ACTA ORTHOPAEDICA SCANDINAVICA
SUPPLEMENTUM NO. 155

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Physical properties and chemical content of canine femoral cortical bone in nutritional osteopenia

Its reversibility and the effect of fluoride

by

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MUNKSGAARD
Copenhagen 1974

Printed in Sweden by
Göteborgs Offsettryckeri AB, Kungälv 1974

ISBN 87-16-01657-2

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PREFACE

This study originates from the Biomechanical Laboratory, Department of Orthopædic Surgery II, University of Göteborg. The late professor Carl Hirsch was the founder and leader of this laboratory unit during 1961 to 1969. Under his guidance a number of investigators contributed to the knowledge of tissue mechanics: Hirsch & Evans (1965), Sedlin (1965), Semb (1966), Sedlin & Hirsch (1966), Viidik (1966), Hirsch & Da Silva (1967), Galante (1967), Tkaczuk (1968) and G. Hirsch (1974). To make use of this knowledge C. Hirsch was in 1968 contacted by a team headed by professor Lennart Krook at New York State Veterinary College, Cornell University, Ithaca, New York. Krook had in many years shown a deep interest in and knowledge of nutritional effects on skeletal metabolism. Studies in dogs of the possibilities to prevent or treat experimental nutritional osteopenia were started. I was then elected to perform, under the guidance of C. Hirsch, the determinations of the physical properties of cortical bone specimens from these dogs. The team consisted of Per Åke Henriksson, Francis Kallfelz, Lennart Krook, Ben Sheffy (New York State Veterinary College, Cornell University), Leo Lutwak (Graduate School of Nutrition, Cornell University), Leonard Bêllangere (Faculty of Medicine, University of Ottawa), Rodney Skogerboe (Colorado State University, Fort Collins). The result of these studies in nutritional osteopenia were reported by Henriksson et al. (1970) and by Krook et al. (1971) in which some of my preliminary results of the mechanical testing were reported. When Carl Hirsch left Göteborg in 1969 to become professor of Orthopædic Surgery and head of the Norrbacka Institute, University of Stockholm, I remained in the Biomechanical Laboratory in Göteborg to finish this study. By his vast international connections prof. Hirsch arranged for me in November 1970 as visitor to the Biomechanical Unit, Department of Mechanical Engineering, University of Surrey (head: professor Josef Zarek) to perform an investigation of the distribution of minerals in bone at the site of a fracture. This technique; electronprobe X-ray microanalyser (Geoscan) was mainly for metallurgic purposes, and had not previously been used on biologic materials. As the result of this investigation was not conclusive it was felt necessary to perform standard chemical analysis of the test specimens. This was performed in various special laboratories.

I am most grateful to the leaders and their staff of these laboratories for their willing co-operation: assistant professor Annika Dahlström, assistant professor Thorild Eriksson, professor Yngve Eriksson and professor Andrus Viidik.

As the late *professor Carl Hirsch* introduced me to orthopædic surgery and especially to the biomechanical aspects of the subject and encouraged me to perform this investigation I will always be grateful to him and regard it as a previlege to have been one of his pupils.

I am also most grateful to my chief professor Bertil Stener for supplying laboratory facilities and especially for his willingness to enter the field of bone biomechanics and his logical criticism in the final stages of this presentation.

I also want to thank associate professor Ian Goldie for facilitating for me to perform this study during my training in orthopædic surgery and for his revision of my English text.

Without the help of a number of persons this investigation would not have come to an end and I am indepted to them, especially:

Eng. Hans Hellström for his excellent and sacrificing technical assistance and Mrs. Birgitta Runze for her patient laboratory assistance

and the following persons who have donated their special skills to this work: Margareta Apelskog, Barbro and Roland Johansson, Lars Johansson, Klas Ljung, Ole Roos, Carl Sandell and Hans Wedel.

Financial support for this investigation was provided by:

Medicinska Forskningsrådets projektnummer B71-17X-565-07/B74-17-565-10
Konung Gustaf V:s 80-årsfond
Göteborgs universitet
Göteborgs Läkarsällskap (Högbergs fond).

INTRODUCTION

The knowledge is scant of the factors in the environment and in the body itself influencing the physical properties of bone. During the last decade there has been an increasing interest in such dietary factors that might affect the whole skeleton especially in human osteoporosis. Clinical fractures as a result of a minor trauma in elderly persons with or without obvious senile osteoporosis is an accelerating clinical problem with increasing average age of the population. Thus it has been of great interest to find possibilities to prevent or treat this condition and/or to strengthen the bone as such. Dietary additions of fluoride and calcium – in recent years in combination with vitamin D (Jowsey et al., 1972) has been of major interest.

The way of measuring the influence of various treatments on the physical properties of bone has been of two types.

In the *clinic* mainly *indirectly* either by determining the fracture incidence in a certain population or by deductive studies of X-ray density. In *experimental* studies in animals *direct* determinations of the physical properties have been performed. Nutritionally induced osteopenia in experimental animals has been used as a model resembling human osteoporosis. Under the leadership of professor Lennart Krook at Cornell University a big team has been concerned with the possibilities to prevent this experimental condition in dogs by adding various doses of fluoride to diet (Henriksson et al. 1970). The same group has also studied the reversibility of this condition (Krook et al. 1971).

This presentation is a report of my studies on the physical properties of the femoral cortical bone material and its chemical composition in these just mentioned dog series. The study is mainly concerned with the bone material as such. The reason for chemical analysis of the pieces submitted to mechanical testing was to confirm that the same changes would appear in strictly cortical bone that had been recorded in the rest of the skeleton by the group at Cornell.

The numerous reports in the literature of studies of physical properties of bone have mainly been of two types; of whole bone or of standardized bone specimens. Most of the studies regarding influence on the physical properties of different experimental conditions such as variation in diet and immobilization of an extremity has been performed on whole bones. The studies of bone specimens have early been concerned with various testing modes and techniques but in recent years the microscopic structure, degree of mineralization and fiber orientation has been the main field of interest. However, very few attempts have been made to correlate the amount of several different chemical components and the physical properties of the same specimen.

The access of bone from different experimental dog groups from the Cornell university seemed to offer an opportunity to perform a combined study of the dietary effect on the physical properties of the cortical bone and its chemical composition. The possibilities to compare the findings in normal dogs with those in dogs treated for nutritional osteopenia seem to have great clinical interest, since fluoride and calcium therapy have been suggested and been given to human osteoporosis - osteopenia. The importance of individual factors as sex, age and body weight seemed also worth while to study.

Thus my purpose was to study and to try to correlate the physical properties and chemical content of the femural cortical bone, especially the changes when trying to reverse nutritional osteopenia by corrected diet and prevent it by fluoride treatment.

REVIEW OF PHYSICAL AND CHEMICAL PROPERTIES OF CORTICAL BONE

A complete survey of the vast literature in the field of bone biomechanics will not be presented since excellent reviews have been published in recent years.[1] For further detailed study in the field of physiology, biology and pathology of bone the reader is referred to the reviews by Bourne (1956), Chvapil (1967), Harris & Heaney (1969), Vaughan (1970), Vittali (1970).

Microscopic structure of bone

One can distinguish two major types of *cortical bone* depending on the orientation of the different components.

Lamellar bone consists of different layers of collagen fibers surrounded by mineral crystals with different direction in each layer.

Haversian bone (secondary bone, osteonic bone) consists of secondary bone after remodelling by vascular canals around which concentric layers of collagen fibers with mineral crystals are deposited. In most of the cortical bone from long bones there is a predominant direction of the osteons which are parallel to the axis of the long bone. The osteons are surrounded by a cement line which is not crossed by any collagen fibers and it is considered to be weaker. Between these osteons there are remnants of lamellar bone or of old osteons. The amount of lamellar bone and osteonic bone varies in different species and different bones from each individual. On a cross section of a long bone lamellar bone can as a rule be found adjacent to the periosteal and endosteal surfaces, with osteons more or less regularly distributed between these layers. It is generally accepted that with increasing age the mineralization of the bone increases and the water content of the bone decreases. The cortical thickness tends to decrease mainly by increase of the internal diameter as well as increasing porosity.

Chemical composition

The chemical composition has been studied by numerous authors.[2] Cortical bone

[1] Bell (1952), Evans (1957), Evans (1961), Kraus (1968), Currey (1970), Frankel & Burstein (1970), Wall (1970), Welch (1970), Yamada & Evans (1970), Swanson (1971), Kummer (1972), Evans (1973).

[2] Rodgers et al. (1952), Eastoe & Eastoe (1954), Sobel et al. (1954), Eastoe (1956), Robinson & Elliott (1957), Robinson (1960), Woodard (1964), Strandh & Norlén (1965), Birkenhäger-Frenkel (1966), Hayek (1967), Lindahl & Lindgren (1967), Birkenhäger & Birkenhäger-Frenkel (1968), Blanton & Biggs (1968), Biltz & Pellegrino (1969).

is composed of three major constituents: water, inorganic substances and organic substances.

Water is present mainly in the interstitial tissue and partly as crystalline water, with a total amount of about 11 % of the wet weight.

Most of the ***organic substances*** (96 %) is collagen in the form of fibers arranged in bundles, with a total amount of about 20 % of the dry weight.

The ***inorganic substances*** are mainly composed of crystals of calcium-hydroxy-apatite $Ca_{10}(PO_4)_6OH_2$ which are arranged along the collagen fibers. Calcium is also present as amorphous calcium-phosphate and as crystals of calcium carbonate. The calcium and phosphorus content is 26 % and 11 % of the dry weight respectively. Minor amounts of magnesium (0.39 %), fluoride (0.1 %), sodium and potassium are also found in the bone.

PHYSICAL PROPERTIES OF BONE

Some basic definitions will be given. For a more comprehensive presentation the reader is referred to standard textbooks on mechanical engineering by e.g. Cottrell (1964) and Hulth (1966) or to reviews of bone biomechanics by e.g. Swanson (1971).

Force is that interaction between bodies which tends to deform or accelerate them.

Stress is the force per unit area of the material.

Strain is the deformation per unit length of the material.

A stress strain curve for the material is the result of the registration of load and deformation of a material when loading to any desired stress or strain. When testing bone the following characteristics are defined:

The elastic region, where the stress strain curve is almost linear.

The elastic modulus or elastic stiffness (Young's modulus of elasticity) is the changing of unit stress per unit strain in the linear portion of the curve.

The elastic limit or the limit of proportionality is the point where the elastic region ends and where the stress strain curve ceases to be linear.

The yield stress is the stress at the limit of proportionality beyond which for practical purposes *the plastic region* extends to the fracture. Removal of stress in this region leaves a permanent deformation of the material.

The ultimate stress is the stress at the fracture, or the point of maximal load.

Cortical bone is an anisotropic, inhomogeneous, composite material which could be defined as a visco-elastic plastic material. It shows pure elastic stiffness when tested to low stresses and thereafter plastic deformation before fracturing. The stiffness (elastic modulus) and the breaking strength (ultimate stress) vary with the rate of deformation (visco-elastic). It shows higher stiffness and higher breaking strength and less deformation at high rates of deformation than at low. It has been compared to composited two phase materials such as concrete or fiber glass (Currey, 1964). The hydroxyapatite-crystals corresponds to the glass with high resistance in compression and the collagen fibers corresponds to the epoxy recine as a glue. Fiber glass is also an unisotropic material with one predominant fiber direction.

FACTORS AFFECTING THE PHYSICAL PROPERTIES OF CORTICAL BONE

A number of different techniques of testing different physical properties of bone has been presented since the first studies of the mechanical properties of bone were reported by Wertheim (1847), Rauber (1876) and Messerer (1880), and there is a large scatter of the results presented. A comparison of the numeric results from different authors can hardly be made since the results depend on the mode of testing: dynamic (vibration, impact) or static (compression, tension, bending, torsion, shear). The amount of bone tested also varies from whole bone as an organ to single osteons. Even when testing small specimens of pure compact bone differencies are seen depending on species, source of origin (femoral, humeral, tibial, fibular) and location within each bone. Testing conditions such as temperature, drying, and embalming also affect the results. Some of these factors will be discussed.

Individual factors

Sexual differences were reported by Evans (1957). He summarized the results of his own and of others and stated that male femoral bone was stronger than female when tested fresh. This was not confirmed by Lindahl & Lindgren (1967) when testing tensile properties of dry cortical strips from human humerus and femur. In compression tests they found (1968) that male femoral bone was slightly stronger than female but this was not true for humeral bone.

Species

Comparison between several species have not been studied by any single author using the same technique and thus no pure comparison can be made, but the values found in the testing of bone from animals (rat, dog, cat and ox) and from human beings are of the same magnitude.

Histological and chemical factors

Source of origin

Differences in physical properties between samples from different bones (fibula, tibia, humerus, and femur) have been found among others by Evans (1958, 1964), Lindahl & Lindgren (1967), Mather (1968). Variations within the same bone have been shown by Valmsley & Schmitt (1957), Forssblad (1959), Sedlin (1965), Melick & Miller (1966), Semb (1966), Amtmann & Schmitt (1968), Da Silva (1970) among others. Differences were shown both along the length of one bone and around its circumference. Samples from human femoral bone seem to be strongest when taken from the lateral quadrant and weakest from the posterior one. The reasons for these variations between various bones and various locations in one bone are still obscure. Longitudinal specimens have higher strength and higher stiffness than those taken radially or tangentially to the cross section of a long bone (Hirsch & Da Silva, 1967, Bird et al., 1968).

Microscopic structure

On the osteonic level Ascenzi & Bonucci (1967) have shown that tensile strength and elastic stiffness in tension were greater and the percentage elongation was lower in such osteons where the collagen bundles had marked longitudinal arrangement. Different types of osteons could be found within the same bone even in the neighbourhood of each other. Primary lamellar bone was stronger than secondary Haversian bone as found by Currey (1959) and Hert et al. (1965), who concluded that the Haversian system makes a bone weaker probably depending on lower mineralization degree of newly formed osteons. Evans (1958) made calculations of the physical properties per the »effective area». The effective area is the cross section area of the specimen minus the area occupied by vascular holes, osteon canals etc. According to Evans these occupied 20 - 28 % of the total area. But still he found differences in tensile strength between specimens from different bones. Further investigations in this field have been published by Evans & Bang (1967) and Evans & Vincentelli (1969). Specimens tested in cyclic loading by Evans & Riolo (1970) showed a contrary picture with increasing »fatigue life» with increasing percentage of osteons and decreasing »fatigue life» with increasing amount of interstitial lamellar bone.

Another feature of the presence of osteons was pointed out by Currey (1962) who suggested that the spread of fracture cracks is delayed by the presence of osteons and that the spreading of cracks followed the cementing lines. Piekarski (1970) found that in slow crack propagation rate the crack travelling distance in the material was lengthened by the crack passing around the osteons following these cementing lines and thus increasing the energy absorption in the material. In high crack propagation rate there was not such an arrest and the crack followed the shortest way between the center of the osteons.

Degree of mineralization

Vose (1962) found a strong correlation between the degree of mineralization in the interstitial bone determined by microradiographic densitometry and strength at the elastic limit. He could not find any correlation between the number and sizes of the Haversian canals and the strength. But he did find that in thinner cortical bone the bone material was stronger than in thicker regions. A strong correlation between the degree of mineralization and tensile properties was also found by Ascenzi et al. (1966). In fully mineralized osteons the stress at fracture and elastic stiffness was nearly twice that of osteons with minimum mineralization. Mather (1968) found in bending test of whole human femur a correlation between the ultimate strength and the specific gravity but not with the ash content. On the other hand the elastic stiffness was correlated with the ash content but not with the specific gravity. Currey (1969) showed a linear correlation between the ash content and the elastic stiffness. Schmitt (1968) found a positive correlation between bone density and strength when testing whole femur in compression.

It is obvious that complete understanding of all factors determining the physical properties of cortical bone is a rather complex problem which needs further investigations.

OSTEOPOROSIS – OSTEOPENIA

Osteoporosis and osteopenia are in this context defined as subnormal amount of bone material, of virtually normal composition, expressed per unit volume.

Nutritional experiments in animals leading to osteopenia

Several authors have studied nutritional effects on bone metabolism. Most of the earlier experiments have been with rachitogenic diets, but in the last decade more interest has been concerned with nutritionally induced conditions that resemble human osteoporosis.

McCance & Widdowson (1966) reported on the composition of cortical bone in undernutrition and found no significant difference in comparison with normal animals. Hammond (1969) has reported on the effect of bone growth and resorption in rats fed meat diet (low calcium, high phosphorus content). The bone formation was slightly reduced and the resorption exceeded 2 to 3 times the rate of bone growth.

Saville & Krook (1969) demonstrated an experimental model of osteopenia in beagle dogs. A low calcium and high phosphorus diet resulted in loss of bone first in vertebræ, then in long bones during a period of 1 year. All changes were explained by nutritionally induced secondary hyperparathyroidism. Nguyen & Jowsey (1970) studied the effect of thyroid-parathyroidectomy in dogs compared with normals. They showed that there were differences in all bone regions but less marked in long bones where the bone-turnover was less. Saville et al. (1969) described an osteopenic condition in one dog which normalized after 63 days of calcium therapy.

Harris et al. (1968) studied the formation of cortical bone and found great individual differences as well as differences depending on time and location within one bone and concluded that a biopsy from one region does not tell anything about the rest of the skeleton of this individual.

PHYSICAL PROPERTIES OF BONE IN OSTEOPOROSIS – OSTEOPENIA

Human osteoporosis (age variations)

Few studies have been reported on the physical properties of cortical bone in human osteoporosis. Care has usually been taken to avoid bones from patients with skeletal diseases when testing physical properties of human bone. The studied variations are thus mainly age influence on the physical properties in persons with apparently normal skeleton.

When bending whole shafts of femur and humerus from human osteoporotics Vose et al. (1961) found a decrease of 40 % of the breaking strength in osteoporotic bones but when studying cortical specimens of the bone an increase of 30 % of the strength. Melick & Miller (1966) found that tensile strength in samples from tibia was significantly lower in individuals over than under 68 years of age. No changes in the ash, calcium or organic contents of the cortical bone were found. They concluded that the internal bone architecture is responsible for the physical properties. They also found variations in strength but not in chemical composition between specimens from different parts of the same bone (tibia). Lindahl & Lindgren (1967, 1968) found decreasing tensile and compression strength with increasing age. Bell & Dunbar (1967) found that with increasing age the decrease in compressive strength was more pronounced than the corresponding decrease in ash weight of human vertebræ.

No age variation could be found by Sedlin (1965). In testing single osteons Ascenzi et al. (1966) could not find any difference between the same type of samples from men of 30 to 80 years of age.

Experimental osteoporosis – osteopenia

The experimental model that has been used for studying osteopenia in animals has been mainly of two types: immobilization or decreasing the calcium content in the diet.

Disuse osteopenia

Allison & Brooks (1921) studied the disuse osteoporosis in dogs produced in different groups by immobilization, denervation, or resection of long bone adjacent to a joint. When testing the whole bone (humerus) they found decreasing bending strength in all three groups compared with normal limbs. On testing cortical bone strips there was no significant difference in the bending strength and they concluded that the disuse osteopenia is a reduction of the amount of bone and not a change of the bone material as such. Gillespie (1954) in a similar study of kittens and rats found that the altered properties in bending were almost entirely due to a loss of the quantity of bone while the quality was only slightly altered. Semb (1966) confirmed this, when testing specimens in bending from immobilized or denervated fore limbs in dogs. Microradiographs showed after 3 weeks osteopenic changes with decrease of cortical thickness and fewer spongious trabeculæ. Nilsson & Smith (1969) studied the breaking force in compression of proximal tibial specimens after tibial fractures and found more pronounced decrease in compression strength than in ash weight.

Low calcium diet

Light & Frey (1941) found spontaneous death of rabbits after 3 - 8 weeks on low calcium and high phosphorus diet partly depending on spontaneous fractures of vertebræ. Bending tests on the femur of rats on different levels of calcium concentration in the food were reported by Bell et al. (1941). The length and the outer diameter of the rat femur were not changed but the inner diameter and thus the medullary canal were narrowed in groups with high content of calcium in the diet. The bending and torsion strength increased to a maximum at the level of 0.36 % calcium in the food. They concluded that it is only the dimensions of the femoral cortical bone that is influenced by the dietary level of calcium and that the chemical and physical structures are not influenced by the diet. The same results were reported by Salomon & Volpin (1972) in growing rats on low calcium diet which gave lighter and thinner long bones and decreased bending strength of metatarsals with increasing deflection in bending.

Weir et al. (1949) found decrease of bending strength and stiffness and increase of deflection in rats on a rachitogenic diet even with supplement of vitamin D. They concluded that the effects were depending of, and correlated with, a corresponding decrease in ash volume.

No reports on the effect of reversibility of different kinds of experimentally produced osteopenic conditions have been found except for the study reported by Krook et al. (1971).

POSSIBILITIES OF FAVOURABLE INFLUENCE ON THE PHYSICAL PROPERTIES OF CORTICAL BONE

Some studies have been reported of the possibilities to increase the breaking strength, the elastic stiffness or the energy absorption. Saville & Smith (1966) report the effect of increased weight-bearing in bipedal rats, which resulted in increasing muscle mass, density and breaking strength of femur. But as this presentation is a report of the dietary effect on the physical properties of bone, the influence of other treatments (for instance training) will not be penetrated.

Interest in recent years have been concentrated on the favourable effects of dietary added fluoride in skeletal diseases.

Fluoride effect on bone

The vast literature on the fluoride effect on bone is excellently reviewed by Faccini (1969) who noted that in the last 35 years over 16.000 papers have been published on the biological effect of fluoride. Some of these effects are also reflected in WHO's »Fluorides and human Health» (1970). The fluoride ion when added to the diet (mostly as NaF) is incorporated in the bone and substitutes the OH group in the calcium hydroxyapatite crystals (Posner et al. 1965). The fluorideapatite crystal is smaller than the hydroxyapatite one and is less soluble in fluids. The incorporation of the fluoride ion in the bone is similar in different animals. In vitro the fluoride ion cathalyses this crystallization of the mineral along the collagen fibers (Münsenberg & Rössler, 1970). Gardner et al. (1959) showed that the concentration of the fluoride was higher in the metaphysis than in the mid part of the long bone and even higher in the tuberosities than in smooth cortical bone (Weidmann et al., 1959). This was confirmed by Henriksson et al. (1970) who found that the accumulation of the fluoride was higher in more spongy bone than in cortical bones. The accumulation seems to be time dependent.

Zipkin et al. (1969) found no effect on calcium or phosphorus concentration in human bones of fluoride administration in the food. There was no influence of the uptake of radiocalcium in the skeleton (Likins et al 1964). The magnesium content seems to increase in the skeleton with increasing fluoride administration (Weidemann et al., 1959, Zipkin et al., 1960, Avioli & Berman, 1968).

Peck et al. (1955) showed reduced collagen synthesis when fluoride was given to rats.

Disturbed balance between osteoblastic and osteoclastic activities was found in rat bone by Röckert (1963): first osteosclerosis was noticed and thereafter great resorption cavities. Epker (1967) found in beagle dogs no effect on appositional rate of Haversian bone formation. An inhibition of bone resorption in rabbit femur was found by Faccini (1967), the femur was thicker and the changes were accompanied by increased activity of the parathyroid glands.

Toxic effect of fluoride

The clinical syndrome of fluorosis reveals two phases: initially a flecky thickening of cortical bone with slight increased density and later irregular periosteal and endosteal thickening with resulting diminishing of the dimensions of the medullary canal and calcification of the attachment of ligaments and tendons (Faccini, 1969). Eulinger has earlier (1964) described the toxic effect of fluoride in experimental animals in three phases:

1. apposition of irregular bone on the periosteal surface of the metaphysis of long bone
2. apposition of irregular bone on the endosteal surface
3. a spongialization of the cortical bone, that is widening of the Haversian canals and remodelling of periosteal osteophytes.

Effect on bone strength by fluoride

Since the fluoride effect on bone is cortical and trabecular thickening it has been supposed to have favourable effect on the bone strength. High levels of fluoride have been claimed clinically to reduce bone pain, restore calcium balance and increase bone density in patients with osteoporosis, Paget's disease and osteogenesis imperfecta as reviewed by Faccini (1969).

In humans

Most studies on the strengthening effect in humans are done indirectly by determining variations of the fracture incidence. Bernstein et al. (1966) reported lower fracture incidence in vertebræ in areas with high concentration of fluoride in the drinking water (4 - 5.8 ppm) compared with those in low concentration areas (0.15 - 0.3 ppm). This opinion is in disagreement with that of Goggin et al. (1965) who found no changes in the incidence of femoral neck fractures 5 years before and 5 years after changing of the fluoride level in the drinking water from 0.1 to 1 ppm. Neither could Alffram et al. (1969) find any differences in the incidence of femoral neck fractures in regions with different concentrations of fluoride in the drinking water varying from 0.1 ppm to 1.2 ppm.

