105 The Relation Between Temporomandibular Joint Internal Derangement, Occlusion and Parafunction. W D SNYMAN*, J C NEL and J DE VRIES, University of Pretoria and Medunsa

Occlusal discrepancies and parafunction have been frequently cited as causes of both condylar and masticatory muscle disorders. It has been postulated that both these factors result in mandibular displacement, causing compression of the intracapsular tissues, micro-trauma, pain, impairment of the blood supply and consequently degenerative changes in the temporomandibular joint. This theory is supported by the fact that alteration to the occlusion is followed by a clinical improvement in a high percentage of patients

The purpose of this study was to determine whether or not an association exists between parafunction, occlusal discrepancies and internal derangement of the temporomandibular joint. A total of 273 patients treated for temporomandibular dysfunction at the TMJ clinic, Department of Restorative Dentisty, University of Pretoria were examined for signs and symptoms of anterior displacement of the menisci, with reduction (ADTMR). The occlusal status was determined by means of an occlusal index previously described. The results indicated a small positive correlation (r_s = + 0.18) between ADTMR and eccentric bruxism ($P < 0.0005$), a higher correlation (r_s = + 0.39) between ADTMR and centric bruxism ($P < 0,0005$), and an even higher correlation between ADMR (r_s = + 0.556) and RC/IC discrepancies ($P < 0,0005$)

It can be concluded that a reasonably high positive correlation exists between internal derangement of the Temporomandibular Joint, occlusal discrepancies and centric bruxism

106 Pilot Study for Comparing two Parallel-sided Root canal Posts, Oval in Diameter with Standard cylindrical posts. N.P.Louw and *I.J.du Toit – University of Stellenbosch, TYGERBERG, 7505.

Standard parallel-sided posts are round in cross section and are difficult to fit into the oval shape of most root canals. Cast posts overcome this problem but cannot be made parallel and therefore suffers from lack of retention. A new commercial product, "Triax" (T), with oval cross-section, was subjected to tensile, shear and torque forces and compared to a commercially available cylindrical post, Parapost Plus (P).

Roots were covered with silicone and mounted in brass containers with acrylic. The root canals of the teeth were prepared for laterally condensed sealing and post-channels were drilled at low speed with the jigs and drills specified by the manufacturers. The different posts with a standardised core was cemented with zinc-phosphate cement according to the A.D.A. specifications and the tooth-post-core assembly tested to failure by means of the three mentioned force variables in a Lloyds Jay-Jay T5001 tensile testing machine.

RESULTS are not statistically significant mainly because an insufficient number of tests could be completed due to problems encountered with jigs and cores.

TENSILE: (P) n=20; force=298,23 N(ewtons) (S.D.=61,75): (T) n=14; force=346,45 N (S.D.=108,36. SHEAR: (P) n=7; force=1105,77 N (S.D.=226,20): (T) n=6; force=1418,53 N (S.D.=325,36). TORQUE: (P) n=4; force= 0,96 Newton Metres (S.D.=0,37). (T) n=13; force=1,59 Newton Metres (S.D.=1,20).

Some changes will be required in the testing protocol but it appears as though the (T) posts have better retention than the (P) posts.

107 The Pattern and Pathology of Skeletal Osteosarcoma in Pretoria.

W.F.P. VAN HEERDEN & A.J. LIGTHELM. University of Pretoria

The modern intensive surgical and chemotherapeutic approach to the treatment of skeletal osteosarcoma necessitates detailed investigation of the prevalence and pathological features of this tumour. This study was undertaken to determine the age and race incidence, the localization, radiological and pathological appearance as well as the histological classification of osteosarcoma.

One hundred and seven cases of this tumour were diagnosed at departments of Anatomical and Oral Pathology of the University of Pretoria. These cases were all reassessed and revised with regard to histological classification. There were 77 black patients, 28 whites and one coloured and Indian patient respectively. Amongst blacks the sex incidence was M:F=1,5:1 and amongst whites M:F=0,5:1. In both races the peak incidence was in the second decade. Affliction of long-bones was found in 76% of cases with the distal femur the most common anatomical site in both race groups. A high incidence (17%) of jaw tumours was found amongst blacks. The general pattern with regard to histological classification is similar to that reported in other series.

The higher prevalence of osteosarcomas amongst blacks in this series correlates with the higher incidence of sarcomas in general amongst blacks in Pretoria. The pattern of osteosarcoma in this series is largely comparable to other series in the literature, with the exception of an inverted sex-incidence amongst whites. The high incidence of jaw tumours amongst blacks further indicates a distinct difference when compared to other series.

108 Cyst Volume and Cyst Growth Potential – An In vitro Evaluation

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University of Pretoria

The increase in size and growth potential of cysts of the jaws play an important role in their behaviour and prognosis. The parameters used to determine the size of cysts include volume, the greatest area and perimeter as well as the diameter. Longitudinal evaluation of cyst growth is the correct way to evaluate the increase in cyst size. At present there is no reliable method in existence neither in vitro nor in vivo, for the determination of cyst growth. "No contact" three dimensional measuring by use of the reflex microscope offers the possibility of quantifying cyst size and hence growth potential.

Histological sections of implantation cysts in rats were evaluated by three operators with respect to the greatest area and perimeter as well as the greatest and smallest diameter. Sections made at 1,2,4,8 and 12 weeks after implantation were examined.

The reflex microscope has a specific accuracy of 4,5 and 15μm in respect of X, Y and Z co-ordinates and the differences between the measurements taken by the three operators of the greatest and smallest diameters were significant ($p < 0.05$: Kruskal-Wallis Test). No significant differences could be shown ($p > 0,05$: Kruskal-Wallis Test) in the measurements of the greatest area and diameter made by the three operators.

By the evaluation of the parameters of area and diameter the size of the cysts could be determined with greater precision and could be repeated in respect of the cysts at the above mentioned times. Consequently, longitudinal evaluation of growth potential in such a model is possible and significant.

109 Cleido-cranial Dysplasia (C.C.D.) in the South Western Cape.
L. BARTMANN, P.H. BEIGHTON, E. HORN, M.E. PARKER, M.G. SAMSODIEN, J. STAZ*, C. WALLIS, U.C.T. and U.W.C.

This autosomal dominant genetic disorder is widespread and well documented, most reports however referring usually to individuals or relatively small groups of cases. Jackson (1951) and Beighton (1978) established its prevalence in the South Western Cape. Jackson recorded 356 descendants of a Chinese seaman from Java who arrived at the Cape in 1896, settled in Somerset West, adopted the name of Arnold, embraced the Moslem Faith and seven wives and had an extensive progeny. Of these at least 70 or 19% exhibited the manifestation of this disorder. The present project is designed to pursue further these observations, to trace more family ramifications, to study in detail selected cases and to note trends, if any in the incidence of this dysplasia. To date (June 1987) 828 descendants, some comprising five generations have been noted of whom 68 or 8% are affected. Sixty one were examined in detail, their ages extending from three months (N.A.D.), nine months (with evidence) up to 79 years (marked evidence). There is no sexual differentiation in transmission. Preliminary findings, subject to biostatiscal evaluation, suggest that in many cases the penetration of C.C.D. is decreasing, especially in the clavicles, with an increase in the number of unaffected descendants.

This study is supported by the Medical Research Council of South Africa and the University of the Western Cape.

110 Ameloblastomas of the Jaws at Ga-Rankuwa Hospital : 1982 - 1987.
M.J.P. HARRIS* and E.J. RAUBENHEIMER. Medical University of Southern Africa, P.O. Medunsa.

Modern literature has indicated that there is a variation in the age, sex and site of occurrence of ameloblastomas in the various population groups.

The purpose of this study was to investigate the clinical and histopathological data of 34 patients with ameloblastomas diagnosed over a period of 5 years at Ga-Rankuwa Hospital (a referral hospital for the northern regions of Southern Africa). The age of the patients varied from 7 years to 80 years with an average of 30 years. The ameloblastomas were histologically confirmed and classified accordingly as either unicystic or solid. The gross patterns of the latter subtype were noted as either plexiform or follicular or a mixed appearance. Any differentiation in the stellate reticulum was also noted.

This tumour occurred mainly in females with an average of 32,5 years. The female to male ratio was 1,8:1 and the average age of the males was 25,5 years. Children under 18 years accounted for 26,5% of the total cases and this parallels the findings of Daramola, et al (1975). The preferred site of occurrence was the mandibular body and ramus which contradicts the study of Akinosi and Williams (1969). Six cases of unicystic ameloblastomas were identified and the average age was 18,2 years. It would appear that ameloblastomas with a plexiform growth pattern occur at a younger age than do the follicular type.

111 Quantitative Bone Changes in Rickets. P. DE VILLIERS*, E. RAUBENHEIMER, J. DAUTH and P. POTGIETER. Departments of Oral Pathology, Chemical Pathology and Orthopaedic Surgery, Medical University of Southern Africa, P.O. MEDUNSA

Microscopic diagnostic criteria in rickets are ill defined. This study was undertaken to determine quantitative microscopic bone changes in rickets and correlate these with biochemical findings.

Standardized transcortical iliac bone biopsies of 9 patients admitted at Ga-Rankuwa Hospital with clinical, biochemical and radiographic evidence of rickets were fixed in 70% alcohol and 3 micrometer thick undemineralized sections prepared and stained accordingly with hematoxylin and eosin, von Kossa and picrosirius techniques. All sections were subjected to image analysis using a VIDS II computerized system. The bone parameters assessed were total trabecular bone volume, mean cortical width, trabecular osteoid volume, mean ostecid seam width, osteoblasts per square millimeter biopsy area, trabecular resorptive surface and osteoclastic resorptive surface. Blood levels of alkaline phosphatase, parathyroid hormone, ionized calcium and inorganic phosphorus were correlated with the histomorphometric findings.

This study demonstrated that a reduction of the mean cortical width (< 570 microns), increased trabecular osteoid volume (> 4,68%), increased mean osteoid seam width (> 11,19 microns) and decreased trabecular resorptive surface (< 3,76%) are the most consistent bone changes in rickets. Furthermore, there appears to be a linear function between the high alkaline phosphatase levels and the number of osteoblasts per square millimeter biopsy area.

112 Histologic Monitoring of Mineralization Activity in Rickets.
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Evaluation of the effect of a supplemented diet on the bone formation rate in rickets is based on subjective clinical and radiographic impressions over prolonged periods. This study was undertaken to determine mineralization activity microscopically in rachitic patients receiving a balanced hospital diet.

Standardized transcortical iliac biopsies of nine hospitalized patients suffering rickets were taken after two three day cycles of tetracycline administration twelve days apart. The tissues were fixed in 70 percent alcohol and undemineralized 5 micron thick sections cut. These were stained with hematoxylin and eosin, von Kossa stain for calcium and a further section mounted unstained. The distribution of mineralization fronts were studied on von Kossa stains and unstained sections were subjected to fluorescent microscopy. The percentage trabecular bone surface labelled and the average distance between tetracycline lines were measured and the bone formation rates or BFR (expressed in microns per day) and mineralization lag time or MLT (expressed in days) calculated. Measurements obtained were compared with accepted normal values (Vigorita, 1984).

Evaluation of the mineralization front was found to be subjective. Determination of BFR and MLT proved measurable and significant. 4 cases showed sufficient mineralization activity, 4 cases required additional Vit. D and mineral supplementation and a bone formation rate of zero prompted further examination and the diagnosis of a malformation syndrome in one case.

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Static and dynamic bone changes in hospitalized patients suffering from rickets — a histomorphometric study

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Static and dynamic bone changes in hospitalized patients suffering from rickets — a histomorphometric study

Aims: The aim of this study was to assess static and dynamic bone changes in patients suffering from rickets. *Methods and results*: Transcortical iliac crest biopsies of 15 hospitalized children with rickets were taken after labelling new bone formation with two cycles of tetracycline administration 10 days apart. Undecalcified sections were prepared, appropriately stained and histomorphometric analysis performed. Static and dynamic bone changes were measured including the volume of bone and osteoid, trabecular and cortical bone dimensions and resorptive and mineralization activities. The results were compared with normal values. The nature of the mineralization fronts was noted. Trabecular osteoid volumes of all but one patient was above the normal range of 1.9% (± 0.4%). This patient suffered rickets associated with the Kwashiorkor–Marasmus syndrome. Tetracycline labelling was found to be more sensitive than subjective evaluation of the nature of the mineralization fronts. Despite a balanced hospital diet, a bone formation rate of zero was found in three cases, indicating a need for vitamin D and mineral supplementation. Seven cases had decreased mineralization lag times, indicating response to the balanced diet. *Conclusions*: This study showed that histometric analysis of labelled bone biopsies is a helpful adjunct to the diagnosis but particularly assessment of response to management of deficiency states in children.

Keywords: rickets, histomorphometry, bone metabolism

Introduction

Rickets is a disease of children characterized by decreased mineralization of osteoid with abnormalities of bone growth. Failure of mineralization occurs when the plasma level of either calcium or phosphate is decreased over a prolonged period. The most common cause of rickets in developing countries is a dietary deficiency of vitamin D or calcium. In developed countries, other causes, notably renal disease, malabsorption states and inherited conditions characterized by increased phosphate loss in the renal tubules or end-organ insensitivity to vitamin D (vitamin D resistant rickets) are more common than nutritional deficiency[1]. The diagnosis of rickets is multidisciplinary. Clinical signs are related to deficient bone mineralization and manifest as skeletal growth retardation with bone loss, enlargement of the metaphysical regions and deformities of weight-bearing bones. Biochemical criteria include decreased plasma concentrations of calcium and phosphate, elevated alkaline phosphatase and chronic acidosis[1].

Bone biopsy is the only reliable technique through which the static bone volume can be established with accuracy. In conjunction with tetracycline labelling, dynamic parameters at cellular level, such as the rate of mineralization, can be expressed and weighted against standardized norms[2]. This study was undertaken to determine the volume of mineralized and unmineralized bone and establish the rate of new bone formation in hospitalized patients with rickets.

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Materials and methods

Two cycles of tetracycline (250 mg three times a day) for

3 days, respectively, were administered at an interval of 10 days to 15 hospitalized patients suffering rickets (Figure 1). All patients received a normal balanced hospital diet without vitamin D and calcium supplementation 2 weeks prior to the taking of the biopsy. Transcortical iliac crest biopsies were fixed in 80% ethanol and embedded in a plastic polymerizing resin. Undecalcified sections of 5 μm were cut and stained with haematoxylin and eosin and the von Kossa technique to evaluate mineralization and the Picrosirius technique to highlight osteoid. One unstained section was prepared to determine tetracycline incorporation with ultraviolet illumination. The sections were viewed with a transmission light microscope attached to an image analyser (Flexible Image Processing System, CSIR, Pretoria, South Africa) and microscopic parameters, as proposed by Vigorita in 1984[2], were quantified (Table 1).

Results

The histomorphometric findings are summarized in Table 2 and the reference values are those for iliac crest bone in children[2]. The trabecular osteoid volume of all cases except case 9 were found to be significantly increased. Microscopic examination of the von Kossa stains of cases[1,2,4,5,9,12–15] showed a sharp interface between mineralized bone and osteoid. A calcification rate (CR) of above 1 μm per day was generally associated with a mottled and broad mineralization front as seen in cases 3, 6, 7, 8, 10 and 11 (Figure 2). Microscopic examination of unstained sections with ultraviolet illumination showed distinct lines of fluorescence of the tetracycline labels at the interface between osteoid and bone and in varying distance apart in all cases except 1, 12 and 15 (Figure 3).

