expressed in square microns. The actual cross-section areas of the periosteocytic lacunae are also measurable using the image analyzing computer (Meunier *et al.*, 1973c).

5. Thickness of iliac cortices. This was measured with a micrometer eyepiece.

6. Marrow adipose volume (M.A.V.). This was measured with the Zeiss I integrating eyepiece and expressed as percentage of the total medullary volume. The absolute volume of the cell population of the marrow (V.C.P.M.) expressed as a percentage of the total bone volume, can be deduced from the marrow adipose volume by means of the following formula:

$$\text{V.C.P.M.} = (100 - \text{M.A.V.}) \times \frac{\text{R.V.}}{100}$$

R.V.·(remaining volume) = 100 — Trabecular bone volume.

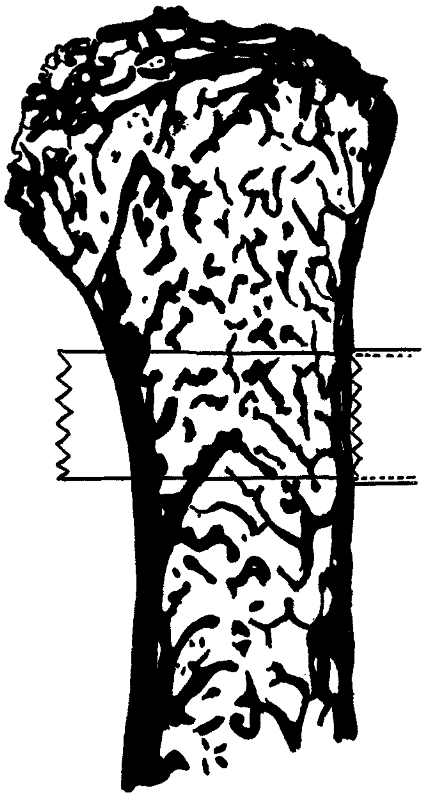

Fig. 1. Site of the biopsy: 2 cm behind the anterior superior iliac spine; 2 cm below the summit of the iliac crest

c) Histodynamical Study with Tetracycline Labelling. Frost's technique of double tetracycline labelling as modified by us (Meunier *et al.*, 1972) was used. In 19 of our 28 patients we gave 600 mg of dimethylchlortetracycline per day for 3 days and again for 6 days after 10 days without medication. Biopsy was performed 8 to 10 days after the end of the second labelling.

This method permits the localization of the borderline between the osteoid tissue and the calcified bone, at the times of the administration of the drug. The calcification rate can be calculated by measuring the distance between the two fluorescent lines. As the interval between the two labelling periods was known, the calcification rate of the osteoid can be calculated and was expressed as μ/day. The calcification rate calculated in trabecular bone was faster than the true calcification rate becuase of the obliqueness of the plane of section of most of the formation foci, but it can be assumed that this overestimation was of the same magnitude in controls subjects and in immobilized patients.

Material

The measurements described above were performed on 34 iliac bone samples from 28 immobilized patients. 22 were suffering from acute post-traumatic cord lesions and 6 due to various other causes: incomplete paraplegia by meningioma, polyradiculitis, bilateral fractures of femora, tibiae, and patellae, trauma of the skull, Schneider's syndrome, cervical myelopathy (1 case of each). The mean age was 33. 19 patients were males and 9 females. The level of the spinal cord lesion was in 25 cases thoracic, 16 lumbar, and 9 cervical. 21 patients had complete anesthesia at the place of the biopsy. The time of the biopsy, in relation with the onset of immobilization, varied from 1 week to 8 years, but less than 20 weeks in 22 cases. Biopsy took place once in 23 cases, twice in 4 cases with an interval from 4 to 26 weeks between biopsies and threetimes in one case (at 1, 3 and 14 weeks after immobilization).

No patient had any biochemical abnormalities indicating the presence of a renal disorder liable to induce bone changes.

Biochemical Studies

Methods

Biochemical studies were performed for three consecutive days either every two weeks or every month. Plasma and urine calcium were measured by a complexometric method (Tronchet, 1958). Phosphate, in both plasma and serum was measured by the method of Briggs (1922). Plasma alkaline phosphatase was estimated as described by Klein *et al.* (1960) and urinary hydroxyproline by the method of Prockop and Udenfriend (1964).

Material

68 patients were investigated biochemically, including 22 of the patients undergoing biopsy. The mean age was 33 years. None of them showed any osteo-articular, metabolic, intestinal or liver diseases, or were on steroid treatment. Their common factor was complete immobilization in the horizontal position for at least 6 weeks with total bed-rest. Complete recuperation (12 cases) or intensive rehabilitation started only after the 6th week.

In some cases, we were able to start the biochemical tests just at the beginning of immobilization, twice a week during the first few weeks. We repeated them over a 52 week period. All our patients had a normal hospital diet.

Statistics

We used the following methods for the statistical processing of the results: comparison of two variances, comparison of two mean values, comparison of two percentages, study of the independance of two quantitative variances, comparison of two regressions, the "smoothing" technique and especially the curve approximation technique under the least square method (Stellmann and Beranger, in press), which has allowed infinitely more accurate curves to be defined.