Experimental studies on animals

Adams & Jowsey (1965) showed in cats that fluoride added to a diet of low calcium content could not prevent bone resorption.

Very few investigations on the physical properties of bone from animals or human beings bed on fluoride rich food have been reported. Jovanovits (1944) studied the effect on the bending strength of the whole femur from growing and adult rabbits supplied a toxic level of fluoride (0.6 - 11 g/body weight) during a period of 96 - 240 days. The breaking strength and elastic stiffness decreased with increasing doses and time on fluoride. The deformation increased. The density of the cortical bone decreased and he concluded that the bone looked thicker and more solid but that the periosteal bone apposition was compensated by endosteal atrophy with increase of resorption cavities and decrease of density. Saville (1967) made compression tests on humerus and femur from rat fed on different levels of fluoride in drinking water. In determining the calcium content he found that this was related to body weight and that it increased in the fore limbs at a dietary level of 20 ppm but not at 2 and 5 ppm. The breaking strength of the humerus and femoral shafts was a linear function of the body weight and unaffected by water fluoridation up to 20 ppm.

Naylor & Wilson (1967) studied the effect of different fluoride concentrations in drinking water from 10 to 500 ppm during a period of 13 to 52 weeks. Breaking strength and deflection of rat femur in bending were determined as well as the ash content. The rats drinking 500 ppm fluoride died within 1 week. The load-deformation was linear to about 3/4 of the breaking stress. They found no significant differences between the breaking stress as well as the ash content of bone in the experimental animals and the controls. However no details are reported and the study is presented only as a short notice. Beary (1969) reports a more extensive study on weaning female rats in two groups: one supplied with low calcium and one with adequate calcium diet but both given three different levels of fluoride in drinking water: 3.4 ppm, 10 ppm and 45 ppm. In the adequate calcium group a significant increase in deflection of whole rat femur tested in bending was found only at the 45.0 ppm level. No significant effect on the breaking strength was found. In the low calcium group a similar significant increase in deflection appeared at 10 ppm level as well. A significant decrease in breaking strength at the two levels was observed and these changes were directly related to the amount of fluoride given. He concludes that the fluoride could not prevent bone disappearance in rats as an effect of the calcium difficiency in the food. Wolinsky et al. (1972) found in a similar study of whole rat femur tested in bending a decrease in breaking strength and elastic stiffness at 500 ppm dose level compared to controls (5 ppm) but no change in energy absorption to the breaking point. In a study of the whole rabbit femur Faccini (1969) found an increase in strength as an effect of fluoride but concluded that this was due to an increase in the width of the cortex and not to a change of the quality of bone, breaking and elasticity being in fact significantly reduced.

The effect of sodium fluoride on healing of fractures is reported by De Gubareff & Platt (1969). They found that fluoride therapy accelerated bone repair of experimental fractures in rats, monkeys and dogs. The same conclusions were earlier reported by Steier et al. (1967) who gave fluoride in combination with vitamin D to rats with experimental fractures.

Smith & Keiper (1965) report on electromagnetic transducer technique of determining visco-elastic properties of small bone specimens. They mention briefly the fluoride effect in human clavicular bone and femoral bone from adult beagle dogs and found no significant difference in visco-elastic properties between specimens of patients from regions with high and low fluoride content in the drinking water. There was no difference in the physical properties of the dog bone in three dogs on a daily intake of 0.3 mg compared to three dogs on 1.0 mg fluoride.

This is the only previous report on the effect of fluoride on physical properties of cortical bone specimens and the dietary conditions are only briefly mentioned. No studies have been found that report the physical properties and the fluoride content in the tested bones.

MATERIAL

As mentioned in the Preface and the Introduction the material consisted of three groups of adult beagle dogs reared at the New York State Veterinary College, Cornell University. The first group of 10 dogs was used to study the influence of dietary added fluoride on nutritionally induced osteopenia and the results have been presented by Henriksson et al. (1970). In the second group of 9 dogs the reversibility of the same condition has been reported by Lennart Krook et al. (1971). The third group consists of ten normal beagle dogs.

In all series adult beagle dogs were used (as decided from closure of the epiphyseal plates). The dogs were raised on a commersial pelleted dog food since weaning. Osteopenia was induced in the first two groups by feeding the beagles a low calcium and high phosphorus diet for 41 weeks. Calcium was supplied at 1/4.5 of the adequate level and phosphorus at 3 times the same. According to National Research Council Standards (1962) these are for calcium 0.54 % and for phosphorus 0.42 %. All other nutritions were supplied in accordance with these standards. Such diet has previously been reported to produce nutritional secondary hyperparathyroidism and osteopenia (Saville & Krook, 1969).

The development of normal beagle dogs has been described by Andersen & Floyd (1963) who found that the inner diameter of the femoral shaft increases until the age of 100 days and the outer diameter until 108 days. The proportion of the outer diameter to the length of the femur is constant (1:12) after the age of 108 days. The water content decreases from 90 % at gestation to 30 % at 250 days of age and is constant thereafter.

Fluoride-prevention of osteopenia

Ten dogs were used. They were divided in five groups, two in each and housed in pairs. Sodium fluoride (NaF) was added to the depletion diet of four groups at the level of 1, 3, 9, 27 ppm respectively, the fifth group thus received no fluoride. Each pair was fed 550 g of the food. The estimated intake of calcium, phosphorus and fluoride per body weight is listed in Table I as well as the signalement of every dog. The dogs will be referred to by one letter and two figures: F for fluoride, the first figure for level of fluoride supplement in ppm and the second for the number of the dog in the pair. This low calcium and high phosphorus diet with added fluoride at different levels were fed in 41 weeks.

TABLE I. Signalement of dogs in fluoride series.
Dog nr, sex, age and body weight at autopsy, and estimated daily intake of Ca, P, F, and fluoride concentration in the food.

Dog nr	Sex	Age		Final Body	Daily intake per kg body weight			F ppm in food
		Years	Days	weight kg	Ca mg	P mg	F ug	
F 0 1	M	1	345	12.7	32	320	0	0
F 0 2	F	1	312	13.2	36	359	0	0
F 1 1	M	1	240	12.7	31	311	26	1
F 1 2	M	1	345	12.7	31	311	26	1
F 3 1	F	1	279	10.5	34	340	85	3
F 3 2	F	3	90	8.2	35	351	88	3
F 9 1	F	1	240	11.4	39	393	295	9
F 9 2	F	3	109	8.6	39	393	295	9
F 27 1	F	1	279	7.3	50	500	1125	27
F 27 2	F	2	13	11.4	37	367	825	27

Reversibility of nutritional osteoporosis

In this series nine dogs were used. For seven of them, after the above mentioned depletion diet had been given for 41 weeks, the diet was corrected and both calcium and phosphorus were supplied two times the adequate levels (see above). The dogs from the fluoride series F-0-1 and F-0-2 were used as controls after the depletion period. The other seven dogs were placed on the above mentioned repletion diet for 4, 8, 12, 16, 20, 24 and 28 weeks respectively. The dogs will be referred to by one letter and one figure: R for repletion and the figure indicating the number of weeks on repletion diet. The estimated intake of calcium and phosphorus per body weight is listed in Table II as well as their signalement.

TABLE II. Signalement of dogs in reversibility series.

Dog nr, sex, age and body weight at autopsy, estimated daily intake of Ca and P in depletion and repletion period respectively. Time of repletion.

Dog nr	Sex	Age		Final Body weight	Daily intake per kg body weight				Time of repletion
					Depletion period		Repletion period		
		Years	Days	kg	Ca mg	P mg	Ca mg	P mg	weeks
R 0[1]	M	1	345	12.7	29	287	-	-	0
R 0[2]	F	1	312	13.2	36	359	-	-	0
R 4	M	2	0	13.6	27	265	202	162	4
R 8	M	2	116	14.6	27	265	191	153	8
R 12	M	2	107	16.0	23	235	173	138	12
R 16	F	2	11	12.3	30	299	226	181	16
R 20	F	2	139	10.5	34	338	267	214	20
R 28	M	2	168	10.9	32	320	252	209	28

[1] Same dog as F 0 1 [2] Same dog as F 0 2

RESULTS FROM THE STUDIES OF THESE DOGS PREVIOUSLY REPORTED BY HENRIKSSON ET AL. (1970) AND KROOK ET AL. (1971)

Some figures from these papers will be presented for comparison with the results of the present study. The two experimental series were studied in similar ways: intravital radiographic examination of the mandibula, microradiographic examination of specimens from mandibula, maxilla, humerus, femur and first lumbar vertebra. Densitometry was made of the right mandibula. Specific gravity was estimated and chemical analysis of bone ash was performed and calcium, phosphorus and fluoride content determined.

Fluoride-prevention of osteopenia

Fluoride added to the diet did not show any effect on the processes leading to loss of bone. The microradiographic examination showed that, independent of the dietary level of fluoride, osteoporosis was more severe in spongious bone than in long bones. There was no difference in loss of bone due to fluoride addition. The specific gravity and the ash content of long bones, vertebræ and frontal bones were not influenced by added amount of fluoride. The bone loss in the mandibula as determined by scanning technique was increased with increasing dietary fluoride. The chemical analysis is showed slight decrease in calcium content and an increased phosphorus content with increasing dietary fluoride, thus giving a decreasing Ca/P ratio. The fluoride content was proportional to the dietary fluoride and the retention in the vertebræ far exceeded that in the long bones.

Reversibility of nutritional osteopenia

Intravital radiograms of mandibula showed pronounced loss of interradicular bone after the depletion period. Re-appearance of bone was seen already within 4 weeks and apparent return to normal after 28 weeks on repletion diet. Large Haversian and Volkmann's canals and soft osteons were found on microradiographs of long bones at the end of the depletion period. Normalization of the condition was found after 28 weeks. Densitometry of mandibulæ showed that remineralization was a linear function of time. Restitution had occurred within 24 weeks. The specific gravity and the ash content showed that the bone loss was more severe in the vertebræ than in the long bones and the return to normal was faster in the vertebrae.

Normal series

Adult beagle dogs were used as normals, five males and five females. The dogs were raised on a commercial pelleted dog food since weaning and no specific manipulation with the food was done. Their signalements are listed in Table III. As the amount and constituents of the food of these dogs have not been fully controlled they can only serve as *normal* dogs and not as strictly *experimental controls.*

The specific gravity and ash content of the right femur of the dogs in the normal series were determined at the Cornell university by the same methods as reported by Henriksson et al. (1970) and Krook et al. (1971). These results are listed in Table III. These figures as well as the body weight of these normal beagle dogs are significantly lower than the normal values reported by Saville (1968) and Saville & Krook (1969).

TABLE III. Signalement, specific gravity and ash content of normal dogs.

Dog nr, sex, age and body weight at autopsy, specific gravity and ash content of whole right femur compared to Saville (1968) and Saville & Krook (1969).

Dog nr	Sex	Age		Body weight	Whole right femur	
		Years	Days	kg	Specific gravity	Ash mg/ml bone
N 1	F	2	100	8.05	1.405	468
N 2	F	2	84	8.05	1.402	478
N 3	F	2	84	9.10	1.357	446
N 4	F	2	80	7.95	1.414	512
N 5	F	2	80	7.80	1.410	472
N 6	M	1	195	9.30	1.311	394
N 7	M	1	195	8.95	1.378	447
N 8	M	1	195	8.73	1.398	466
N 9	M	1	195	9.03	1.419	511
N 10	M	1	195	9.21	1.386	484
Mean					1.388	467.8
S D					±0.033	±34.26
In normal Beagles Saville 1968					1.4326 ±0.0223	
Saville & Krook 1969					1.45	540

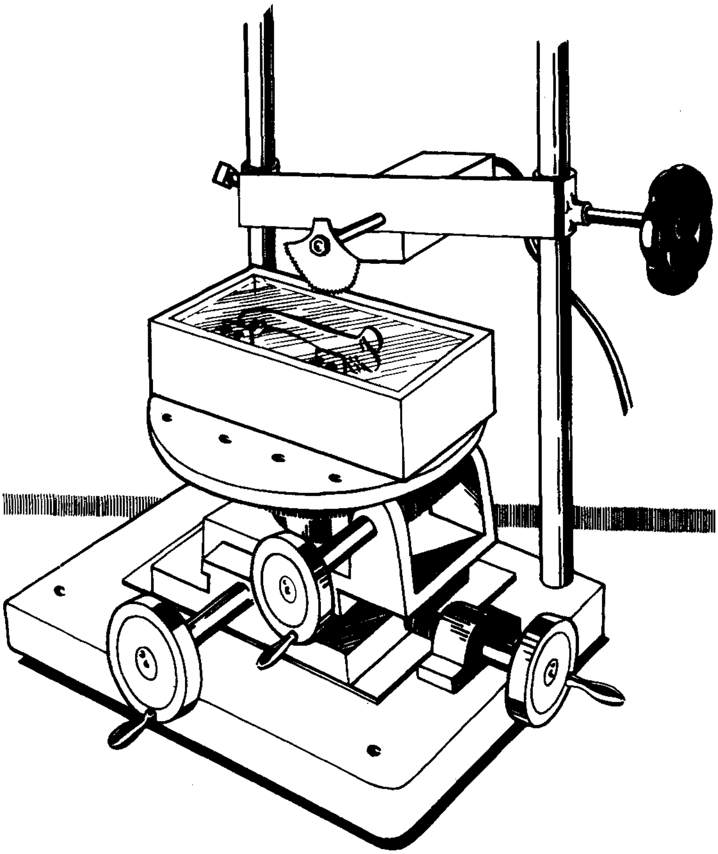


Fig. 1. Equipment used to cut even bone strips from dog femur under water

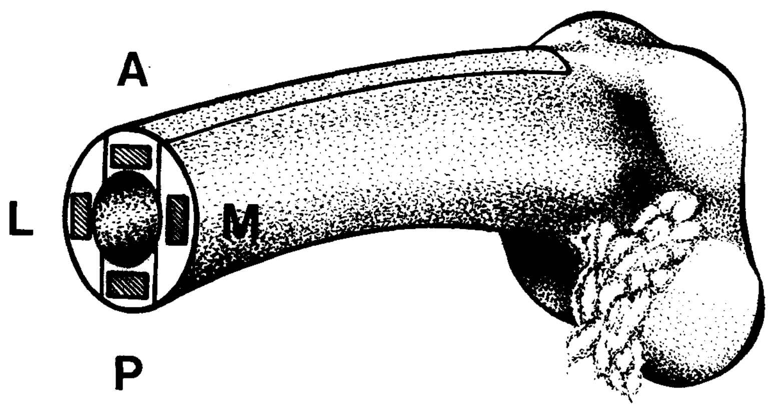


Fig. 2. Distal half of the left femur from beagle dog, showing saw cuttings and at the cross section the dimensions of the specimens (1 x 2 mm). Anterior (A), medial (M), posterior (P), and lateral (L) quadrants.

METHODS

The distal half of the left femur of the dogs from the experimental groups and the whole left femur from the normal group were available for the present study. After necropsy in the United States the left hind limb was amputated in the middle of the thigh and deep frozen, packed with dry ice and transported by flight from Cornell University to Göteborg. At the arrival there was still dry ice left in the box and the specimens were quite hard. (Unfortunately the specimens from dog R-24 was lost during the transport.) The femurs were cleaned from soft tissues under water and radiographs were taken for registration purposes as well as to determine the cortical bone thickness. The specimens were kept deep frozen in a plastic container with Ringer's solution until required for further processing or testing. All preparations were performed in Ringer's solution or under running water. The tests were made in 100 % humidity at 23 to 25^{o}C.

TESTING OF PHYSICAL PROPERTIES

The specimen preparation

A special apparatus was designed to obtain pieces from exactly identical areas of the circumference of the femoral shaft from the animals (Fig. 1). This consists of a stand with a plate and screws which can rotate and displace the plate in sagittal and transversal directions. On the top of the plate a water-tight perspex box is placed. A metal rod to which the bone has been screwed is fastened to the floor of the box which contains Ringer's solution. Above this box is an oscillating circular bone saw placed which can be raised and lowered. This equipment was used to cut even strips from the cortex 3 - 4 mm wide and 3 - 4 cm long. The strips were cut from the anterior, posterior, medial and lateral quadrants respectively of the distal femoral shaft (Fig. 2).

Great care was taken to cut these strips parallel to the long axis of the femur. The strips were stored in Ringer's solution in deep freezer until further preparation. The strips were ground under running water to a final dimension of approximately 1 x 2 mm using two rotating disks with different grades of abrasive paper (Knut's rotor, Struers, Denmark). During this procedure the specimens were attached to a modified version of the grinding fixture described by Sedlin & Hirsch (1966). This modified version gives possibilities of grinding to several different thicknesses of the specimens with very small intervals (Fig. 3). The need of this was due to the small amount of cortical bone available (Fig. 2). The resultant test pieces were marked and measured at three points 1 cm apart during the grinding until the differences over this length of 2 cm were 0.03 mm or less. The measurements were made with a CEJ friction release micrometer (accurate to 0.005 mm).

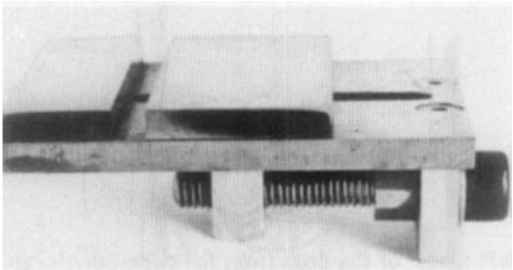


Fig. 3. Fixture for holding bone specimens when grinding to different dimensions. The cut bone strip is fixed between the jaws and can be elevated by putting metalrods of different dimensions under the specimen, thus enable grinding off various amounts of bone until a rectangular cross section is obtained.

Testing equipment

The mechanical testing of the specimens were performed in a modified standard material testing machine: Alwetron universal testing machine model T - 2000, Fig. 4. (In part earlier described by C. Hirsch 1968, Tkaczuk 1968, C. Hirsch 1971, Nordwall 1973, G. Hirsch 1974). The registrations were performed according to the CGS system but will here be converted to and reported according to the Systeme International (SI) of units, according to which force is expressed in Newton (N).

The testing part of this machine consists of four post-stands fastened on a table plate, underneath which an electric motor engine system is placed (A in Fig. 4). On top of the four post-stands a load cell is mounted to a plate (B). The deformation of the specimen to be tested is transmitted by a cross head (C) moving at a selected speed upwards and downwards between the four post-stands by a telescopic screw system. The load cell and the cross head could be supplied with different specimen holders. The variation in speed is provided by an electromagnetic gear box which gives the machine a steppless deformation speed varying from 0.5 to 500 mm/min. The motor is running at a constant speed all the time and the various operations are performed by electromagnetic couplings and breaks, thus eliminating acceleration and deceleration errors or reducing them to negligable values. The compartment between the cross head and the top plate has been closed by a climate chamber of perspex (D) in which temperature and humidity is controlled. The temperature was kept constant at 23 to 25°C by means of a thermostate (E) which controlled the temperature of water saturated air from a humidifyer (F). The humidity was controlled by a standard hair hygrometer (G) inside the chamber and kept at 95 - 100 %. A permanent fog-like atmosphere and condensation on the walls in the box indicated a constant saturation of the air.

Registration of load and deformation

The load was measured by a strain-guage load cell at the top of the box. Two different load cells 9.81 N (1 Kp) and 490 N (50 Kp) load cells transducer with an accuracy of ± 1 % (Lorenzon & Wettre) were used. The deformation was registrated with a differential transformer (Bofors type RLK-1) connected with the moving cross head and the non-moving side-columns at one side outside the climate chamber (H). The core of the differential transformer was fixed to a micrometer enabling calibrations and adjustments to zero position. Two differential transformers with different range of measurements ± 12 mm and ± 3 mm with an accuracy of 0.5 % (Bofors, Sweden) were used. The load cell and the differential transformer were coupled to a measuring bridge and amplifier (I), the output of which was coupled to a X-Y-recorder (J) (Moosely type 705 AM, Hewelitt-Packard) with an accuracy of ± 0.20 %. Calibrations were performed between different types of tests.

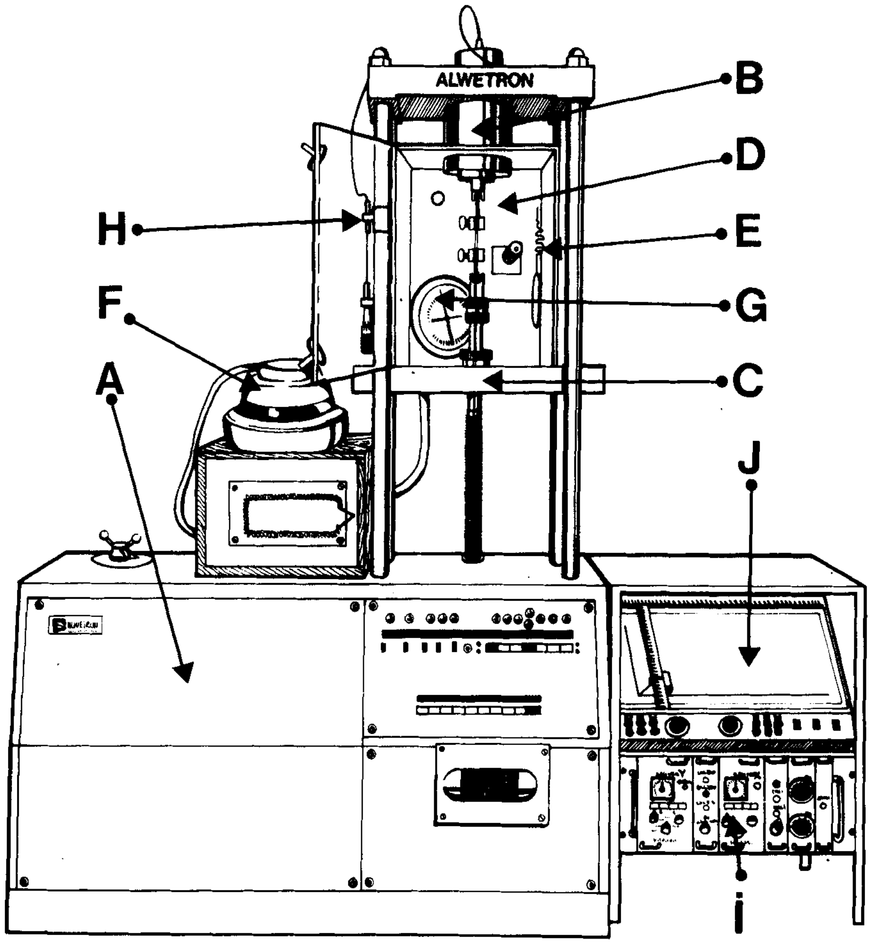


Fig. 4. Testing equipment, Alwetron T-2000, with climate chamber and recording unit. Motor engine and gear (A), load cell (B), moving cross head (C), perspex climate chamber (D), thermostate (E), humidifyer on air heater (F), Hygrometer (G), differential transformer (H), amplifyer (I), X-Y recorder (J).

Bending tests

Bending tests to low stresses (2.45 N) were performed on all specimens from all four quadrants. The specimens were freely suspended end-supported beams and loaded centrally (3 point bending). The distance between the two points of support was 20 mm. The 9.81 N load cell and the ± 12 mm differential transformer were used. The tests were performed to a load of 2.45 N (0.25 Kp) at a constant deformation rate of 0.5 mm/min. Three tests were performed with 2 minutes relaxation pause between. The load-deformation curves achieved were parallel and straight. The second curve was used for calculations.

When testing in bending to fracture the equipment was slightly changed and the load was transmitted to the beam on two symmetrical placed points (4 point bending). The distance between the loading points was chosen to 6 mm, leaving 7 mm on each side (Fig. 5). To control that the fractures occurred between the loading points these were marked with ink which was transmitted to the specimens when loading. All fractures were located between, or at the loading points. The 490 N load cell and the ± 12 mm differential transformer were used. The deformation rate was increased to 10 mm/min.

Tension tests

Tension test to low stresses (49.0 N) was performed on all specimens. The test length was 15 mm since the constant cross section area was limited to a length of 20 mm. Two clamps were supplied with abrasive paper for proper fixation of the bone specimens. The lower clamp was freely suspended to avoid pretension of the specimen when applying it and to ensure proper alignment of the specimen (Fig. 6). The specimens were marked at the edge of the clamps to check for slipping, which was not observed. Three tests were performed to a load of 49.0 N (5 Kp) at a constant deformation rate of 0.1 mm/min. The 490 N load cell and the ± 3 mm differential transformer were used. The second and third curve obtained were parallel and were used for calculations.