Discussion

The taking of a bone biopsy for screening purposes in patients with suspected metabolic bone disease has little justification; less invasive methods such as radiology and blood biochemistry can be employed for this purpose. However, most of the drugs involved in the treatment of rickets are expensive and serious complications are frequently seen from vitamin D intoxication[3]. The taking of a bone biopsy to determine the response to initial therapy is justified as it offers the possibility to quantify the volume of osteoid and establish the rate of new bone formation, thereby providing important information on the prognosis of a specific therapeutic regime. This procedure identifies non-responders during the initial stage of therapy which consisted in our study of a normal balanced hospital diet. The success of

Figure 1. Clinical appearance of case 4. Note the bowing of weight-bearing bones.

dynamic bone histomorphometry relies on the labelling of new bone formed over a set period before taking the biopsy. This is done by administering two 3-day cycles of tetracycline at least 10 days apart. The iliac crest is the site most frequently used for biopsy because it is easily accessible, non-weight bearing, and therefore not susceptible to stress-induced skeletal changes[4]. Biopsies are fixed in 80% ethanol to prevent loss of tetracycline which occurs during formalin fixation[2]. The technique requires special image analysis equipment and expertise and clinicians are advised to, before taking a biopsy,

Table 1. Histomorphometric parameters determined

Term	Abbreviation	Definition
Trabecular bone volume	TBV	The percentage of the medullary cavity occupied by mineralized and unmineralized bone.
Mean trabecular width	MTW	The average width of all trabecular bone spicules.
Mean cortical width	MCW	The mean thickness of both cortices.
Trabecular osteoid surface	TOS	The percentage of bone surface covered by osteoid.
Trabecular osteoid volume	TOV	The osteoid area expressed as a percentage of trabecular bone area.
Mean osteoid seam width	MOSW	The osteoid area divided by mm of bone surface covered by osteoid.
Trabecular resorptive surface	TRS	The percentage of bone surface showing Howship's lacunae.
Osteoclastic resorptive surface	ORS	The percentage of bone surface lined by osteoclasts.
Osteoclasts per mm of trabecular perimeter	OTP	The number of osteoclasts per mm of bone perimeter.
Calcification (apposition) rate	CR	The distance between the middle of all double tetracycline labels divided by the number of days between the administration of two labels.
Mineralization lag time	MLT	The mean osteoid seam width divided by the bone formation rate.
Bone formation rate	BFR	The calcification rate times the percentage of trabecular surface labelled.
Percentage of trabecular surface labelled	TSL	The percentage of bone surfaces labelled by tetracycline labels.
Mineralization front	MF	The nature of the line of mineralization between osteoid and mineralized bone.

ascertain whether the histopathology laboratory is equipped to perform this investigation.

In contrast to osteoporosis, the bone in rickets and its adult counterpart, osteomalacia, is qualitatively abnormal and characterized by a failure of mineralization. The earliest bone change occurring in rickets is the disappearance of mineralization fronts. The border between osteoid and mineralized bone loses its ill-defined granular nature and is replaced by a distinct line separating unmineralized and mineralized bone. This change is associated with impairment of mineralization of osteoid and impacts on the dynamic measurements as a decreased uptake of the tetracycline label. The percentage of trabecular surface labelled (TSL) subsequently decreases and the calcification rate (CR) falls below 0.54 μm per day[2,5]. These parameters are also the first to respond to effective treatment. The sharp mineralization fronts (as seen in cases 1, 2, 4, 5, 9, 12–15) are indicative of a lack of sufficient mineralization of osteoid. Tetracycline labelling, however, appears to be more sensitive, as in a number of these cases (2, 4, 5, 9, 13 and 14) the rate of mineralized bone formation (BFR) was found to be adequate despite sharp mineralization fronts. The BFR, which is decreased in untreated rickets, brings the CR into perspective by expressing the dimensional volume of new bone formed more accurately. This parameter prognosticates the potential outcome of a therapeutic regime and may obviate the necessity to introduce vitamin D and mineral supplementation. The three cases in our study with BFRs of zero warrant vitamin D and mineral supplementation. Failing response, further investigations

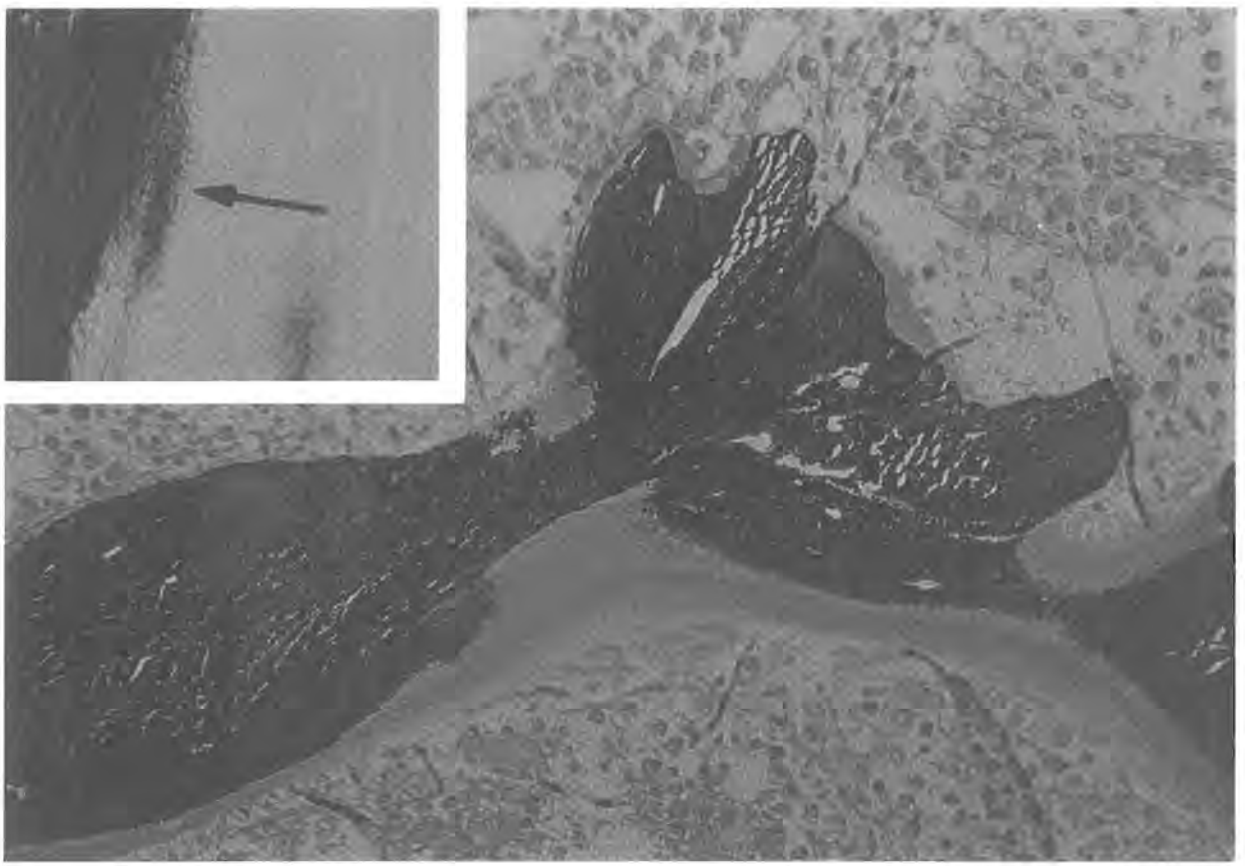

Figure 2. Photomicrograph of a mineralized bone trabeculum (black) covered by a wide osteoid seam (von Kossa stain, ×100). The inset shows a higher magnification of a wide and mottled mineralization front (arrow) (von Kossa stain, ×250).

should be employed in order to exclude poor compliance to therapy or establish a non-nutritional cause for their bone deficient state. In the event of not finding any other cause, the status of the osteoid matrix may be so abnormal as to prevent mineralization[6]. The mineralization lag time (MLT), which is generally longer in rickets than in other bone deficiency states, is an indication of the discrepancy between the process of osteoid and mineralized bone formation. This parameter is generally lengthened in rickets and a decrease thereof is an indication of response to therapy, as achieved in seven of our cases.

After mineralization has ceased, the second pathognomonic microscopic feature of rickets is introduced during which the osteoblasts continue to produce osteoid which remains unmineralized, and an increase in the volume of osteoid occurs. This change manifests as a widening of the osteoid seams and reflects as an increased mean osteoid seam width (MOSW). Eventually, the osteoblasts cease to deposit osteoid, dedifferentiate and osteoblasts elsewhere on the bone surface lay down osteoid which also thickens and subsequently becomes inactive. The bone surface covered by osteoid increases and the trabecular osteoid surface (TOS) and volume of osteoid (TOV) subsequently rises. This phase continues until large areas of bone surface are covered by abundant osteoid. The increase in the trabecular bone volume (TBV) in rickets is therefore a result of osteoid formation. Extensive osteoid coverage prevents osteoclast mediated bone resorption and reflects as a decrease in the osteoclast-associated parameters[2,5]. In most of our cases, the osteoclastic resorptive surface (ORS) and osteoclasts per mm of trabecular perimeter (OTP) were found to be elevated despite abundant osteoid. This is probably indicative of the osteoclast activating effect of secondary hyperparathyroidism. In the atrophic (or porotic) form of rickets, osteoid is, however, reduced in volume. This manifestation is seen in the Kwashiorkor–Marasmus syndrome associated with rickets and indicates the coexistence of two deficiency states in the same child[7]. Our case 9, which exhibited narrow osteoid seams and a low trabecular osteoid surface, falls in this category. These changes in

Table 2. Histomorphometric findings in 15 patients treated for rickets

	Ref. Value (SD)[2]	Case 1 Male 13 years	Case 2 Female 15 years	Case 3 Male 6 years	Case 4 Male 5 years	Case 5 Male 7 years	Case 6 Male 6 years	Case 7 Male 3 years	Case 8 Male 5 years	Case 9 Male 5 years	Case 10 Male 4 years	Case 11 Male 4 years	Case 12 Female 13 years	Case 13 Male 5 years	Case 14 Male 5 years	Case 15 Female 3 years
TBV	22.5% (3.5)	15.4	23.6	23.3	14.1	36.2	21.2	11.5	29.7	22.9	20.8	14.9	16.2	24.4	35.4	26.5
MTW	213 μ (65)	128.3	186.1	187.2	121.5	258.1	126.4	127.5	152.4	130	155	203.8	158.5	59.4	159.8	110
MCW	909 μ (98)	104.9	570.4	394	416.3	1157	419	120	379.4	421	344	748	579	103.4	271.1	186
TOS	18.9% (5)	29.3	8.1	68.8	34.3	55.9	20.9	24.0	21.3	14.2	31.8	10.4	15.5	95	64	45.5
TOV	1.9% (0.4)	35.3	10.3	9.4	9.9	14.8	11.0	11.3	4.7	0.6	6.1	3.9	8.5	57.8	28.9	21.8
MOSW	9.7 μ (0.4)	44.1	27.2	29.2	23.7	31.8	29.5	13.7	18.2	5.0	10.2	10.8	16.1	33.5	40.4	16.9
TRS	5.1% (0.6)	6.5	1.3	2.4	3.2	5.4	2.0	0.7	3.8	1.7	3.6	1.3	2.1	3.9	4.2	4.6
ORS	0.13% (0.6)	0.8	0.3	0.5	0.8	1.4	0.1	0.2	1.4	0.2	0.6	0.3	0.1	0.2	0.6	2.5
OTP	0.03 (0.01)	0.1	0.07	0.1	0.1	0.4	0.06	0.04	0.4	0.04	0.1	0.06	0.04	0.05	0.8	5.1
CR	0.64 μ (0.1)	0	0.9	1.6	0.6	0.8	1.3	1.4	1.9	0.9	1.6	1.6	0	0.7	0.2	< 0.1
MLT	29 days (3)	> 100	0.8	0.5	3.3	5.6	0.5	0.2	3.6	0.4	0.4	1.5	> 100	0.8	33.7	> 100
BFR	0.44 μ (0.04)	0	35.8	63.2	7.1	5.7	59.0	57.7	5.1	12.8	24	7	0	42	1.2	0
TSL	12.8% (2.3)	0	39.3	39.5	11.8	7.1	45.4	41.2	13.2	14	15	5	0	60	6	0

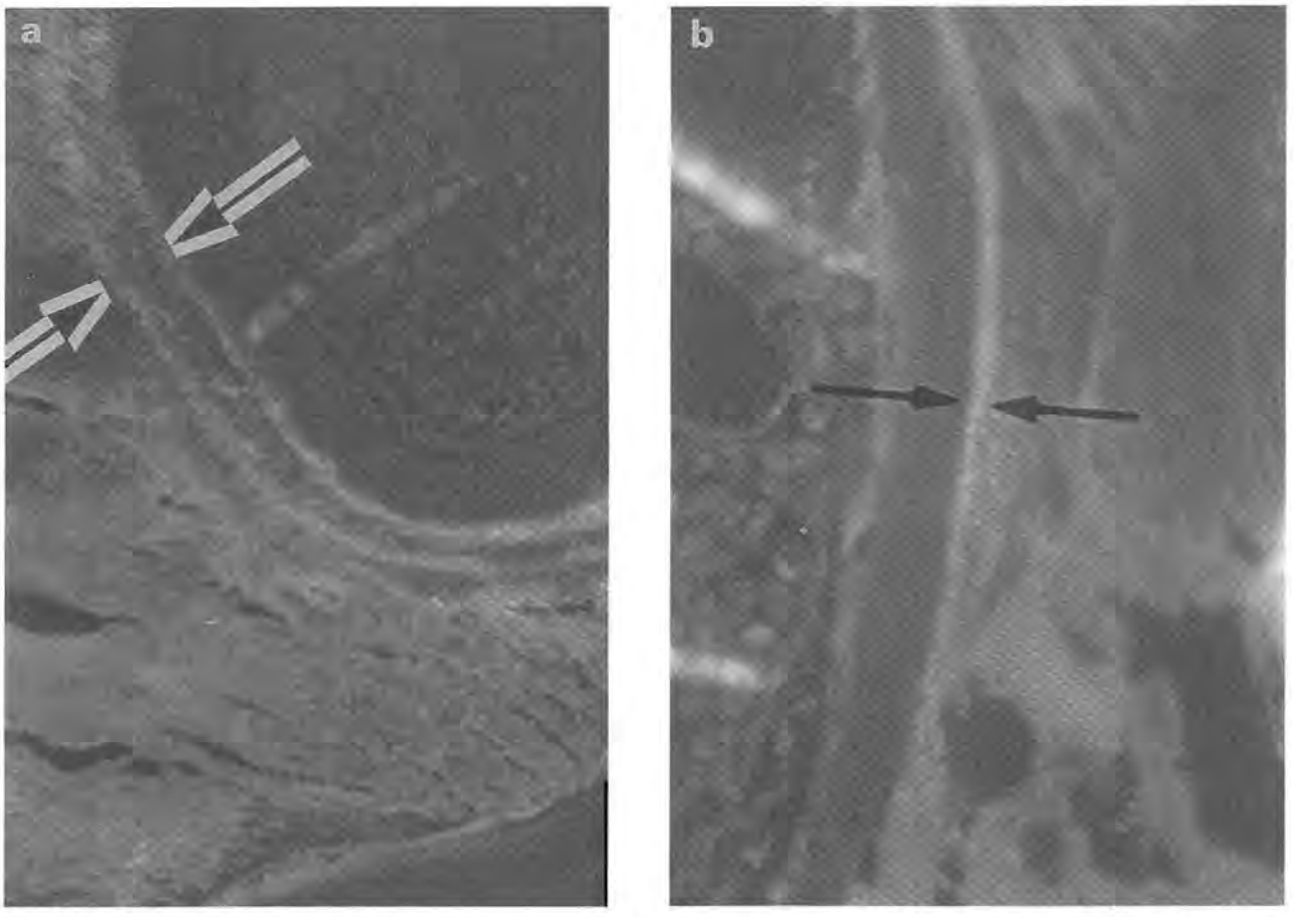

Figure 3. Fluorescing tetracycline labels. **a**, The arrows show spacing between the fluorescing lines in a case with a normal CR. **b**, The superimposed lines on the right (arrows) indicate inadequate mineralization activity (unstained sections viewed under ultraviolet light, ×20).

rickets indicate the need for a more extensive therapeutic regime addressing protein and calorific malnutrition.

Histomorphometric analysis of bone is a helpful adjunct to the diagnosis and therapeutic management of bone deficiency states. The refinement which it introduces contributes to accurate diagnosis and limit the usage of broad and non-specific terminology like juvenile osteoporosis. Labelling of dynamic bone changes and the quantification of the response to a normal balanced hospital diet obviates the need for expensive and potential toxic vitamin D supplementation in most patients with rickets. It furthermore contributes significantly to the early identification of those in whom additional investigations are required to diagnose a non-nutritional cause for the bone deficiency state.

Acknowledgement

Mrs C.S.Begemann is thanked for secretarial assistance.

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This presentation will enunciate the following:
1. What is telepathology
2. Classification, description, advantages and disadvantages of different telepathology systems
i Static telepathology
ii Robotic (Dynamic) telepathology
3. Requirements of a telepathology system: The hardware and the software
4. Transmission of images
5. Causes of failure of telepathology systems
6. Results of a study of the use of telepathology in the field of oral pathology

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THE ROLE OF HISTOMORPHOMETRY IN THE DIAGNOSIS AND MANAGEMENT OF METABOLIC BONE DISEASES.