Results

Histological and Histodynamic Data

Histological Data

a) Iliac Trabecular Bone Volume (T.B.V.). Without taking into account the duration of immobilization, in 22 out of 25 biopsies the values of T.B.V. were lower than the control values collected from 236 normal subjects all of whome suffered violent deaths (Courpron *et al.*, 1973). Nevertheless, all of them except one remained above the vertebral fracture threshold i.e. T.B.V. α 11% (Meunier *et al.*, 1973b; Fig. 2).

To evaluate any correlation between the immobilization time and the trabecular bone volume, without taking the age of the patients into account, we calculated a global correlation coefficient and two partial correlations (from 0 to 25 weeks and after 25 weeks), and constructed a global "smoothed" curve. T.B.V. depends on the immobilization duration up to the 25th week. Beyond that time, the T.B.V. was constant, meaning that a stable new value was reached in immobilized bone at about the 25th week. This value corresponds to a T.B.V. of $12.1 \pm 1.77\%$.

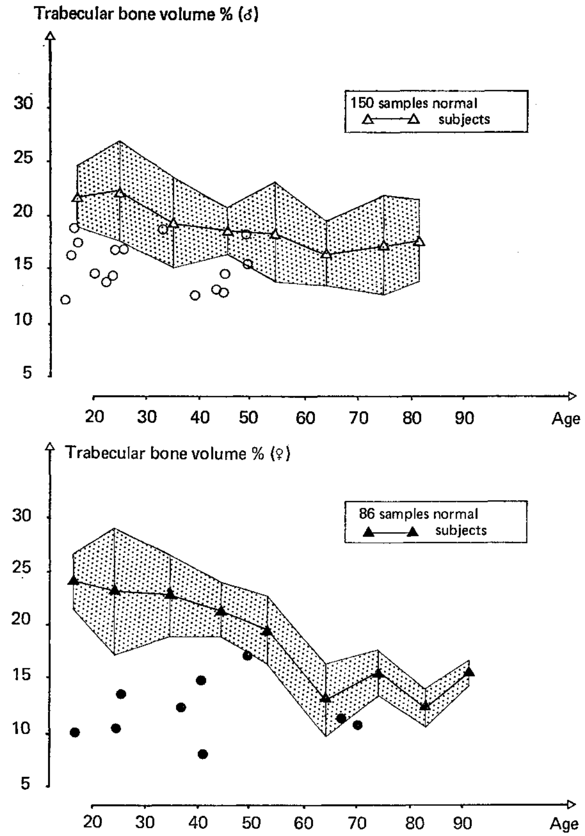

Fig. 2. Trabecular bone volume of 25 immobilized patients (males ∘ and females •) compared with 236 samples from normal subjects of all ages (150 males and 86 females)

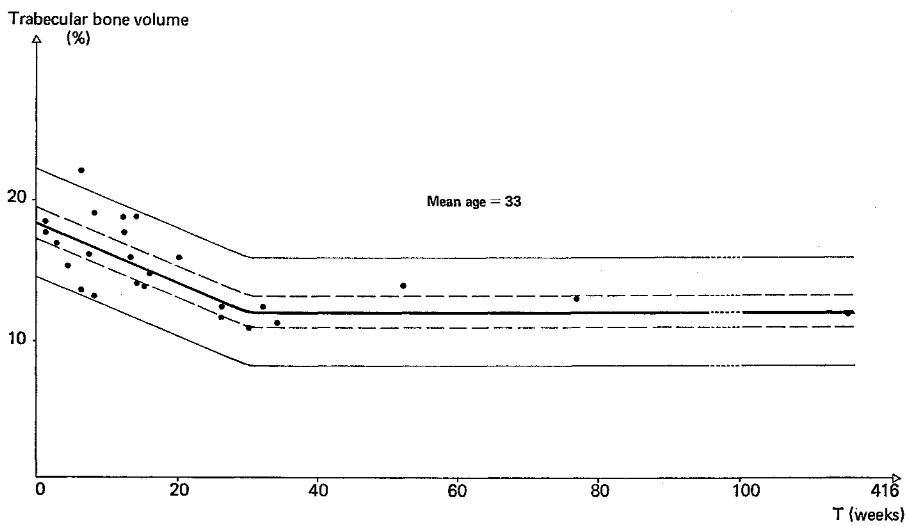

Fig. 3. Trabecular bone volume changes with the time of immobilization

We then evaluated any correlation between the T.B.V. and the duration of immobilization after taking the ages of the patients into account. Due to the fact that they began their immobilization period with a T.B.V. closely depending on their ages at that time, we corrected the T.B.V. of the patients immobilized for less than 25 weeks by extrapolating it to a mean age of 33 (Courpron *et al.*, 1973). The correlation between bone loss and duration of immobilization became more significant (increasing from $r = 0.647$ to $r = 0.760$) (Fig. 3). Thus the variation of the T.B.V. during the first 25 weeks appears to be real, rather than an artefact due to the varying ages of the patients.

b) Osteoid Volume of Cancellous Iliac Bone. 1. Relative osteoid volume. We were not able to obtain early measurements since all the samples taken during the first five weeks were partially decalcified. We noted an early and distinct decrease in the relative osteoid volume in all our subjects (on an average of 0.05% at the 10th week), whatever the cause of immobilization, followed by a rise to normal values (2%) and eventual stabilization at slightly lower values (1.2%).