When testing in tension to fracture the same experimental set-up was used but the deformation rate was increased to 1 mm/min. A paleness of the specimens was easily visible at the area of fracture just before this occurred as described by Burstein et al. (1972).

Choosing of specimens for different tests

The bending and tension tests to low stresses were performed on specimens from all the four quadrants.

Testing to fracture in bending was performed on all the specimens from the anterior and posterior quadrants in the fluoride and reversibility series and the N-series except for N-2 and N-9. In addition the medial and lateral quadrants from N-1 and N-10 were also tested in bending to fracture.

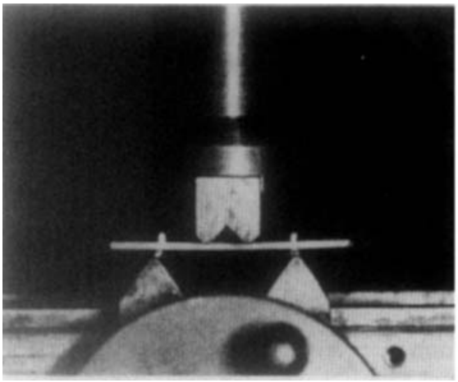
Tension tests to fracture were performed on all the specimens from the medial and lateral quadrants in the fluoride and reversibility series and the N-series except for N-1 and N-10. In addition the anterior and posterior quadrants from N-2 and N-9 were tested in tension to fracture.

Measured variables

Bending (Fig. 7)

In three point bending (3p-B) to low stresses:

Elastic stiffness (Young's modulus of elasticity) »E - 3p-B» was calculated to a load of 2.45 N.



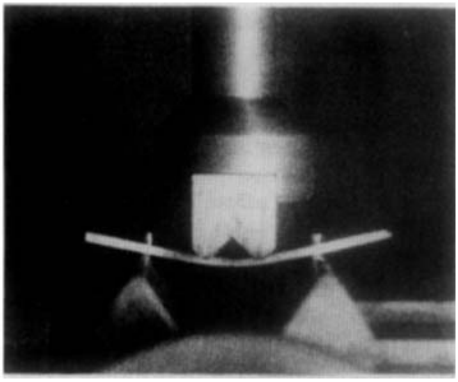


Fig. 5. **Testing set-up in four point bending test.** Fig. 5 a – before loading, and b – during loading.

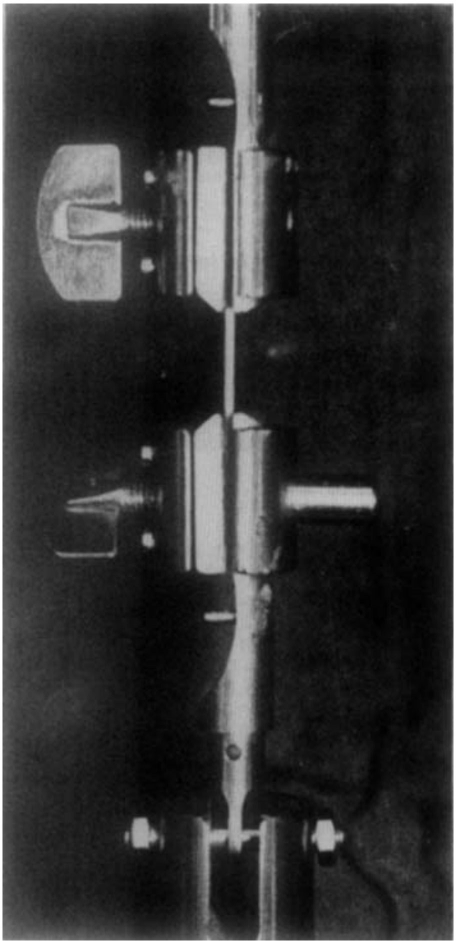


Fig. 6. Specimen in clamps in tension tests. The lower clamp is freely suspended by a bolt through a hole which exceeds the bold diameter with 1 mm. To avoid pretension when tightening the claps and to asure proper alignment.

In four point bending (4p-B) to fracture:

Elastic stiffness (Young's modulus of elasticity) »E - 4p-B» was calculated from the linear part of the load deformation curve.

Stress at the elastic limit »S_y»

Ultimate bending stress »UBS», was calculated using the above mentioned formulæ which can not be applied strictly since the load deformation curve is not linear to the point of maximal stress (– this will be discussed later).

Total energy absorption in bending to fracture »T», was calculated using planimetric measurements of the area under the load deformation curve. (If no fracture occurred the area was calculated to the point of maximal deflection (D_m) possible in the testing equipment, approximately 5.5 mm.).

Energy absorption to the point of maximal stress »A_1», was calculated as the area under the curve to the point of maximal stress.

Energy absorption after the point of maximal stress »A_2», was calculated as the area under the curve between the point of maximal stress and fracture.

The ratio of energy absorption to the point of maximal stress to total energy absorption »A_1/T» was calculated and is expressed in %.

The ratio of energy absorption after the point of maximal stress to total energy absorption »A_2/T» was calculated and is expressed in %.

Tension (Fig. 8)

In tension to low stresses:

Elastic stiffness (Young's modulus of elasticity) was calculated to a load of 45 N.

In tension to fracture:

Elastic stiffness (Young's modulus of elasticity) was calculated from the linear part of the load-deformation curve.

Stress at the elastic limit »S_y»

Ultimate tensile stress »UTS» was calculated at the fracture point of maximal stress in tension.

Total energy absorption in tension was calculated as the area under the load deformation curve.

Maximal strain »D_m/L» was calculated to the fracture point and is expressed in %.

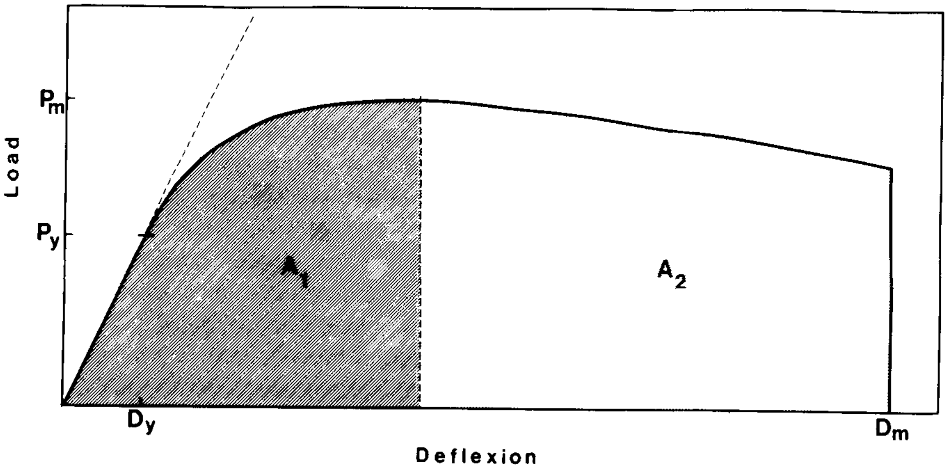


Fig. 7. Load-deformation curve in four point bending test to fracture. Load (P_y) and deflextion (D_y) at the elastic limit, maximal load (P_m), maximal deflexion (D_m), energy absorption before maximal stress corresponds to the area under the curve (A_1), after maximal stress (A_2). The total energy absorption to fracture (T) is the sum of A_1 and A_2. The dotted line indicates the slope of the linear portion of the curve and was used for calculating the elastic stiffness (changing of unit stress per unit strain).

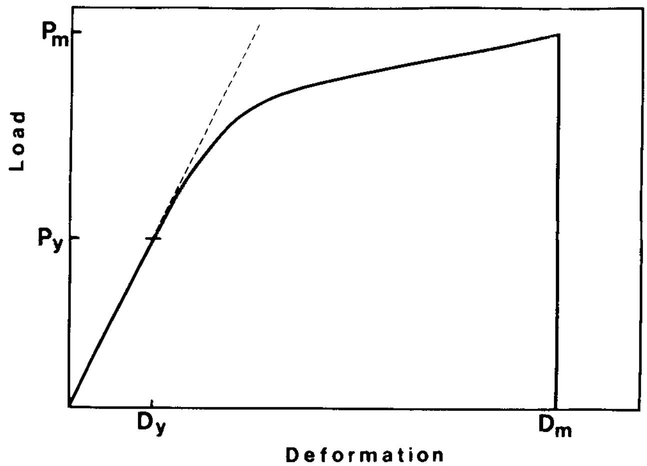


Fig. 8. Load deformation curve in tension test to fracture. Load (P_y) and deflexion (D_y) at the elastic limit, maximal load (P_m), maximal deformation (D_m). The energy absorbtion corresponds to the area under the curve. The dotted line indicates the slope of the linear portion of the curve and was used for calculating the elastic stiffness (changing of unit stress per unit strain).

The ratio of deformation at elastic limit to maximal deformation »D_y/D_m» is expressed in % (Proportion of elastic defomation to total deformation, Gamma by Frankel & Burstein 1972).

Formulæ used in calculation of different physical properties

Symbols used in calculation of physical properties

E = modulus of elasticity
S = stress
e = strain
I = moment of inertia
M= maximum moment
P = load

A = cross section area of specimen
a = distance from end support to one of the loads in 4 p bending
b = breadth of specimen
d = depths of specimen
c = distance from neutral axis to fiber of greatest stress
D = deflexion in bending, deformation in tension
L = test length of specimen

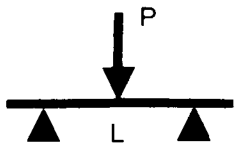
Standard formulæ were used and adopted from textbooks in mechanical engineering (Lundberg 1955, Cottrell 1964, Hulth 1966) and testing of polymers (Loveless (1966).

Bending:

In three point bending the following standard formulæ were used:

$E = \frac{PL^3}{48ID}$; $S = \frac{Mc}{I}$; $M = \frac{PL}{4}$; $I = \frac{bd^3}{12}$; $c = \frac{d}{2}$

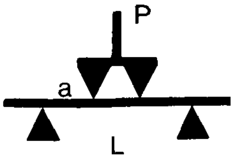
which gives $E = \frac{PL^3}{D4bd^3}$



In four point bending the following formulæ were used:

$E = \frac{PLa^2}{DI}(1 - \frac{4a}{3L})$; $S = \frac{Mc}{I}$; $M = \frac{Pa}{2}$; $I = \frac{bd^3}{12}$; $c = \frac{d}{2}$

which gives $E = \frac{Pa^2\,(3L-4a)}{Dbd^3}$ and $S = \frac{Pa^3}{bd^2}$



Tension

Hook's law: $E = \frac{S}{e}$; $S = \frac{P}{A}$; $A = bd$; $e = \frac{D}{L}$ which gives $E = \frac{PL}{Dbd}$

Units

According to the Systeme International (SI) the following units will be used:

forces – Newton, N.
stresses – Mega Newton per square metre, MN/m^2 (= 10^6 N/m^2 = 0.102 Kp/mm^2).
elastic stiffness (modulus) – Giga Newton per square metre, GN/m^2 (= 10^9 N/m^2).
energy absorption – Nm/mm^3 of the tested bone.

For comparison with the CGS units conversion might be useful: 10 GN/m^2 = 1020 Kp/mm^2; 100 MN/m^2 = 10.2 Kp/mm^2.

Comments on testing procedures

Specimen preparation and size

Most of previous authors who have tested bone specimens with approximately the same cross section area as in this study (1 x 2 mm) have obtained these from bones with greater dimensions; such as long bones from human or oxen. The length of the distal half of the left femur of the beagle dog was approximately 5 cm, with an outer diameter of approximately 10 mm (Fig. 2); thus, it was obvious that a special apparatus was needed as there was not much bone to give up. As even such a small angle as 10° of the specimens from the long axis of the bone effects the properties when testing (Hirsch & Da Silva, 1967) and as one could expect different results from the different regions around the circumference of the bone, great care was taken when cutting the bone strips out of the cortical part of the femur.

The effect of drying has been tested by a large number of authors who all have shown that dry bone is stiffer and has higher breaking strength than wet bone and less deformation to failure (Demster & Liddicoat 1952, Koo 1953, Smith & Walmseley 1959, Evans 1964, Ascenzi et al. 1966, Sedlin & Hirsch 1966). All preparations and tests were performed under water or in 100 % humidity and great care was taken in the sawing of the specimens to prevent heat which could dry the bone or denaturate the collagen. For this purpose an oscillating bone saw was used.

Bending tests

The difficultes in these tests and the sources of error are discussed by Swanson (1971) who pointed out that minor vascular holes and the non-uniformity of the material might effect the crack propagation in bending tests with non-uniform stress distribution in the material. However, the three point bending test was felt to be safe for low stress. To eliminate these difficulties the four point bending test was adopted when testing to fracture. This mode of testing gives a uniform stress between the two loading points and is preferred according to Loveless (1966), Sonnerup (1969), Zarek (1970), Burstein & Frankel (1971). The ends of the supports and the force transmitting loading points were rounded to avoid friction. As the maximal deflection in bending was limited by the experimental set-up at 5.5 mm the energy absorption in those specimens, which had not fractured into two pieces at this deflection, was calculated as the area under the curve to this point of deflexion.

Tension tests

The great difficulty in tension tests of small specimens is to avoid slipping of the specimen in the clamps and stress concentrations at the edge of the clamps. This some authors have come across by diminishing the dimensions in a central section of the specimen between the clamps. This was not possible here as the original cross section was too small (1 x 2 mm). As a fact some of the fractures in tension occurred at the edge of one clamp. The optimal way of determining the deformation is averaging extensometers applied on the specimen (Swanson, 1971). Such were not available and measurements of the movement of the cross head was the way to determine the deformation. A possible slipping of the clamps in this study will be discussed after the results obtained with the different tests. (Se page 70.)

Swanson (1971) discussed the effect of applying the load not exactly along the axis of the specimen. This will result in bending stresses and a load excentricity of 1 % of the specimen diameter will give a maximal stress of 8 % higher than the intended uniform stress. Therefore great care was taken to aligne the specimen vertically and in addition the lower clamp was freely suspended. It was fixed to the moving cross head with a bolt through a hole with a diameter that exceeded the bold diameter with 1 mm. In this way the specimen was to some extent self aligning when the load was applied.

Source of origin of the specimens

The preliminary study in both bending and tension to low stresses revealed great differences between specimens from the different quadrants in the same femur – in fact greater than between specimens of the same quadrant from different experimental dogs. Therefore it was decided to test half of the material for fracture in bending and the other half to fracture in tension. As in these pilot tests the elastic stiffness of the anterior quadrant always exceeded that of the posterior as did also the medial quadrant in comparison to the lateral, the anterior and posterior quadrants were choosen for the bending tests to fracture and the medial and lateral ones for the tension tests to fracture. To check that the physical properties as a result of testing to fracture varied between the different quadrants in the same way as the elastic stiffness had done in the preliminary study, in two of the normal dogs all the four quadrants were tested to fracture in bending, and in two other of the normal dogs all the four quadrants were tested to fracture in tension.

Error of the method

The error of the different methods could not be determined for the destructive tests. Nine bone specimens of the same size from the radius of other dogs (Romanus & Garmer, 1974) were tested to low stresses in 4 point bending. The same procedure was performed after the specimens had been kept for one week in deep freezer and gave an error of the elastic stiffness of 2.84 %.

STATISTICAL METHODS

Although the material in this study (from some aspects) has to be considered as rather small and a statistical analysis may be doubtful, it was felt necessary to undertake some calculations to discover any major significant differences or correlations between the measured variables.

Standard statistical methods were used (Kemp & Nielsen 1959, Ciba-Geigy 1970).

In the comparison of mean values between different quadrants, sexes or dog groups, F distribution tests were used.

When examining the possible influence of one measured variable on any other (as diet, mechanical, chemical) the linear regression was calculated using the least square fit method. The coefficient of correlation (r) will be given.

In some comparisons of the results from two different quadrants the sign test was used. It reveals determination of a difference in a pair as positive or negative.

In analysis of the possible effect of some chemical components of the bone on some physical properties, multiple correlation analysis were used.

The following symbols will be used:
n = number of observations
r = coefficient of correlation between two variables, when negative marked with –.

Significant levels will be marked as follows:

$2\,p<0.05$ = +	»significant»
$2\,p<0.01$ = ++	»highly significant»
$2\,p<0.001$ = +++	» »

Error of methods

The error of the different methods used was calculated from duplicated determinations (standard error of the means SEM) using the formula $SE = \sqrt{\frac{\Sigma d_1^2}{2n}}$

where d_1 is the difference between duplicate measurements. The error was then expressed as the percentage of the mean of all determinations

$$\frac{x_1 a + x_1 b + x_2 b + \ldots x_n a + x_n b}{2n}$$

CHEMICAL ANALYSES

Weight, volume and density

After finishing the mechanical test to fracture, two pieces of 5 mm length from each test specimen were cut off near the fracture site and stored in Ringer's solution at room temperature for at least 24 hours. Then the weight was determined with a standard microwave (Mettler H20-T) with an accuracy of 0.01 mg. Before weighing the bone pieces were superficially dried with a cloth. The humidity and the temperature in the weight chamber were kept constant (60 - 70 %, 19 - 23^{o}C respectively). The accuracy was determined by repeating the procedure two days later on 64 of the specimens at the same time of the day and the error of the method was 0.18 %. The wet volume was determined by measuring the dimensions of the test piece with the friction release micrometer. The error of this determination of volume was 0.85 %. This was done by putting 28 pieces after drying back in Ringer's solution for two days and measuring the dimensions once more. The density at room temperature was calculated for the two pieces from each specimen and expressed in mg/mm^3. The mean of these two pieces was used as the density of the specimen. The error in this determination was 1.13 % in 104 pairs.

The results of all chemical analysis were calculated as percentage of wet weight, dry weight and mg or μg per mm^3 wet bone. Ratios between the different components were calculated.

Water

For determination of the water content the specimens were dried in vacuum (0.05 mm Hg) at 43^{o} C for 16 hours. The dry weight was measured with the same microwave, keeping the humidity as low as possible (30 - 40 %, by putting phosphorus pentoxide in the room and in the microwave). During the first two hours after the drying the hygroscopic water absorption in the test piece was negligible. The water content was calculated for the two pieces from each specimen and expressed as percentage of wet weight and as mg/mm^3 wet bone. The mean of these two pieces was used as the water content of the specimen. The error in this determination was 3.7 % in 104 pairs.

One of the two pieces from each test specimen was hydralized in HCl and parts of the solution were taken for determination of hydroxyproline, calcium, magnesium and phosphorus content. The other piece was used for ash and fluoride determinations.

Collagen

The analyses of collagen were performed by determination of hydroxyproline according to Stegemann (1958), modified by Bergman & Loxley (1963) as employed by Viidik (1971) and performed at the Anatomic Department, University of Göteborg by his experienced technical assistants.

The specimen with a weight of approximately 20 mg was hydrolized in 1.5 ml 6-N HCl in sealed Duran 50 tubes at 130° C for 3 hours. The content was diluted with distilled water to 25 ml and neutralized by means of NaOH with one drop of methyl-red as indicator. 1 ml of this solution was taken to determination of the hydroxyproline content and finally read in a Beckman B spectrophotometer at a wave length of 5680 Å. The light transmission in the spectrophotometer was read in a standard curve which gave the hydroxyproline content as mg/ml in the solution. The error of this method is less than 0.5 % (Viidik, 1971). The amount of hydroxyproline in proportion to collagen content in the tissues was 1/7.46 according to Neumann & Logan (1950).

Calcium

One ml of the above mentioned hydrolysate was taken for determination of calcium with atomic absorption spectrophotometer using a Perkin Elmer model 403 at a wave length of 4227 Å. 0.1 ml was taken with an Eppendorf micropipette from the solution and diluted with 5 ml 1 % lantanchloride (La Cl) in 5 % HCl solution. Standard solutions were made in the same way containing 5, 4, 3, 2, 1 mg Ca/ml. Between each sample a control was made with a blank and between every third sample with the standard solutions. The absorption was counted as a mean of 100 counts. The above mentioned dilutions gave calcium concentrations in the solution of 3 - 6 mg/ml. This is well below 7 mg/ml which is the upper limit for the linear working range given by the manufacturer. The error of the method was 0.88 % in 7 double tests.

Magnesium

The determination of magnesium content was performed in the same way as for calcium analysis but at the wave length of 2852 Å and with the standard solutions containing 0.5 and 0.2 mg Mg/ml. The dilution gave the magnesium concentration in the solution of 0.03 - 0.09 mg/ml. This is well below the upper limit of the linear working range of 0.5 mg/ml which is given by the manufacturer. The error of the method was 8.7 % in 7 double tests.

Comments on calcium and magnesium determinations

In a solution with lantanchloride (La Cl) in excess this gives stronger bindings to phosphate and other ions (silicon, aluminium, sulphate) thus they do not diminish the sensitivity for calcium and magnesium which otherwise would be the case.

Phosphorus

The determination of phosphate (PO_4) was performed with the standard calorimetric method according to Fiske & Subbarow (1925) as employed by Thorild Eriksson (1972), at the Laboratory for Clinical Chemistry, Faculty of Odontology, University of Göteborg.

This involves a complex binding of Molybden. 0.2 ml of the test solution was taken and mixed with 2 ml of the solution of Mo-reagens: (90 g Mo O_3 dissolved in 250 ml 5-N NaOH diluted with distilled water to 2000 ml and filtered, mixed with the same quantity of 10-N H_2SO_4), and 5 ml distilled water and finally 5 ml of a reduction solution prepared from: 0.6 g ELON (p-methylaminophenolsulphate), 6.0 g Na_2SO_4 and 100 ml distilled water. The absorption at a wave length of 700 Å was read in a Beckman B spectrophotometer after 35 minutes and compared with standard solutions containing 1, 2.5, 5, 10 and 15 mMol PO_4/ml and the concentrations in the specimen were read from the curve from the standard solutions. Double samples were taken from each test solution. The mean of the double tests was taken as the value for phosphorus content in the specimens. The error of this method was 2.33 % in 104 double tests.

Fluoride and ash

The determination of fluoride and ash content was performed according to McCann (1968) as employed by Y. Eriksson (1971) and performed at the Institution of Cariology, Karolinska Institutet, Stockholm, by his experienced technical assistant.

The bone specimens were dried at 105° C for 14 - 20 hours and then weighed. Ashing of the bone specimens was done in a metal melting pot for 2 hours at 550° C and then the ash weight was determined, The ash of the specimens were then dissolved in 0.5 ml 0.5-M $HClO_4$ and 1 ml 2-M trinatriumcitrate which gives a pH of about 6. Analyse was performed with four fluoride-ion selective electrodes (Orion) coupled to a calibrating unit and read with a pH meter. Standard solutions were made at different concentrations and gave a straight line on a semilogarithmic paper which was used for reading of the concentrations in the specimens. The high citrate concentration gives a complex binding of magnesium, calcium and other ions which could otherwise interfere with the result by bindings to the fluoride ions. The error of this method is in the pH meter less than 3 % and the total error of the method is well below 5 % (Y. Eriksson, 1971). The concentration in the specimens was calculated as ppm in ash, as well as the previously mentioned concentrations. One bone piece was lost (N-4 anterior) before this determination.

Comments on chemical determinations

In reporting chemical composition of bone different units have been used. The simplest way is to determine the ash content by heating the bone to 500 - 600° C and express this in percentage (%) of the original weight of wet or most often dried bone. The rest is then estimated as organic substances. When performing analyses of the different components the previously and still often used way is to express the content as % of dry bone weight. Expression in % of wet bone weight is only used by those authors who perform a determination of the water content. The drying is standardized for each author but not between different authors in most cases at 105° C to constant weight.

In reports on both physical properties and bone chemistry the most often used unit is % of bone ash weight. Since the excellent papers of Robinson & Elliot (1957) and later Strandh & Norlén (1965) an increasing number of authors report their results on a volume basis which of course is more relevant, especially in studies of conditions of bone loss. Osteoporosis is diagnosed on a volume basis, e.g. on a two-dimensional area basis (histological sections or microradiograms). As the effect of the dietary changes of calcium, phosphorus and fluoride were to be investigated in this study it was felt most appropriate to determine the different minerals as well as the organic material. The chemical components were calculated not only as % of wet and dry bone weight but also as weight per unit wet volume. The reason for this was the scant reports in the literature of normal values for dogs expressed on volume basis. The normal values for bone are for instance in the Scientific Tables (Ciba - Geigy, 1970) expressed as % of dry fatfree tissue. The different correlations between the chemical contents and other parametres revealed similar results in the three different ways of calculating one chemical component, and i many cases a higher correlation when calculating on the volume basis. The result on a wet volume basis will thus be used throughout this study, but in some cases the % of wet and dry bone weight will be presented for comparison.