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Objectives: The objective of this study was to determine the role of dynamic microscopic changes in the diagnosis and management of metabolic bone diseases.
Methods: Biopsies were taken of tetracycline labeled bone of 30 patients suffering metabolic bone diseases of diverse etiologies. The biopsies were fixed in 80% alcohol, embedded in resin, sectioned and stained with the picrosirius-, von Kossa- and H&E staining techniques. Unstained sections were viewed with ultraviolet illumination. Static and dynamic bone parameters of osteoblastic -, osteoclastic – and mineralization activities were measured with the aid of an image analysis system linked to a light microscope. Histomorphometric findings of each case were matched with reference values, biochemical results, the final diagnosis and therapeutic responses.
Results: The study showed that the responses to mineral supplementation of patients suffering rickets and osteomalacia were greatly influenced by the volume of osteoid. Cases in which more than 80% bone surfaces were coved by osteoid were characterized by hypocalcemia. Rickets associated with the kwashiorkor – marasmus syndrome showed low osteoid volumes and a poor clinical response to mineral supplementation. Active tunneling resorption was found to be the benchmark of hyperparathyroidism and diletescence rather than osteoclastic activity the cause of bone loss in disuse osteopenia. In chronic liver failure, hypogonadism and osteogenesis imperfecta all osteoblast – and mineralization activities were found to be reduced. Spindle shaped osteoblasts distinguished the bone changes in osteogenesis imperfecta from those of liver failure and hypogonadism. Renal osteodystrophy could be classified on histomorphometric parameters in hyper- and hypo dynamic subtypes and provided useful information on the need for parathyroidectomy.
Conclusion: Histomorphometry plays a central role in the diagnosis and management of metabolic bone diseases.

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HUMAN PAPILLOMAVIRUS-ASSOCIATED ORAL LESIONS IN HIV-PATIENTS RECEIVING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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Oral papillomatous lesions are a manifestation of human papillomavirus infection that have been noted infrequently in HIV-infected persons. In the era of HAART the incidence of oral warts in HIV-seropositive patients appears to be increasing, whereas the prevalence of oral lesions strongly associated with HIV-infection have been noted to be decreasing.
Objective: Aim of this study was to characterize oral papillomatous lesions and their relapses in patients with HIV infection treated with HAART on their clinical aspects, to determine the presence of HPV-DNA by PCR and to specify the HPV genotypes by sequencing analysis.
Material and method: 25 biopsies of oral papillomatous lesions diagnosed according to accepted features were obtained from six HIV-patients receiving HAART for > 6 months (homosexual males, median age 36.5 year. [29–68 year.], median CD4 ± cells 238/μL [32–460 μL], median viral load < 50/mL [<50–12000/mL]). 8/25 biopsies were taken from recurrent lesions. Clinically the lesions were diagnosed as verruca vulgaris (7/25), condyloma accuminatum (16/25) and focal epithelial hyperplasia (FEH) 2/25. In two patients the lesions were multiple and spread throughout the entire mucosa. Routine histopathology was performed, additionally, biopsies were tested for the evidence of HPV-DNA by PCR using L1 consensus primers and subjected to sequencing analysis.
Results: 22/25 biopsies harboured HPV-DNA. Sequencing analysis revealed HPV-32 in 10/21 specimens, HPV-72 in 6/21, HPV-55 in 1/21 and HPV-7 in 1/21 cases. In one patient an unknown HPV-type was identified. In condylomata accuminata HPV-32 and -72 was detected, in verrucae vulgares HPV-7 and 32, in FEH HPV-55 and one HPV-genotype not yet identified. In one patient the biopsy obtained from recurrent lesion clinically diagnosed as verruca vulgaris revealed two different types of HPV (-7 and -32).
Discussion: Studies on HPV-associated oral lesions have suggested a correlation between FEH and HPV-13 and -32, verruca vulgaris with HPV-2 and -4 and condyloma accuminatum with HPV-6 and -11. However, in HIV-patients unusual HPV types such as HPV-7 have been identified from oral warts. In the present study HPV-DNA of a single type could be found in oral papillomatous lesions of different clinical appearances and different HPV-genotypes could be identified in lesions of similar appearance. HPV-55 which was associated with FEH in this study is mainly related to lesions of the genital tract. So far, HPV-72 has only once been identified in an oral wart with atypia in a HIV-positive patient. In our study HPV-72 was evident in two patients with multiple condylomata. This study suggests that (1) HIV-infection seems to predispose individuals to oral infection with uncommon as well as new HPV-types and (2) the increased risk of HPV infection in patients receiving HAART may represent a form of immune reconstitution syndrome occuring in response to improved cell-mediated immune function.

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DETECTION OF TP53 MUTATIONS IN ORAL LEUKOPLAKIA BRUSH BIOPSY

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Histopathologic Changes in Metabolic Bone Disease

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Summary: Metabolic bone disease encompasses a heterogeneous group of disorders that influence skeletal metabolism and structure. They are generally diagnosed at an advanced stage and manifest clinically with stunted skeletal growth in children and pathological fractures in adults. Biochemical markers for bone metabolism are equivocal and microscopic examination of labelled bone remains the gold standard for the diagnosis and accurate monitoring of the response to therapy. This article reviews the role of microscopic bone changes in the diagnosis and management of metabolic bone disease.

Keywords: metabolic bone disease, rate of bone formation, histomorphometry

INTRODUCTION

Metabolic bone disease encompasses a heterogeneous group of disorders that affect skeletal collagen or mineral deposition. These include malnutrition, malabsorption, ageing, immobilization, endocrine and neoplastic diseases, drugs and toxins, genetic conditions, kidney disease and HIV infection. In developed countries the most common associations are old age, immobility and the use of drugs whereas in the developing world, nutritional factors are more frequently implicated. Metabolic bone disease generally progress sub-clinically and are diagnosed late when clinical manifestations focus attention on end stage skeletal debilitation. During childhood, growth of the skeleton is retarded and although all bones are affected, most changes impact on the growth centres of long bones. Significant mortality, morbidity and societal expense result in adults where pathological fracture is the key manifestation.

DISCUSSION

Assessment of bone metabolism

Until recently, the only available biochemical markers for bone turnover were total serum alkaline phosphatase for the monitoring of bone formation and urinary hydroxyproline for bone resorption. Both are not specific to bone metabolism and unable to detect small changes in bone turnover. The lack of sensitive markers for bone metabolism has directed efforts to develop new and more specific biochemical assays. Bone GLA-protein (BGP), also called osteocalcin, is the only protein found to be specific for bone and dentin. It is produced by osteoblasts and might play a role in the recruitment of osteoclasts thereby being one of the mediators that couple the activities of these cells. A fraction of newly synthesized BGP is released into circulation, where it can be measured by radioimmunoassay. Serum BGP measurements should be interpreted with caution as many unresolved questions remain on the volume distribution, metabolic clearance, influence of vitamin D deficiency, circadian rhythm and breakdown thereof by osteoclasts (1). Urinary excretion of pyridinium cross-links is the most tangible improvement in the search for a specific biochemical marker for bone resorption. Hydroxylysylpyrinoline (HP) is widely distributed in type I collagen of bone and the type II collagen of cartilage, but is absent in skin collagen. Lysylpyrinoline (LP) is present

only in type I collagen of bone (2). An increase in urinary HP and LP has limited application and has been associated with hyperparathyroidism (3), menopause (1), rheumatoid arthritis and osteoarthritis (4). Finding a sensitive biochemical marker for bone resorption remains a challenge, the outcome of which may lie in studies on the multitude of peptides released during osteoclast induced bone resorption.

Refinements of histological methods not only established microscopy as the gold standard in the diagnosis of metabolic bone disease (Table 1), but also contributed significantly to the understanding thereof (5,6). In order to assess sub clinical bone changes at cellular level, a transcortical biopsy of a non-weight bearing bone, like the iliac crest, is advised (5). The biopsy is performed 2 days after the last of two 3-day cycles of tetracycline administration, which are at least 10 days apart (7). The presence of both cortical and trabecular bone in the biopsy is important, as significant differences exist in the metabolism of these two bone compartments. Cortical bone, which makes up 85% of the total bone mass of the body, is exposed to the periosteum and is remodelled internally through an extensive network of Haversian and Volkmann canals. Trabecular (or cancellous) bone constitutes the remaining 15% (8), is enveloped in fat and marrow, each spicule is remodelled on its surface and is relatively avascular internally. Fixation of the biopsy in 90% ethanol minimizes loss of tetracycline. Undecalcified sections are stained with the Von Kossa- and Picrosirius techniques which highlight mineral and osteoid content respectively. Unstained sections are examined with ultraviolet illumination in order to assess the extent of tetracycline incorporation. Static and dynamic bone changes are measured with the aid of an image analyzer. The static changes represent the state of the bone at the time at which the biopsy was taken. These include indices reflecting bone mass, volume of osteoid and osteoclastic resorption. The dynamic parameters reflect the rate and extent of incorporation of mineral (tetracycline) into bone between administrations of the labels. Static and dynamic histomorphometric measurements are compared with standardized norms in young healthy adults (Table 2)(5) and correlated with clinical, radiologic and biochemical findings thereby accurately assessing all aspects of bone metabolism. A recommended diagnostic flow chart is reflected in Figure 1.

Factors influencing bone metabolism

Malnutrition

Failure of mineralization occurs when plasma concentrations of either calcium (Ca) or phosphate (P) are decreased over a prolonged period. The most common causes thereof in developing countries are dietary deficiencies of Vitamin D (Vit D) or Ca whereas in developed countries, other factors like renal disease and malabsorption states are more frequently implicated (9). In growing children these conditions lead to rickets and after skeletal growth has ceased, osteomalacia. Rickets is amongst other clinical features characterized by cranial deformities including frontal bossing and craniotabes (softening of the skull), softening of the metaphysis and bowing of weight bearing bones (Fig. 2). With Vit D and /or Ca deficiencies stimulation of PTH secretion is aimed at maintaining normal serum Ca levels. These biochemical changes are associated with quantifiable microscopic alterations to the morphology of bone. In contrast to osteoporosis, bone in rickets is qualitatively abnormal and characterized by excessive osteoid deposits (reflected as an increase in indices of osteoid) and a lack of mineralization (manifesting as a decrease of indices of mineralization). Secondary hyperparathyroidism, which occurs only in Ca and Vit D deficiency states (10) leads to increased osteoclast induced resorbtive activity. Normal blood Ca concentrations are maintained as long as exposed bone surfaces show resorbtive facets (Fig. 3). When all surfaces are covered by osteoid, further osteoclast-mediated bone resorption is prevented and hypocalcemia develops. The term 'hypertrophic' rickets has been used to describe patients with broad osteoid seams lacking mineralization (9). These patients usually respond favorably to mineral and Vit D supplementation. A broad mineralization front and increased dynamic measurements are therefore indicators of a good response to therapy (Fig. 4). In the 'atrophic' form of rickets osteoid is significantly reduced in volume, dynamic measurements are low and mineralization fronts narrow. This manifestation is often seen as part of the kwashiorrkor-marasmus syndrome (or protein calorie malnutrition) complicated by Ca and P deficiencies. This complex deficiency state is resistant to Vit D and mineral administration alone and requires additional amino acid supplementation in order to provide nutritients for osteoid production (7). The histomorphometric findings in rickets and osteomalacia are diagnostic (Table 3).

Diets high in fibre may adversely affect Ca retention (11) and decrease Ca absorption from the gastrointestinal tract (12). This is the result of the high Ca binding capacity of wheat bran (13). Moreover there is evidence that the Vit D mediated mechanism for active absorption of dietary Ca may be diminished by dietary fibre (14). The diets of vegetarians must be carefully monitored, as they are inadvertently Vit D deficient due to their low fat content (15). Osteomalacia in vegetarians is characterized by a severe reduction of trabecular bone volumes, an increase in the trabecular surfaces covered by osteoid, severely thickened osteoid seams and reduced mineralization activities. Serum markers are of no value as the majority of patients have normal Ca, Vit D and alkaline phosphatase concentrations (16). Magnesium (Mg) deficiency is an important risk factor for osteoporosis in humans. Several studies have reported significant reductions in serum - and bone Mg concentrations in postmenopausal women with osteoporosis. Elderly women who consumed less than 187 mg Mg per day were found to have significantly lower bone mineral densities than women whose average dietary Mg intake exceeded this figure. Western diets have intakes of Mg which are significantly below recommended levels. This leads to reduced bone formation and subsequent reduced bone volume with increased skeletal fragility (17). Vitamin C deficiency results in a decreased bone formation rate with normal resorption, resulting in a decreased bone volume (18). Failure to respond to dietary supplementation should prompt further investigations to exclude vitamin D resistant rickets, malabsorption syndromes, debilitating disease states or poor patient compliance. Causes of malabsorption include gluten sensitive enteropathy, gastrectomy, gastrointestinal tuberculosis, Crohn's disease, sarcoidosis and conditions leading to a deficiency of bile salts in the intestine. Bile salts are an absolute requirement for optimal Vit D and Ca absorption in the gastrointestinal tract (15).

Ageing

Although age related bone changes are not classifiable as disease, they are being recognised as a major health and medical economic problem (19). The overall direct cost, including rehabilitation, of osteoporosis (which is defined as quantitively reduced but qualitively normal bone) in both men and women were estimated in 1997 to be 52.5 million US$ per million of the population of the United States annually (20). During puberty bone mass increases about 3-fold over a few years (21). Peak skeletal bone mass is achieved between 25 and 35 years of age (22). It is generally accepted that those who achieve a higher peak bone mass are less at risk of developing osteoporotic fractures in later life. Between 60% and 80% of variance in

bone phenotypic expression at any age is genetically determined and the effect of lifestyle and hormonal factors modifying the remaining. The former include diet, exercise, alcohol consumption and tobacco use amongst a range of others that are less well characterized. Excessive ingestion of common salt, phosphorus or caffeine and the use of tobacco and alcohol have been associated with increased fracture incidence in epidemiological studies. Dietary intake of Ca has been a major focus in age related osteoporosis. Intakes of 1200 – 1500 mg/day have been recommended around puberty and after menopause and 800 – 1000 mg/day suggested for other stages of life (23). The administration of Ca and Vit D reduces the incidence of hip fracture among elderly women in nursing homes (24). Estrogen hormone replacement therapy in estrogen deficient postmenopausal women leads to a gain in alveolar bone density (25) as well as a significantly lower risk for tooth loss than age matched non-estrogen users (26).

Histomorphometric studies are valuable in diagnosing and monitoring the response to therapy of patients suffering osteoporosis (Table 4). Men, like women, loose trabecular bone more rapidly than cortical bone and several studies have shown that the trabecular bone volume of the iliac crest declines with age at approximately equal rates in men and women (27). Data indicate that age related osteoporosis occurs predominantly by a process that removes entire structural elements and is characterized by trabecular bone loss rather than thinning (Fig. 5). The trabeculae that remain are more widely separated and some may even undergo compensatory thickening (28). The concept of two osteoporotic syndromes in women, type I with predominantly trabecular bone loss related to gonadal steroid deficiency and type II with cortical bone loss greater or equal to trabecular bone loss due to ageing (29) has not yet been demonstrated in men. The lower incidence of complications of osteoporosis in men may be due to a higher peak bone mass at skeletal maturity (30), shorter life expectancy and the absence of a distinct menopause equivalent with accelerated bone loss in men (31).

The causes of age related osteoporosis in healthy individuals are certainly multifactorial. A recent study suggests that preserved osteoclast activity and decreased osteoblast function are the cellular events responsible for bone loss during ageing (31). Osteoblasts of both genders appear to deposit inadequate volumes of new bone insufficiently balancing sustained osteoclast resorption. This results in loss of bone trabeculae at foci of resorption. The trabecular bone volume is reported to reduce by 40% between the ages of 20 to 80 years in

healthy individuals and the osteoblast to osteoid interface and osteoid labelling for mineral depositioning each by more than 18% (27). However not all bone is lost through osteoclastic activity. Bone loss from quiescent lamellar bone surfaces, without osteoclastic activity, termed delitescence, could partly be responsible for age related osteoporosis (32). The standardized norms reflected in Table 2 should in practice be adapted to the age of the patient.

Immobility

Physical activity is important for the maintenance of skeletal bone mass. In the absence of mechanical stimulation, the surface area of bones decrease significantly when compared to those subjected to mechanical stimulation (33). Disuse osteoporosis may be generalized or confined to immobilized skeletal segments. The loss of skeletal mass could be as high as 25 – 45% over periods as short as 30 – 36 weeks. Orbital space flight, which results in unloading of the skeleton, leads to bone loss approximating 20% of the skeletal mass in some astronauts during short-term weightlessness (34). The resulting bone loss can be arrested by estrogen replacement (35). Disuse osteoporosis results from a combination of a moderate decrease in osteoblastic activity and a marked increase in osteoclastic resorption of trabecular bone. Bone loss is primarily trabecular and subperiosteal scalloping of cortical bone may be evident. Hypocalcemia, hypercalcuria, ectopic calcifications and hydroxyprolinuria frequently occur in disuse osteoporosis. Phosphorus supplementation and corticoids may prove effective in reversing the hypocalcemia and hypercalcuria associated with immobilization and prevent metastatic calcifications from occurring (34).