2. Osteoid volume related to specimen sample volume. Figure 4 demonstrates a marked decrease in osteoid volume at the beginning of immobilization.

c) Trabecular Osteoclastic Resorption Surface. The global comparison between the resorption surfaces of our immobilized patients and that of a series of 130 normal subjects (Courpron *et al.*, 1973) showed a significant difference ($m = 4.2 \pm 0.5$ and $m = 3.6 \pm 1.1\%$; $t = 2.00$) (Fig. 5). The correlation between the duration of immobilization and the resorption surfaces required 3 partial correlations (0–15 weeks, 15–34 weeks, 34 weeks or more) and the global correlation after smoothing was significant ($r = 0.58$).

The resorption surface, therefore, depends on the duration of immobilization according to approximately three periods: an increase in the first 16 weeks from normal values (3%) to high values (5.3%), then a return to normal values by the 40th week, and stabilization afterwards at a normal level.

d) Size of the Periosteocytic Lacunae. The size of the periosteocytic lacunae was identical in both sexes and did not vary significantly with age, its mean value being $50.71 \pm 5.52\ \mu^2$ (Fig. 6). We limited the study to the first 26 weeks of immobilization. The correlation between this and the duration of immobilization appeared significant ($r = 0.422$). The periosteocytic lacunae were slightly, but significantly, enlarged during the course of immobilization. This enlargement occured fairly late (12th week) and was temporary. The apparent shrinkage of the lacunae at the onset of the immobilization was not significant.

e) Thickness of Iliac Cortices. We studied the evolution of each of the two cortices (internal and external) in reference to the duration of immobilization. They become thinner, earlier with the external cortex and later with the internal. The eventual total decrease of thickness was similar for each of them: about 50%, from 1200 μ (normal value) to 500 μ, over a period of 40 weeks.

f) Volume of Adipose Tissue and Absolute Volume of the Cell Population of the Marrow. 1. Volume of marrow adipose tissue: The correlation between this and the

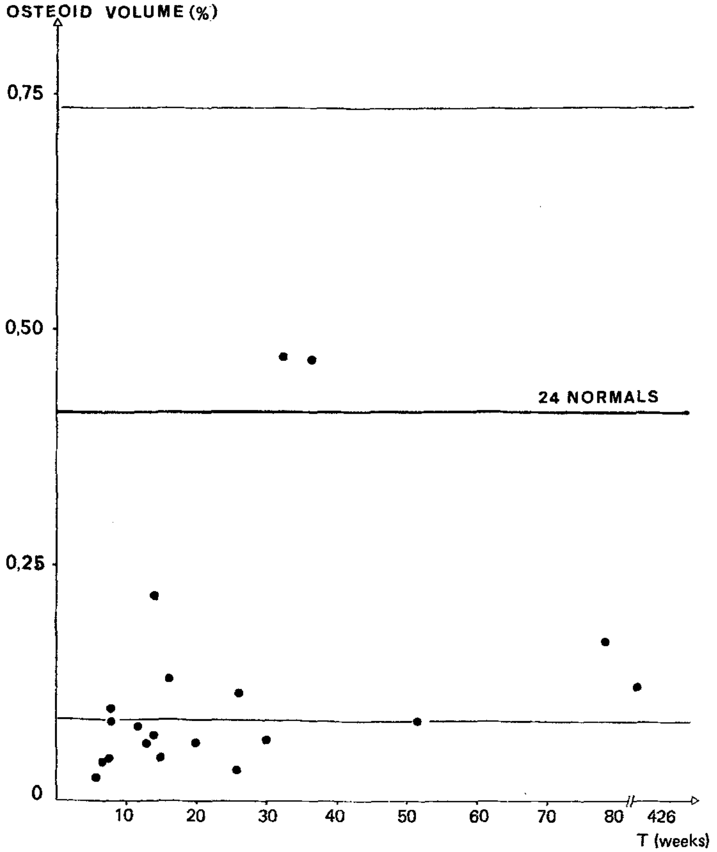

Fig. 4. Changes in osteoid tissue volume with time of with immobilization

duration of immobilization was significant ($r = 0.602$). There was an early and brief collapse in the volume of marrow adipose tissue, followed by an increase to high values (twice the normal value on the 20th week), and a final stabilization at values close to normal conditions (about 30%) (Fig. 7).

2. Absolute volume of the cell population of the marrow. This calculation checks whether the variations of the marrow adipose tissue are due to the atrophy of the bone trabeculae or not. There was a global depression of this parameter in the course of immobilization but, in fact, it remained within the normal range (42 to 52%). However, the volume of the cell population of the marrow of patients with thoracic spinal cord lesions appears to resist a change due to immobilization. In patients with cervical cord lesions, on the other hand, the cell volume decreased to about 30% until the 20th week, then returned slowly to normal values (Fig. 8).