Another important factor in deciding for weight per volume unit as the basic one, was the fact that all variables determined in mechanical testing is expressed in force unit per cross section area of the specimen or per volume of the specimen (energy absorption), in this particular study per wet area or per wet volume bone.

Water determination

The determination of water content in tissues that has been deep frozen is considered uncertain (Biltz & Pellegrino, 1969). One reason for doing this in the present study was the necessity of drying the specimens to obtain chemical contents expressed as % of dry bone weight (the most often published way previously). Measuring of the dry volume was time consuming and therefore the hygroscopic property of the dried bone pieces would enter as an undeterminable error. Anyhow the dry volume was determined in some 15 specimens and a shrinkage of 20 % of the wet volume occurred after a dehydration of about 12 % of the wet weight.

X-RAY EXAMINATION

Radiograms from all the received femurs (except for R-4, F-27-1 and F-27-2) were taken with a focus of approximately 90 cm. An antero-posterior projection and a lateral projection were made. The cortical thickness, the outer and inner diameter at the mid-point of the femur and the femoral length in the normal series were measured with a micrometer on the radiographs. The error of these measurements was checked with repeated examinations and was: for the cortical thickness 3.4 % in 92 double tests; for the outer diameter 0.8 % and the inner diameter 1.6 % in 46 double tests; and for the femural length in the normal series 0.3 % in 10 double tests. The inner diameter used for further calculations was the mean of the measurements on the antero-posterior and the lateral projections.

EXAMINATION OF DISTRIBUTION OF CALCIUM, PHOSPHORUS, AND FLUORINE OVER A CROSS SECTION OF THE SPECIMEN

This was performed at the University of Surrey, England, under the guidance of Prof. Josef Zarek. Bone pieces from one of the fractured ends of the specimens were imbedded in methylmethacrylate and the cross section was polished, on standard metallographic polishing rotating disks (Struers model DP 10) using different grades of diamantpaste, until a smooth surface was achieved. These polished cross sections were photographed in a standard reflected light microscope as registrations and as check for scratches during the polishing procedure. The surface was then prepared with coating for study in the electrone-probe X-ray microanalyser (Geoscan, Cambridge instrument comp. Ltd, England). The purpose was to study the amount, and distribution over the cross section of calcium, phosphorus and fluorine atoms.

The result was not conclusive since great difficulties were met and as previous experience of this technique on biological material was lacking.

1. Fluorine atoms were present in too low concentration (< 1 %) to be detected.
2. In high magnification the energy of the electrone beam had to be increased to a magnitude at which it penetrated about 0.5 mm down in the cross section and thus burned and destroyed the bone.
3. In low magnification the distribution of Ca and P atoms were too uniform, and the result was an unexact determination of the amounts present.
4. Uncontrollable sources of errors were most likely forthcoming since vacuum had to be achieved before each examination of a specimen and extensive calibrations had to be performed.

HISTOLOGICAL EXAMINATION

The photograms taken through a microscope with reflected light of the polished cross section of the specimens near the fracture were used in an attempt to classify the microscopic outlook of the bone. The pictures from all four quadrants from the fluoride and reversibility series (except R-4) were shown to three different examiners: an engineer without medical education (A), the author (B) and an orthopædic surgeon (C). They were asked to separate the 60 pictures in different groups and then describe the characteristics of these groups. Then they were asked to try to find similarities between groups in order to diminish the number of groups to as few as possible. The signalement (dog number and quadrant) of the photographs were registrated for each group to look for any similarity between the photographs within each group.

RESULTS

The presentation of the results will mainly be in form of diagrams showing the influence of different factors on the physical and chemical parameters. The mean values of the whole material of these parameters will be presented for comparison with the results from other authors.

The original tables with the results from the different determinations in this study is not presented here. They are deposited at the University Library in Göteborg and can be obtained on request from the author. (Dept. of Orthopædic Surgery II, University of Göteborg, Sweden).

EFFECT OF VARIATION OF FLUORIDE CONTENT IN THE DIET ON THE PHYSICAL PROPERTIES AND CHEMICAL CONTENT OF CORTICAL BONE STRIPS

Linear regression correlations were calculated for fluoride levels in the food and physical properties and chemical content of the different four quadrants and for the mean of the four quadrants. Some of the measured variables are plotted against fluoride level in the food to illustrate the correlation. The mean and 95 % confidence limit for the normal series are given for comparison.

Physical properties

The correlation coefficients and significant levels are listed in Table IV.

Bending

Elastic stiffness both in three point and in four point bending (Fig. 9) was not influenced by the dietary adding of fluoride. Nor were there any significant effect on *stress at the elastic limit* (Fig. 10) or at the *ultimate bending stress* (Fig. 11). A slight increase was seen at the 1 and 3 ppm levels for the anterior quadrant only.

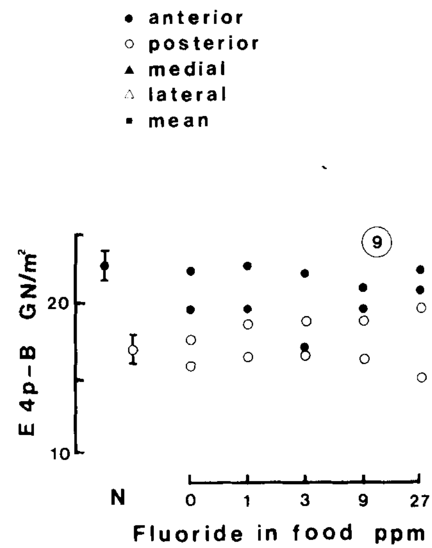
Total energy absorption was significantly increased for the anterior quadrant even at the lowest level (1 ppm) of dietary fluoride (Fig. 12). The absence of increase with increasing dietary fluoride could partly be explained by the fact that the anterior specimens from seven of the eight dogs that had received fluoride supplement did not fracture in bending at the maximal deflection possible in the experimental set-up (5.5 mm). (Only two of the specimens in the other series did not fracture at the maximal deflection). This increase in energy absorption was only true for the anterior quadrant and as seen from Fig. 13 there was a significant increase in the *energy absorption to the point of maximal stress* (A_1) for this quadrant. Fig. 14 shows the same feature as Fig. 12 describing the *energy absorption after maximal stress*, the upper limit of which could

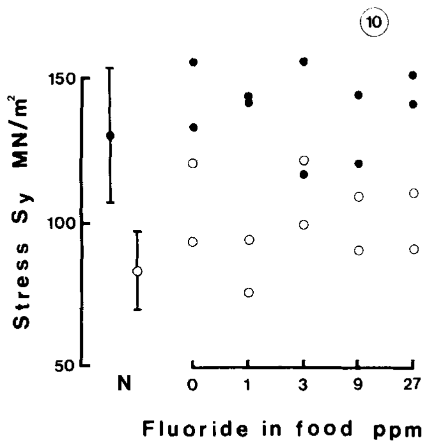
TABLE IV. Effect of dietary added fluoride on the physical properties of cortical bone

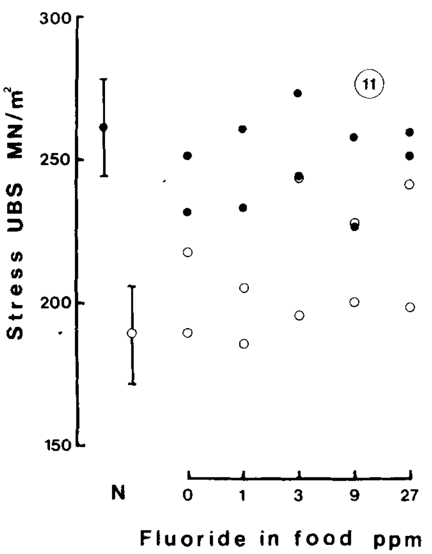
Linear regression correlations between fluoride level in the food and the physical properties of femoral cortical bone specimens in 10 beagle dogs were calculated and the correlation coefficients (r) and significant levels (+) are listed for the four different quadrants and the mean of the four quadrants ($\bar{X}$).

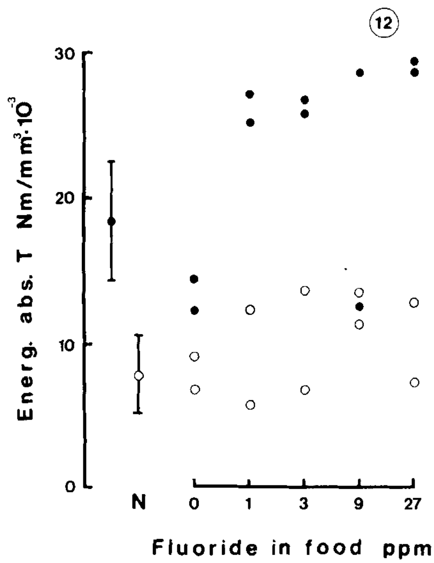
	Ant.	Post.	Med.	Lat.	$\bar{X}$	Fig. nr
Bending						
Elastic stiffness (3p-B)	.15	.07	-.26	.56	.19	
Elastic stiffness (4p-B)	.14	.05	-	-	.12	9
Stress at elastic limit (S_y)	.10	0	-	-	.06	10
Ultimate bending stress (UBS)	.18	.29	-	-	.28	11
Total energy absorption (T)	.44	.17	-	-	.47	12
Energy absorption to maximal stress (A_1)	.70+	.34	-	-	.73++	13
Energy absorption after maximal stress (A_2)	.27	-.12	-	-	.22	14
Ratio of energy absorption to max. stress to total energy absorption (A_1/T)	.04	.15	-	-	.14	
Ratio of energy absorption after max. stress to total energy absorption (A_2/T)	-.04	-.15	-	-	-.14	15
Tension						
Elastic stiffness (low stress)	.27	.05	-.34	.14	.07	
Elastic stiffness (fracture test)	-	-	-.24	.16	.07	16
Stress at elastic limit (S_y)	-	-	-.14	.24	-.03	
Ultimate tensile stress (UTS)	-	-	.15	.28	0	17
Energy absorption	-	-	.05	.30	.15	18
Maximal strain (D_m/L)	-	-	.11	.20	.16	19
Ratio of deformation at elastic limit to max. deform. (D_y/D_m)	-	-	-.18	-.19	-.20	

not be measured. The *ratio of energy absorption* before (A_1/T) and after maximal stress (A_2/T_1, Fig. 15) related to total energy absorption was not influenced by the fluoride level.









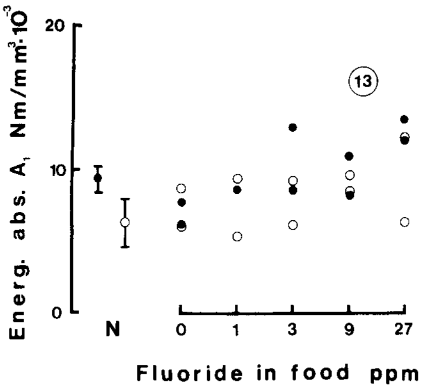
Figs. 9 to 19. Physical properties for the different quadrants are plotted against fluoride level in the food as well as the mean and the 95 % confidence limit for the different quadrants from the normal series (N).

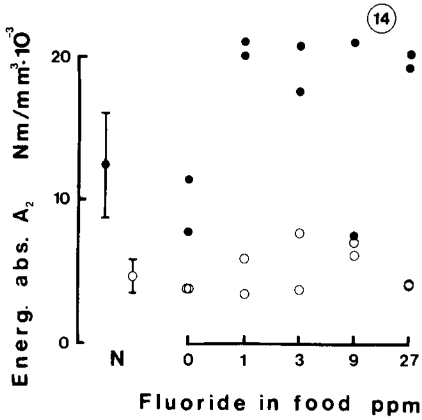
Fig. 9. Elastic stiffness (E 4p-B)

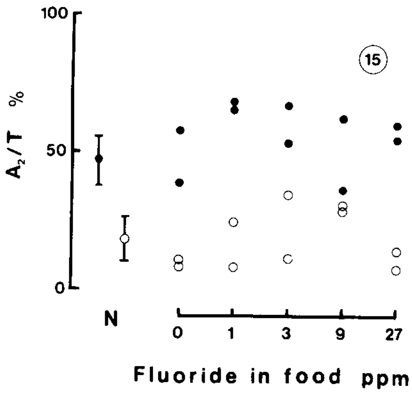
Fig. 10. Stress at elastic limit in bending (Stress S_y)

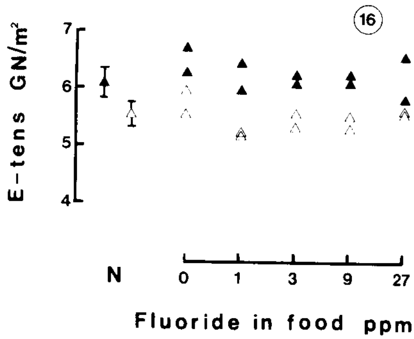
Fig. 11. Ultimate bending stress (UBS)

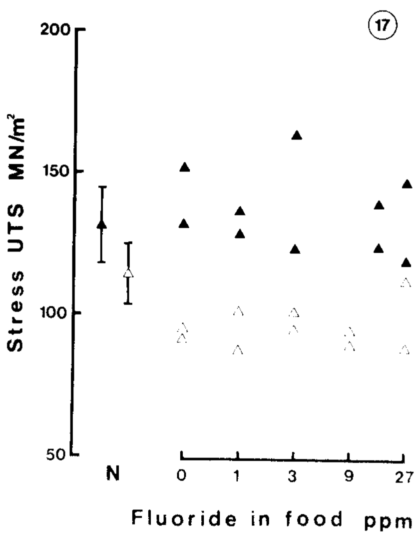
Fig. 12. Total energy absorption in bending (Energ. abs. T)











- ● anterior
- ○ posterior
- ▲ medial
- △ lateral
- ▪ mean

Fig. 13. Energy absorption to maximal stress (Energ. abs. A_1) r ant. .70+

Fig. 14. Energy absorption after maximal stress (Energ. abs. A_2)

Fig. 15. Ratio of energy absorption after maximal stress to total energy absorption (A_2T)

Fig. 16. Elastic stiffness in tension to fracture (E-tens)

Fig. 17, Ultimate tensile stress (UTS)

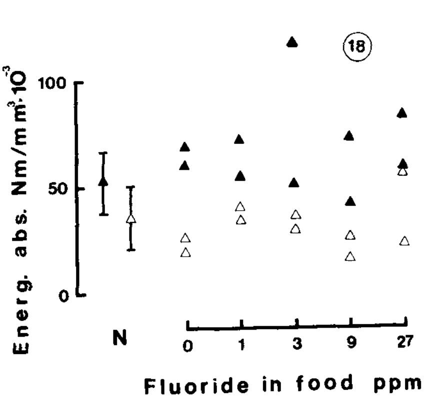


Fig. 18. Total energy absorption in tension (Energ. abs.)

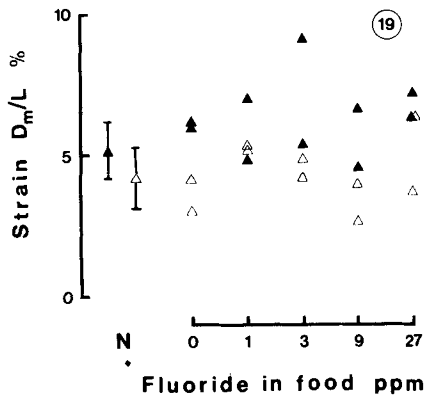


Fig. 19. Maximal strain in tension (D_m/L)

Comments

The predominant impression is the great differences between the results from the anterior and from the posterior quadrants. The increased energy absorption was reflected by the increased maximal deflection in bending, resembling clinical green stick fractures. This was only true for the anterior quadrant tested in bending.

Tension

The *elastic stiffness* in tension to low stresses as well as to fracture showed no effect of additional fluoride in the diet (Fig. 16), nor was there any effect on the *stress at the elastic limit* or the *ultimate tensile stress* (Fig. 17) or the *energy absorption* (Fig. 18) or *maximal strain* (Fig. 19).

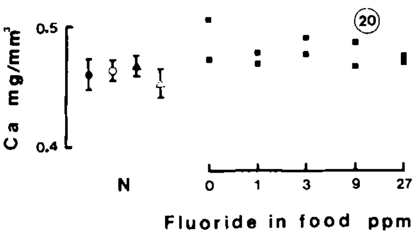
Comments

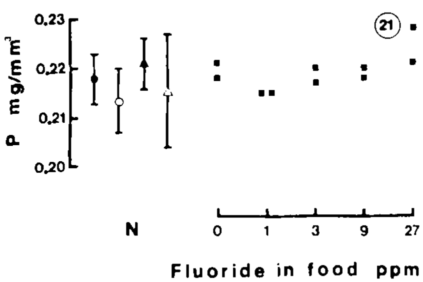
The pattern of the results achieved in tension tests was similar to the pattern of those in bending. No effect of fluoride level in the food was registrated. There was, however, a marked difference between the results from the medial and lateral quadrants as seen from these diagrams.

Chemical composition and femoral shaft dimensions

The correlation coefficients and significant levels are listed in Table V. Some of the mean values for the four quadrants are plotted against fluoride level.

Density of the cortical bone was not affected by the dietary level of fluoride and this was true even for the *ash* and *water* contents. The *calcium* content with increasing fluoride level decreased slightly but not significantly (Fig. 20). The *phosphorus* content on the other hand increased slightly, but significantly only for the anterior quadrant and for the mean of the four quadrants (Fig. 21). As expected the *fluoride* content increased in all the four quadrants (Fig. 22). There was a slight decrease in the *magnesium* content but significant only for the posterior quadrant and a more marked decrease in the collagen content which was highly significant for the posterior quadrant and significant for the mean of the four quadrants (Fig. 23). The *Ca/P* ratio showed marked decrease, highly significant for the medial and the lateral quadrants and significant for the mean of the four quadrants (Fig. 24). As the fluoride content increased, the *Ca/F, P/F, Mg/F* and *collagen/F* ratios decreased but significantly only for one or two quadrants. However, a consistent decrease in all four quadrants was found for the *collagen/P* ratio, significant for the anterior and posterior quadrants and highly significant for the mean of the four quadrants (Fig. 25). The *cortical thickness* showed a slight thinning with increasing fluoride level, but significant only for the lateral quadrant (Fig. 26), this in spite of a marked decrease of the *inner diameter* as reflected in Fig. 27.

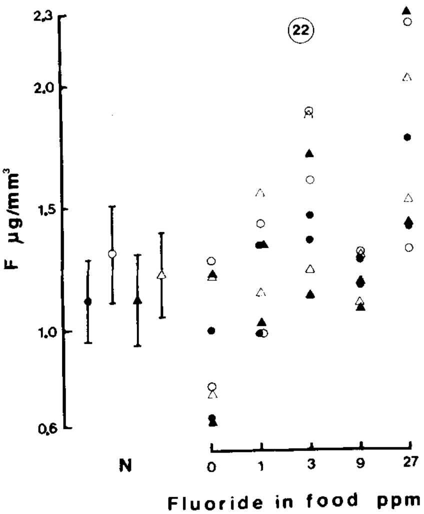


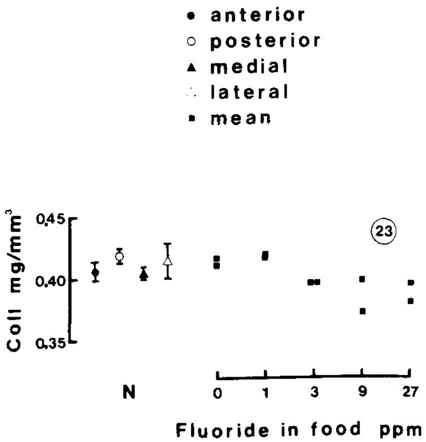


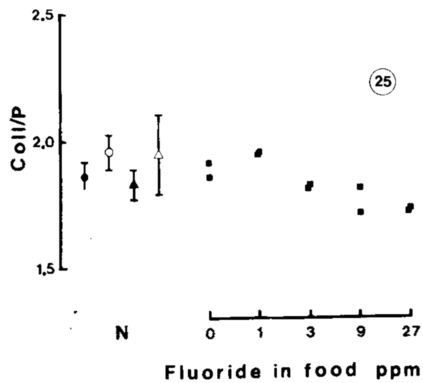
Figs. 20 to 27. Chemical content and femoral shaft dimensions (the mean values plotted against fluoride level in the food as well as the mean and 95 % confidence limit for the different quadrants from the normal series.

Fig. 20. Calcium content

Fig. 21. Phosphorus content r x̄ .73++







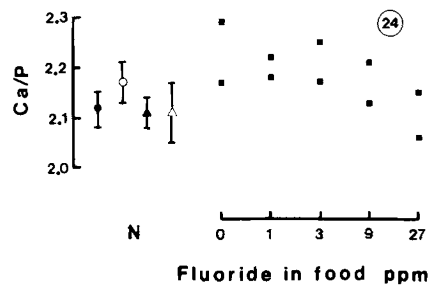


Fig. 22. Fluoride. r $\bar{x}$ .60 +

Fig. 23. Collagen. r $\bar{x}$ −.62 +

Fig. 24. Calcium/phosphorus (Ca/P) r $\bar{x}$ −.70 +

Fig. 25. Collagen/phosphorus (Coll/P) r $\bar{x}$ −.77 ++

Comments

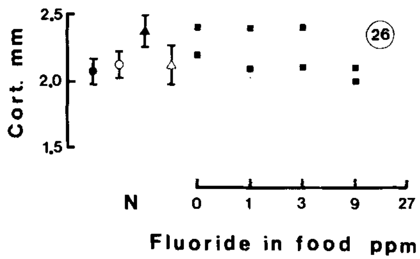
Summarizing the effects of rising dietary levels of fluoride reveals very few changes in the chemical compositions except a consistent increase of the phosphorus and thus a consistent decrease of the Ca/P and collagen/P ratios. It also seems to be a decrease in the amount of collagen as earlier shown by Peck et al. 1965. However, the number of dogs at each fluoride level and the total number are too small for any certain conclusions. The results are in good agreement with the fluoride effect on the chemical composition of the right femur and other parts of the skeleton as reported by Henriksson et al. (1970) from the same animals. An interesting comparison can be made between these results regarding the fluoride accumulation in different parts of the skeleton and the mean values of the four strips from the different quadrants. Fig. 28

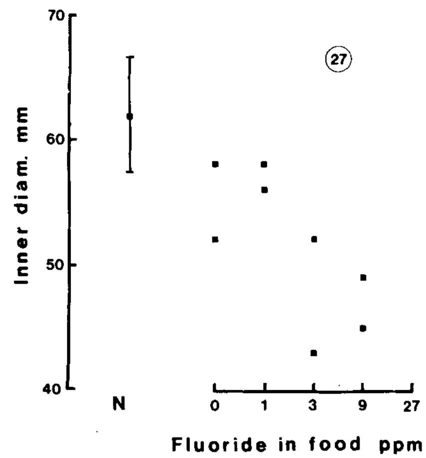
TABLE V. Effect of dietary added fluoride on chemical content and femoral shaft dimensions.

Linear regression correlations between fluoride level in the food and the chemical contents and femoral shaft dimensions of femoral cortical bone in 10 beagle dogs were calculated and the correlation coefficients (r) and significant levels (+) are listed for the four different quadrants and the mean of the four quadrants ($\bar{X}$).

	Ant.	Post.	Med.	Lat.	$\bar{X}$	Fig.nr
Density	.05	-.24	.13	.24	.04	
Ash	.15	-.06	-.19	.22	.06	
Water	-.11	-.49	.02	.04	-.19	
Calcium	-.22	-.14	-.42	-.05	-.35	20
Phosphorus	.73 ++	-.08	.47	.58	.73 ++	21
Fluoride	.66 +	.48	.65 +	.55	.60 +	22
Magnesium	-.10	-.64 +	-.45	-.29	-.51	
Collagen	-.50	-.78 ++	-.35	.09	-.62 +	23
Ca/P	-.44	-.13	-.73 ++	-.74 ++	-.70 +	24
Ca/F	-.48	-.48	-.53	-.44	-.50	
Ca/Mg	.03	.68 +	.46	.23	.44	
Ca/Collagen	.28	.44	.16	-.25	.29	
P/F	-.51	-.43	-.48	-.40	-.47	
P/Mg	.20	.69 +	.62 +	.39	.56	
Collagen/P	-.66 +	-.69 +	-.43	-.41	-.77 ++	25
Mg/F	-.45	-.66 +	-.55	-.47	-.54	
Collagen/F	-.52	-.51	-.55	-.48	-.53	
Collagen/Mg	-.08	.49	.16	.22	.30	
Cortical thickness [1]	-.20	-.20	-.56	-.83 ++	-.61	26
Inner diameter[1]	-	-	-	-	-.66 +	27

[1] In 8 femurs.