Endocrine factors

Growth hormone (GH) regulate bone resorption and bone formation and has a direct effect on osteoblasts via GH binding sites as well as an indirect effect via insulin–like growth factor (IGF-1). Estrogen facilitates the GH – IGF-1 axis whereas glucocorticoids appear to suppress GH induced action of IGF-1. Although little success has been achieved in the identification of GH receptors on osteoclasts, it has been proven that GH regulates osteoclast formation in bone marrow cultures. This explains the biphasic action of GH. GH secretion initially results in increased bone resorption with a concomitant bone loss followed later by increased bone formation. The point where bone formation balances bone resorption, is usually reached after 6 months. Net bone mass gain after GH therapy may take some time as

the initial decrease in bone mass must first be replaced. Both cortical and trabecular bone parameters are increased by prolonged GH secretion. Conversely, cortical and trabecular bone measurements are reduced in pituitary dwarfism (36).

In normal subjects 0.2 osteclasts should be present per mm^2 trabecular bone and 5% of bone surfaces show Howships lacunae (Table 2). Recent onset hyperparathyroidism is characterized microscopically by foci of tunnelling resorption (Fig.3) as well as an elevation of all indices of resorbtive and osteoblastic activities. Subperiosteal osteoclasts are rare, and when present indicative of a high resorbtive state as seen in hyperparathyroidism. The development of brown tumors of hyperparathyroidism is, unlike generally believed, a rare event and occurs only in a small percentage of cases with increased parathyroid activity. Fibrous marrow scars may be indicative of healed or long-standing hyperparathyroidism (7). The rate at which osteoid is deposited in empty Howships lacunae after restoration of normal parathyroid function, is indicative of the efficiency of recruitment of new osteoblasts. Skeletal changes similar to those seen in hyperparathyroidism but associated with normal PTH concentrations are indicative of Jansens disease, a condition characterized by normal PTH secretion with overactive PTH receptors on osteoclast (37).

Sixty seven percent of men with spinal osteoporosis suffer an identifiable cause, which may include ethanol abuse, hypogonadism or hypercortisolism (38). Hypogonadism in males appear to affect mainly the bony cortex (39) whereas gonadal steroid deficiency in females leads predominantly to cancellous bone loss (29). Corticosteroid deficiency manifests as cancellous osteopaenia whereas excess results in decreased bone growth, except in growing males (40). Decreased bone growth is aggravated by corticosteriodal inhibition of gastrointestinal absorption of Ca, which leads to secondary hyperparathyroidism and bone resorption. Excessive thyroid hormone replacement therapy causes accelerated bone loss with subsequent osteoporosis due to thyroid hormone induced osteoclastic activity (41).

Neoplastic disease

Malignancy is frequently associated with significant skeletal changes. An excellent summary of the effect of malignant disease on bone was recently published (8). By far the most common causes of hypercalcemia are primary hyperparathyroidism and malignancy, the latter being one of the more common paraneoplastic syndromes. Malignancies most frequently

associated with hypercalcemia are carcinomas of the lungs (35%), breast (25%), hematological system (14%) and the head and neck region (6%). Hypercalcemia associated with malignancies may be due to a combination of humoral factors secreted by the neoplastic cells and that act systemically on target organs, local factors released by neoplastic cells in bone and that directly stimulate bone resorption and coexisting primary hyperparathyroidism. The secretion of an immunologically distinct factor with PTH-like biological activity, better known as PTHrP, has been identified in several neoplasms, including renal carcinoma, squamous cell carcinoma and carcinoma of the breast. PTHrP may also mediate lactation associated bone loss as it is expressed in lactating mammary tissue. Approximately 80% of hypercalcemic patients with solid tumors have detectable plasma PTHrP concentrations. PTHrP may also play a role in hypercalcemia of patients with non-Hodgkin's lymphoma, of these 62% had increased PTHrP concentrations. PTHrP increases renal tubular absorption of Ca, reduces renal phosphorus uptake and increases osteoclastic bone resorption. It differs from the action of PTH by decreasing serum concentrations of $1,25(OH)_2$ Vit D, an important distinction from patients with primary hyperparathyroidism where the opposite applies. Hypercalcemia of malignancy is usually associated with suppressed PTH concentrations and ectopic production of PTH by neoplasms remain a rare event. It differs from hypercalcemia secondary to hyperparathyroidism by the uncoupling of bone formation from bone resorption, two processes that are generally linked. In primary hyperparathyroidism both osteoclastic- and osteoblastic activities increase, whereas in patients with hypercalcemia of malignancy only osteoclastic activities increase (Table 5). Serum osteocalcin concentrations are significantly lower in patients with bone metastases. PTHrP is thought to be responsible for these biochemical changes. Biphosphonates have become the most useful anti-resorbtive agent for the treatment of malignancy-induced hypercalcemia. The action of these drugs is discussed elsewhere in this review.

The mechanism responsible for the hypercalcemia associated with hematological malignancies is multifactorial and includes the secretion of local bone active cytokines, such as IL-6, IL-1 and lymphotoxin or TNBFβ. In the early stages of multiple myeloma both the recruitment of osteoblasts and activity of osteoclasts are enhanced. Stimulated osteoblasts produce IL-6, a potent myeloma cell growth factor and a critical cytokine for the formation of osteoclasts in bone marrow. In overt myeloma, osteoblastic activity becomes significantly reduced resulting in net bone loss (42). Uncoupling of osteoblastic and osteoclastic activities appears to be a

key event in bone loss of myeloma, the latter of which is aggravated by high dose glucocorticoid therapy (43).

Drugs and toxins

Drugs and chemical substances may exert profound influences on bone metabolism. Glucocorticoid therapy directly depresses bone formation and inhibits gastrointestinal Ca absorption, leading to hypocalcemia and secondary hyperparathyroidism with increased bone resorption (44). Osteoporosis is a well known complication of ethanol abuse. It results from complex nutritional deficiencies (implicating Ca, Vit D and protein), decreased exposure to sunlight and testosterone deficiency (31). The effect of ethanol on cortisol metabolism (the pseudo Cushing's syndrome) as well as direct inhibition of osteoblastic function by ethanol may contribute to the net bone loss experienced by alcoholics. Cigarette smoking is a risk factor for osteoporosis in both genders (31) and may relate to smoking induced reduction of Ca absorption in the gastrointestinal tract, accelerated estrogen metabolism, decreased testosterone concentrations in men and earlier menopause in women. Diphenylhydantoin and phenobarbital may cause a decrease in Vit D concentrations due to hepatic breakdown of Vit D, secondary hyperparathyroidism and accelerated cortical bone loss (31). Long term intake of slow release sodium fluoride and calcium citrate increases bone mass, improves bone quality and significantly reduces vertebral fracture rate in osteoporotic patients. These improvements are reflected as increases in bone density and mineral apposition rates, reduced trabecular spacing and an increase in the mean number of nodes in cancellous bone (45). People living in communities with high levels of fluoride in their water supply (2.5 mg/l or more) have significantly higher bone mineral densities than their counterparts from communities with low (0.03 mg/l) and moderate (0.7 mg/l) fluoride concentrations. Exposure to fluoride at levels considered to be optimal to prevent dental decay (0.7 - 1.2 mg/l) appears to have no significant impact on bone mineral density (46).

A group of drugs, collectively known as the bisphosphonates and extensively reviewed by Fleisch in 1998 (47), inhibit both ectopic mineralization and bone resorption. The former is due to a direct physicochemical mechanism during which the formation and aggregation of Ca-P crystals from clear solutions are inhibited, transformation of amorphous Ca-P into hydroxyapatite blocked and aggregation of apatite crystals delayed. The inhibiting effect on the formation of calcium salts are valuable in the treatment of diseases with ectopic

mineralization such as atherosclerosis. Prevention of the formation of dental calculus deposits has already been proven as a potential benefit of these drugs. Bisphosphonates are powerful inhibitors of bone resorption. They play an important role in the management of diseases characterized by bone resorption, such as Pagets disease of bone, bone resorption associated with neoplastic disease, hyperparathyroidism and osteoporosis. A decrease in bone loss and increase in bone mineral density have been reported in subjects with postmenopausal osteoporosis and corticosteroid induced bone loss and who were treated with bisphosphonates. When given in high dosage, bisphosphonates impact on the mechanical properties of the skeleton. Strong inhibition of bone resorption can lead to bone fragility as a result of the inability to replace old bone and repair micro cracks (Fig. 6). In humans, bisphosphonates inhibit tumor induced bone resorption, correct hypocalcemia, reduce pain, prevent development of new osteolytic lesions and fractures and improve quality of life. They are now the treatment of choice in hypocalcemia of malignancy. At the cellular level, bisphosphonates inhibit osteoclast recruitment, adhesion and lifespan and decrease their activity. This is reflected histologically as a decrease in osteoclast numbers and shallower than normal resorbtive facets on bone surfaces. As cells of the osteoblastic linage control the recruitment and activity of osteoclasts under physiological and most pathological circumstances, bisphosphonates may act through the modulation of the interaction between osteoclasts and osteoblasts.

Children treated with chemotherapeutic agents experience a decrease in skeletal growth. These agents have a direct effect on the skeleton itself and its effect is not the result of a disturbance in GH secretion. Disruption of the columnar arrangement and a decrease in the number of chondrocytes within the growth plates occur. Some workers reported a permanent deficit whereas others demonstrated a catch-up growth, with only minimal loss in final body height. In addition to chemotherapeutic drugs, glucocorticoids are frequently used in the treatment of certain childhood malignancies. These drugs reduce linear growth, which is variably balanced by GH therapy, as their action is at least in part caused by antagonism of GH's action (48).

Utilizing double tetracycline labelling before biopsy, Compston and co-workers demonstrated reduced bone formation in farm workers exposed to organophosphate pesticides (49) and Gulf war veterans (50). In the latter study a significant reduction in the volume of trabecular

bone was found to be associated with decreased osteoblast activity. Resorbtive facets on bone surfaces were increased, a phenomenon explained on the basis of failure of the suppressed osteoblasts to cover eroded bone surfaces rather than increased osteoclast activity. Nearly all veterans have a history of exposure to organophosphates and pyridostigmine which could explain the suppression of osteoblasts. Another possible cause is that changes in lifestyle of these subjects, including tobacco, excessive alcohol consumption and reduced levels of physical activity may be the key to their bone changes.

Genetic conditions

Racial differences could account for slight variations in the histomorphometric norms reflected in Table 2. American blacks have a greater bone mass and a lower incidence of osteoporosis and hip fractures than their white counterparts. The rate of bone turnover is lower in blacks than in whites, an observation supported by the significantly lower level of serum GLA in blacks. This provides a mechanism to explain the decrease loss of bone mass in blacks compared to whites during ageing (51).

Osteogenesis imperfecta is transmitted as a dominant autosomal trait. The severity of its skeletal manifestations divides affected individuals into a congenita (multiple fractures occur in early life and even in utero) or tarda (fractures generally occur after weight bearing) subtypes. Blue sclera, dental involvement (dentinogenesis imperfecta) and hyper extensile joints are frequent findings, signifying a collagen deficiency as the basis of the disease (52). Microscopy shows a highly variable picture. Many cases show large osteocytic lacunae, an increased proportion of primary lamellar bone and a marked lack of secondary Haversian systems. The osteoblasts are densely staining and resemble fibroblasts (Fig. 7). Sheets of connective tissue lie adjacent to new sites of bone formation.

Vitamin D resistant rickets (or hypophosphatasia) is a rare disorder caused by an autosomal recessively inherited defect in P reabsorption in the proximal renal tubules. Cases differ in clinical severity, ranging from fractures in utero to a mild form of rickets. Biochemical abnormalities include hypophosphatasia and hypocalcemia with hypercalcuria (52). The underlying defect appears to correlate with a lack of mineralization activity. The bone has wide osteoid borders, which lack mineralization activity and a diagnosis of hypertrophic rickets is generally proposed on static bone measurements. Analyses of dynamic

measurements however show a lack of mineral deposition despite dietary mineral supplementatioin.

Since it was described by Albers-Schönberg nearly 100 years ago, **osteopetrosis** or marble bone disease has remained one of the most dramatic bone dysplasias affecting humans. It presents in various forms of inheritance and severity, extending from lethal varieties (malignant osteopetrosis) to forms that manifest with mild hematological and neurological symptoms. The clinical manifestations of the mild forms are due to compression of nerves and displacement of marrow by excessive bone. The rate of formation of bone has been reported to be normal or excessive and bone resorption highly suppressed. Osteoclasts are abnormal and exhibit absence of ruffled borders (53,54) (Fig. 8). Cortical width and trabecular volumes are significantly increased resulting in a reduction in the volume of the bone marrow (54). Unresorbed cartilage inclusions and lack of Haversian systems (56,57) are other characteristic microscopic features. Repopulation of the bone marrow with T-cell depleted HLA compatible bone marrow transplants appear to be successful in establishing a normal osteoclast population (54) and reversal of net bone formation.

The mean age of survival of patients with **cystic fibrosis** has increased dramatically to over 30 years during the past decades. The increased life span has resulted in new complications amongst which bone loss and osteoporosis feature. It is well known that patients with cystic fibrosis are growth retarded and young adults have short and narrow bones. Because of a significant decrease in bone volume, these patients may develop osteoporotic fractures prematurely (58).

Renal disease

Kidneys play an important role in bone metabolism by producing active Vit D metabolites and through the filtration- and reabsorption of divalent ions. An early and virtually universal feature of chronic renal failure is a rise in serum PTH accompanied by hyperplasia of the parathyroid glands. The stimulus is hypocalcemia and several factors amongst which decreased production of active Vit D, P retention and skeletal resistance to the action of PTH lead to this. The pathological classification of renal bone disease is based on static and dynamic histomorphometric evaluation and divides the bone changes in high turnover - and low turnover uremic bone disease (Table 6). The former is characterized by osteitis fibrosa

and mixed osteopathy. Osteitis fibrosa results from PTH hyper secretion and is characterized by an elevated rate of bone formation, increased number of osteoblasts and osteoclasts, abundant resorbtive facets, increased osteoid production and bone marrow fibrosis (hence the name of the disease) (59). The mixed osteopathy is characterised by increased medullary fibrosis with an increased osteoid volume (60). The major difference from a nutritionally induced osteomalacia is an increased osteoid thickness and not volume for a given rate of bone formation (61). Low turnover uremic bone disease is characterized by a low bone formation rate and a normal or decreased osteoid seam thickness. These changes are also referred to as adynamic bone disease and the mechanism leading to its primary low bone formation rate is complex (59). Accurate assessment of the bone formation rate is essential before a decision is made to perform a parathyroidectomy on a patient in renal failure (62). Once dialysis begins, the bony changes can be complicated by aluminium bone disease, if aluminium is present in the water supply (dialysis patients are exposed to 300 - 400 litres of water across the dialysis membrane per week). Aluminium poisons osteoblasts, as it is readily absorbed and deposited in osteoid and acts in many ways opposite to the action of PTH. Employing the tricarboxilic acid or the solochrome azurine staining method facilitate detection of aluminium in osteoid. An iron stain must be used in conjunction as positive results may be caused by iron deposits (28). Beta 2 microglobulin deposits are furthermore frequently present in the periosteum of patients on prolonged hemodialysis. Of these, the majority develop femoral neck fractures. Periosteal β2 microglobulin may therefore be helpful in predicting a renal dialysis patient's susceptibility to femoral fracture (63).

HIV infection

HIV-1 infected patients show a notable decrease in bone turnover. This change appears to correlate with the severity of the disease, according to the CDC classification and the number of CD4+T lymphocytes. All osteoblastic parameters (osteoid volumes, osteoid seam widths and mineralization activity) as well as the number of osteoclasts are reduced. Although the exact mechanism is not yet clear, it has been possible to infect continuous human osteoblast cell lines with different strains of HIV-1, suggesting a direct effect of the virus on bone forming cells (64).