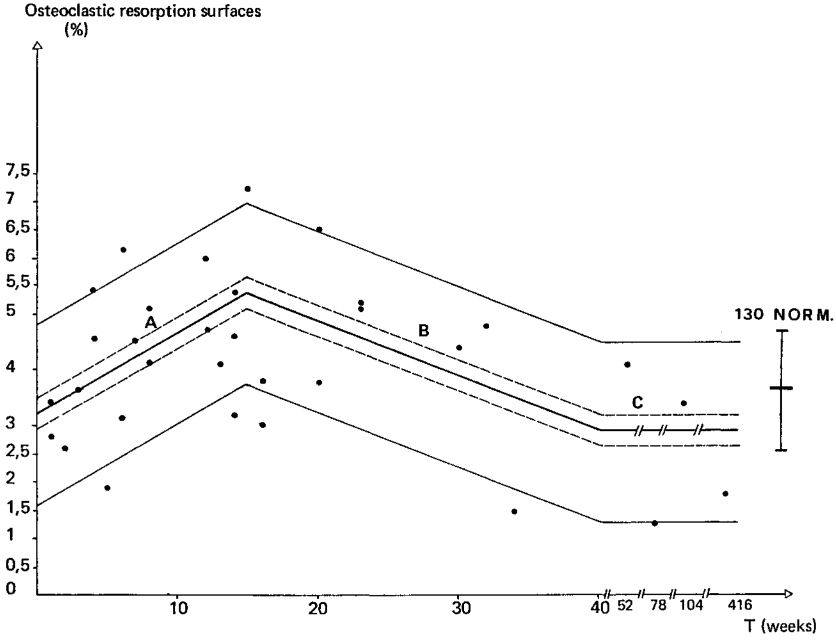

Fig. 5. Changes in trabecular osteoclastic resorption surfaces with time of immobilization

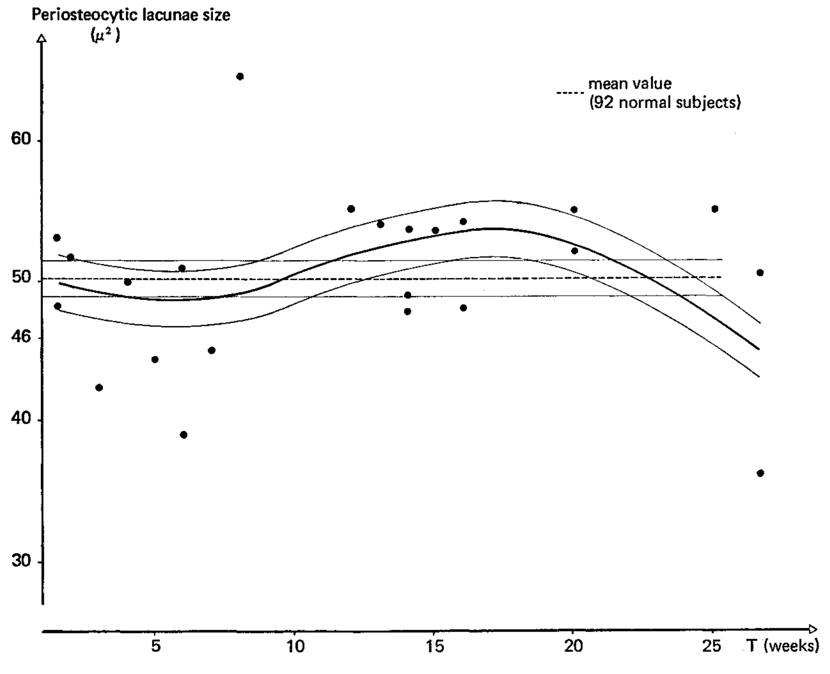

Fig. 6. Periosteocytic lacunae size and time of immobilization

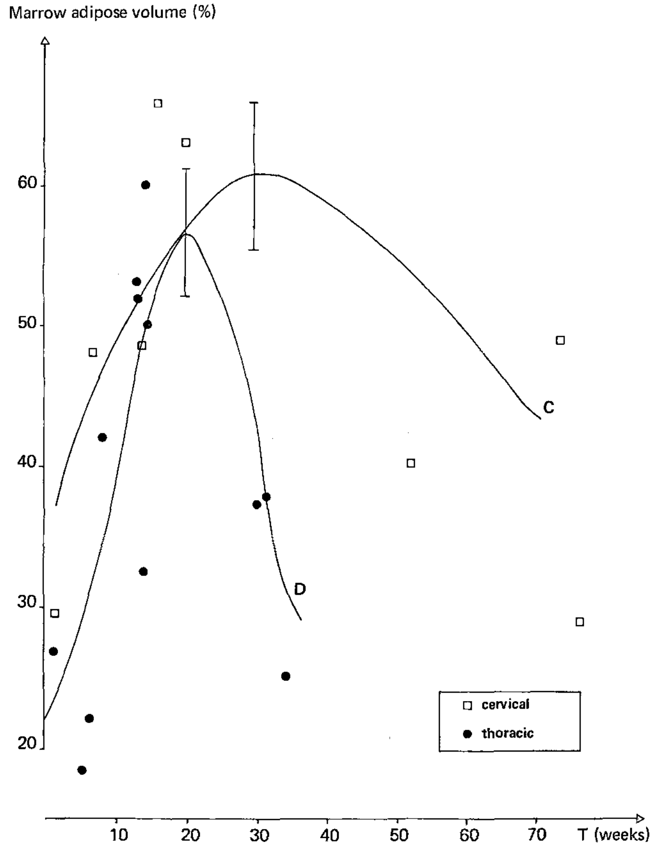

Fig. 7. Correlation between marrow adipose volume and time of immobilization

Thus the decrease of the volume of the cell population of the marrow is not merely due to the widening of the marrow cavities. It is also due to an active phenomenon, although small in magnitude, the replacement of hematopoietic tissue by adipose tissue.