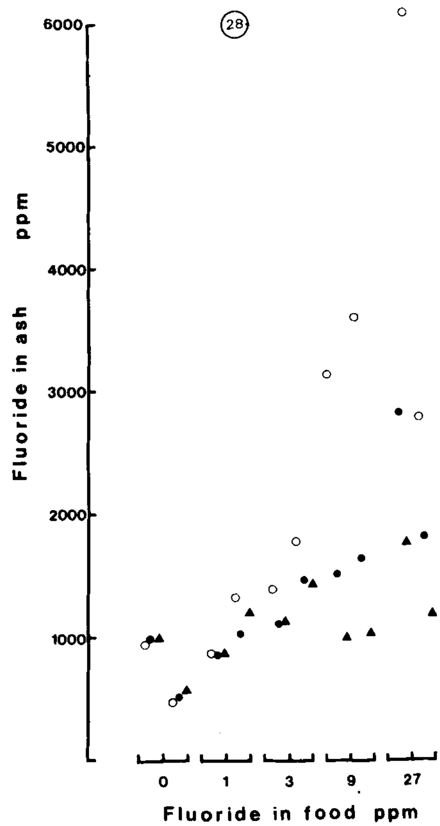


Fig. 26. Cortical thickness (Cort.) r x̄ −,61

Fig. 27. Inner diameter of mid femoral shaft (mean of the diameter in the antero-posterior and lateral projections. r x̄ −,66 +

Fig. 28. Fluoride in ppm in bone ash for two beagle dogs at each fluoride level in the food (vertebræ ○, whole right femur ●, and the mean of the four cortical quadrants of the distal half of the left femur ▲).

shows the fluoride in ppm in bone ash plotted for each dog in the pairs on different levels of dietary fluoride. From this it is seen that the fluoride concentration in the control dogs is practically identical in the bones of different location and composition: vertebræ (mainly spongy bone), right femur (cortical long bone with spongious ends), and the mean value of the four cortical strips (pure cortical bone). In the 1 and 3 ppm levels incorporation of the fluoride in the different bones was practically the same, but at the 9 and 27 ppm levels there was a more pronounced increase in the incorporation in spongy bones such as the vertebræ than in the right femur and the cortical strips from the left femur.

Summary of effects of fluoride addition

The fluoride addition to the food could not prevent the occurrence of nutritionally induced osteopenia as reported by Henriksson et al. (1970). The result of this study of the physical properties and chemical content of cortical bone from femur showed no effect on most of the parametres studied in bending or tension test of bone strips. Only for one quadrant (anterior) there was a marked increase in energy absorption even at the lowest level of fluoride addition (1 ppm). This was mainly due to increased flexibility. No effect was thus seen on the elastic stiffness, stress at elastic limit or ultimate stress, in bending or tension tests. Nor were there any effect on the proportion of the total energy absorption before and after the maximal stress in bending or the total energy absorption in tension. No effect was seen on the maximal strain or its proportion of elastic strain in tension. Although the material is rather small the differences between different quadrants in one individual were greater than between specimens from dogs that had received 0 ppm or 27 ppm fluoride in the food.

These results are in good agreement with the findings of Naylor & Wilson (1967) and Beary (1969) on tests of whole rat femurs. Wolinsky et al. (1972) found on the other hand a decreasing bending strength and elastic stiffness at high dose level (500 ppm). Similar findings were earlier presented by Jovanovits (1944) with decreased breaking strength and elastic stiffness with increasing doses and time of fluoride addition. Smith and Keiper (1965) found no effect on the visco-elastic properties determined by electromagnetic transducer technique of small bone specimens.

No change of the calcium content was found in this study, as previously shown by Likins et al. (1964), Zipkin et al. (1969). The increase of phosphorus in this study was not found by previous authors but from different parts of the skeleton in the same dogs as reported by Henriksson et al. (1971). As shown previously one would expect magnesium content to increase with increasing fluoride in the food (Weidmann et al. 1959, Zipkin et al. 1960, Avioli & Bearman 1968). The slight decrease found in this study was not significant and the error of the method determining magnesium content was perhaps too high (about 8 %). The accumulation of fluoride in the femoral cortical bone segment showed highest values in the lateral and posterior quadrants, the later being muscular attachment (linea aspera). This is in good agreement with results earlier presented by Gardner et al. (1959) and Weidmann et al. (1959). As expected there was a narrowing of the medullary canal by decreasing inner diameter of the femoral shaft as reported by several authors, as well as a slight decrease in the cortical thickness due to diminishing outer diameter with increasing fluoride content in the food.

EFFECT OF THE TIME OF REPLETION ON THE PHYSICAL PROPERTIES AND CHEMICAL CONTENT OF CORTICAL BONE STRIPS

Linear regression correlations were calculated for the time of repletion and the physical properties and chemical contents of the different four quadrants and for the mean of the four quadrants. Some of the measured variables are plotted against time of repletion to illustrate the correlation. The mean and the 95 % confidence limit for the normal series are given for comparison.

Physical properties

The correlation coefficients and significant levels are listed in Table VI.

Bending

The *elastic stiffness* showed, both in three point and four point bending (Fig. 29) a slight increase with increasing time of repletion but significant only for the lateral quadrant in three point bending. There was a slight increase of *stress at elastic limit* (Fig. 30), and a more marked increase of *ultimate bending stress* (Fig. 31) with increasing time of repletion, but not significant for any single quadrant. The *total energy absorption* to fracture increased in the anterior quadrant but the change was not significant (Fig. 32). No effect was seen on the *energy absorption to maximal stress* (Fig. 33), but a marked increase in the *energy absorption after maximal stress* was observed in the anterior quadrant, but not significant (Fig. 34). The *ratio of the energy absorption* before (A_1/T, and after maximal stress (A_2/T, Fig. 35) in relation to the total energy absorption was not influenced by the increasing time of repletion.

Comment

A slight increase of elastic stiffness and stress at the elastic limit and a marked increase of ultimate stress was found with increasing time of repletion. Total energy absorption increased in the anterior quadrant, mainly due to plastic deformation after maximal stress. As in the fluoride series great differences between the different quadrants were found.

Tension

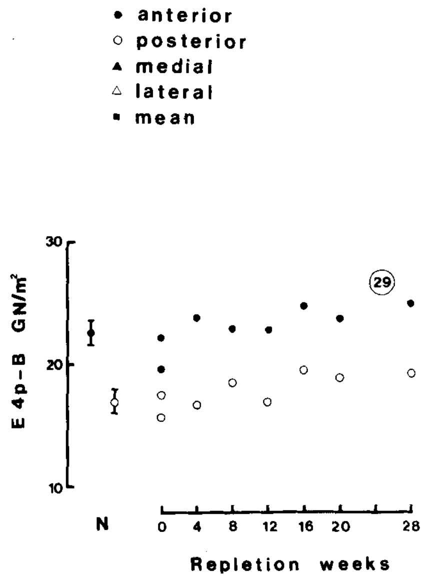
The *elastic stiffness* showed, in tension to low stresses as well as to fracture, an increase with increasing time of repletion, but significant only for the posterior quadrant (Fig. 36). The *stress at elastic limit* was slightly increased, but highly significant only for the lateral quadrant. No consistent effect was seen on the *ultimate tensile stress* by increasing time of repletion (Fig. 37). There was no certain effect on the *energy absorption* in tension (Fig. 38) or at the *maximal strain* (Fig. 39) of increasing time of repletion diet.

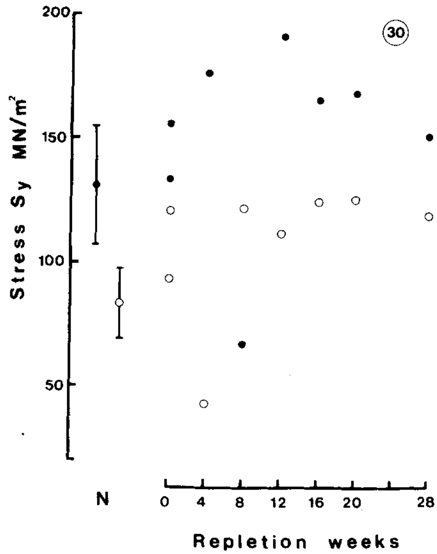
TABLE VI. Effect of increasing time on repletion diet on the physical properties of cortical bone.

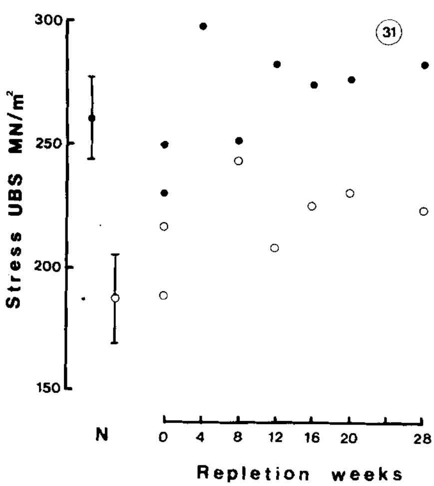
Linear regression correlations between time of repletion and the physical properties of femoral cortical bone specimens in 8 beagle dogs were calculated and the correlation coefficients (r) and the significant levels (+) are listed for the four different quadrants and the mean of the four quadrants ($\bar{X}$).

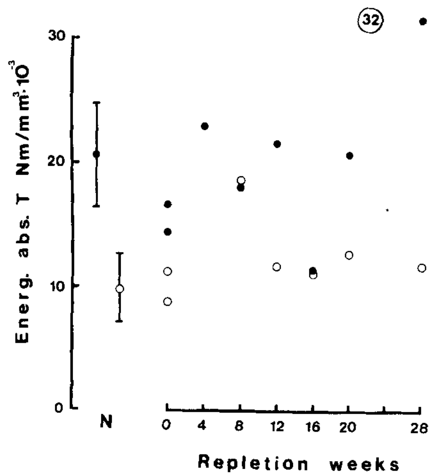
	Ant.	Post.	Med.	Lat.	$\bar{X}$	Fig. nr
Bending						
Elastic stiffness (3p-B)	.42	.43	.40	.72+	.67+	
Elastic stiffness (4p-B)	.74+	.78+	-	-	.87++	29
Stress at elastic limit (S_y)	.18	.44	-	-	.49	30
Ultimate bending stress (UBS)	.53	.52	-	-	.82++	31
Total energy absorption (T)	.57	.17	-	-	.58	32
Energy absorption to maximal stress (A_1)	.09	.29	-	-	.31	33
Energy absorption after maximal stress (A_2)	.61	.03	-	-	.58	34
Ratio of energy absorption to maximal stress to total energy absorption (A_1/T)	-.43	-.11	-	-	-.39	
Ratio of energy absorption after maximal stress to total energy absorption (A_2/T)	.43	.11	-	-	.39	
Tension						
Elastic stiffness (test to low stress)	.54	.71+	.23	.44	.77+	
Elastic stiffness (test to fracture)	-	-	.16	.32[1]	.19[1]	36
Stress at elastic limit (S_y)	-	-	.47	.87++[1]	.75+[1]	
Ultimate tensile stress (UTS)	-	-	.07	.33	.24	37
Total energy absorption	-	-	.28	.31	.47	38
Maximal strain (D_m/L)	-	-	.21	.29	.43	39
Ratio of deformation at elastic limit to maximal deformation (D_y/D_m)	-	-	.09	.01[1]	.13[1]	

[1] 7 specimens.









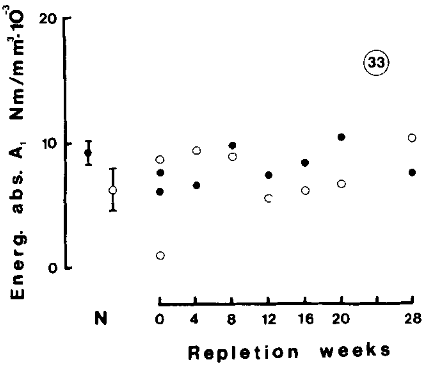
Figs. 29 to 39. Physical properties for the different quadrants plotted against time on repletion diet, as well as the mean and the 95 % confidence limit for the different quadrants from the normal series (N).

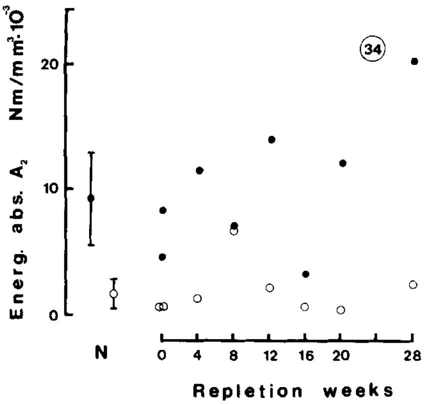
Fig. 29. Elastic stiffness in four point bending (E 4p-B) r ant .74 +; post .78 +

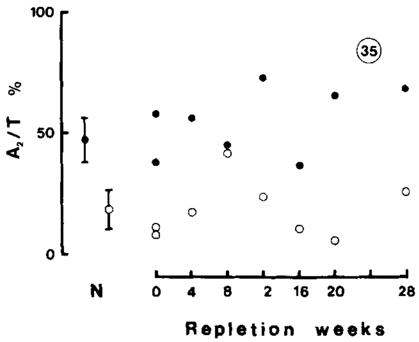
Fig. 30. Stress at elastic limit in bending (Stress S_Y)

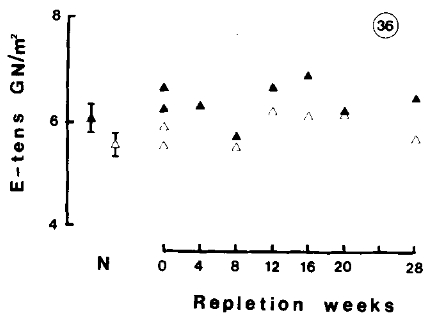
Fig. 31. Ultimate bending stress (UBS)

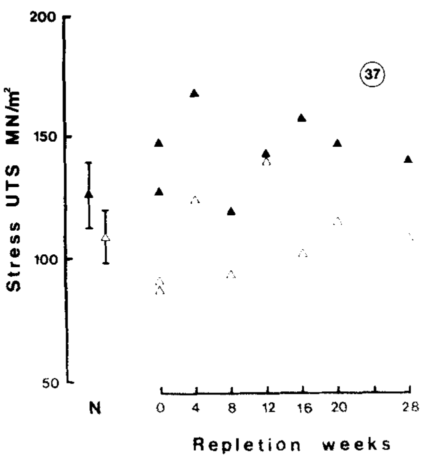
Fig. 32. Total energy absorption in bending (Energ. abs. T)











- ● anterior
- ○ posterior
- ▲ medial
- △ lateral
- ▪ mean

Fig. 33. Energy absorption to maximal stress (Energ. abs. A_1)

Fig. 34. Energy absorption after maximal stress (Energ. abs. A_2)

Fig. 35. Ratio of energy absorption after maximal stress to total energy absorption (A_2T).

Fig. 36. Elastic stiffness in tension test to fracture (E-tens)

Fig. 37. Ultimate tensile stress (Stress UTS)

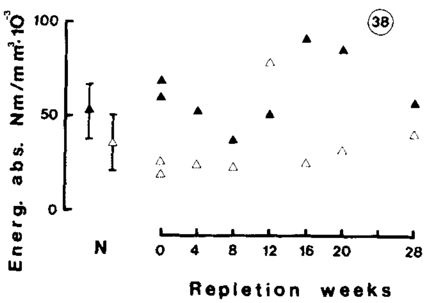


Fig. 38. Total energy absorption in tension (Energ. abs.)

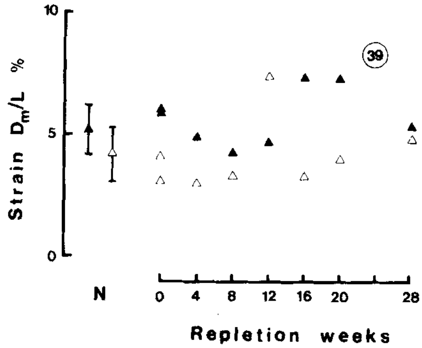


Fig. 39. Maximal strain in tension (D_m/L)

Comment

An increase in elastic stiffness and stress at the elastic limit was found with increasing time of repletion diet. The same pattern showing great differences between the different quadrants was seen also in the tension tests.

Chemical composition and femoral shaft dimensions

The correlation coefficients and significant levels are listed in Table VII. Some of the mean values for the four quadrants are plotted against the time of repletion.

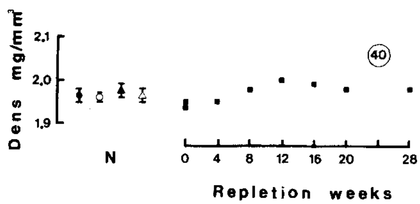
The *density* of the cortical bone slightly increased in all four quadrants but significantly only for the lateral and for the mean of the four quadrants (Fig. 40). The same was the fact for the *ash* content, significant for the anterior and lateral quadrants and highly significant for the mean of the four quadrants (Fig. 41). There was no consistent effect on the *water* content. A slight, but not significant decrease of the *calcium* content (Fig. 42) was found with increasing time of repletion. On the contrary the *phosphorus* content increased significantly for the mean of the four quadrants (Fig. 43). The *fluoride* content increased, significantly only in the anterior quadrant (Fig. 44). It is interesting to note the rather constant but slight decrease in the *collagen* content in all four quadrants, being significant only for the medial (Fig. 45). As the calcium and collagen content seemed to decrease and the phosphorus and fluoride content seemed to increase with increasing time of repletion diet the consequence was a decrease in the *Ca/P* (Fig. 46), *Ca/F*, *collagen/P* and *collagen/F* ratios as well as a decrease in the *P/F* ratio. None of these changes were significant. There was a slight, but not significant, increase of the *cortical thickness* (Fig. 47).

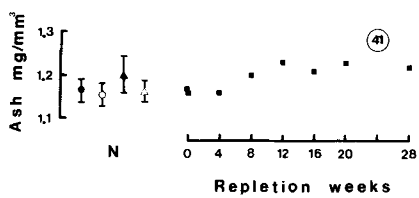
TABLE VII. Effect of increasing time on repletion diet on chemical content and femoral shaft dimensions.

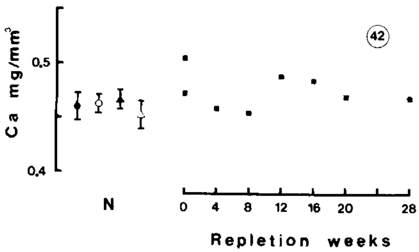
Linear regression correlations between time of repletion and chemical contents and femoral shaft dimensions of cortical bone in 8 beagle dogs were calculated and the correlation coefficients (r) and significant levels (+) are listed for the four different quadrants and the mean of the four quadrants ($\bar{X}$).

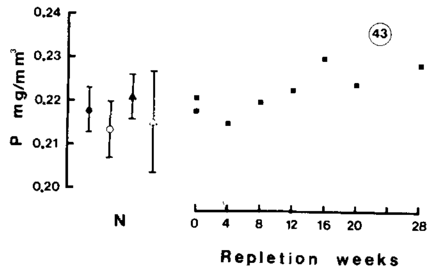
	Ant.	Post.	Med.	Lat.	$\bar{X}$	Fig. nr
Density	.44	.56	.65	.72 +	.72 +	40
Ash	.75 +	.59	.58	.66 +	.84 ++	41
Water	-.76 +	.31	.12	.46	.01	
Calcium	-.16	-.54	.49	-.42	-.21	42
Phosphorus	.29	.03	.27	-.20	.77 +	43
Fluoride	.66 +	.51	.27	.32	.48	44
Magnesium	-.08	-.50	.23	-.39	.21	
Collagen	-.45	-.11	-.75 +	-.37	-.36	45
Ca/P	-.38	-.61	.37	-.42	-.42	46
Ca/F	-.60	-.60	-.30	-.38	-.49	
Ca/Mg	.04	.35	-.12	.33	.24	
Ca/Collagen	.11	-.04	.65	.16	.13	
P/F	-.64	-.57	-.33	-.36	-.50	
P/Mg	.16	.43	-.19	.37	.22	
Collagen/P	-.39	-.14	-.57	-.12	-.40	
Mg/F	-.47	-.62	-.25	-.40	-.45	
Collagen/F	-.65	-.56	-.41	-.40	-.54	
Collagen/MG	0	.28	-.41	.26	-.07	
Cortical thickness [1]	.42	.56	.52	0	.62	47
Inner diameter[1]	-	-	-	-	.32	

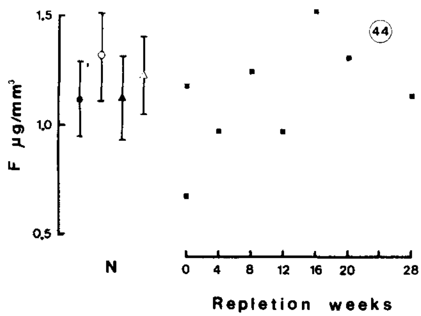
[1] In 7 femurs.

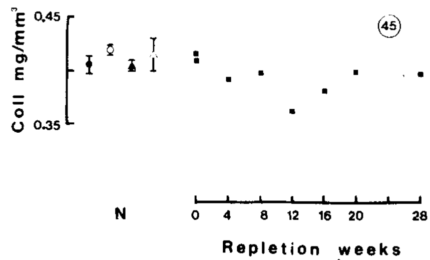


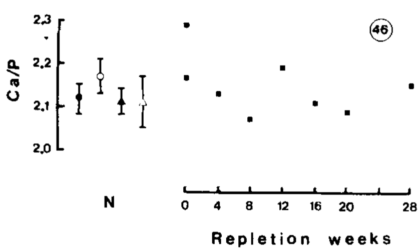


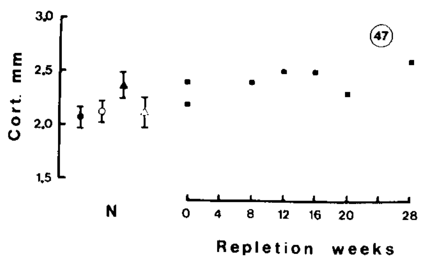












Figs. 40 to 47. Chemical content and femoral shaft dimensions (the mean values) plotted against time on repletion diet as well as the mean and 95 % confidence limit for the different quadrants from the normal series (N).

Fig. 40. Density of wet bone (Dens.) r $\bar{x}$ .72 +

Fig. 41. Ash content r $\bar{x}$ .84 ++

Fig. 42. Calcium content

Fig. 43. Phosphorus content r $\bar{x}$ .77 +

Fig. 44. Fluoride content

Fig. 45. Collagen content

Fig. 46. Calcium/phosphorus (Ca/P)

Fig. 47. Cortical thickness (Cort.)

Comment

The number of dogs in this series was too small to demonstrate significance for more than a few of the correlations. This was especially true for the effect on the chemical composition. The increase in density and ash content with increasing time on repletion diet is in good agreement with the results reported from the same material in the right femur and other bones in these dogs as reported by Krook et al. (1971).

Summary of effects of repletion diet

Few studies have earlier been reported on the reversibility of nutritional induced osteopenia. The effects on the rest of the skeleton of increasing time of repletion showed almost complete reversibility after 28 weeks of repletion as reported by Krock et al. (1971). The result of the study of physical properties and chemical content showed few significant correlations between time on repletion and different physical properties mainly due to small number of experimental animals (8). However, from the diagrams is seen an increase of elastic stiffness, stress at elastic limit and breaking strength both in bending and tension tests with increasing time of repletion. The energy absorption in bending also increased for one quadrant mainly depending on increased flexibility after maximal stress. This was not seen in tension. The increase in the physical properties was though not greater than the difference between two quadrants from the same femur.

As previously reported by Krook et al. (1971) there was a slight increase in ash content and density but no effect on the calcium content. There was an increase of phosphorus and fluoride content with increasing time on repletion. However, there was a slight decrease of collagen content with increasing time on repletion even when calculated on a volume basis.