Ideopathic metabolic bone disease

A small percentage of patients suffer progressive bone loss with either a speculative or unidentifiable cause (34). **Juvenile idiopathic osteoporosis** is related to reduced bone formation rather than increased resorption and disturbances in GH or IGF-I production have been implicated (65). Although the exact mechanism of osteopenia in patients suffering **ankylosing spondilitis** remains to be determined, both trabecular thinning and loss of structural bony elements are involved (66). The **fragile bone syndrome** is characterised by bone fragility, calvarial and/or gnathial fibro-osseous lesions and metaphesial under modelling of the tubular bones. Bone histomorphometry has shown increased osteoid surfaces and osteoid volumes, making it distinctly different from osteogenesis imperfecta (67). The early phase of **Paget's disease of bone** is characterized by bone resorption (osteoclastic parameters are 10 – 20 times increased). This phase is soon followed by an osteoblastic response with bone depositioning. Both resorption and formation occur in small areas adjacent to each other. Small pieces of bone rather than complete osteones are subsequently formed. The marrow spaces generally show foci of increased spindle shaped cells with mild fibrosis, indicative of increased recruitment of undifferentiated precursor cells. The net result is broadening of the bone with irregular cemental lines referred to as a mosaic pattern (52).

CONCLUSION

Recent advances in the study of dynamic bone changes provide valuable information on the morphogenesis of metabolic diseases. Early diagnosis and accurate monitoring of these debilitating skeletal states are now within reach of the Pathologist.

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Legends:

FIG. 1. Outline of a diagnostic flow chart for metabolic bone disease.

FIG. 2. Clinical features of rickets.

FIG. 3. Trabecular bone in rickets showing extensive coverage of bony surfaces by osteoid. Note the focus of tunnelling resorption, indicative of hyperparathyroidism (arrows) (Picrosirius stain, X150).

FIG. 4. Active mineralization of osteoid as depicted by the extent of - and the distance between the two fluorescent lines (arrows) (unstained section viewed with UV light, X250).

FIG. 5. Stereo micrograph of trabecular bone in a patient with osteoporosis. Note the loss of structural elements depicted by the blunt ending trabeculae (arrows) (Bar = 0.5 mm).

FIG. 6. Stereomicroscopic view of trabecular micro cracks in a patient on high dosage bisphosphonates (Bar = 0.3 mm).

FIG. 7. Microscopic features of trabecular bone in osteogenesis imperfecta. Note the large osteocytic lacunae and fibroblast – like osteoblasts (H&E stain, X250).

FIG. 8. Osteoclast in a Howships lacuna in osteopetrosis. Note the absence of a ruffled cytoplasmic border (arrows) (Bar = 15 microns).

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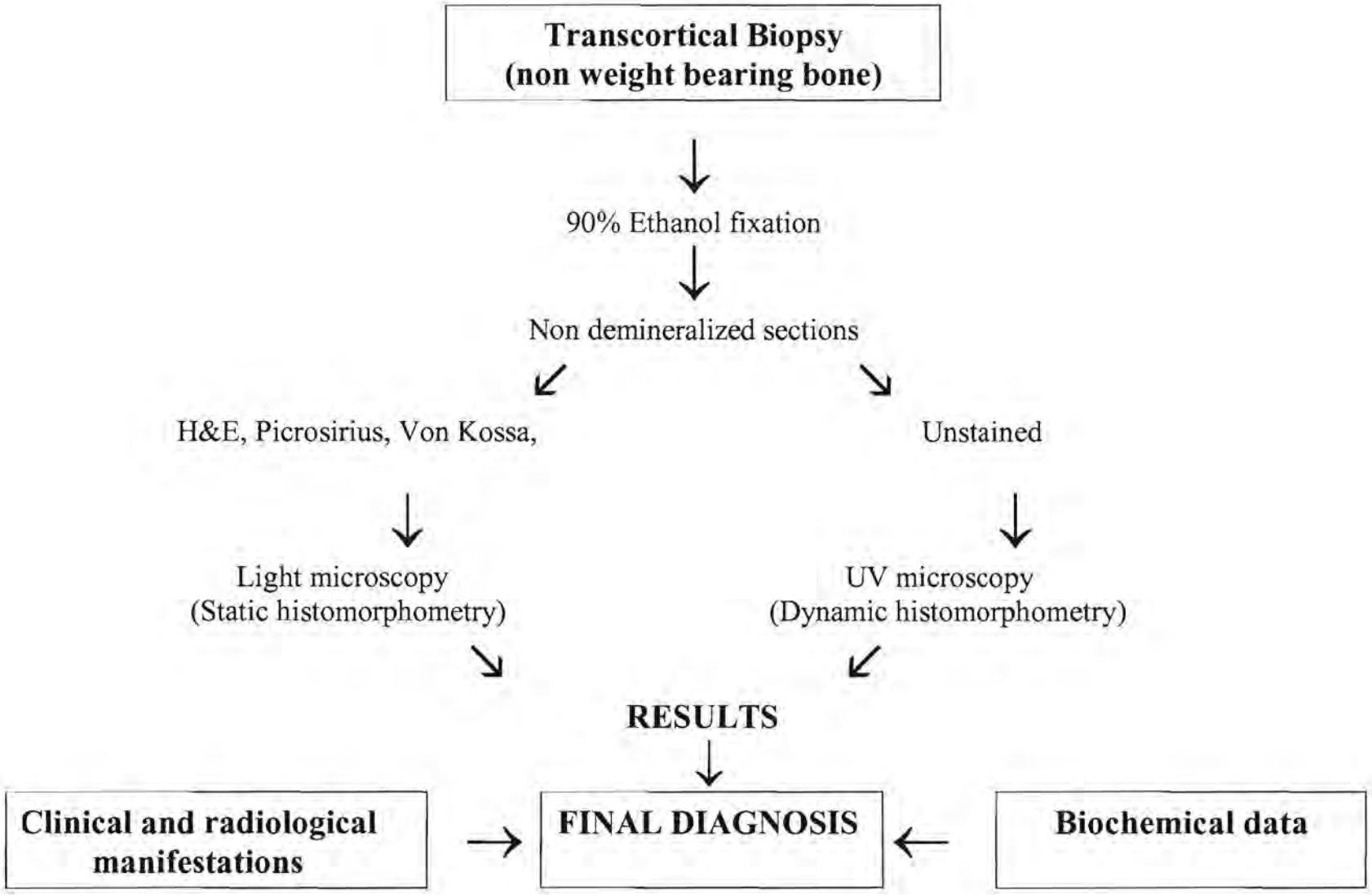
Transcortical Biopsy
(non weight bearing bone)
90% Ethanol fixation
Non demineralized sections
H&E, Picrosirius, Von Kossa,
Unstained
Light microscopy
(Static histomorphometry)
UV microscopy
(Dynamic histomorphometry)
RESULTS
Clinical and radiological manifestations
FINAL DIAGNOSIS
Biochemical data

TABLE 1: *Conditions commonly diagnosed and managed through bone histomorphometry*

1.	Rickets/Osteomalacia - acquired type - Vit D resistant type
2.	Osteoporosis - female type - male type - immobility
3.	Hyperparathyroidism
4.	Osteopetrosis
5.	Uremic bone disease
6.	Paget's disease

TABLE 2: *Histomorphometric terms and reference values (5).*

Term	Definition	Reference Values Mean ± SD
Indices of Bone Mass		
Trabecular bone volume	% of medullary cavity occupied by mineralized and unmineralized bone	22.5 ± 3.5%
Mean trabecular width	Average width of all trabecular bone spicules	213 ± 65 microns
Mean cortical width	Mean thickness of both cortices	909 ± 98 microns
Indices of Osteoid		
Trabecular osteoid surface	% of bone surface covered by osteoid	18.9 ± 5.0%
Trabecular osteoid volume	Osteoid area expressed as a % of trabecular bone area	1.9 ± 0.4%
Mean osteoid seam width	Osteoid area divided by the millimetres of bone surface covered by osteoid	9.7 ± 0.4 microns
Indices of Resorption		
Trabecular resorptive surface	% of bone surface showing Howship's lacunae	5.1 ± 0.6%
Osteoclastic resorptive surface	% of bone surface lined by osteoclasts	0.13 ± 0.6%
Osteoclasts per mm of trabecular perimeter	Number of osteoclasts per millimetre of bone perimeter	0.11 ± 0.04%
Indices of Mineralization		
Calcification (apposition) rate	Distance between double tetracycline labels divided by the number of days between administration of labels	0.03 ± 0.01%
Mineralization lag time	Mean osteoid seam width divided by the bone formation rate	0.20 ± 0.04%
Bone formation rate	Calcification rate times % of trabecular surface labelled	0.64 ± 0.10 microns/day
Percentage of trabecular surface labelled	% of bone surfaces labelled by tetracycline	12.8 ± 2.3%
Mineralization front	Nature of line of mineralization between osteoid and mineralized bone	73.4 ± 26.5%

TABLE 3: *Characteristic histomorphometric features of rickets/osteomalacia.*

Feature	Status
Mineralized bone mass	decreased
Indices of osteoid	increased* or decreased**
Indices of resorption	increased
Indices of mineralization	decreased

*hypertrophic rickets/osteomalacia

**atrophic rickets/osteomalacia

TABLE 4: *Characteristic histopathologic features of osteoporosis*

Feature	Status
Trabecular bone volume	decreased
Mean trabecular width	normal or increased
Mean cortical width	normal* or decreased**

* female type I

** female type II

TABLE 5: *Histopathologic differences between bone changes induced by primary hyperparathyroidism (PTH related) and malignancy induced (PTHrP related).*

	PTH	PTHrP
Indices of osteoid	increased	normal
Indices of resorption	increased	increased

TABLE 6: *Histomorphometric findings in renal bone disease*

	High turnover renal bone disease	Low turnover renal bone disease
Indices of osteoid	increased	normal or reduced
Indices of resorption	increased	normal
Bone marrow fibrosis	prominent	inconspicuous

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SHORT COMMUNICATION

AMINO ACID COMPOSITION OF DENTINE IN PERMANENT HUMAN TEETH

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(Accepted 20 August 1991)

Summary—Dentine of permanent mandibular incisors from nine individuals was hydrolysed and the amino acid composition determined by ion-exchange chromatography against a standard calibrant of 41 amino acids. Nineteen amino acids were detected, including small quantities of 1-methylhistidine and asparagine, two amino acids whose existence had apparently not been recorded before in human dentine. The total content of hydroxylysine plus lysine varied between 2.6 and 3.3 residues per 100 (SD, 0.74) in different teeth, which therefore did not support previous studies that had proposed a constant total value. This and other quantifiable differences between present and previous findings may be the result of the different methods and the influence of dietary and other regional factors on dentinogenesis.

Key words: human dentine, amino acids.

Dentine is the major component of teeth, responsible for most of the weight, volume and overall shape (Butler, Munksgaard and Richardson, 1979). Ninety per cent of the organic matrix of human dentine consists of collagenous proteins (Jones and Leaver, 1974) and the remaining 10% is made up of non-collagenous proteins, proteoglycans, glycoproteins and lipids (Avery, 1987). Various analytical methods, some of which are historic, have been used for determination of amino acids in dentine, namely: microbiological assay (Hess, Lee and Neidig, 1952), quantitative paper chromatography (Battistone and Burnett, 1956) and ion-exchange chromatography (Eastoe, 1963).

Our objective now was to determine the amino acid composition of human dentinal matrix with a modern technique and to compare it with previously published data.

Permanent mandibular incisors were extracted from nine bodies of known age and sex in the Forensic Medicine mortuary of Ga-Rankuwa Hospital, situated 32 km north of Pretoria. Before processing, the crowns and cementum were removed with a dental bur, and the pulp with an endodontic file. Pieces of radicular dentine of approx. 0.20 g were washed, dried and hydrolysed in 6 M hydrochloric acid (HCl) for 24 h at 110°C. The hydrolysates were neutralized with neutralizer (Spitz, 1973) and citrate buffers in the ratio 1:2:2, filtered (Millex-GS 0.22 μm) and then diluted further 1:1 with the citrate buffer. Calibrants containing 41 amino acids were prepared and diluted as above. The amino acids of dentine and the calibrants were separated in duplicate by ion exchange on a Beckman 6300 amino acid analyser which incorporates a 25-cm lithium column and a four-buffer system. Chromatograms thus obtained were integrated and quantitated with a Hewlett-Packard 3390A integrator and the results expressed as a per cent residues detected. The results were tabled as the average of the total number of residues per 100 and the SD for each amino acid was calculated.

Nineteen amino acids were detected (Table 1). Asparagine and 1-methylhistidine, which have not previously been identified in human dentine, were present in small quantities. Asparagine was present in all our hydrolysates. In a serial study of hydrolysis, asparagine was detected only after 16 h and remained present in all acid hydrolysates for 24 h. In hydrolysates stored at 4°C, asparagine could be detected over as long as 12 months. No explanation for this phenomenon could be found. Acid hydrolysis of a pure mixture of aspartic acid, asparagine, glutamic acid and glutamine showed complete hydrolysis of asparagine and glutamine within 30 min. The concentration of aspartic acid and glutamic acid increased and high levels were detected under these conditions. It is suggested that asparagine in dentine may be 'protected' against complete hydrolysis. Furthermore, our monitoring system may have been more sensitive and with improved resolution as larger quantities of aspartic acid, glutamic acid, arginine, leucine, iso-leucine and valine were found than previously reported (Table 1).

Linde (1984) reported that the total content of hydroxylysine plus lysine in dentine is constant at 3.5 residues per 100. In our study, we found a variation between 2.6 and 3.3 residues per 100 (SD, 0.74), supporting Eastoe's (1963) finding of a variation in the total content of hydroxylysine and lysine over a

Table 1. Comparison of our findings (average of nine cases, with SD) with those of previous investigations (expressed as residues per 100)

Human dentine amino acids	Hess *et al.* (1952)	Battistone and Burnett (1956)	Eastoe (1963)	Linde (1984)	Present study (1992)	
					$\bar{X}$	SD
Aspartic acid	4.4	5.4	5.5	4.5	5.9	0.06
Hydroxyproline	10.3	11.6	10.1	9.6	10.4	0.24
Threonine	2.7	2.0	1.9	1.8	2.1	0.11
Serine	3.4	3.0	3.8	4.1	4.0	0.28
Asparagine	—	—	—	—	0.3	0.08
Glutamic acid	7.4	7.6	7.3	7.2	8.8	0.15
Proline	14.5	9.7	11.5	11.9	11.8	0.28
Glycine	30.9	31.3	31.9	33.4	30.1	0.30
Alanine	9.8	11.2	11.2	10.2	8.6	0.31
Valine	2.6	2.5	2.5	2.3	3.0	0.15
Methionine	0.35	0.46	0.52	0.7	0.5	0.05
Iso-leucine	1.0	*	1.0	1.0	1.3	0.00
Leucine	2.8	*	2.6	2.4	3.0	0.04
Phenylalanine	1.2	*	1.4	1.4	1.5	0.05
Hydroxylysine	0.64	0.71	0.84	1.5	1.1	0.11
Lysine	2.4	2.2	2.3	2.0	2.1	0.17
1-Methylhistidine	—	—	—	—	0.3	0.07
Histidine	0.54	0.43	0.53	0.4	0.3	0.19
Arginine	4.4	5.0	4.7	5.2	5.6	0.17

*Total of leucine, iso-leucine and phenylalanine is 6.4.

comparatively narrow range (2.91–3.35 residues per 100). The presence of two amino acids that have not hitherto been reported in dentine as well as other differences between our study and earlier ones may, amongst other factors, be the result of different methods. The more efficient buffer system and the modern lithium columns that we used facilitate the separation of isomers and increase the resolution of the various amino acids. The reported differences in the amino acid content of human dentine may also reflect dietary and other regional factors that may influence the formation of dental hard tissues.

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9 The Effect of Modern Dentine Bonding Systems on Human Dentine.
F A DE WET* and M R FERREIRA, Faculty of Dentistry, University of Pretoria, Pretoria, South Africa.

Most modern dentine bonding systems contain primers or cleansers which are used to remove or alter the smear layer or dentine before resin application. The purpose of this study was to assess the effect of four modern dentine bonding systems (DBS), three with primers/cleansers and one without, on the appearance of human dentine.