Histodynamic Data

19 patients underwent double tetracycline labelling. Only a few osteons retained tetracycline in our patients and in 5 cases, no sites were labelled. In one case only, the calcification rate was normal ($1.1 \pm 0.36\ \mu$/day for controls between 30 and 39 years). In all other cases, it was extremely low because the two labels were either confused in a thin line or extremely close. Later measurement did not show any rise of the calcification rate. These data give evidence of a large decrease of the osteoblastic appositional rate (Fig. 9).

Biological Data

Plasma calcium was measured 187 times in our 68 patients. The statistical processing of the results did not show any tendency to hypercalcemia.

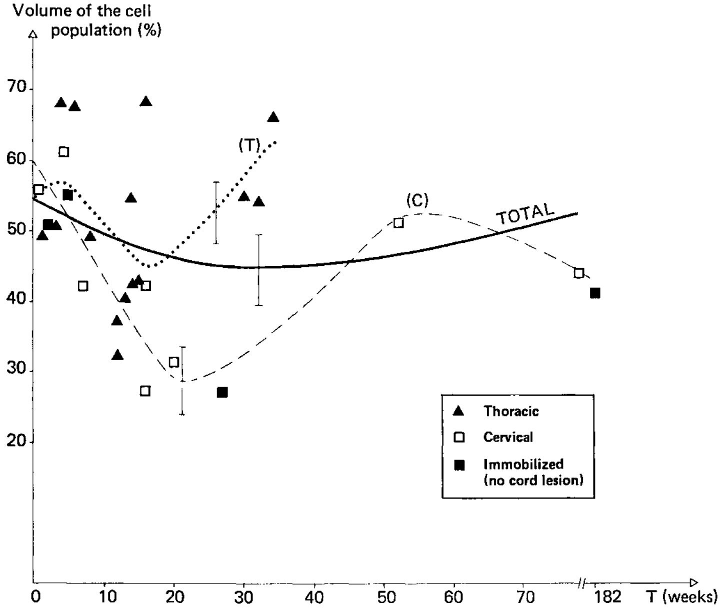

Fig. 8. Correlation between volume of the cell population of the marrow and time of immobilization (total, cervical and thoracic)

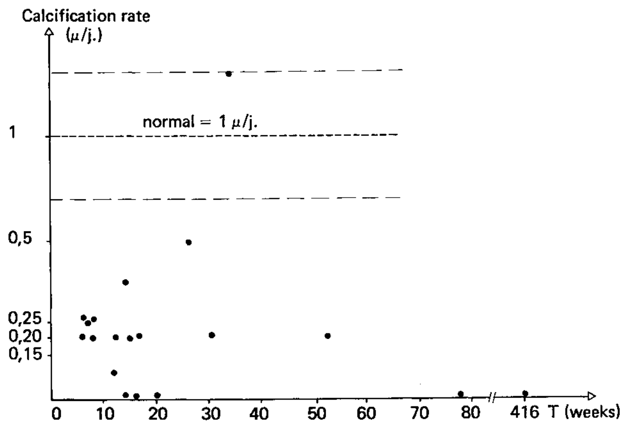

Fig. 9. Calcification rate compared with time of immobilization

Hypercalciuria (Fig. 10) appeared immediately after the onset of immobilization and reached its maximum in the 10th week, averaging at this time 390 mg/24 h. It then decreased slowly and appeared to stabilize towards the 15th week, at still at an elevated level. No significant correlation was obtained for measurements performed after 20 weeks.

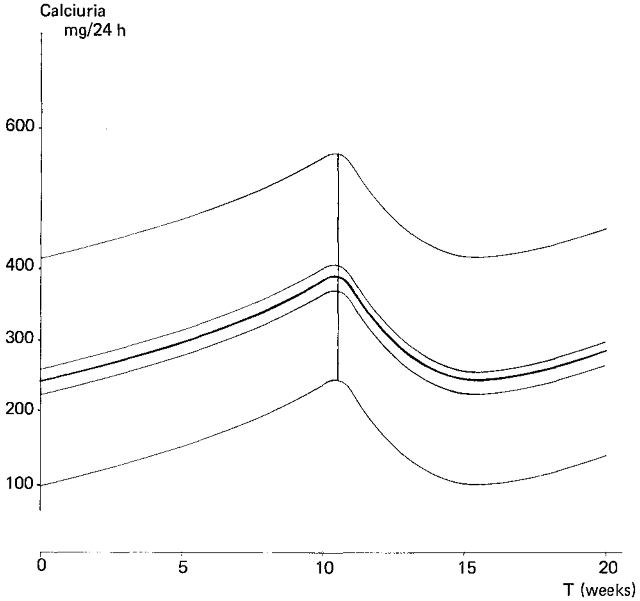

Fig. 10. Correlation between calciuria and time of immobilization (smoothing from 2 curves)