VARIATION WITH AGE OF PHYSICAL PROPERTIES AND CHEMICAL CONTENT

It was not felt justified to calculate any variation in the physical properties and chemical contents with varying age, since efforts were made to choose dogs of the same age for the present study. Several of the dogs were littermates (F-0-1 & F-1-2; F-3-1 & F-27-1; R-4 & R-28; N-2 & N-3; N-4 & N-5; N-6, N-7, N-8, N-9 & N-10). The youngest was 1 year and 6 months and the oldest was 3 years and 3 months (Fig. 48).

VARIATIONS WITH BODY WEIGHT OF PHYSICAL PROPERTIES AND CHEMICAL CONTENT

Linear regression correlation was calculated in the whole material to evaluate if any marked influence was caused by the body weight of the dog.

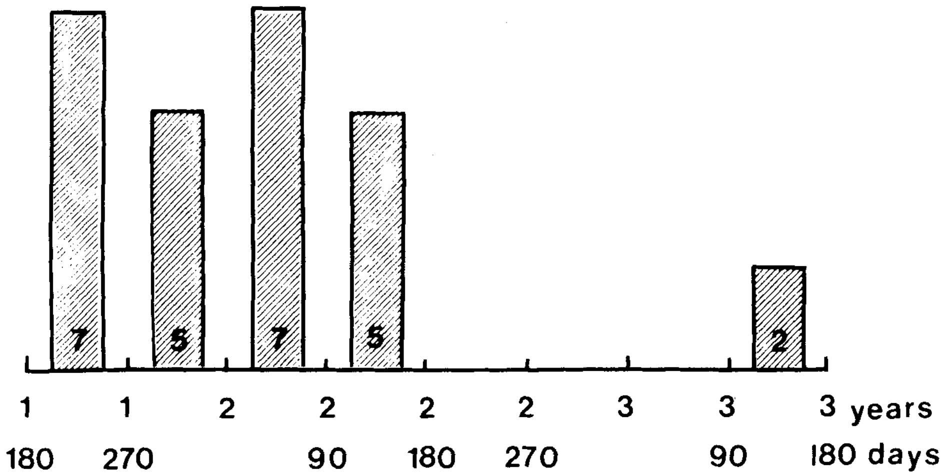


Fig. 48. Age distribution of 26 beagle dogs in three experimental groups).

Physical properties

The correlation coefficients and significant levels for some physical properties are listed in Table VIII. The only statistically significant effect of increasing body weight was a slight decrease in elastic stiffness in tension to low stresses. This was highly significant for the anterior quadrant (Fig. 49). A large scatter is seen and no certain conclusions can be drawn. A similar effect was seen on the proportion of the elastic deformation to total deformation (D_y/D_m) in tension but significant only for the lateral quadrant.

Chemical composition and femoral shaft dimensions

The correlation coefficiencies for some chemical content are listed in Table IX. The variation with body weight of the chemical compositions as for the physical properties showed very few significant correlations. A slight increase in calcium content was found, significant only for the anterior quadrant. A slight decrease in fluoride content with increasing body weight was seen in all the four quadrants but not significant for any. A similar slight decrease with increasing body weight was found of the collagen content and this was significant for the posterior quadrant and for the mean of the four quadrants. A slight increase in the Ca/collagen ratio was thus found in all quadrants but significant only for the lateral. A moderate increase in cortical thickness was found, significant for all four quadrants and highly significant for the medial quadrant and the mean of the four quadrants (Fig. 50).

Comments

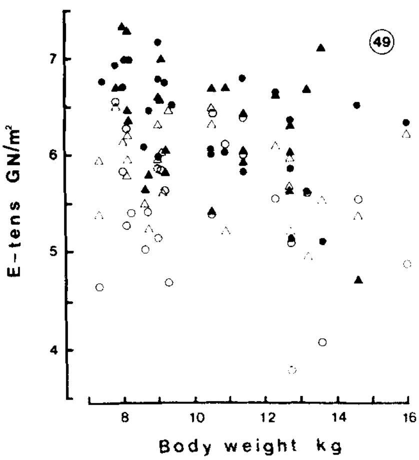
In conclusion it can be stated that few correlations were found between the body weight of the experimental animals and the physical properties and the chemical composition of cortical bone strips from the femur.

TABLE VIII. Body weight and physical properties of cortical bone.

Linear regression correlations between body weight and the physical properties of femoral cortical bone specimens in 26 beagle dogs were calculated and the correlation coefficients (r) and significant levels (+) are listed for the four different quadrants and the mean of the four quadrants ($\bar{X}$).

	Ant.	Post.	Med.	Lat.	$\bar{X}$
Bending					
Elastic stiffness (3p-B)	.03	.02	.34	-.34	0
Ultimate bending stress (UBS)	-.03	.23	-	-	.10
Total energy absorption (T)	-.16	.22	-	-	-.05
Tension					
Elastic stiffness (test to low stress) [1]	-.55++	.30	-.27	-.22	-.47+
Ultimate tensile stress (UTS)	-	-	.35	.15	.32
Total energy absorption	-	-	.07	.25	.22
Maximal strain (D_m/L)	-	-	.02	.28	.21
Ratio of deformation at elastic limit to maximal deformation (D_y/D_m)	-	-	-.21	-.46+[2]	-.41+[2]

[1] Fig. 49. [2] In 23 specimens.



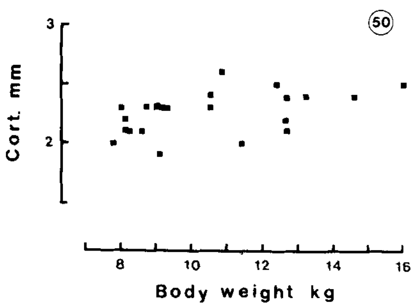


Fig. 49. Elastic stiffness to low stress plotted against body weight (E-tens)
r ant −.55 ++

Fig. 50. Cortical thickness (mean) plotted against body weight (Cort.)
r $\bar{X}$ .51 ++

TABLE IX. Body weight and chemical content and femoral shaft dimensions.

Linear regression correlation between body weight and chemical content and femoral shaft dimensions of cortical bone specimens in 26 beagle dogs were calculated and the correlation coefficients (r) and significant levels (+) are listed for the four different quadrants and the mean of the four quadrants ($\bar{X}$).

	Anterior	Posterior	Medial	Lateral	$\bar{X}$
Density	.10	.07	.10	-.12	.06
Ash	.13	.03	.23	.04	.16
Water	.06	.20	-.11	.20	.11
Calcium	.43 +	-.21	.14	.30	.26
Phosphorus	.20	.08	.14	.03	.16
Fluoride	-.32	-.30	-.32	-.30	-.32
Magnesium	.24	0	.03	.27	.25
Collagen	.02	-.38 +	-.12	-.32	-.32
Ca/P	.29	.29	0	.29	.12
Ca/Mg	0	.13	0	.19	.09
Ca/Collagen	.30	.29	.10	.40 +	.37
Cortical thickness [1] [2]	.42 +	.46 +	.54 ++	.40 +	.51 ++
Inner diameter[1]	-	-	-	-	-.08

[1] In 23 femurs. [2] Fig. 50.

SEX INFLUENCE ON PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION

All the measured variables were tested for any differences between male and female. Calculations were performed in the different series but the dogs were too few in number for any conclusions. The result is presented in Table X as the means of all specimens in the whole material for male and female respectively.

TABLE X. Differences between sexes of physical properties, chemical content and femoral shaft dimensions.

Mean value and standard deviations for cortical bone specimens from 12 male and 14 female beagle dogs. The degrees of freedom and the corresponding F-value and its significant level are listed for the determined parameters that showed significant difference between male and female specimens.

	Male		Female				
	Mean	(SD)	Mean	(SD)		F-value	Sign. level
Elastic stiffness (3p-B) GN/m^2	17.90	(2.43)	19.06	(2.18)	(1-102)	6.51	+
Elastic stiffness (low stress) GN/m^2	5.80	(0.71)	6.21	(0.59)	(1-102)	10.29	++
Water mg/mm^3	0.2306	(0.0119)	0.2199	(0.0117)	(1-102)	21.45	++
Ash % of wet weight	59.85	(2.06)	60.94	(1.37)	(1-101)	10.36	++
Fluoride ug/mm^3	1.152	(0.241)	1.327	(0.374)	(1-101)	7.75	++
Cortical thickness mm	23.14	(2.42)	21.75	(2.43)	(1-90)	7.52	++
Femoral length mm	123.2	(7.1)	115.8	(5.7)			

Only the variables with significant difference between male and female are presented. Compared to the female specimens the male had: lower elastic stiffness in both bending and tension tests, higher water content, lower ash and fluoride content, greater cortical thickness and femoral length.

Comments

This material of 26 beagle dogs consisted of 12 male and 14 female dogs.. For most of the parametres studied there was no significant difference between the male and female specimens. Evans (1957) summarizes that male femoral bone was stronger than female when tested fresh. Lindahl & Lindgren (1967) could not confirm this in tensile tests of cortical strips. In compression (1968) they found higher strength in male femur but not in humerus. Higher water content was found in the male and lower ash content which could be expected since water is replaced by mineral crystals with increasing degree of mineralization.

COMBINED RESULTS OF MECHANICAL TESTING IN ALL SERIES

The three point bending and tension tests to low stresses were performed on all the four quadrants in all 26 femurs.

The four point bending tests to fracture were performed on all anterior and posterior quadrants from the fluoride and reversibility series and N-3 to N-8, and all four quadrants from N-1 and N-10 (24 anterior and 24 posterior specimens and 2 medial and 2 lateral specimens).

Tension tests to fracture were performed on all medial and lateral quadrants from the fluoride and reversibility series and N-3 to N-8 and all four quadrants from N-2 & N-9 (2 anterior and 2 posterior specimens and 24 medial and 24 lateral specimens).

The mean values and standard deviations of the whole material are listed in Table XI. The results of the bending tests are in good agreement with several previous authors as summarized by Swanson (1971). This is not the fact with the result from the tension test as for example the elastic stiffness is here significantly lower than in previous reports, in this material only about 1/3 of the elastic stiffness in bending. This will be discussed later (page 70).

Differences between different quadrants

From the mean values listed it is obvious that there are great differences between the different samples in most of the measured variables depending on the origin from the femoral circumference. This is also shown in the previously presented result in the fluoride and repletion series (Figs. 9 to 19 and 29 to 39). It is even more striking when performing a sign test, the result of this is listed in Table XII. Some significant differences will be discussed.

Bending

Elastic stiffness both in three point and four point bending tests revealed higher stiffness for the anterior than for the posterior quadrants in all femurs (24 - 0). The stress at elastic limit and ultimate bending stress was higher for the anterior quadrant than for the posterior except in one case (23 - 1). The energy absorption was much higher in the anterior than the posterior quadrant (21 - 3). All the nine specimens that did not fracture at the maximal deflexion possible in the testing set up (5.5 mm), were from the anterior quadrant. This difference is depending on the energy absorbed after maximal stress as there was no difference in the energy absorption to maximal stress between the two quadrants (15 - 9). The proportion of energy absorption after maximal stress to total energy absorption was thus higher in the anterior quadrant than in the posterior in every tested pair (24 - 0). The two tested pairs of medial and lateral specimens followed the same patterns.

TABLE XI. Physical properties in bending and tension tests for femoral, cortical bone.

The mean value and standard deviation (SD) in each quadrant from 24 beagle dogs as well as from all four quadrants are listed. Specimens from the medial and lateral quadrants from only two dogs are tested to fracture in bending and specimens from the anterior and posterior quadrants from only two dogs were tested in tension to fracture. The elastic stiffness in 3 p–B and in tension test to low stress were calculated from tests performed on specimens from all 26 dogs.

	Anterior		Posterior		Medial		Lateral		All quadrants	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Bending										
Elastic stiffness (3p-B) GN/m^2	20.471	(1.34)	16.20	(1.45)	20.36	(1.50)	17.05	(1.27)	18.52	(2.36)
Elastic stiffness (3p-B) GN/m^2	22.12	(2.02)	17.38	(1.49)	22.16	-	19.12	-	19.82	(2.92)
Stress at elastic limit (S_y) MN/m^2	140.5	(27.3)	96.3	(21.6)	130.6	-	107.1	-	118.5	(31.9)
Ultimate bending stress (UBS) MN/m^2	260.2	(21.7)	205.8	(24.9)	259.2	-	226.7	-	233.8	(34.8)
Total energy absorption (T) $Nm/mm^3 x 10^{-3}$	20.80	(6.73)	9.58	(3.48)	17.60	-	13.59	-	15.22	(7.60)
Energy absorption to max. stress (A_1) Nm/mm^3 x 10^{-3}	9.11	(2.10)	7.57	(2.24)	8.76	-	8.15	-	8.35	(2.26)
Energy absorption after max. stress (A_2) Nm/mm^3 x 10^3	11.70	(5.60)	2.02	(1.75)	8.85	-	5.44	-	6.87	(6.21)
Ratio of energy absorption to maximal stress to total energy absorption (A_1/T) %	46.8	(12.9)	81.7	(11.0)	51.0	-	63.5	-	63.7	(20.7)
Ratio of energy absorption after maximal stress to total energy absorption (A_2/T) %	53.2	(12.9)	18.3	(11.0)	49.0	-	36.5	-	36.3	(20.7)
Tension										
Elastic stiffness (test to low stress) GN/m^2	6.35	(0.56)	5.51	(0.72)	6.35	(0.59)	5.86	(0.45)	6.02	(0.68)
Elastic stiffness (test to fracture) GN/m^2	6.43	-	5.33	-	6.22	(0.37)	5.60	(0.34)	5.91	(0.49)
Stress at elastic limit (S_y) MN/m^2	80.22	-	53.94	-	69.53	(9.32)	58.55	(7.85)	64.24	(10.49)
Ultimate tensile stress (UTS) MN/m^2	147.5	-	95.0	-	138.1	(17.6)	107.4	(16.4)	122.5	(14.1)
Total energy absorption NM/mm^3 x 10^{-3}	69.78	-	27.36	-	59.47	(20.69)	33.98	(16.76)	46.65	(13.84)
Maximal strain (D_m/L) %	6.30	-	3.90	-	5.75	(1.41)	4.28	(1.34)	5.02	(1.55)
Ratio of deformation at elastic limit to maximal deformation (D_y/D_m) %	20.10	-	29.00	-	20.48	(5.60)	26.43	(8.86)	23.55	(7.91)

TABLE XII. Differences of physical properties between different quadrants.

The result of a sign test and significant levels (+) are listed below. The number of highest value for one property when comparing two quadrants in the same femur is listed below.

	Ant–Post	Signif.	Med–Lat	Signif.
Bending				
Elastic stiffness (3p-B)[1]	26 – 0	++	24 – 2	++
Elastic stiffness (4p-B)[1]	24 – 0	++	2 – 0	
Stress at elastic limits (S_y)	23 – 1	++	2 – 0	
Ultimate bending stress (UBS)	23 – 1	++	2 – 0	
Total energy absorption (T)	21 – 3	++	2 – 0	
Energy absorption to maximal stress (A_1)	15 – 9		1 – 1	
Energy absorption after maximum stress (A_2)	24 – 0	++	2 – 0	
Ratio of energy absorption to max. stress to total energy absorption (A_1/T)	0 – 24	++	0 – 2	
Ratio of energy absorption after maximum stress to total energy absorption (A_2/T)	24 – 0	++	2 – 0	
Tension				
Elastic stiffness (low stress)[2]	24 – 2	++	21 – 5	++
Elastic stiffness (fracture test)	2 – 0		22 – 1	++
Stress stiffness (fracture test)	2 – 0		20 – 3	++
Ultimate tensile stress (UTS)	2 – 0		22 – 2	++
Total energy absorption	2 – 0		20 – 4	++
Maximal strain (D_m/L)	2 – 0		19 – 5	++
Ratio of deformation at elastic limit to maximal deformation (D_y/D_m)	0 – 2		4 – 18	++

[1] A–M: 13–13; P–L: 5–21 + + [2] A–M: 15–11; P–L: 11–5.

Compared to the posterior and lateral quadrants, the anterior and medial quadrants had the following properties: higher elastic stiffness, higher stress at elastic limit and ultimate bending stress, greater energy absorbing capacity, depending on greater deflection after maximal stress. Nine of the 24 tested anterior specimens did not fracture at the maximal deflection possible in the testing machine (5.5 mm).

Tension

A similar difference between the specimens from different quadrants was found in the tension test.

The elastic stiffness in tension tests to low stress as well as to fracture revealed higher values for the medial than the lateral quadrants (21 - 3 and 22 - 2 respectively). The same was true for the stress at the elastic limit and the ultimate tensile stress (20 - 3 and 22 - 2 respectively). The ratio of the elastic deformation to total deformation was greater in the lateral quadrant (18 - 4) and the plastic deformation and the energy absorption was thus greater in the medial quadrant (20 - 4) as well as the total deformation (19 - 5). The two tested pairs of anterior and posterior specimens followed the same pattern.

Compared to the lateral and posterior quadrants, the medial and anterior quadrants had the following properties: higher elastic stiffness, higher stress at elastic limit and ultimate tensile stress, and greater energy absorbing capacity.

Summarizing this it was found that the anterior and medial quadrants have higher stiffness, higher stress at the elastic limit, higher ultimate stress, more plastic deformation and higher energy absorption in both bending and tension tests than the posterior and lateral quadrants. In bending and tension tests to low stresses of all four quadrants and the tests to fracture of N-1, N-2, N-9 and N-10, where a comparison between the anterior and medial and between the posterior and lateral quadrants was possible, the only other significant difference was that the lateral specimens had higher elastic stiffness than the posterior ones in three point bending (21 - 5). A typical load-deformation curves from anterior and posterior specimens tested in bending to fracture is seen in Fig 51 and a typical load-deformation curve from a tension test on a medial and lateral quadrant is shown in Fig. 52.

Correlations between different physical properties

The linear regression correlation was calculated for some of the different physical properties measured in bending and tension tests.

In bending test

The correlation coefficiencies are listed in Table XIII; some of these will be discussed in detail. The highly significant correlation between the elastic stiffness and ultimate bending stress in both the anterior and posterior quadrants is shown in Fig. 53. The total energy absorption was significantly greater for the anterior than for the posterior

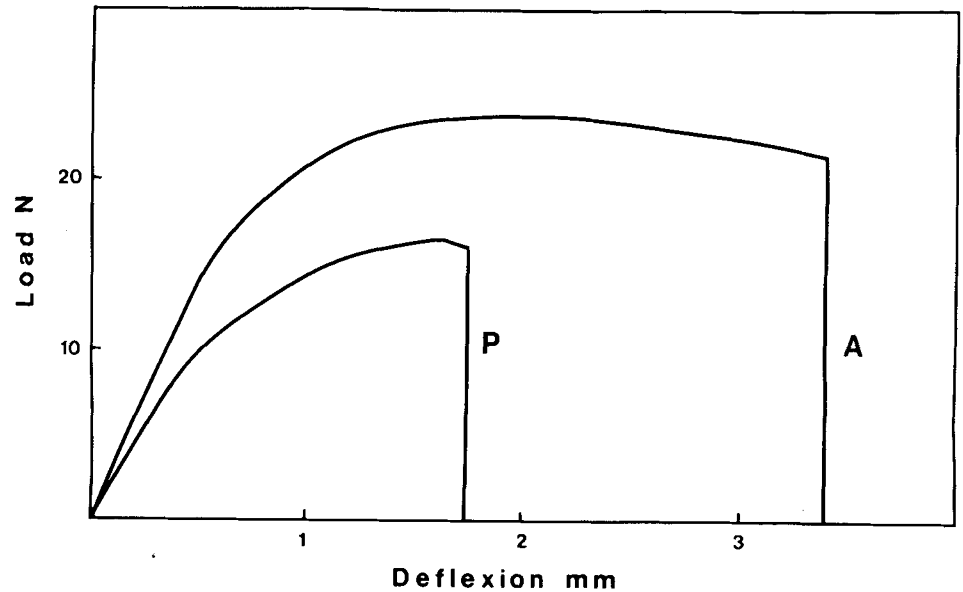


Fig. 51. Load deformation curve in four point bending for anterior (A) and posterior (P) quadrants, illustrates the great difference in elastic stiffness (slope of the linear portion of the curve), breaking strength, maximal deflexion and energy absorption (area under the corresponding curve).

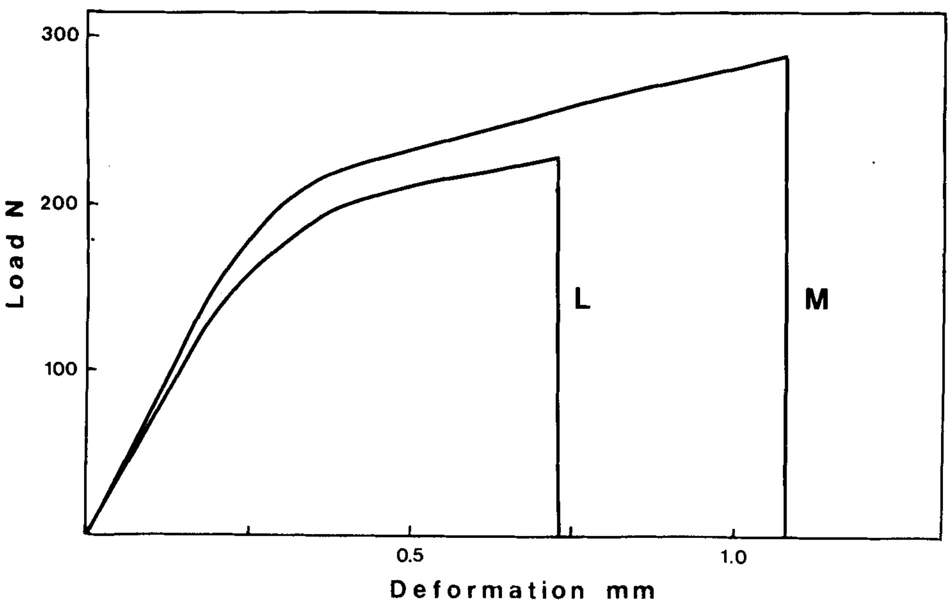


Fig. 52. Load deformation curve in tension test of medial (M), and lateral (L) quadrants, illustrates the great differences in elastic stiffness (slope of the linear portion of the curve), breaking strength, maximal deformation and energy absorption (area under the corresponding curve).

TABLE XIII. Correlation between different physical properties in bending.

Linear regression correlation between different physical properties in bending was calculated for femoral cortical bone specimens in 24 beagle dogs. The correlation coefficients (r) and significant levels (+) are listed for the different quadrants.