Denthesive Bond (D, Kulzer), Pertac Universal Bond (P, E.S.P.E.), Prisma Universal Bond 3 (PUB3, Dentsply) and Syntac (S, Vivadent) were included in the study. The crowns of 80 sound, human, molar teeth were imbedded in rings, leaving the occlusal surfaces projecting. These surfaces where ground wet on 220 grit SiC paper to expose superficial dentine. Fifteen dentine surfaces were then treated with each of the four DBS. Cylinders of matching composites were thereafter bonded to these surfaces, using a rubber split mould. After 24 hours storage in water the specimens were stressed to failure using a shear load in an Instron. Fracture sites were evaluated in a SEM. Five abraded dentine surfaces were treated with the respective primer/cleanser of each of 3 DBS. Dentine treated with the D and S cleanser/primer, and debonded specimens of D and S, displayed open dentinal tubules. Subsequent application of an adhesive closed almost all the tubules. Pieces of fractured dentine were attached to some composite stubs, showing resin tags clearly penetrating deep into, and even through the dentine fragments. It can be concluded that only 2 of the 4 DBS tested opened the dentinal tubuli.

10 Evaluation of the Effectiveness of an Oral Health Preventative Programme. S.Dhansay*, H. Lalloo, A. Bawa, M.H. Moola. University of the Western Cape

This study was designed to measure the effectiveness of an oral health preventative programme based in a school population in t Cape Peninsula. The objective of the study was to measure the difference between schoolchildren who were exposed to the programme and children who were not.

Three experimental (programme) and two control schools were selected for the study. A total of 110 children in the experimental group and 102 in the control group were examined (Total = 212) in the age group 11-12 years. The examiners were calibrated for reproducibility using WHO (1986) criteria for dental caries. The results of the study showed a mean DMF(S) o 7.8 (±3.1) for the experimental schools and 24.5 (±8) for the control schools. 84% of the experimental group and 60% of control group were caries free. 98% of the experimental group and 65% of experimental group had an M component of 0.42% of the control group; 8% of experimental group had at least one 1st molar tooth decayed. These results clearly showed that the programme is successful in a community based school dental service.
This project is supported by an MRC grant.

11 Film Thickness Evaluation - Implementing the BENCOR MULTI-T System. C.H. DRIESSEN*, F.A. DE WET and W.J.C. COETZEE. Faculty of Dentistry, University of Pretoria, South Africa.

Various techniques have been described for the determination of film thickness of dental cements. The purpose of this study was to assess the effectivity of the BENCOR MULTI-T system, and to explore the effect of load variation on the film thickness data. Four luting cements were included in this study i.e. X-R IONOMER (X), a glass ionomer; Mirage-FLC (M), a dual cure resin; UNITY (U), a self curing resin; and POLY-F Plus (P), a polycarboxylate. The BENCOR MULTI-T device (Driessen, 1990) was used for testing. The system is based upon direct force application derived from any calibratable source through it's active rod onto the specimen material placed between two 22mm0 glass discs. The latter is covered with teflon discs for the purpose of: (1) simulating oral temperature, (2) transfering visible light (if needed) for the curing of a VLC material and (3) protecting the 160μm glass discs from direct metal impact. Measuring of the specimens was done by electronic digital calipers with control by reflex microscope data and SEM image observation. Ten samples of each product were tested using 10Kg and 15Kg forces in order to assess the effect of different loads on the film thickness. All data were statistically analysed. It was found that the technique enabled operators to measure film thickness of dental cements accurately, easily, fast and with only small variations. Statistical analyses showed a significant difference ($p<0.05$) between application of 10Kg and 15Kg load of (X)[25,76:19,60μm] and (U)[28,60:14,20μm] but not for (M)[31,14:30,80μm] and (P)[38,00:34,00μm]. It can be concluded that the BENCOR MULTI-T system is able to easily assess the film thickness of dental cements, and that a 15Kg load reduces film thickness compared to the 10Kg load.

12 Cross-Infection Risks Associated with High-speed Dental Handpieces. C.H. HAUMAN.* Department of Oral Pathology, Faculty of Dentistry, University Stellenbosch, Tygerberg, South Africa

Dental handpieces are particularly prone to contamination with patient material, which can then transmitted to the next patient. The common approach of disinfecting handpieces by external chemic wiping in combination with flushing may pose unacceptably high risks to those individuals treated soc after infected patients.
The aim of this study was to evaluate the efficacy of chemical disinfection of high-speed handpiece Autoclavad high-speed handpieces were contaminated with an overnight culture of *Staphylococc aureus* and dried in a hot air oven for 90 minutes. The outer surfaces of equal numbers of the handpieces were wiped with 70% alcohol, alcohol-in-hibitane and Asepsyx (iodophor). The front, bac and sides of the heads of these handpieces were pressed onto the surface of blood agar plates. addition, contaminated handpieces were attached to the dental unit and water was flushed through th handpiece onto the surface of blood agar plates for 2 seconds. Handpieces used in the Tygerber Dental Hospital were tested in a similar way after routine lubrication and alcohol swabbing. Residu contamination of handpieces after flushing for specific periods of time were also tested. Aft overnight incubation, growth was recorded. Although the numbers were reduced, *S. aureus* could st be cultured from the outer surfaces of artificially contaminated handpieces after wiping with all thre disinfectants. Handpieces used in the clinic yielded virtually no growth from the external surfaces aft routine cleansing. Confluent or 2+ growth was obtained with samples from the interior surfaces both artificially contaminated handpieces and handpieces from the clinic. *S. aureus* was still preser on the internal surfaces of artificially contaminated handpieces after flushing for 5 minutes.
Sterilisation of both the internal and external surfaces of handpieces is necessary to exclude the risk cross-infection in the dental surgery.

13 Effect of Respiratory Acidosis on Faecal Fluoride Excretion in Rats. S.D. JANSE VAN RENSBURG* and C.A. VAN DER MERWE. University of Pretoria, and Medical Research Council, Pretoria, South Africa.

Respiratory acidosis is characterised by a primarily increased PCO_2 with a compensated increased $[HCO_3^-]$ which may effect the pH of blood. As the permeation of fluoride (F) through epithelia is dependent on the pH, as well as F concentration, respiratory acidosis could have an effect on faecal F excretion, and therefore on the F balance of the body. Sixteen young adult female Sprague-Dawley rats were used in this study. They were divided into a control group (Group A; receiving a normal atmospheric gas mixture) and an experimental group (Group B; subjected to an atmospheric CO_2 content of 7%). All the rats were fed a low F diet (< 1ppm) and received water with a fluoride content ([F]) of 20ppm *ad lib* for 7 weeks. Water and food consumption were monitored daily. F intake/rat/day via water and food was calculated from the data. After sedation, blood was collected anaerobically in heparinised syringes from the descending aorta. Faeces were collected from the large intestine. The [F] of the faeces was determined potentiometrically after HMDS diffusion. Blood gas analysis were done using the ABL blood gas analyser. The data were subjected to the Mann-Whitney procedure to detect differences between the groups, and to multiple regression analysis to explain the variation in the [F] of the faeces. Although the F intake via the water differed significantly ($p<0,05$) between the groups, there was no significant difference between the F intake via food ($p=0,197$) or the total F intake ($p=0,071$). There was a significant difference ($p<0,05$) in the [F] of the faeces (A:$\bar{x}$= 148,33 ppm; B:$\bar{x}$= 213,20 ppm) between the groups. Taking the [F] of faeces as the dependant variable, 68,89% of the variation in the [F] of faeces in the experimental group could be explained by the combination of the independent variables H_2CO_3 or PCO_2 and total F intake. A 46,92% association between the variables was found in the control group. Respiratory acidosis enhanced faecal excretion of fluoride in rats. The higher [F] of the faeces in the experimental group could possibly be due to net secretion of fluoride into the gut lumen. This project was partially supported by a grant from Afrox Pty Ltd.

14 Inorganic Contents of Opaque and Translucent Radicular Dentine. F.S. NKHUMELENI*, E.J. RAUBENHEIMER, W.F.P. VAN HEERDEN, M.L. TURNER and M.J. DREYER. Depts. Oral Pathology and Chemical Pathology, MEDUNSA, P.O Medunsa.

This study was undertaken to compare the calcium, magnesium, phosphorus, zinc and fluoride contents of opaque and translucent radicular dentine. Twelve mandibular incisors were utilized. The crowns and cementum were removed using a dental bur. The specimens were then hydrolysed individually in 1M perchloric acid. Calcium, zinc and magnesium were determined utilising the ammonium phosphomolybdate calorimetric method and fluoride by ion selective electrode method. The mean values (mg/g) of opaque and translucent dentine respectively were :

Calcium (Ca)	244.65	(SD ± 3.60)	and	244.17	(SD ± 4.10);
Magnesium (Mg)	8.25	(SD ± 0.42)	and	6.97	(SD ± 0.69);
Fluoride (F)	0.22	(SD ± 0.01)	and	0.27	(SD ± 0.02);
Phosphorus (P)	127.70	(SD ± 2.43)	and	125.00	(SD ± 2.33);
Zinc (Zn)	0.14	(SD ± 0.01)	and	0.20	(SD ± 0.03).

The Mann Whitney test showed that there was a significant difference between the Mg, F and Zn contents of opaque and translucent dentine ($p<0.05$). Our findings do not support those of Moore and Leaver (1974) who found that only Calcium values were significantly lower in translucent dentine.

15 Enamel Surface Roughness after CO_2 Laser Radiation, in vitro Assessment. S.H. PAN*, C. BAKER, J. DE VRIES, P.J. BECKER and S.S. MASHELE. Dept. of Operative Dentistry, Faculty of Dentistry, MEDUNSA, S.A.

Recently, a CO_2 laser has been used successfully to enhance dental bonding. Knowledge of surface roughness is essential to reduce bond failures. The objectives were to: 1) determine the surface roughness after conventional drilling with/without laser radiation and with/without acid etching using the Bendix Profilometer. 2) examine under Scanning Electron Microscope (SEM) the ultrastructure of the enamel surface after CO_2 laser radiation. Thirty-two human maxillary central incisor teeth were selected and stored in 10% buffered formalin. Conventional drilling and combined treatment with laser radiation were randomised on the labial enamel surface in the vertical dimension of 3 x 5mm. Laser radiation was set at a repeat pulse energy intensity of below 3W for a period of 10 seconds. Acid etching was performed on half of the specimens and the surface roughness was measured with the Bendix Profilometer. The SEM assessment was also noted. The experiment was designed as a randomised block but test results showed that roughness caused by laser produced the maximum measurable roughness of 10 micron for each sample point. The analysis then focused on conventional high/low speed drilling and no significant difference was found between acid etching techniques. ($p = 0,4270$) or between drilling speeds ($p = 0,7355$). Compared to laser the roughness after conventional drilling was clinically less.
An improved understanding of roughness caused by CO_2 laser radiation on enamel surfaces may lead to possible clinical application in aesthetic restorative dentistry.

16 Relation Between Blood, Molar, Cortical Bone and Trabecular Bone Lead Levels of Rats. R.J. ROSSOUW* and S.R. GROBLER, Faculty of Dentistry, University of Stellenbosch, Tygerberg, South Africa

Bone is the major reservoir of body lead stores. Loosely and deeply bound lead compartments in bone provide short- and long-lived sources of this toxic element to blood and soft tissues.

Several groups of inbred BD-IX rats were continuously exposed to nebulized aerosols of lead for different time periods. Furthermore, the effect of different post-exposed periods on the lead concentrations were also investigated. The lead concentrations were determined in blood, molars, tail vertebra and iliaca crest of the rat. The graphite furnace atomic absorption spectrophotometer was usad.

The blood, molar, tail and iliaca crest lead levels differed significantly ($p<0,05$) among the exposed groups. However, only the blood and iliaca crest lead concentrations differed significantly ($p<0,05$) in the post-exposure periods.

It is concluded that lead is absorbed in the apatite crystal of different kinds of bone. Furthermore, cortical bone is identified as long term storage reservoirs of lead in the body.

This study was supported by the MRC.

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Composition of Tubular and Intertubular Areas in Translucent Dentine. P.S. NKHUMELENI*, E.J. RAUBENHEIMER and M.L. TURNER, Faculty of Dentistry, Medical University of Southern Africa.

Most researchers seem to agree that the changes which cause root dentine to appear translucent occur in the lumina of dentinal tubules. The purpose of this study was to compare the occluding material and adjacent intertubular dentine. The radicular translucent dentine of mandibular incisors from nine subjects were coated with a 10-12nm gold-film. The specimens were examined in an ISI SX30 SEM equiped with a Link Systems EDAX-analyser. Two areas were investigated, namely material occluding the tubules and areas not more than 5 microns from the edge of the obliterated tubule. The mean values of occluding material and intertubular dentine (%) respectively were as follows:

Calcium	(Ca)	67.4 (SD = 12.4)	and	60.9 (SD = 7.9)
Phosphorus	(P)	40.7 (SD = 8.7)	and	33.9 (SD = 8.4)
Ca:P ratio		1.7 (SD = 0.5)	and	1.8 (SD = 0.6)

There was a significant difference between the calcium and phosphorus content of the occluding material and adjacent intertubular dentine ($p < 0.05$). Our findings did not support those of Brinkmann and Hartmann (1980) who found no correlation between dentine translucency and its mineral content.

The material occluding the dentinal tubules is more mineralized than intertubular areas in translucent dentine.

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Comparing the Shear Bond Strength of One, Two and Three Step Dentine Bonding Systems. P.J. VAN DER VYVER, F.A. DE WET, W.R. VORSTER and J. OOSTHUYSEN*, Faculty of Dentistry, University of Pretoria, Pretoria, South Africa.

Some of the most important features when chosing a dentine bonding system is the ease to use, and the time required to apply the system. The purpose of this study was to compare the shear bond strengths of one, two, and three step dentine bonding systems.

One hundred and twenty sound, human, molar teeth were collected and the crowns embedded in rings. The occlusal surfaces were ground wet on 220 grit SiC paper in order to expose superficial dentine. The samples were randomly assigned to 6 test groups consisting of 20 teeth each. One group was used to evaluate each of the following materials: Pertac Bond (PB), Tokuso Light Bond (TLB), Art Bond (AB), Prisma Universal Bond 3 (PUB3), Scotchbond Multi-Purpose (SMP) and Optibond (OB). Of the products, two were one-step systems i.e. PB and TLB, another two were two-step systems i.e. AB and PUB3, and the last two were three-step systems i.e. SMP and OB. SMP etchant was used with OB system to achieve total smear-layer removal, making it a three-step system. Twenty dentine surfaces were treated with each of the 6 bonding systems, and cylinders of matching composites thereafter bonded to the surfaces, using a rubber split mould. All samples were stored in distilled water at 37°C for 24 hours before they were stressed to failure using a shear load in an Instron. Data were analyzed statistically (ANOVA). The bond strengths for the different products (MPa) were as follows: PB: 11,21 ± 1,54; TLB: 6,7 ± 1,7; AB: 15 ± 2,3; PUB3: 14,3; SMP: 23,6 ± 2,5 and OB: 24,1 ± 2,5.

The three-step bonding systems showed higher bond strengths to dentine than the 1 and 2 step systems. The two-step systems also had a higher bond strength than the one-step systems.

Amongst the six systems tested, the three step systems provided the best shear bond strength values.

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Laser Effects on Pulpal Floor Thickness and Intercuspal Tooth Strength. S.H. PAN[*1] and C. BAKER[2] and J De Vries[3]. Faculty of Dentistry[1&3], MEDUNSA & MRC/WITS, DRI[1]; E M Unit, MEDUNSA[2], R.S.A.

Carbon dioxide (CO_2) lasers have been successfully used to fuse the apatite of enamel with that of dentine. In order to assess the effectiveness of this fusion, it is essential to ascertain that strength of a premolar tooth. The aim of this study was to determine the optimal pulpal floor thickness after CO_2 laser irradiation. Repeat pulsed CO_2 laser with energy densities of 10-15 J.cm^{-2} was applied to 10 conventionally prepared M-O-D cavities. The remaining 10 teeth were prepared by conventional drilling only. All of the teeth were fitted with the indirect cast resin inlay restorations (Isosit-N) and luted with resin cement (Vivadent). Teeth were subjected to a stress (± 20-50 MPa) causing them to fracture. Compressive strengths of teeth from the experimental and controlled groups were analysed. Measurements of pulpal floor thickness were carried out by image Analysis and SEM. For the overall observation vector (ANOVA), it was found that treatments differed significantly ($p=0,0084$), thickness (≥ 2mm; < 2mm) differed significantly ($p=0,0028$), while no interaction was found between treatment and thickness ($p=0,1164$).

For optimal intercuspal tooth strength, the pulpal floor thickness must be ≥ 2mm, since modern aesthetic dental treatment may involve a combination of conventional drilling and laser irradiation.

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Evaluation of Optibond when used as a Fissure Sealant. P.J. VAN DER VYVER, F.A. DE WET and J. OOSTHUYSEN*. Faculty of Dentistry, University of Pretoria, Pretoria, South Africa.