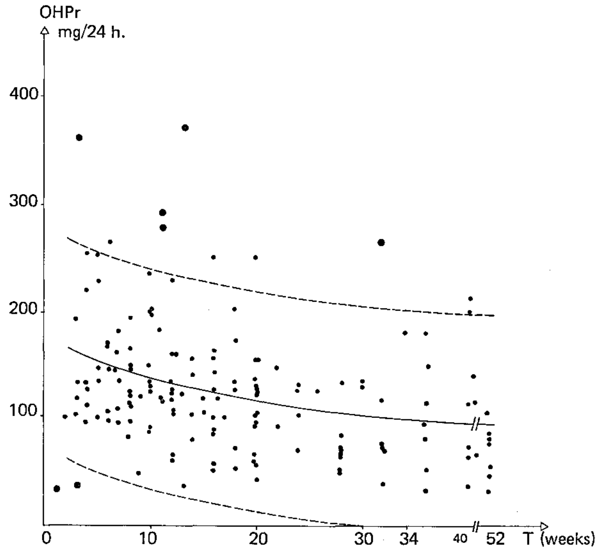

Fig. 11. Changes of hydroxyprolinuria with time of immobilization (7 samples • are excluded because corresponding to developing osteomas)

Hyperphosphoremia occured early with a maximum in the 8th week (48 mg/l) followed by a gradual decrease over about 40 weeks and a slight rise at that time.

Phosphaturia increased rapidly during the first few weeks, sometimes reaching 2500 mg/24 h, but it was impossible to say with any accuracy what the further evolution would be, because of the extreme disparity of the values. Phosphaturia appeared to remain high during at least the first 30 weeks of immobilization.

Hydroxyprolinuria. (Fig. 11) The correlation between the time of immobilization and hydroxyprolinuria was studied. The smoothed correlation was significant ($r = 0.361$): hydroxyprolinuria was high as early as the first few weeks, above 100 mg/24 h, then decreased steadily to stabilize at a mean level of 90 mg/24 h by the 52nd week.

Alkaline Phosphatase. It was not possible to evaluate this parameter during immobilization because of the presence of neurogenic osteoma. Values above 15 Bodanski units corresponded in every case to a developing osteoma. In all the other cases of spinal cord lesions, there was, at some time, a rise of the serum alkaline phosphatase above 5 Bodnaski units, even without ectopic bone formation. The phosphatase remained normal in patients without spinal cord lesions. Elevated alkaline phosphatase may be a sign of the presence of growing ectopic bone, as suggested by Furman *et al.* (1970).

Discussion

Magnitude and Limits of Bone Rarefaction

The trabecular bone volume (T.B.V.) had decreased as early as the 4th week. It should be remembered that, as early as the 2nd week of space flight, astronauts showed small but significant decreases in bone mass (Mack and Lachance, 1967; Kazarian and Von Gierke, 1969). This finding was confirmed experimentally in immobilized rats from the 12th day (Landry and Fleisch, 1964), in rabbits from the 15 day (Hardt, 1972) and in dogs from the 3rd week (Burckhart and Jowsey, 1967).

The magnitude of this bone rarefaction in the case of total immobilization is proven by the consistant decrease of the iliac bone mass which averages 33%. In one case, it reached 60%, falling from 16.8 to 7.3% in 18 weeks. The relative loss of bone depends on the subject's bone capital at the onset of the immobilization period: in a 17 year old subject, for example, the T.B.V. showed a 50% decrease, while Vignon *et al.* (1970), in 30 bed-ridden patients age 70 to 90, noted a mean T.B.V. decrease of 25% compared with the mean values of 46 non-immobilized subjects of the same age. This variability of the relative and absolute bone losses is explained by the fact that the T.B.V. does not seem to decrease below a *threshold* at which it tends to stabilize. This threshold in our subjects was reached in 25 weeks at a mean value of T.B.V. of 12.1%. In pathological bone rarefaction, vertebral collapses occur when the T.B.V. is under the "fracture threshold" i.e. 11% (Courpron *et al.*, 1973).