	Ultimate bending stress (UBS)			**Elastic stiffness (4p-B)**		
	Ant	Post	Ant + Post	Ant	Post	Ant+Post
Elastic stiffness (4p-B)	.74+++	.77+++	.90+++	-	-	-
Total energy absorption (T)	0	.73+++	.67+++	.35	.13	.54+++
Energy absorption to max. stress (A_1)	-.03	.73+++	.49+++	.38	-.39	.25
Energy absorption after max. stress (A_2)	0	.51++	.63+++	-.22	.20	.57+++
Ratio of energy absorption after max. stress to total energy absorption (A_1/T)	0	.42+	.71+++	.12	.18	.67+++
Stress at elastic limit (S_y)	.63+++	.80+++	.85+++	.44+	.65+++	.77+++
Elastic stiffness (3b-P)	-	-	-	.53++	.81+++	.88+++

	Total energy absorption T			**Energy absorption to max. stress (A_1)**		
Ratio of energy absorption after max. stress to total energy absorption (A_2/T)	.74+++	.72+++	.88+++	-	-	-
Energy absorption after max. stress (A_2)	-	-	-	.40+	.52+	.49+

	Elastic stiffness (3p-B)				
	Ant	Post	Med	Lat	Ant+Post+Med+Lat
Elastic stiffness (low stress)	.24	.51++	.51++	.52++	.64+++

quadrants. The difference was mainly depending on energy absorption after stress (A_2) due to plastic deformation before the fracture occurred. There was no significant dif-

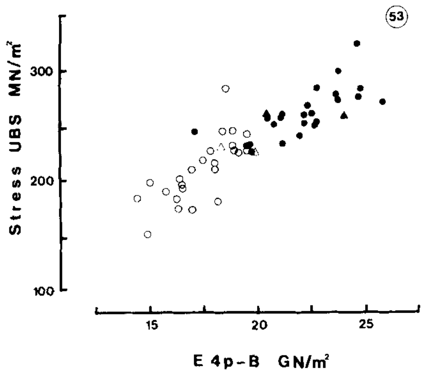


Fig. 53. Correlation between ultimate bending stress and elastic stiffness in four point bending (Stress UBS – E 45-B)

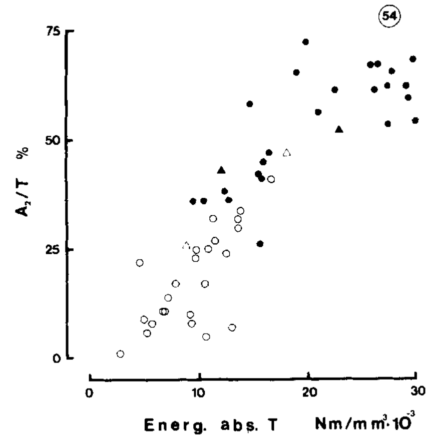


Fig. 54. Correlation between the ratio of energy absorption after maximal stress to total energy absorption and total energy absorption (A_2T – Energ. abs. T)

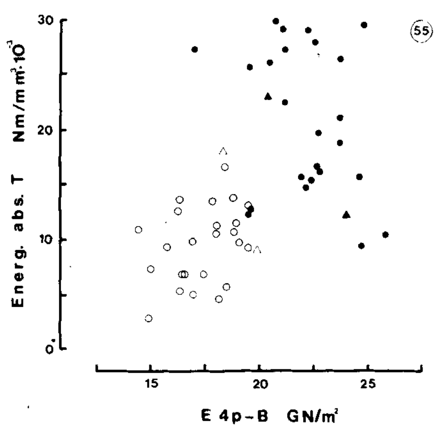


Fig. 55. Correlation between total energy absorption in bending and elastic stiffness in four point bending (Energ. abs. T – E 45-B)

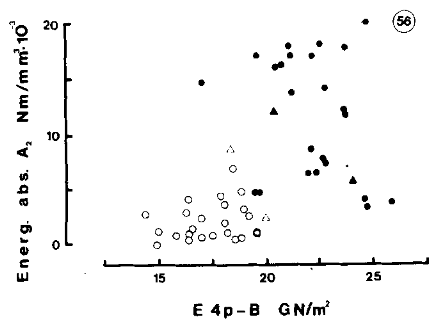


Fig. 56. Correlation between energy absorption after maximal stress and elastic stiffness in four point bending (Energ. abs. A_2 – E 4p-B)

ference in the deformation to maximal stress between the anterior and posterior quadrants. These aspects are reflected in Fig. 54, which shows high correlation between the total energy absorption and the proportion of the total energy absorption that appeared after maximal stress (A_2/T), which was true for both the anterior and posterior quadrants. This curve also shows great differences between the anterior and posterior quadrants. The total energy absorption (T , Fig. 55),the energy absorption after maximal stress (A_2, Fig. 56) and the ratio of the energy absorbed after maximal stress to total energy absorption (A_2/T, Fig. 57) shows no clear cut correlation to the elastic stiffness.

In tension tests

The correlation coefficiencies between some different properties are listed in Table XIV. Some of these will be discussed in detail.

The highest correlation was obtained between the energy absorption and the strain (D_m/L), which is shown in Fig. 58. Some correlation was found between most of the tested variables. The correlation between the ultimate tensile stress and the elastic stiffness is shown in Fig. 59. Fig. 60 indicates that energy absorption in tension is depending on the ultimate tensile stress and on the strain, which was not the fact in bending where the deflexion was responsible for most of the variations in the energy absorption.

All the presented diagrams showed that great and consistent difference were found between the different quadrants. The findings in the two pairs of femur in which all the four quadrants were submitted to the same type of testing to fracture (N-1 and N-10 in bending and N-2 and N-9 in tension) confirmed this pattern.

TABLE XIV. Correlation between different physical properties in tension.

Linear regression correlation between different physical properties in tension was calculated for femoral cortical bone specimens in 24 beagle dogs. The correlation coefficients (r) and significant levels (+) are listed for the different quadrants.

	Ultimate tensile stress (UTS)			Elastic stiffness (fracture test) T tens fail		
	Med	Lat	Med + Lat	Med	Lat	Med + Lat
Elastic stiffness (fracture test)	.67+++	.34	.74+++	-	-	-
Stress at elastic limit (S_y)	.62+++	.57++	.74+++	.49+	.48+	.67+++
Energy absorption	.74+++	.73+++	.83+++	.39+	.06	.53+++
Maximal strain (D_m/L)	.62+++	.58++	.71+++	.25	-.08	.37++
Ratio of deform. at elastic limit to max. deform. (D_y/D_m)	-.63++	-.27	-.53+++	-	-	-
Elastic stiffness (low stress)	-	-	-	.44+	.22	.50+++

	Energy absorption		
	Med	Lat	Med+Lat
Maximal strain (D_m/L)	.98+++	.97+++	.97+++

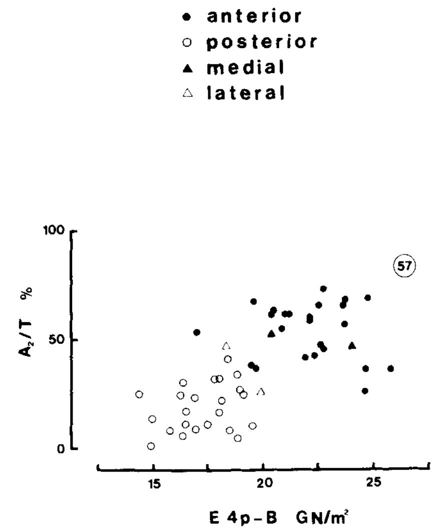


Fig. 57. Correlation between the ratio of energy absorption after maximal stress to total energy absorption and elastic stiffness in four point bending (A_2T – E 4p-B)

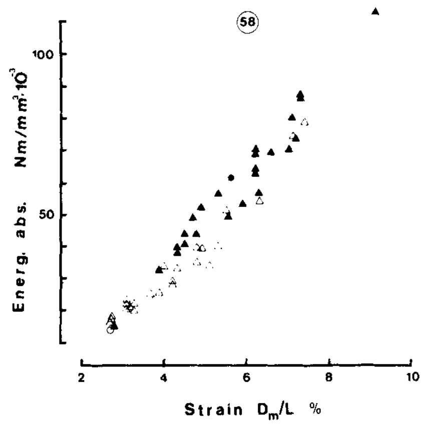


Fig. 58. Correlation between energy absorption in tension and maximal strain (D_m/L) (Energ. abs. – strain D_mT)

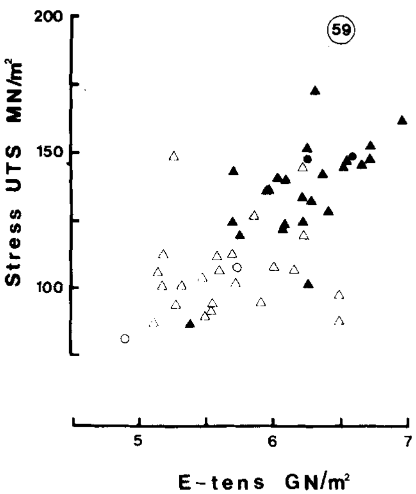


Fig. 59. Correlation between ultimate tensile stress and elastic stiffness in tension to fracture (Stress UTS – E-tens)

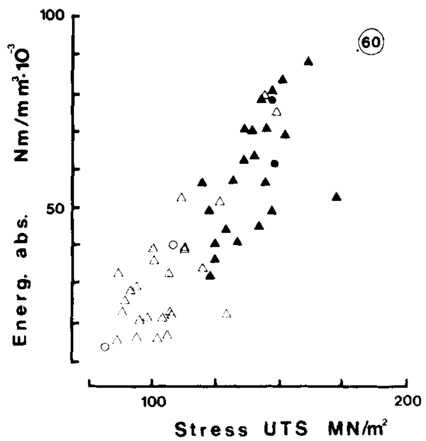


Fig. 60. Correlation between energy absorption in tension and ultimate tensile stress (Energ. abs. – Stress UTS)

Comments on results from mechanical testing – especially sources of error

Bending – tension

The results from the different tests are listed in Table XI as the mean values and standard deviations for the four different quadrants and the mean of all quadrants. There were great differences between the results obtained in bending and in tension tests, some of these will be discussed.

Energy absorption

The difference in energy absorption in bending and in tension is mainly due to the anisotrophy of the material (the specimens have a predominant fiber direction) and very different forces are needed to fracture the specimens in bending (20 - 30 N) than in tension (200 - 300 N).

Ultimate bending (UBS) and tension stress (UTS)

As is previously mentioned the values of ultimate bending stress are too high, owing to the fact that the formula used is not applicable beyond the elastic limit (yield point). Burstein et al. (1972) have developed an equation for the plastic region of the stress strain curve obtained in bending. This necessitates determination of the properties in tension as well as in compression of the same specimens or from almost identical regions. This was impossible to realize here because of the small amount of cortical bone available in these femurs from dog. An attempt was made to calculate according to the mentioned equation. Assuming that the dog bone in my test had the same relation between stress at the elastic limit in compression and in tension as their bovine bone (η = 1.65) and using the mean of D_y/D_m from the 48 medial and lateral quadrants and the 4 anterior and posterior quadrants tested in tension (0.24 ± 0.08 compared to 0.29 ± 0.10 in bovine femur from Burstein et al. 1972), this gave a theoretical relation between UBS/UTS of 1.81. As totally only 4 anterior and posterior specimens were tested to fracture in tension and 4 medial and lateral specimens were tested to fracture in bending, the mean of these 4 respectively were compared with the mean of the 48 anterior and posterior tested in bending and the mean of the 48 medial and lateral tested in tension. This calculation gave a UBS/UTS of 1.91, which was in surprisingly good agreement with the theoretical result (compared to Burstein et al. (1972) who found for beams with a square cross section theoretically a ratio of about 1.7 and from tests 1.56).

Elastic stiffness in bending and tension

The mean falue of elastic stiffness was in bending 18.52 ± 2.36 $GN/_m2$ and in tension 6.02 ± 0.68 $GN/_m2$ which gives a ratio of about 3. Sedlin & Hirsch (1966) reported about the same difference using similar testing technique but a different testing machine and different clamps. The elastic stiffness in bendings is in good agreement with the findings of other authors as reviewed by Swanson (1972).

A too low elastic stiffness in tension can be obtained when slipping occurs of the specimen in the clamps. The load deformation curve was linear, however, both when tested to low loads (49 N) and to fracture (up to about 100 N). Slipping would probably be seen as a marked non-linearity. One would suspect increased slipping at increasing loads and the curve would show too long plastic deformation. The strain at fracture in this material ($D_m/L = 0.066 \pm 0.011$) is higher than reported by other authors. The ratio of elastic strain to total strain (D_y/D_m) would be lower if slipping occurred in the plastic region. It was in this study 0.236 ± 0.079 which is good in accordance with 0.29 ± 0.10 as reported by Burstein et al. (1972).

Any other source of error such as unaxial loading and stress concentration at the clamp could hardly be of the magnitude of 300 % and still give results with a consistent pattern in the differences between the different quadrants. The reason for the different results obtained in bending and in tension as to elastic stiffness and stress at elastic limits is thus unknown to me. Some consistent error in the testing machine might be responsible. Anyhow, it was felt safe to perform a comparative study using the same technique on all specimens.

RESULTS OF CHEMICAL DETERMINATIONS AND X-RAY EXAMINATIONS

For comparative reasons the chemical components will be reported as % of wet and dry bone weight as well as weight per wet volume in Table XV. This also enables a comparison with other authors for adult human cortical bones (Eastoe, 1962 as reported in Scientific Tables Ciba - Geigy 1970) and from normal cortical dog bone as reported by Biltz (1969). The results are in good agreement with others (Rogers et al., 1952, Eastoe & Eastoe, 1954, Robinson & Elliott, 1957, Woodard, 1964).

Differences of chemical compositions between different quadrants

The results of the sign test is presented in Table XVI. This shows that the density and ash content was highest in the medial quadrant as well as the concentration of calcium and phosphorus. The fluoride content was highest in the posterior and lateral quadrants as was also the collagen content. This gave low Ca/F and Ca/collagen ratios in these quadrants, and the collagen/P ratio was highest in the posterior and lateral quadrants and lower in the medial than in the anterior quadrant. The collagen/Mg ratio was highest in the lateral quadrant. The cortex was thickest in the medial quadrant and thinnest in the lateral and anterior quadrants.

TABLE XV. Chemical content and femoral shaft dimensions of cortical bone.

The mean and standard deviations of the different determinations of all four quadrants in 26 beagle dogs are listed for three different units: % of wet bone weight, % of dry bone weight, and mg/wet bone volume. For comparison results are adopted from Bilz & Pellegrino (1969) and Ciba (1970). Femoral shaft dimensions compared to Andersen & Floyd (1963).

	% in wet bone		% i dry bone		mg/mm^3 wet bone	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Density	-	-	-	-	1.961	(0.028)
Ash	60.43	(1.80)	68.23	(1.82)	1.184	(0.048)
Water	11.47	(0.71)	-	-	0.2248	(0.0129)
Calcium	23.92	(1.03)	27.02	(1.11)	0.4697	(0.0214)
Phosphorus	11.14	(0.41)	12.59	(0.43)	0.2189	(0.0098)
Fluoride[2]	0.0636	(0.0170)	0.0718	(0.0191)	1.245	(0.329)[1]
Magnesium	0.314	(0.042)	0.355	(0.046)	6.177	(0.239)[1]
Collagen	20.45	(1.34)	23.11	(1.59)	0.4014	(0.239)

[1] $\mu g/mm^3$ [2] ppm in ash: 1.052 ± 0.278 [3] of wet weight

	Mean	(SD)
Ca/P	2.148	0.90
Ca/F	403.2	111.9
Ca/Mg	77.04	8.08
Ca/Collagen	1.180	0.105
P/F	187.6	49.6
P/Mg	35.94	4.08
Collagen/P	1.839	0.158
Mg/F	5.294	1.656
Collagen/F	345.1	96.2
Collagen/Mg	66.11	9.42

	Femoral dimensions mm		
	Mean	(SD)	Adult Beagles (Andersen & Floyd, 1963)
Cortical thickness	2.24	0.25	
Inner diameter	5.64	0.82	6
Outer diameter	10.04	0.87	10
Length of femur[5]	119.6	7.6	120
Length/outer diam.[5]	11.40	0.57	12

[5] In 10 normals.

% in dry dog bone (Biltz 1969)	% in dry adult human bone (Ciba 1970)
-	-
69.8	-
-	12.2[3]
26.7	26.4
12.1	11.3
-	0.094-0.270
0.39	0.39
	18.67[4]

organic content: dry ox femur (Eastoe & Eastoe 1954)

Summarizing this one could describe the four quadrants as follows:

The anterior quadrant: *low* content of magnesium and thin cortex
high collagen/F, Ca/Mg and Ca/F ratios

The posterior quadrant: *low* content of ash, Ca/Mg, Ca/F, collagen/F, P/F ratios
high content of collagen, magnesium, fluoride

The medial quadrant: *low* content of collagen, Ca/P, collagen/P, collagen/Mg
high content of ash, phosphorus, Ca/collagen, Ca/F, P/F and thick cortex

The lateral quadrant: *low* density and low content of phosphorus, Ca/collagen, Mg/F, thin cortex
high content of collagen, Ca/Mg, collagen/P, collagen/Mg.

TABLE XVI. Differences of chemical content and femoral shaft dimensions between different quadrants.

The result of a sign test and significant levels (+) are listed below. The number of highest value for one property when comparing two quadrants in the same femur is listed below.

	Ant–Post	Signif.	Med–Lat	Signif.
Density	14 – 12		20 – 6	++
Ash	14 – 11		20 – 5	++
Water	11 – 15		11 – 15	
Calcium	12 – 14		21 – 5	++
Phosphorus	12 – 14		21 – 4	++
Fluoride	5 – 19	++	6 – 19	++
Magnesium	9 – 17		10 – 16	
Collagen	5 – 21	++	5 – 21	++
Ca/P	7 – 19	+	8 – 18	
Ca/F	21 – 4	++	22 – 4	++
Ca/Mg	17 – 9		9 – 17	
Ca/Collagen	17 – 9		22 – 4	++
P/F	21 – 4	++	22 – 4	++
P/Mg[1]	18 – 8		11 – 15	
Collagen/P[2]	7 – 19	+	4 – 22	++
Mg/F	14 – 11		21 – 5	++
Collagen/F	19 – 6	+	15 – 11	
Collagen/Mg	14 – 12		6 – 20	++
Cortical thickness[3]	7 – 16		22 – 1	++

[1] P–L: 6–20 ++ [2] A–M: 21–5 ++ [3] P–M: 1–22 ++

Comments

When summarizing the results from the mechanical testing and the chemical determination and analysing the interquadrantial differences as the results from the sign test one might conclude that the following chemical content was common for specimens with high elastic stiffness, stress at elastic limit, ultimate stress and energy absorption: high content of Ca/collagen, Ca/F, collagen/F.

On the contrary the specimens with low values of the physical properties seem to have high content of collagen/P.

Important chemical factors for the physical properties of the medial and lateral quadrants seem to be collagen/P, Ca/collagen, P/F.

Important factors for the physical properties in the anterior and posterior quadrants seem to be Ca/F, collagen/F. These ratios were however not submitted to any statistical analysis to correlate them with different physical properties.

As seen from this above mentioned assumptions it seems that the *collagen content* is important for the physical properties and not only the direction of the collagen fibers have excellently been studied by Evans & Vincentelli (1969) and Ascenzi et al. (1966).

CORRELATION BETWEEN PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION IN THE DIFFERENT QUADRANTS

The variation in physical properties with varying levels of fluoride in diet and varying time of repletion was small and significant only in some properties. The difference in the physical properties between the different quadrants was greater in any single femur tested than between the lowest and highest level of fluoride in the diet and between no repletion and 28 weeks of repletion. It thus seemed justified to study if this interquadrantial differences could be explained by a co-variation in the chemical composition of the tested specimens.

Density and ash content

Correlations between specific gravity, ash content and various physical properties have previously been reported (Mather 1968, Schmitt 1968, Currey 1969). In this study linear regression correlation was calculated for density and ash content and some of the physical properties which had shown great difference between different quadrants and the coefficients are listed in Table XVII and XVIII. There was a high correlation between the density and the ash content for all four quadrants as could be expected, but the determination coefficients (r^2) were not over 0.5 in any quadrant.

TABLE XVII. Density and physical properties of cortical bone specimens.

Linear regression correlation between density and physical properties of femoral cortical bone specimens in 24 beagle dogs was calculated and the correlation coefficients (r) and significant levels (+) are listed for the different quadrants.

	Ant	Post	Med	Lat	Ant + Post	Med + Lat
Bending						
Elastic stiffness (4p-B)	.67+++	0.64+++	-	-	.48+++	
Ultimate bending stress (UBS)	.62+++	0.43+	-	-	.42++	
Total energy absorption (T)	- .21	0.06	-	-	0	
Tension						
Ultimate tensile stress (UTS)	-	-	.59++	0.25		0.55+++
Correlation between Density and Ash content	.70+++	.67+++	.55++	.64+++		

Bending

There was significant high correlation between the elastic stiffness for four point bending and density as well as ash content for both the anterior and posterior quadrants. A highly significant correlation was found between the density, as well as the ash content, and the ultimate bending stress for the anterior quadrant and a significant one for the posterior quadrant. No correlation was found between density and total energy absorption in bending, nor between this and ash content.

Tension

Highly significant correlation was found between density and ultimate tensile stress for the medial quadrant and a significant correlation between ash content and stress at elastic limit for the lateral quadrant.

These variables (density and ash content) could explain only a part of the variations in the physical properties, the determination coefficient (r^2) being too low (0.5 - 0.2). These variables only reflect the total mineral and organic components in the cortical bone.

Water, collagen, calcium, phosphorus and fluoride

Multiple regression analyses were calculated on the variation in these five chemical components and some of the physical properties.

TABLE XVIII. Ash content and physical properties of cortical bone.

Linear regression correlation between ash content and the physical properties of femoral cortical bone specimens in 24 beagle dogs was calculated and the correlation coefficients (r) and significant levels (+) are listed for the different quadrants.

	Ant	Post	Med	Lat	Ant + Post	Med + Lat
Bending						
Elastic stiffness (4p-B)	.53++	.70+++	-	-	.49+++	
Stress at elastic limit (S_y)	.24	.45+	-	-	.35+	
Ultimate bending stress (UBS)	.52++	.49+	-	-	.44+++	
Total energy absorption (T)	-.12	0	-	-	.08	
Tension						
Elastic stiffness (test to fracture)	-	-	.38	.50+		.56+++
Stress at elastic limit (S_y)	-	-	.24	.45+[1]		.47+++
Ultimate tensile stress (UTS)	-	-	.23	.21		.43++
Total energy absorption	-	-	.15	.06		.32+
Maximal strain (D_m/L)	-	-	.09	.03[1]		.23

[1] 23 specimens.

Bending

The T-value, significant level and multiple correlation coefficient are listed in Table XIX. There was a positive correlation between the phosphorus content and the elastic stiffness, both in three point and four point bending, and the ultimate bending stress but significant only for three point bending to low stresses for the anterior, medial and lateral quadrants. There was a negative correlation between fluoride content and elastic stiffness and ultimate bending stress. These correlations were significant but only highly for the medial quadrant for the elastic stiffness in three point bending.

Tension

The T-value, significant level and multiple correlation coefficients are listed in Table XX. There was a positive correlation between the water content and elastic stiffness in tension to fracture, ultimate tensile stress, energy absorption and maximal strain but significant only for the medial quadrant for energy absorption and strain. There was positive correlation between phosphorus content and many of the properties tested for the medial quadrant but significant only for ultimate tensile stress and highly significant for elastic stiffness to fracture. A more consistent result was found for fluoride correlation

TABLE XIX. Water, calcium, phosphorus, fluoride, collagen and physical properties in bending.

Multiple regression correlation analyses were performed and the T-value and multiple correlation coefficients and significant levels are listed for the different quadrants and some physical properties.

	Elastic stiffness (4p-B)		**Ultimate stress (UBS)**		**Energy absorption after maximal stress (A_2)**	
	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
Water	-0.63	-0.42	0.09	-0.40	-0.43	0.03
Calcium	-0.47	-0.19	-0.40	0.57	-0.63	0.57
Phosphorus	1.10	1.44	1.21	1.28	-0.23	0.76
Fluoride	-1.40	-0.42	-1.25	-0.36	1.22	-0.72
Collagen	-1.13	0.07	-1.56	-1.10	0.68	-0.60
Multiple r	.50	.38	.51	.46	.37	.28
Number	23	24	23	24	23	24

	Elastic stiffness (3p-B)			
	Anterior	Posterior	Medial	Lateral
Water	-1.71	-1.46	0.55	0.62
Calcium	-0.60	0.47	1.88	-1.33
Phosphorus	2.23+	1.50	2.40+	2.16+
Fluoride	-1.51	-0.04	-3.60++	0.43
Collagen	-1.58	0.44	-0.02	1.30
Multiple r	.75	.61	.79	.47
Number	25	26	26	26

which was negative and highly significant for elastic stiffness in tension to fracture and ultimate tensile stress for the medial quadrant.

Comments

No certain conclusions can be drawn from this scatter of correlations, the multiple cor-

TABLE XX. Water, calcium, phosphorus, fluoride, collagen and physical properties in tension.

Multiple regression correlation analyses were performed and the T-value and multiple correlation coefficients and significant levels are listed for the different quadrants and some physical properties.

	Elastic stiffness (fracture test)		**Ultimate stress (UTS)**		**Energy absorption**		**Strain D_m/L**	
	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral
Water	0.49	0.46	1.51	1.33	2.38+	1.47	2.52+	1.46
Calcium	1.16	0.33	0.56	-0.07	1.07	1.27	0.98	1.67
Phosphorus	3.49++	-0.40	2.69+	-0.46	1.83	-1.06	1.23	-1.27
Fluoride	-3.35++	-0.52	-3.77+++	-0.10	-1.46	0.89	-0.81	1.15
Collagen	0.78	-1.32	0.13	-0.96	-0.54	0.05	-0.74	0.27
Multiple r	.81	.36	.75	.43	.66	.48	.61	.52
Number of specimens	24	23	24	24	24	24	24	24

	Elastic stiffness tension (low stress)			
	Anterior	Posterior	Medial	Lateral
Water	-0.11	0.80	0.69	-0.42
Calcium	-0.67	-1.23	0.64	-1.23
Phosphorus	0.20	-0.14	1.36	1.25
Fluoride	0.23	0.64	-1.49	-0.41
Collagen	-1.07	1.47	0.84	0.31
Multiple r	.42	.36	.48	.33
Number	25	26	26	26

relation coefficient (r) and thus the determination coefficients (r^2) being to low in most cases.