The Optibond bonding system has multiple uses, including bonding to enamel, dentine, porcelain, composite and metal. Since the system contains a 48% filled, radiopaque and fluoride-releasing, dual-curing catalyst paste, it might have ideal properties for usage as a fissure sealant. The purpose of this study was to evaluate the shear bond strength (SBS) of Optibond (Dual Cure system) when used as a fissure sealant on human dental enamel.

Fourty freshly extracted, sound, human, molar teeth were collected, the roots and pulps removed and the crowns embedded in rings. The exposed occlusal surfaces were ground wet on 220-grit SiC paper to produce a flat enamel surface. All enamel surfaces were etched for 30 seconds with 37% phosphoric acid, rinsed and dried. The Optibond primer was then applied according to manufacturers' instructions and light-cured for 20 seconds. Optibond dual-cure (consisting of a catalyst with resinous liquid and a filled accellerator paste) was mixed, and cylinder stubs of the material then bonded to the treated enamel surface using a silicone rubber split mould. All samples were cured for 1 minute from the occlusal direction, and an additional 2 minutes after removal of the split mould (1 minute each from 2 different directions). Twenty bonded samples were stressed to failure 15 minutes after bonding, by using a shear load in an Instron and the remainder stored for 24 hours in distilled water at 37°C prior to testing. The mean SBS was calculated for each group, the data analysed using the Student-T test and several fracture sites examined in the SEM.

The SBS after 15 minutes and 24 hours were 15,96 ± 3,59 MPa and 20,32 ± 2,71 MPa respectively ($P<0,01$). SEM investigation demonstrated mixed cohesive/adhesive fractures.

It can be concluded that the SBS of Optibond to enamel after periods of both 15 minutes and 24 hours are adequate for usage in clinical fissure sealing situations, with the highest bond strengths being obtained after 24 hours.

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Determination of Patient Trends, Logistics and Appointment Preferences. P.J. COMBRINK* and W.A. WILTSHIRE. Department of Orthodontics, University of Pretoria, Pretoria.

In a concerted effort to continue to improve the service rendered by the Orthodontic Department of the University of Pretoria, it was the purpose of this study to survey patients attending the orthodontic clinic. An anonymous questionnaire which included a variety of questions was completed by 65 patients or parents. The responses were statistically analysed employing the Fishers exact test and the log linear model on frequency tables. Of the 65 patients only 30 were members of medical aid funds and were prepared to pay ±R160 out of their own pockets for treatment. Patients without medical aids were prepared to pay ±R311 for orthodontic treatment. Thirteen respondents were prepared to pay as much as needed to complete treatment. More patients (69.84%) used private transport than public transport (15.87%) to attend the clinic. No correlation was evident between mode of transport and frequency of cancelled ($p=0,363$) or missed ($p=0,292$) appointments. No significant correlation ($p=0,175$) between geographical abode and missed or cancelled appointments was apparent either. 17% of patients regularly missed appointments, 64% maintained that a 24 hour cancellation would suffice and 42.28% used the appointment card in order to remember their appointments. 77% felt a fine should be imposed for broken appointments. There was a highly significant correlation between patients who regularly kept their appointments and who suggested imposition of a fine ($p=0.000$). 60% of patients preferred the 7h15 appointment time to the 14h00 time slot. Patient complaints included: long waiting lists (28.4%), inconvenient times (18%), long appointments (17.2%) and student turnover (10.3%). Regular managerial audits is necessary in order to access patient requirements and protect the patient service at a dental training hospital.

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Frequency of Preventable Malocclusions in the Mixed Dentition: A Preliminary Report. E.R. De Villiers* and W.A. Wiltshire, Department of Orthodontics, Faculty of Dentistry, University of Pretoria, Pretoria

Specific defined malocclusions, where early recognition and simple interceptive treatment may minimise or eliminate the need for complex appliance therapy, have been identified and certain ages of special vigilance in the developing dentition are recognised. The aim of this longitudinal study is to determine the percentage of malocclusions at the age of 10-11 years that could have been prevented or minimised, if causative factors had been diagnosed and treated from the age of 8-9 years. A total of 961 children, aged 8 and 9 years were examined by 3 investigators at 9 schools in the Pretoria suburbs of Pretoria West and Atteridgeville to collect baseline data on the current status of occlusion in these groups. An average of 67,9% of cases presented with a Class I molar relationship; 10,9% with a Class II; 7,45% with a Class III and 12,1% with a cusp-to-cusp molar relationship. Early loss of second primary molars occured in the maxilla (5,5%) and in the mandible (7,5%). First primary molars were lost in 5,4% of cases in the maxilla and in 11,3% in the mandible. Space was sufficient in 71,9% of cases, but 18,3% showed a shortening of arch length. Ectopic eruptions, ankylosis of primary molars, supernumerary teeth were very rare. Single tooth anterior crossbites were evident in 7,2% of cases and 7,1% had more than one tooth in anterior crossbite. Posterior, single tooth crossbites were found in 2,5% and multiple tooth crossbites in 0,6% per quadrant. 45% of children had a normal overbite, 30,1% had a deep bite, 8,8% had an edge-to-edge bite and 14,7% presented with an anterior openbite. The majority of children had Class I malocclusions with sufficient arch length and a seemingly normal developing occlusion.

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Airborne Lead Exposure and Lead Levels in Rat Tissues. R.J. ROSSOUW* and S.R. GROBLER. Faculty of Dentistry, University of Stellenbosch, Tygerberg.

Lead has no metabolic role in the human body and its presence in teeth is associated with various toxic effects. The purpose of this study was to indicate the importance of the combined effect of airborne lead concentration and length of exposure. Experimental work was done on Rattus rattus norvegicus. Groups of 20 inbred rats were exposed to: (1) "clean air" (0.05 µg Pb/m^3) for 70 days; (2) 77 µg Pb/m^3 for 70 days; (3) 249 µg Pb/m^3 for 28 days; and (4) 1 546 µg Pb/m^3 for 30 days. Half the rats in each group were then killed and the other half kept in "clean air" until the blood lead of groups 1-3 had returned to normal. Tail vertebrae, iliaca crest and epiphyses' radius, and blood were analyzed for lead by atomic absorption spectrophotometry. In the rats killed immediately after exposure, the lead levels of blood or iliaca crest or epiphyses or tail vertebrae differed significantly ($p<0.05$) among all 4 groups, except between groups 2 and 3 for the tail vertebrae. For the post-exposure rats, the blood showed no significant differences ($p>0.05$) among groups 1, 2 and 3. However, for tail vertebrae or iliaca crest or epiphyses no significant differences were found between groups 2 and 3.

It can be concluded that lead turnover had the following sequence: blood (soft tissue) > iliaca crest (trabecular bone) > epiphyses (trabecular and compact bone) > tail vertebrae (compact bone). Blood lead became supplemented through the process of bone remobilization and different bone compartments exist.

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Longitudinal Study on the Oral Yeast Carraige in Children. E. BLIGNAUT, R. SENEKAL* and J. KROON. Faculty of Dentistry, University of Pretoria, Pretoria.

Yeasts are amongst the very first microorganisms to colonise the oral cavity of the newborne. Since yeasts occur widely in nature, it is inevitable that humans should come into contact with them and previous studies have shown yeasts to be amongst the normal oral flora of 50%-70% of healthy individuals. It was also thought that the tongue is the most likely place of residence for oral yeasts and statistics from previous studies are based mainly on a once only specimen taken from the tongue or saliva of a particular individual.

In order to establish whether the occurance of yeasts are merely incidental at the time of sampling, 172 children ranging in age between 3 and 8 years were studied over a 2 year period. Specimens were taken from both plaque and saliva and immediately plated onto chalk agar. While yeasts were isolated from an average of 22,6% of children at one time or the other, only 2,9% of the children were yeast positive on both occasions of which one had different species from the one year to the next. During the 1992 study, four children had yeasts in both saliva and plaque of which 3 exhibited different species while only one was positive for both specimens during the 1993 study.

From these results it can be concluded that the presence of yeasts in the oral cavity of an individual at a particular point in time, does not implicate a carrier state.

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Geographic variations in the composition of ivory of the african elephant *(Loxodonta africana)*

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Abstract

Tracing the source of origin of illegal ivory will contribute to the identification of poorly managed game parks and facilitate steps taken to prevent the African elephant from becoming extinct. This study was aimed at establishing a database on the composition of ivory obtained from elephant sanctuary areas in Southern Africa. Fragments of elephant ivory from seven geographically distinct areas in South Africa, Namibia and Botswana were analysed for inorganic and organic content. A total of 20 elements was detected in the inorganic fraction of ivory, some in concentrations as low as 0.25 μg/g. The concentrations of calcium, phosphate, magnesium, fluoride, cobalt and zinc showed statistically significant differences ($p < 0.007$) between ivory obtained from different regions. Analyses of the organic fraction identified 17 amino acids. Ivory from arid regions showed significantly lower proline plus hydroxyproline content and under-hydroxylation of lysine residues. This study indicates that chemical analyses of ivory could be beneficial in tracing the source of illegal ivory.

Keywords: Elephant ivory; Composition; Environment; Organic and inorganic analyses

1. Introduction

The illegal harvesting of ivory is generally restricted to poorly managed game-sanctuary areas in Africa and, if unabated, will lead to the eradication of elephant from large parts of the continent (Armstrong and Bridgland, 1989; Ottichilo, 1986). The rapid decrease in elephant numbers was met by a listing of the African elephant as a protected species by the Convention on Trade in Endangered Species (CITES). Despite doubt about the future of the African elephant, their numbers in protected areas in certain African countries are indisputably growing (Armstrong and Bridgland, 1989; Hall-Martin, 1992).

Dentine (or ivory) forms the bulk of the substance of most teeth of mammals and is composed of organic and inorganic fractions. The inorganic composition of the ivory of the elephant has not yet been investigated in detail except for work done on its isotope composition. The carbon isotope ratios (^{13}C:^{12}C) of ivory distinguish between elephant roaming the woodland and those in dense forests (Van der Merwe, et al., 1988). Similarly, nitrogen isotope ratios (^{15}N:^{14}N) in the ivory of the African elephant are related to water stress or rainfall. Overlap in the distribution of carbon and nitrogen isotopes can be expected when dealing with ivory of elephant from similar environments. In such cases, strontium isotopes may be helpful in making a distinction, as ^{87}Sr:^{86}Sr ratios in tusks reflect the local

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geology of a particular region (Van der Merwe et al., 1990). No scientific information is available on the organic composition of elephant ivory.

Science can contribute to the exposure of those areas in which illegal ivory harvesting is taking place by establishing a databank on geographical variations in the chemical composition of ivory. This would contribute significantly to the identification of the source of origin of illegal ivory, making more specific intervention in poorly controlled elephant sanctuaries possible.

Our purpose now was to determine variations in the inorganic and organic composition of ivory obtained from seven distinct elephant-sanctuary areas in Southern Africa.

2. Materials and methods

Sixty-four fragments of ivory were obtained through the National Parks Board of South Africa from the following areas: north-western Namibia (Kaokoveld), northern Namibia (Etosha National Park), north-eastern Namibia (Caprivi), northern Botswana (Kavango), north-eastern South Africa (Kruger National Park), northern Natal (Tembe Elephant Park) and eastern Cape (Addo Elephant Park) (Fig. 1). The habitats vary between arid (Kaokoveld), African woody savannah (Kruger National Park and Caprivi), savannah with salt pans (Etosha), subtropical forests (Kavango), dense Karoo shrub (Addo Elephant Park) and coastal dune forest (Tembe Elephant Park). All ivory was obtained within 7 days of death from animals that had died of natural causes or as part of the population-control programmes employed in the respective areas.

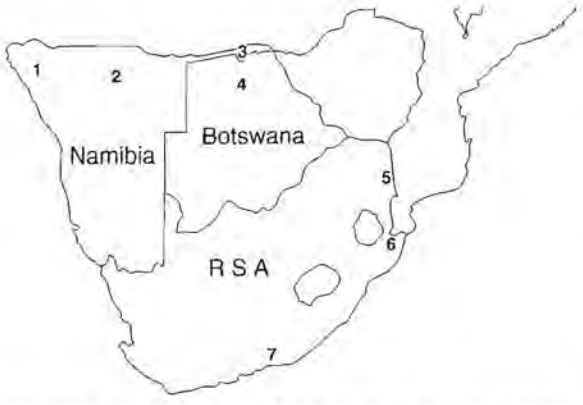

Fig. 1. Origin of ivory (1, Kaokoveld; 2, Etosha National Park; 3, Caprivi; 4, Kavango; 5, Kruger National Park; 6, Tembe Elephant Park; 7, Addo Elephant Park, RSA, Republic of South Africa).

2.1. Inorganic analyses

The dry weights of all fragments were determined accurately after carefully removing the ensheathing layer of cementum with a rotating diamond disc. The specimens were agitated in a weak acid (0.1 M HCl) for 10 min to remove traces of metal that may have contaminated the ivory during sample preparation. The fragments were washed for 15 min in distilled water and demineralized in 1 M perchloric acid at room temperature for 3 weeks. Complete demineralization was confirmed microscopically by embedding, sectioning and staining of the organic residue with von Kossa. The inorganic composition was determined by atomic absorption spectrophotometry (Perkin Elmer 500; Norwalk, CT, U.S.A.), Astra 8 analyser (Beckman Instruments Inc., Brea, CA, U.S.A.) and ion-selective electrodes (Radiometer, Copenhagen, Denmark). The mean values obtained per site of origin as well as the SD were expressed in mg/g dry weight and tabulated. The level of significance between the findings for each element and at each site was determined with the aid of the student's t-test for unequal variances after standard distribution curves had been established between paired values.

The trace-elemental composition of 27 fragments was determined with an ARL 34000 inductively coupled plasma optical emission spectroscope (ARL, Boston, MA, U.S.A.) consisting of inductively coupled argon plasma operating at 27 MHz with direct reading on a 29-channel spectrometer. Perchloric acid (1 M) was used as control in order to monitor the possibility of contamination of the demineralizing solution. The mean concentrations and SD were expressed in μg/g ivory and tabulated. The level of significance between the concentrations of each element at different geographical sites was determined by the student t-test for unequal variances.

2.2. Organic analyses

Fragments of peripulpal ivory devoid of cementum and weighing between 1 and 3 grams were prepared from five tusks of the Kruger National Park, six of Kaokoveld elephant, fifteen from Etosha, four from Tembe and two from Addo Elephant Park. The solid particles were hydrolysed in sealed tubes containing 5 ml 6 M HCl at 110°C for 24 h. The hydrolysates were neutralized, filtered (Millex-GS, 0.22 μm) and diluted 1:1 with citrate buffer (pH 2.2). Calibrants of a standard of 43 amino acids were prepared, and the amino acids of the hydrolysates and calibrants were separated in a Beckman 6300 amino acid analyser (Beckman Instruments Inc., Palo Alto, CA, U.S.A.), which incorporated a 25-cm lithium column and a four-buffer system. The chromatograms were inte-

grated and quantitated with a Hewlett-Packard 3390A integrator (Hewlett-Packard Co., Palo Alto, CA, U.S.A.). The analyses were done in duplicate in order to eliminate methodological errors. The results were tabulated as the average of the total number of residues per 100 and the SD for each amino acid calculated. The differences in the amino acid compositions between the regions with a sample size of five or more were analysed with the student *t*-test assuming unequal variances.

3. Results

3.1. Inorganic composition

The fragments of ivory weighed between 1.101 and 3.570 g after drying and Von Kossa staining of all specimens demineralized for 14 days showed complete loss of calcium. The concentrations of calcium, phosphate, magnesium and fluoride are expressed per region and a sample mean is given for each element in Table 1. Statistical analyses showed that the differences between the respective elements in the following geographical locations were highly significant ($p < 0.002$):

For calcium: Addo vs all other locations, Etosha vs Caprivi, Etosha vs Kavango, Caprivi vs Tembe.

For phosphate: Kruger National Park vs Kaokoveld, Addo vs Kaokoveld.

For magnesium: Addo vs Kruger National Park, Caprivi and Tembe vs Addo and Kaokoveld vs Caprivi.

For fluoride: Kaokoveld vs all other locations except Etosha and Etosha vs all other locations except Kaokoveld.

The respective concentrations of the trace elements are shown in Table 2. Due to the size of the samples, statistical analyses could be made only on the Addo, Etosha and Kruger National Park groups of specimen. Differences in the concentrations of the following elements between the respective groups were found to be significant:

For cobalt: Addo vs Kruger National Park ($p < 0.007$).

For zinc: Addo vs Kruger National Park ($p < 0.002$).