On the other hand, the 30 bed-ridden old patients studied by Vignon *et al.* (1970) also showed a mean T.B.V. of above 11%. The only subject of our series whose T.B.V. fell below 11% showed obvious radiological signs of vertebral fractures during the course of rehabilitation. There is therefore a steady state where bone mass ceases to decrease. This state is reached in an average of 25 weeks and corresponds to a 6% decrease of T.B.V. per month. Since, every 10 years, a normal male subject loses 5% of his T.B.V. merely through physiological ageing (Meunier *et al.*, 1973b), the surprising rapidity of evolution of disuse bone

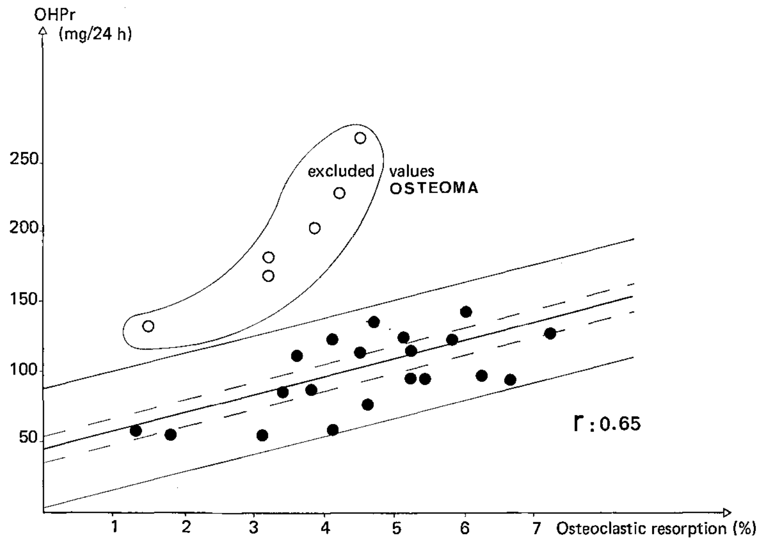

Fig. 12. Correlation between trabecular osteoclastic resorption surfaces and hydroxyprolinuria

rarefaction can thus be appreciated. The term *acute osteoporosis* seems well justified here.

Histological Bone Changes and Comparison with Biological Data

Heaney (1962) and Burckhart and Jowsey (1967) showed that immobilization involved an hyper-resorption of bone culminating in the 10th week. According to our results, the resorption surfaces are increased in man early in the course of immobilization, reaching a maximum on the 15th week. This increase coincides with a marked hyper-calciuria and hyper-hydroxyprolinuria. Hydroxyprolinuria, measured in 26 of our 34 biopsies, was plotted against the resorption surfaces. The correlation appeared significant ($r = 0.650$; Fig. 12), 6 values corresponding to developing osteoma proven by clinical examination, X-rays and Sr-87 scanning had to be excluded. Hydroxyprolinuria depends mainly on increased-resorption and destruction of collagen, but it may depend, also, on the anabolic phenomenon in an evoluting neurogenic osteoma. Klein *et al.* (1968) have stated that the rise in hydroxyprolinuria occurs earlier than hypercalciuria in acute immobilization. This fact does not appear clearly from our results, though calciuria seems slower to rise than hydroxyprolinuria.

Besides osteoclastic resorption, there is a distinct and early hypoosteoidosis, revealed by the study of the osteoid volume. These data rejuvenate Albright's hypothesis that the main factor of disuse bone rarefaction was the diminution, even a suppression, of bone apposition (Albright *et al.*, 1941). Furthermore the tetracycline labelling experiments demonstrate that the calcification rate is extremely low. Since both osteoid volume and calcification rate are low, the bone apposition rate will itself be very low.

The enlargement of periosteocytic lacunae suggests osteocytic hyperactivity of parathyroid origin (Meunier *et al.*, 1973a, c). In our immobilized patients, there was a periosteocytic enlargement of small but significant magnitude which accured only after the 11th week. Onwards the maximum enlargement (11th week) corresponds to the lowest level of phosphoremia. It may therefore be suggested that the two phenomena, comparable in magnitude, are linked and correspond to a small secondary hyperparathyroid reaction.

The absolute volume of the cell population of the marrow decreases regularly with ageing. There is a distinct parallelism between the involution of the osteoporotic cancellous bone and the rarefaction of the cell population of the marrow (Meunier *et al.*, 1973b). There is nothing comparable in the case of disuse bone rarefraction: the fall in the curve of the absolute volume of the cell population of the marrow is barely significant. Only patients with cervical spinal cord lesions showed a distinct fall in their marrow cell volume, which was temporary. This is where disuse bone rarefaction differes fundamentally from physiological bone ageing: the bone marrow is only slightly influenced by immobilization, except temporarily in the case of quadriplegia with respiratory disorder.

Attempt at Histodynamic Interpretation

In the light of Frost (1972) works, it is possible to analyze the influence of immobilization on bone remodeling. Four possibilities can be considered at the level of bone Basic Multicellular Units (BMU):

1. a decrease of their activation (or 'birth-rate'),
2. an increased 'longevity',
3. or both, i.e. decreased 'birth-rate' and increased 'longevity',
4. an abortion of forming centers, i.e. a resorption phase not followed by a formation phase or followed by a shortered formation phase.

The second hypothesis seems to be the most suitable one to explain the effect of immobilization on the bone (Fig. 13). The increased longevity of BMU creates a transient, consisting of a transitory increase of osteoclastic resorption together with a bone hypoformation, then a return to normal bone formation, and the final onset of a new 'steady state' characterized by a bone that has become 'lazy' in that it produces far less bone per unit time than it did before. If we compare this hypothesis with the periods defined by our biological and quantitative histological studies of calciuria, hydroxyprolinuria, osteoclastic resorption surface and osteoid volume, the hypothesis appears to fit the data. Thus the initial collapse in the T.B.V. can be understood better, as can its subsequent stabilization on the 25th week when the immobilized bone has reached its new steady state.