When summarizing one might conclude that the anterior and medial quadrants showed higher correlation between the chemical composition and some physical properties than the posterior and lateral in which some other factors seemed to influence the physical properties more than the chemical compositions. The only chemical component that seemed to have positive correlations with the physical properties was the phosphorus content. A negative correlation was found between the fluoride content and some physical properties.

RESULTS OF HISTOLOGICAL EXAMINATIONS

The result of an attempt to classify the microscopic picture is presented for the three different examinors as the number of pictures of different quadrants in different groups with the characteristics of the group as given by the examinor.

Examinor A (engineer without medical education)

	Ant.	Post.	Med.	Lat.
Laminar	3	3	2	0
Mixed (elevated and lowed craters)	2	3	9	0
Diffuse picture without relief	8	4	2	0
Wavy regions, low regularity	1	3	1	7
Low regularity, marked relief	1	2	1	4

When diminishing the number of groups the second and third were put together and the fourth together with the fifth and thus gave the following three groups.

	Ant.	Post.	Med.	Lat.
Laminar	3	3	2	0
Mixed	10	7	11	4
Unregular	2	5	2	11

Examinor A some months later classifying to three groups directly

	Ant.	Post.	Med.	Lat.
Laminar	3	3	2	0
Regular craters	11	7	10	1
Marked vallies	1	5	3	14

Examinor B (the author)

	Ant.	Post.	Med.	Lat.
Fig. 61				
a) Lamellar bone	4	1	2	0
b) Osteon rich and regular, no relief	9	6	9	1
Mixed lamellar and osteon bone, no relief	2	0	3	0
c) Sparse osteons, much collagen bundles, very marked relief	0	2	0	13
d) Mixed osteons and collagen bundles, marked relief	0	6	1	1

a b c d

Fig. 61. Polished cross section of femoral cortical bone specimens with a dimension of 1 x 2 mm. Fig. 61 a shows lamellar bone from the anterior quadrant of animal R 12. Fig. 61 b, osteon (Haversian) bone from the medial quadrant of animal F-0-1. Fig. 61 c sparse osteon, marked relief from the lateral quadrant of animal F-1-1. Fig. 61 d, mixed osteons and lamellar with marked relief from the posterior quadrant of animal F-1-1.

When diminishing the groups the second and third were put together and the fourth together with the fifth and gave the following three groups.

	Ant.	Post.	Med.	Lat.
Lamellar bone	4	1	2	0
Osteon + lamellar bone, no relief	11	6	12	1
Few osteons much collagen bundles, marked relief	0	8	1	14

Examinor C (orthopædic surgeon) had great difficulties in classifying the pictures in less than seven groups.

	Ant.	Post.	Med.	Lat.
1. Many cross cut osteons, little orientation	1	4	4	0
2. Mainly orientation	2	2	2	0
3. Motley (variated) picture, mainly longitudinally cut lamellar, not orientated	0	1	0	12
Partly type 1 and 2	4	3	3	0
Partly type 2 and 3	0	1	2	0
Between type 1 and 3	3	1	3	1
Resembles type 2, some orientation	4	2	1	1

He could not classify the rest and he could not diminish to three groups.

Comments

Evaluation of histological picture of polished cross sections of the specimens as done in this way is hardly possible. This attempt was made to seak for common factors within each of the four different quadrants. It seems obvious that three different persons could recognize pictures of specimens from the lateral quadrant which seemed more irregular, free from regular osteons and wavy with a marked relief. Some agreement was found for many of the anterior and medial quadrants which showed a regular pattern of osteons without relief, even in a third group good agreement was found in identifying lamellar bone. In this group no lateral quadrants were selected.

It thus seemed as the anterior and medial quadrants had regular, osteonic and lamellar bone with a »hard» outlook without relief (Figs. 61 a & b). The posterior and lateral quadrants were more irregular and looked »soft» with marked relief (Figs. 61 c & d).

GENERAL DISCUSSION

Material

The material in this investigation is fairly small: only two experimental animals at each fluoride level in the diet and only one dog on each four week interval of repletion diet (twice the normal amount of calcium and phosphorus). One cause for this restriction is that experiments with nutritional osteopenia had started early at the Cornell University. In these investigations densitometry, microradiography and determination of the chemical content of the bone ash were used and gave such clear cut results showing that fluoride treatment could not prevent the appearance of the nutritional osteopenia but that the osteopenia was reversible when giving repletion diet. To professor C. Hirsch and myself it seemed of importance to examine also the physical properties and chemical content of cortical bone material in these well planned series. When judging the possibilities to affect the physical properties in nutritional osteopenia when adding fluoride to the diet or treating it during varying time of repletion diet a limiting factor was of course that in my study a control series of similar dogs with untreated nutritional osteopenia was lacking – especially regarding the final results. However, a study of this kind had already been performed at the Cornell University published by Saville & Krook (1969). It seemed to offer great difficulties to repeat such a study only to be able to complete it with determination of the physical properties of cortical bone. In the literature there are several reports showing that both in osteopenia by disuse and in such caused by low calcium – high phosphorus diet there has been no effect on the physical properties of the bone material as such but only on the amount of bone substance present. Therefore, I decided to refrain from completing my series with such a material.

To be able to perform a comparison a series of 10 normal dogs (5 males and 5 females) was included. These normal dogs were of the same age as the experimental dogs but had lower body weight and lower specific gravity and ash content of the whole right femur as compared to those of normal dogs published earlier (Saville 1968, Saville & Krook 1969). It was therefore with greatest care that the results obtained in the study of these dogs were used as normal experimental controls.

The main interest of my studies has been to judge if any positive or negative correlation could be found in the bone as a material between results from mechanical tests as well as the chemical determinations on one side and the fluoride level in the food and also the time of repletion on the other. To make possible any comparison of the different parametres between the experimental series and normal dogs the results from the normal series have mainly been presented in the diagrams of the experimental series.

Determining physical properties of bone

The methods in this study were to perform bending and tension tests on bone specimens from the distal half of the left femur from the experimental dogs. One half of the left hind limb was the only source available for these studies. The importance of studying specimens of bone material when determining the physical properties has earlier been emphasized by some authors as there are great difficulties to draw conclusions regarding any variation of the material if a whole bone is tested. The variations within one single bone have been shown to be great and the dimensions of a long bone are not constant along its length nor between different individuals. Many hindrances met when I tried to prepare identical bone specimens from a source of origin with so small dimensions as the distal half of the beagle femur (the mid femur outer diameter is about 10 mm, the cortical thickness is about 2.4 mm and the total femoral length is about 12 cm). Few reports have been published on testing of bone specimens from an experimental animal of such a small size. A special technique had therefore to be designed which I described on page 23.

The testing equipment used in this study is standard material testing machine (Alwetron T-2000) which had been modified with a climate chamber to obtain constant temperature and humidity. This testing apparatus has previously been used for several investigations under the leadership of professor C. Hirsch.

I decided to perform a non-destructive test on all bone specimens from all four quadrants since it was not known if the fluoride treatment or the time on repletion would affect the bone properties in bending in the same way as in tension. Thus a non-destructive bending and tension test was performed on all specimens to low stresses approximately below 25 % of ultimate fiber stress. Sedlin (1966) had previously shown that this did not alter the physical properties of the specimens. Regarding a suitable length of the specimens Sedlin showed that the physical properties were constant when using a proportion of the specimen thickness to the test length of 1:15 to 1:30. For my specimens with an accessible maximal thickness of 1 mm thus a suitable test length of 20 mm was adopted. These dimensions are also in good agreement with those used in testing of other anisotopic material such as fiber glass (Loveless 1966). As the result from the testing in bending and tension to low stresses revealed great differences between the different quadrants, half of the material was tested to fracture in bending and the rest to fracture in tension. A corresponding pair of the quadrants was thus choosen for the same test; anterior and posterior in bending, and medial and lateral quadrants in tension. The four point bending test was adopted to avoid uneven stress distribution in the deflected specimen.

In the present investigation both bending and tension tests have been performed. Compression tests were impossible depending on technical difficulties and the very small amount of available bone material. The bending test is however a combination of tension and compression test and therefore gives some information even of the compression strength which is generally accepted to be higher than the tension strength. Thus in the bending test the material fractures first in tension on the convex surface of the tested beam and thereafter the crack propagates to the concave surface. The great deflextion after maximal stress in bending tests of the specimens from the anterior quadrants in the fluoride series and two other anterior specimens in the reversibility series could perhaps be explained by decreased strength in compression and thus, a fracture might have been arised in compression on the concave surface. This would explain the steplike decrease of stress in the material after maximal stress (Burstein 1972). These types of fractures seem to be similar to green stick fractures in children.

Chemical determinations

The results of the mechanical testing revealed only small differences in the physical properties from animals that had received no fluoride addition or had had no repletion diet and those animals that had received 27 ppm fluoride in the food or had been on repletion for 28 weeks. Moreover the results published by Henrikson et al. (1970) showed that the accumulation of fluoride was lowest in the long bones. Because of the obvious possibility that the cortical bone specimens were of normal chemical composition containing no increased amount of fluoride it was felt necessary to undertake chemical determinations of the tested specimens to reveal if the fluoride treatment or the repletion period had influenced the tested specimens. Therefore the chemical determinations were performed on the same specimens that had been submitted to mechanical testing. Another reason for doing this was to study if the great differences between the physical properties of the quadrants were correlated to similar differences in the chemical composition. As few studies of the physical properties and the collagen content have been reported it was also felt motivated to add determination of the collagen content to the often used determination of ash content of calcium, phosphorus and fluoride. The determination of collagen necessitated a drying of the specimens with a technique that did not denaturate the collagen. Thus drying could not be performed at the usually used temperature of 105° C but was made in vacuum at 43° C. The reason for expressing the chemical content as amounts per volume wet bone has been discussed on page 36.

Microstructural examination

An attempt was made to study the distribution of different minerals over the cross section of the specimen near the fracture site through X-ray micro-analysing technique. However, great difficulties were met and the result was not conclusive. Since the preparation for this study comprised polishing of the cross section of the bone specimens and checking for the absence of scratches over this area, in a reflected light microscope, it gave possibilities to photograph the polished cross sections. I then found that these photograms showed marked difference depending on variations in the softness of the material. This resulted in varying degree of relief of the surface – after polishing with standard metallurgic polishing methods – just as a raised pattern. An attempt was therefore made to classify this histological outlook which resulted in ability for three different persons to recognize and separate at least one of the quadrants from the femoral shaft.

Results

Fluoride addition

The physical properties and the chemical content of the cortical bone specimens showed few and small changes with increasing fluoride addition in the food. It was only the energy absorption in bending test of the anterior quadrant that showed a marked increase compared to the normal dogs even at the lowest level of fluoride. This seemed mainly to depend on increased flexibility. A slight increase in phosphorus content was found as well as a slight decrease in collagen content. However, a consistent and great increase of the fluoride incorporation correlated to the fluoride level in the food was seen even in strictly cortical bone material but not as marked as in more spongious regions. *Thus no beneficial effects were seen of increasing fluoride in the food or in the bone material.*

Repletion diet

The reversibility series showed marked effect of increasing time of repletion. A number of the determined physical properties showed positive variation with increasing time of calcium and phosphorus rich food to previously osteopenic animals, as e.g. increased breaking strength and elastic stiffness. The density and ash content of the cortical bone also increased with increasing time of repletion. Few changes were seen in the rest of the chemical content depending on the time of repletion.

Differences between quadrants

The predominant impression of the results is, however, the great and consistent variation in the physical properties between specimens from different locations within the same bone. This investigation seemed to show that the effect of fluoride addition and increased time on repletion had different effects on the different quadrants of the femoral circumference. For instance the fluoride incorporation was highest in the posterior quadrant (even in normal dogs fluoride content was highest here). This quadrant is a pronounced muscular attachment site and is thicker than the rest of the circumference (moreover it showed the lowest value of most physical properties). These findings are in good agreement with earlier published results, showing that the fluoride incorporation is highest in the tuberosities (Weidmann et al. 1959) and the skeletal turnover is highest in the muscular attachments (Henriksson et al. 1974).

Physical properties – chemical content

The correlation between the physical properties and the chemical content in the different quadrants was calculated and the only positive correlations were between the phosphorus content and some of the physical properties and more consistent negative correlations were found between the fluoride content in the specimens and some physical properties – this even from those dogs that had received no fluoride addition.

The chemical composition of the bone seems to be of less importance for the physical properties of the material even if some parameters such as Ca/collagen, Ca/F and collagen/F seem to be of higher values in specimens from quadrants with higher elastic stiffness, breaking strength and energy absorption. The *amount* of collagen seems to be important and not only the *direction* of the collagen fibers as has earlier been shown. Moreover the microscopic structure seems to be of great importance as previously shown by several authors. Ascenzi et al. (1966) found different properties in adjacent osteons from the same bone source.

All this illustrates the difficulties to get a summarized picture of the factors that determine the physical properties of the bone material. It is also obvious that it is very difficult to draw conclusions from testing of whole bones as both the physical properties and the chemical content varies remarkably between the different regions and that even the dietary effect is different in different regions. Thus an examination of a whole long bone can only be a summary of different quadrants and of various segments and give information of the variation in dimensions which of course is of great importance from a clinical standpoint. But without knowledge of the variation in properties of the material the conclusions from the variations in dimensions are very doubtful.

However, an investigation of this kind with controlled diet to experimental animals with small variations in body weight and age, with determination of physical properties in bending as well as in tension and an examination of different mineral content as well as collagen content and water in the tested cortical bone specimens has not been found in the literature.

Having performed this study some personal thoughts on bone biomechanics arose and it was felt suitable to present some of these.

Which chemical properties are of importance for the skeleton?

This is a difficult question since the function of the skeleton is very complex. The skeleton is the support of the body and is the origin and attachment for muscle groups necessary for locomotion. The function of the bone mass as being a reservoir for the minerals, especially calcium, is certainly at least of the same importance. Maintaining isocalcemia is necessary for continued existence. Bone resorption and bone apposition are physiologic processes.

Can a demineralization occur in the skeleton without seriously disturbing the supporting function?

The results both from previous studies and from my own show no effect on the strength of the bone material from animals with induced osteopenia. Clinical experience also shows that after immobilization a rapid demineralization of different skeletal regions occurs without clinically observable negative effects.

Which physical properties of the bone as a material are of greatest importance during life?

The resistance against trauma is interesting mainly when a fracture occurs as a result of minimal trauma. Probably demineralization and osteopenia can proceed to a fairly marked degree without having any injurious effect on the physical properties of the material. Trauma with high velocity (impact trauma) generally means an amount of energy that far exceeds the absorbing capacity of the skeleton. Thus in such situations a decreased bone strength would not be of any major importance. The properties of bone material reveal at dynamic tests with high velocity or vibration have greatest relevance in judging the resistance to fracture as a result of fall from height or of trauma with high velocity. Good correlations have been shown in testing at low and at high speed but the breaking strength and elasticity is considerably higher with high deformation rates (McElhaney 1966). Thus when studying conditions resembling human osteoporosis or osteopenia of such a degree that there is risk for fracture from minimal trauma it seems applicable to conduct determinations of the physical properties of the material at low deformation rate or static loading. Such studies are needed before any certain conclusions can be drawn from measurements of diminishing amounts of bone.

The importance of *the capacity of bone material to absorbe energy* has been discussed by Swanson (1970) but has been studied in very few investigations. The difference in energy absorption at low and at high deformation rate has been studied by Piekarski (1970). He showed that the energy absorption is increased in low deformation rate where the fracture is a result of pulling apart the material and is dependent on exceeded possible deformation. At high crack propagation rate the maximal stress in the material seems to be the limiting property and the absorbed energy is lower.

An investigation of this type with determination of the physical properties of bone specimens and of the chemical content especially of the organic component collagen, of the same piece of bone as done in this study seems to be of value as an analysis of the quality of bone as a material. Investigation of bone specimens from different pathomorphologically well defined conditions would be of greater interest since the variations with various diets and immobilization of healthy experimental animals have not revealed any great differences in the physical properties or chemical content of bone. Diseases such as marked clinical osteoporosis, osteogenesis imperfecta, osteopetrosis, Paget's disease and skeletal fluorosis which are known sometimes to decrease or to increase the strength of the bone as an organ would be an important source for studying variations in the bone material itself. When doing such investigations this study has shown it to be of greatest importance to take samples from very well defined locations within one bone, preferably from such parts of the skeleton that normally are subjected to great load and risk of fracturing (i.e. rather from the long bones in the lower extremity than from the ribs). The importance to study the physical properties of bone as such, trying to correlate these findings with biological factors such as different diseases have earlier been pointed out by some authors (Hirsch 1968, 1970, Currey 1970) but such studies do not seem to have been put into practice.

SUMMARY

Fractures in human osteoporosis and in higher ages constitute an increasing clinical problem. Experimental studies have therefore been performed in which nutritionally induced osteopenia in animals has served as a model of human osteoporosis. The effect has been studied of various treatments in this experimental condition. The determination of the physical properties of bone in these conditions has mainly been performed by using bending tests of whole bone such as femur or metatarsals. However, there are great difficulties in evaluating these results and in making conclusions regarding the effect of various treatments on the physical properties of bone material as such. No study has been found reporting the effect on the physical properties and chemical content of the same test specimens from cortical bone, nor on the effect of fluoride, calcium and phosphorus addition to the food intake in nutritional osteopenia.

Material

This is a report of the physical properties and chemical content of femoral cortical bone strips in studies of fluoride effect on and reversibility of nutritional osteopenia. The effects on various other parameters from this dog material have previously been reported by Henriksson et al. (1970) and Krook et al. (1971). Nutritional osteopenia was induced by feeding adult dogs low calcium and high phosphorus diet for 41 weeks. Fluoride was given as a supplement at 0, 1, 3, 9, 27 ppm levels in the food to two dogs each. Reversibility was studied by feeding six adult dogs, calcium and phosphorus enriched food, after 41 weeks on depletion diet, for 4, 8, 12, 16, 20, 28 weeks respectively. Ten normal kennel beagles were used as controls.

Testing of mechanical properties

After necropsy four cortical strips (from the anterior, posterior, medial and lateral quadrants) from the distal half of the femur were cut and ground to a final cross section of 1 x 2 mm and then used for the present study. The specimens were tested to low stresses as end supported beams in three point bending and in tension. Then the specimens from the anterior and posterior quadrants were tested to fracture in four point bending (to obtain more even stress distribution in the material) and from the medial and lateral quadrants to fracture in tension. The elastic stiffness, stress at elastic limit, ultimate stress and energy absorption were calculated from the load deformation curves in both bending and tension tests. Maximal strain and its proportion of elastic strain were calculated in tension tests. All preparations and tests were performed under water, in Ringer's solution or in 100 % humidity. The tests were performed in a modified standard material testing machine (Alwetron T-2000) supplied with a climate chamber to obtain constant temperature and humidity.

Chemical analysis

Pieces from the tested specimens were taken for determination of density and the content of ash, water, calcium, phosphorus, fluoride, magnesium and collagen using standard techniques. Concentrations in % of wet and dry bone weight were calculated but the content per unit wet volume of the different components was choosen as the basic unit in studying the osteopenic condition.

Correlations were calculated between fluoride content in the food, time on repletion diet and the physical properties of and chemical content in the cortical bone specimens. Correlations were also calculated between different physical properties as well as between physical properties and the chemical content of the corresponding specimens.

Fluoride effect on nutritional osteopenia

The fluoride addition could not prevent the appearance of this condition of bone-loss as reported by Henriksson et al. (1970). The only effect on the physical properties was an increase in energy absorption in the specimens from the anterior quadrant in the fluoride treated animals. Only very slight changes with increasing fluoride level in the food were seen in the chemical composition of the cortical bone, such as a not significant decrease of calcium and magnesium, a significant increase of phosphorus, a significant decrease of collagen, a significant decrease of Ca/P and collagen/P ratios. The incorporation of fluoride in cortical bone increased with increasing fluoride in the food but to a lesser extent than in the whole femur and in vertebræ. The cortical thickness decreased as well as the inner diameter of the femoral shaft.

Effect of repletion diet on nutritional osteopenia

The osteopenic condition was reversible after 28 weeks of repletion as reported by Krook et al. (1971). An increase of the elastic stiffness, stress at elastic limit and breaking strength was found in both bending and tension tests with increasing time of repletion. The energy absorption in bending increased for the anterior quadrant. The changes were not significant for many of the properties, mainly depending on the small number of animals in this series.

Only very slight and few significant changes were seen in the chemical composition of the cortical bone specimens, e.g. a significant increase of density and ash content, a significant increase of phosphorus, a not significant increase of fluoride, a not significant decrease of collagen content and Ca/F, P/F, collagen/P and collagen/F ratios. There was a slight but not significant increase of cortical thickness.

Variation with age

The age variation was too small (1.5 to 3 years) to be subjected to any analysis of the changes with increasing age of the determined properties.

Variation with body-weight

Some of the physical properties showed a slight variation with increasing body-weight. The elastic stiffness in tension seemed to decrease as did the elastic proportion of the total deformation in tension. No changes were seen in the chemical content but the cortical thickness showed moderate but significant increase with increasing body-weight.

Differences between sexes

Differences were found between specimens from male and female dogs regarding the elastic stiffness in bending and tension: the specimens from the females having higher stiffness, lower water content, higher ash and fluoride content and less cortical thickness and shorter length of femur.

Result of mechanical testing in all three series

The results showed differences between the properties in bending and tension tests, the elastic stiffness in tension being about 1/3 of that in bending. This could not be explained by errors in the testing techniques such as slipping or miss-alignment of the specimens. The difference in ultimate fiber stresses (UBS and UTS) corresponded well with the use of a formula not strictly applicable for calculating stresses beyond the elastic limit in bending. High correlation was found between different properties in bending test. The energy absorption seemed mainly to be dependent on the total deflexion and not on ultimate stress or elastic stiffness. This was not the fact in tension where the energy absorption was highly dependent of maximal strain as well as ultimate stress and elastic stiffness.

The predominant result from the mechanical tests was the great and consistent interquadrantial difference. The anterior and medial quadrants as compared with the posterior and lateral ones had higher elastic stiffness, stress at elastic limit, ultimate stress, plastic deformation and energy absorbing capacity. These differences between the quadrants were greater than between the same quadrants from the different experimental animals irrespective of the given diet.

Result of chemical analysis in all three series

The chemical content varied considerably between the different quadrants, the fluoride incorporation being highest in the lateral and posterior quadrants, the latter being a muscular attachment having higher skeletal turnover.

When comparing the physical and chemical characteristics of the different quadrants it seemed as the specimens with high elastic stiffness, stress at elastic limit and ultimate stress (anterior and medial) had high: Ca/collagen, Ca/F and collagen/F ratios. On the other hand the specimens with low figures for physical properties (posterior and lateral) had high collagen/P ratio.

Correlation between physical properties and chemical content

High correlation was found between *density* and *ash* content on one side and ultimate stress and stress at elastic limit in both bending and tension on the other.

Multiple correlation analyses were performed to evaluate the influence of *water, calcium, phosphorus, fluoride* and *collagen* content on the physical properties. The only consistent correlation was a positive one for phosphorus content and some physical properties and a negative for fluoride content. The variation of the chemical content could not explain more than 25 - 50 % of the variation in physical properties. The highest correlation was found for the anterior and medial quadrants.

Conclusions

Fluoride added to a diet inducing osteopenia did not influence the physical properties or the chemical content of cortical bone from the femur to an appreciable extent. The fluoride content in the bone followed that in the diet.

Increasing time of calcium and phosphorus enriched diet to osteopenic dogs had a favourable influence on the physical properties of femoral cortical bone and increased the density and ash content but did not change the determined chemical contents.

Great and consistent differences both in physical properties and in chemical contents were found between specimens from different quadrants of the distal half of the dog femur. The different diets showed different but not consistent effects on the properties of the different quadrants.

There was no consistent correlation between the chemical content and the physical properties. The density, ash and phosphorus contents were positively correlated and the fluoride content was negatively correlated to some physical properties.

Difficulties are met when trying to obtain normal values for physical properties of bone material as there are great differences within the circumference of such a small bone as the dog femur. When studying the influence, on the physical properties, of various treatments great precision has to be undertaken when taking out specimens for mechanical testing to ensure that they derive from comparable origin within the same bone.

Finally some personal comments are given from the experience of this work regarding the applicability of static bone tests and chemical analyses. A better source for studies of properties of bone is bone from individuals with skeletal disease, as nutritional and disuse osteopenia does not seem to change the physical properties of the bone material to any major extent.

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