Twice the sample mean or higher concentrations were analysed in ivory from a single tusk in the following locations (sample mean in parentheses):

Olifantsbad (Etosha)—*copper* = 7.5 (2.2) μg/g;
Tembe—*manganese* = 11 (0.6) μg/g;
Ombika (Etosha)—*manganese* = 1.5 (0.6) μg/g;
Kavango—*iron* = 8.8 (4.4) μg/g;
Ombika (Etosha)—*iron* = 13 (4.4) μg/g;
Olifantsbad (Etosha)—*zinc* = 40 (20) μg/g;
Ombika (Etosha)—*zinc* = 54 (20) μg/g;
Koinseb (Etosha)—*aluminium* = 23 (6.2) μg/g.

3.2. Organic composition

Ivory obtained from the Kaokoveld was more brittle than that from other regions. Complete hydrolysis of Kaokoveld and Etosha ivory was obtained in less than half the time than that required for ivory from other regions. The amino acid composition of hydrolysed ivory is expressed per site of origin in Table 3. Due to the small number of samples, statistical analyses were made on ivory obtained from the Kruger National Park ($n = 5$), Kaokoveld ($n = 6$) and Etosha ($n = 15$) only. Highly significant differences ($p < 0.001$) were found in the respective amino-acid compositions of ivory from the following regions:

Hydroxyproline Kruger ivory (10.2 parts/10^6, SD 2.6) significantly higher than Etosha ivory (9.2 parts/10^6, SD 0.7);

Proline Kruger ivory (13.1 parts/10^6, SD 0.5) significantly higher than Etosha ivory (11.6 parts/10^6, SD 0.8);

Table 1
Inorganic composition of ivory per site of origin (average mg/g dry wt, SD in parentheses)

Origin	Number of specimens	Ca	PO_4	Mg	F
KNP[1]	13	195.8(17)	115.5(5)	14.6(3.2)	0.08(0.01)
Kaoko[2]	9	193.7 (15.9)	118 (2.6)	18.2 (4.2)	0.11 (0.02)
Etosha	26	192 (16)	116 (3.5)	15.3 (4.2)	0.12 (0.03)
Caprivi	6	208.9 (6.4)	114.7 (4)	13.1 (0.9)	0.07 (0.01)
Kavango	4	205.9 (1.8)	115.3 (3)	12.2 (2.7)	0.06 (0.01)
Tembe	3	191.1 (8.9)	113.1 (5)	14.7 (1.2)	0.05 (0.01)
Addo	3	170.8 (2.5)	113 (1.4)	17.3 (0.4)	0.03 (0.02)
Sample mean	64	195.6 (15.7)	115.5 (4)	16.4 (4.4)	0.09 (0.04)

[1] Kruger National Park; [2] Koakoveld.

Table 2
Trace elemental composition of ivory per site of origin (mean (μg/g dry wt, SD in parentheses)

Origin	No. Spec.	As	Cd	Cr	Co	Cu	Pb	Mn	Hg	Ni	Fe	Zn	Mo	Al
KNP	5	8.5(0.7)	0.44(0.03)	4.1(0.2)	0.8(0.07)	2.06(0.3)	8.9(1.0)	0.3(0.09)	1.5(0.2)	0.9(0.08)	2.3(0.3)	20.4(3.0)	0.6(0.02)	3.8(0.4)
Kaoko	3	7.2(0.6)	0.4(0.02)	3.4(1.0)	0.77(0.05)	1.9(0.2)	9.7(0.4)	0.44(0.3)	1.5(0.2)	1.0(0.08)	4.0(1.1)	17.0(8.4)	0.56(0.08)	6.5(0.2)
Etosha	6	8(1.4)	0.4(0.03)	3.8(0.4)	0.7(0.09)	2.8(2.4)	8.6(1.2)	0.61(0.4)	1.4(0.2)	0.9(0.1)	6.6(4.5)	27.8(17)	0.55(0.06)	8.8(6.6)
Caprivi	1	6.0	0.37	2.6	0.75	2.0	11.0	0.25	1.2	0.79	1.6	13.0	0.47	3.6
Kavango	3	8.2(1.3)	0.44(0.03)	4.0(2.0)	0.68(0.09)	2.3(0.4)	9.1(2.7)	2.43(2.9)	1.5(0.1)	0.9(0.13)	5.8(4.2)	24(12.7)	0.58(0.0)	8.5(5.0)
Tembe	3	9.3(2.4)	0.4(0.2)	3.9(0.3)	0.7(0.02)	2.2(0.6)	8.4(0.3)	2.2(4.3)	1.6(0.1)	0.9(0.05)	5.2(2.3)	22.0(7.0)	0.6(0.03)	6.2(1.1)
Addo	4	7.8(2.0)	0.36(0.04)	3.1(0.5)	0.64(0.06)	1.9(0.5)	8.0(1.4)	0.39(0.1)	1.3(0.2)	0.8(0.1)	3.3(1.5)	16.6(5.8)	0.5(0.07)	4.6(1.6)
Sample mean	25	8.0(1.4)	0.4(0.04)	3.7(0.6)	0.72(0.1)	2.2(1.2)	8.7(1.2)	0.6(0.9)	1.4(0.2)	0.89(0.1)	4.4(3.2)	20(10.8)	0.56(0.06)	6.2(4.3)

Origin abbreviations as in Table 1.

Table 3
Total amino-acid composition of hydrolysed ivory (expressed as the average of the total number of residues per 100 above, SDs below)

Origin Specimens	Sample size	Asp	Hypro	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ileu	Leu	Phe	Hylys	Lys	His	Arg
KNP	5	5.3	10.2	2.2	4.2	8.3	13.1	30.6	9.8	2.6	0.3	1.1	3.0	1.5	0.7	1.4	0.8	4.2
		0.2	2.6	0.4	0.3	0.2	0.5	0.5	0.3	0.3	0.1	0.1	0.3	0.2	0.1	0.3	0.4	0.5
Kaoko	6	5.0	9.8	2.1	4.0	8.2	12.5	30.7	10	2.5	0.2	1.2	3.0	1.5	0.4	2.9	0.6	4.5
		0.3	0.8	0.2	0.1	0.2	1.0	0.7	1.1	0.3	0.1	0.1	0.2	0.2	0.2	0.6	0.1	0.5
Etosha	15	5.0	9.2	1.9	4.0	8.3	11.6	31.3	10.8	2.3	0.5	1.2	2.9	1.5	0.4	3.0	0.7	4.6
		0.3	0.7	0.2	0.2	0.2	0.8	1.2	1.2	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3
Tembe	4	5.0	11.3	2.0	4.0	8.1	12.5	30.1	9.3	1.9	0.4	1.1	3.0	1.5	0.6	1.6	0.8	4.3
		0.2	0.2	0.1	0.2	0.1	0.1	0.4	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.4
Addo	2	5.0	11.4	2.0	3.9	8.2	12.4	30.0	9.2	1.9	0.5	1.1	3.0	1.6	0.3	3.3	0.6	4.9
		0	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0	0	0	0.1	0	0	0	0	0
Sample mean	32	5.1	9.9	2.0	4.0	8.0	12.2	30.8	10	2.3	0.4	1.2	3.0	1.5	0.4	2.7	0.7	4.6
		0.3	1.0	0.2	0.2	1.3	1.2	1.0	1.2	0.3	0.4	0.1	0.1	0.1	0.2	0.7	0.2	0.4

Asp, aspartic acid; Hypro, hydroxyproline; Thr, threonine; Ser, serine; Glu, glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ile, isoleucine; Le, leucine; Phe, phenylalanine; Hylys, hydroxylysine; Lys, lysine; His, histidine; Arg, arginine.

Lysine	Etosha (3 parts/10^6, SD 0.2) and Kaokoveld (2.9 parts/10^6, SD 0.6) significantly higher than Kruger ivory (1.4 parts/10^6, SD 0.3);
Hydroxylysine	Kruger ivory (0.7 parts/10^6, SD 0.1) significantly higher than Etosha (0.4 parts/10^6, SD 0.1) and Kaokoveld ivory (0.4 parts/10^6, SD 0.2).

The difference in the hydroxylysine content between Kruger and Kaokoveld ivory was significant ($p < 0.01$). Although the number of tusks analysed from Addo and Tembe was too small for statistical analyses, the concentrations of the mentioned amino acids in these regions tended to follow the pattern observed in Kruger ivory and appeared to differ from those of ivory obtained from Etosha and the Kaokoveld in the mentioned respects.

4. Discussion

During the formation of dentine (or ivory), which is essentially a biological apatite deposited on an organic matrix, over 45 elements compete for incorporation (Wetherell and Robinson, 1973). It is, however, not clear whether all these elements are structural substitutes in the hydroxyapatite crystal or whether they are absorbed onto the crystal surface. The inorganic composition of ivory reflects greatly the composition of an animal's diet (Posner and Tannenbaum, 1984). Unlike bone or any other tissue, the composition of ivory remains stable after its formation as it is not subject to turnover and remodelling throughout life. This phenomenon can be exploited to monitor environmental pollution and identify the site of origin of a tusk.

An extensive databank on the composition of ivory from the different conservation areas in Africa could assist in tracing the origin of ivory and might play a key role in identifying regions in which illegal tusk harvesting is taking place. This study, as well as others (Van der Merwe et al., 1988; Van der Merwe et al., 1990; Vogel et al., 1990), clearly indicates the realistic possibility of tracing the source of ivory on its chemical composition. The techniques used in the trivariate isotope analyses reported by those groups are expensive and the equipment is not readily available. Our study indicates that the concentrations of elements such as calcium, phosphate, magnesium, fluoride, cobalt and zinc are of potential value in identifying the site of origin of Southern African ivory. Addo, Kaokoveld and Etosha ivory in particular have unique compositions: Addo ivory is distinguished by its low calcium content and Kaokoveld and Etosha ivory by a high fluoride content. Cobalt, zinc and magnesium content hold great potential in facilitating a distinction between ivory from the Kruger National Park and Addo Elephant Park, phosphate to distinguish between Kaokoveld and Kruger ivory, and calcium and magnesium between Caprivi and Tembe ivory. The high concentrations of copper, manganese, iron, zinc and aluminium in selected tusks obtained from different regions in Etosha are interesting and warrant further investigation. The area is characterized by many salt pans. Rain is sporadic and, owing to evaporation, the salinity of water in most of the pans is high. High iron concentrations in ivory should, however, be interpreted with caution as haemolysis or pulpal haemorrhage during death could theoretically lead to an increase in iron. The overlap in composition of ivory obtained from the Kaokoveld and Etosha supports the fact that the geochemistry of the regions is similar in many respects and that elephant migrate between these regions.

The geochemical composition of a particular region is subject to change. Following the rapid economic growth experienced in urban areas after the Second World War, diseases associated with environmental pollution increased due to delayed implementation of countermeasures preventing spillage of heavy metals and other elements in the environment (Kagawa, 1994). The determination of the elemental load in the tissues of man and animals will play an increasing part in future preventive environmental medicine. For reasons already mentioned, the study of the composition of ivory (or dentine) is ideal for this purpose. The concentrations of the 17 elements identified in elephant ivory in this study reflect the chemical nature of the environments in which these animals roam. Most of these elements occur in higher concentrations in heavy industrialized and polluted areas (Hirano and Suzuki, 1996; Andersen et al., 1996; Kusaka et al., 1996) and our findings could serve as baseline values to monitor future pollution of natural resources in Africa.

The presence of minute quantities of mercury and lead in ivory is proof that these elements, which are generally regarded as pollutants, are present in natural ecological systems. In 5000-year-old premolars from Nubia and in 500-year-old teeth from Greenland the lead concentrations were between 0.39 and 3.4 μg/g or less than 25% of the sample mean of ivory in our study. Modern teeth from the same regions contain 10–100 times more lead (Grandjean and Jorgenson, 1990). Many elements occur in the foliage of plants and seasonal fluctuations in the concentrations of cadmium, lead, copper, zinc, manganese and selenium in the liver and kidneys of reindeer in Svalbard in Norway have been reported (Borch-Ionson et al., 1996). Lead is present in plants and soils, and its metabolism follows closely that of calcium, particularly

its deposition in mineralized tissue (Browning, 1969a), and over 90% of the body's burden of lead is located in the skeleton (McDonald et al., 1951). Mercury occurs in minute quantities in nearly all foods and is widely distributed as free metal in soils, dust and water (Browning, 1969b). Arsenic is present in all soils in amounts varying from less than 10 to 500 parts/10^6 and is stored in all tissues, particularly the hair and nails (Browning, 1969c). Most mammals, including man but excluding chimpanzees, methylate arsenic to methylarsonic acid, which is rapidly excreted in the urine (Vahter et al., 1995). The presence of arsenic in all the ivory samples studied is indicative of the elephant's inability to methylate and detoxify arsenic. Cadmium is present in the order of 1 part/10^6 in many plant and animal tissues and occurs in abdominal organs and in smaller quantities in bone and teeth (Browning, 1969d). All our ivory samples had a cadmium content of below 1 part/10^6. A concentration of 0.2 μg/l was found in the drinking water in Germany (Muller et al., 1996) and certain plants accumulate all available cadmium from the medium in which they grow (Devi et al., 1996). The vegetation of a particular region could therefore concentrate the available cadmium and contribute to dietary overload. The cadmium content of rice samples from various areas in the world varied between 0.88 and 133.2 ng/g (Watanabe et al., 1996).

Chromium occurs in nature as chrome iron ore and is found in small quantities in soils and plants (Browning, 1969e). Cobalt is present in complex chemical compounds in ore as well as plants growing in cobalt-containing soil. Cobalt is essential to animal nutrition as anaemia develops in its absence (Browning, 1969f). Copper is widely distributed in animal tissues and human ingestion is estimated to be in the region of 2 mg/day. It occurs free as native copper or in ores, and is found in trace amounts in plants (Browning, 1969g). Manganese is essential for the nutrition of plants and animals, and is widely distributed in nature as an oxide, a sulphide, a carbonate, a silicate or in other ores (Browning, 1969h). Nickel constitutes approx. 0.016% of the earth's crust and is widely distributed in plants, especially green leafy vegetables (Browning, 1969i). The chief storage depots are the spinal cord, brain, lungs and heart (Ware et al., 1954). Zinc is estimated to represent 0.004% of the earth's substance and is 25th in order of occurrence, approaching that of iron and greater than copper or manganese (Lutz, 1926). Zinc is required for enzymatic function and the highest amounts have been found in the liver, voluntary muscle and bone, with a total body zinc content of 1.36–2.32 g in man (Browning, 1969j). Molybdenum is essential for all nitrogen-fixing higher plants, and legumes, cereal grains and green leafy vegetables are good sources. Its metabolism in animals is closely linked to that of copper, with which it appears to have reciprocal antagonism. The concentration of molybdenum is similar to that of manganese, where it is highest in the liver (1–3 parts/10^6) (Browning, 1969k). Aluminium is present in a natural diet in amounts that are very low in animal products but high in plants. A significant proportion of aluminium is inhaled, and the amount of aluminium in the organs, blood and urine is small (Browning, 1969l).

The increased brittleness and rapid rate of hydrolysation of ivory from the Kaokoveld and Etosha compared to the other regions are of interest. The annual rainfall in the Kaokoveld and parts of Etosha is below 200 mm/year. The aridness of the region, characterized by dry savannah and shrub, has led to the term 'desert elephant' for those elephant that were driven into the region by human inhabitation of the more arable land (Viljoen, 1987; Pauw, 1990). There is good reason to believe that the diet of these elephant differ significantly from those in the other regions studied. Analyses of ivory from the Kaokoveld and Etosha show the highest fluoride concentrations, lowest total proline plus hydroxyproline content and under-hydroxylation of lysine as unique characteristics. The high fluoride content is probably the result of the water that collects in the closed systems of the salt pans and becomes concentrated by evaporation. Although the substitution of the hydroxyl group with fluoride in the hydroxyapatite crystal increases its resistance to dental caries, it softens the mineral phase of the ivory significantly (Lavelle, 1975), thereby weakening the crystal. The low proline plus hydroxyproline content, as well as the under-hydroxylation of lysine, are likely to be the result of malnutrition and may also have a profound effect on the strength of the collagen scaffold of mineralized tissue (Chatterjee, 1978). Vitamin C, iron and oxygen are cofactors required for the enzymatic hydrolysation of lysine during biosynthesis of the tropocollagen molecule (Anderson, 1992). Insufficient dietary vitamin C is likely to be the main cause of synthesis of the under-hydroxylated lysine-containing collagen in Kaokoveld and Etosha ivory. This is supported by the craving of the Kaokoveld elephant for fresh fruit, especially citrus, to the dismay of many travellers through the region.

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