The fourth hypothesis may be also suitable. If it is assumed that some relatively prompt effect of the paralysis also caused bone forming centers to slow, this would then leave resorption in great excess over formation. Furthermore, it would lead to an irreversible loss of bone.

Disuse bone rarefaction may be encountered in any immobilized subject, in orthopaedics as well as in neurology and whatever the cause of immobilization. Of the four factors we mentioned as possibly affecting this bone rarefaction (mechanical, endocrine, neurological or vascular), the mechanical factor appears

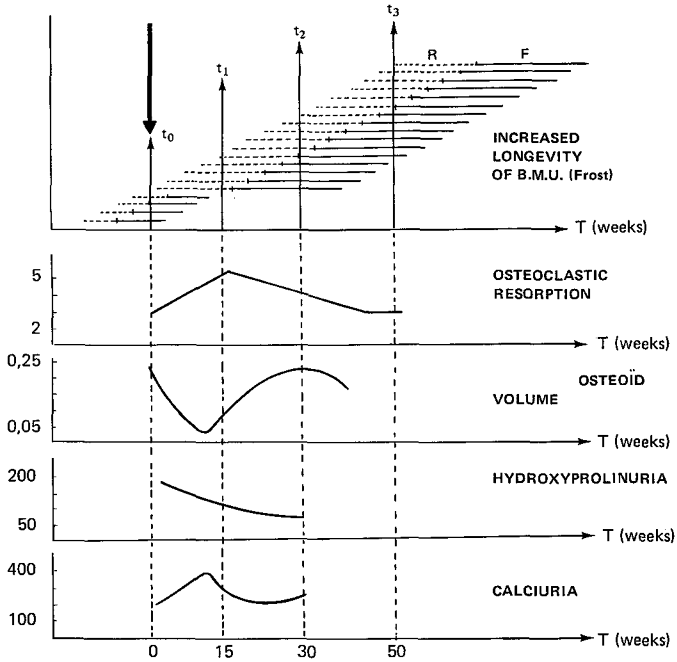

Fig. 13. Histodynamic hypothesis retained and biological and histological changes with time of immobilization

to be distinctly dominant. Hypo-osteoidosis, the earlier thinning down of the external cortex of the iliac bone, the fact that the only normal calcification rate was observed with a subject showing unilateral recuperation of the lower limb on the same side as the biopsy, put it clearly into light. The works of Bassett (1968), Kazarian and Von Gierke (1969), Epker and Frost (1965) have stressed the importance of the mechanical factors. In the light of Frost's work and the hypothesis that derives from it, Albright's hypothesis of the mechanical stimulus indispensable to osteoblasts seems to be supported, in that mechanical stresses seem necessary to keep up a resorption-formation sequence of normal balance and duration. Loss of that stimulus could delay the coming into action of osteoblasts.

Our study did not allow us to evaluate the reversibility of this bone rarefaction. The fact that we found no hyperosteoidosis at any time nor any return towards normal of the calcification rate, suggests to us that reversibility cannot appear spontaneously, as Mattson (1972) stated. Only one of our subjects, on the way to recuperation on the side of biopsy (right), showed normal osteoid volume and calcification rate after 8 months, while the T.B.V. was very low (11.1%). It would be interesting to perform a comparative quantitative histological study over a longer period, with subjects on the way to recuperation and others still immobilized. One may, however, wonder whether disuse bone rarefaction does not play a part in some subjects in the later aetiology of pathological bone rarefaction, due to the fact that they have never regained a normal T.B.V. for their age, in spite of apparently good rehabilitation in other respects.

An endocrine factor does not seem to be fundamental but the participation of parathyroid does look real, possibly as a reaction to an excessive loss of calcium. This does not exclude the permissive role of parathyroid hormone in the development of disuse bone rarefaction which Burckhardt and Jowsey (1967) and Cates *et al.* (1970) have shown. It should however be noted that Sevastik and Mattson (1971) have reported no significant modification at the level of the gland itself. Arnstein *et al.* (1973) have found high levels of parathyroid hormone in 8 out of 17 cases of quadriplegia and paraplegia, but their results were not related to the duration of immobilization.

A neurological factor remains for discussion. Indeed disuse bone rarefaction occurs as well with patients showing spinal cord lesions as with patients immobilized for multiple fractures or bone tuberculosis. On the other hand, in the course of immobilization caused by neurological disorders, the highest degree of bone rarefaction may come simply from the 'perfect' mechanical immobilization caused by the neurological disorder.

It is difficult to evaluate the importance of any vascular factor, for there is no conclusive link between the increase of the bone blood flow noted by Semb (1966) and Hardt (1972), and the bone cell phenomena observed by us. The preservation of the marrow cell populations in our patients would appear to weigh against an important vascular factor.

From the therapeutic point of view, only an early program of mobilization similar to that of aerospace training would, for the time being, seems to offer any chance of preventing the appearance of disuse bone rarefaction.

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