The Journal of Bone and Joint Surgery

CONCERNING OSTEOPOROSIS

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The word osteoporosis is often used in medical language by radiologists and orthopaedic surgeons but sometimes it is used mistakenly as a synonym for decalcification, bone atrophy, rarefaction of bone or osteolysis. Few have asked themselves whether these terms are really synonymous. Furthermore, one often hears of the diagnosis of osteoporosis or bone decalcification even when the porosis of bone is clearly no more than a symptom, and no more than one manifestation of a disease. These terms should not be used indiscriminately one for another. Osteoporosis is not at all the same as bone decalcification. Osteoporosis and decalcification are not synonymous—though it might perhaps be agreed that the terms osteoporosis and bone rarefaction are to be regarded as similar.

THREE TYPES OF BONE ATROPHY

Osteoporosis is an anatomical lesion of bone characterised by progressive thinning of the osseous trabeculae which normally maintain a constant organic and mineral structure. As a result of this thinning, the spaces between trabeculae become larger, and compact bone is transformed into spongy bone with more open texture. The total amount of bone tissue is decreased. Despite thinning of bone trabeculae and loss of fine trabeculation, histological evidence shows a normal degree of calcification but with marked reduction in the number of cells. These anatomical changes are accompanied by normal levels of serum calcium, serum phosphorus and serum alkaline phosphatase.

Osteomalacia—Decalcification of bone does of course occur in osteomalacia, the elementary lesion of bone which at one time was confused with osteoporosis and even today may present difficulty in clinical diagnosis. In osteomalacia the structure and composition of bone changes because there is qualitative disturbance in bone maintenance, the bone becoming soft from reduced and insufficient calcification of its organic structure. A striking histological feature of osteomalacia is the abundance of osteoid tissue. Moreover, unlike osteoporosis, osteomalacia is characterised by disturbance of the mineral metabolism of body fluids with deficiency of calcium, deficiency or excess of phosphorus, excess of alkaline phosphatase and hypocalcuria.

Osteoclastic resorption is different again from osteoporosis and osteomalacia. *Osteitis fibrosa cystica* is characterised by osteoclastic resorption of bone with a secondary and quite intense medullary fibrosis. This bone disease, arising from primary hyperparathyroidism, is characterised by a high calcium level in the blood, low serum phosphorus, excess of alkaline phosphatase, hypercalcuria and hyperphosphaturia.

If we are to have one term to cover these three primary disorders of bone—osteoporosis, decalcification as in osteomalacia, and osteoclastic resorption as in *osteitis fibrosa cystica*—we should speak simply of “bone atrophy.” In this brief survey I will try to emphasise the difficulties of this study, its great interest, and the many problems that still remain to be solved. When orthopaedic surgeons study patients with bone disease of this type they must consider all possible causative mechanisms, remembering that the decrease in bone mass is usually a

symptom of general medical disorder: the skeletal change is often no more than a reflection of morbid states in other organs or systems. It is my purpose to emphasise particularly the types of bone atrophy of practical importance—namely pre-senile and senile osteoporosis—and to review the generalised bone diseases that give rise to it.

From the radiographic point of view these bone disorders have in common an increased translucency of bone. There is thinning of cortex, decrease in the number of bone trabeculae, and enlargement of marrow spaces. But although there is such similarity in the radiographic aspect the diseases are very different from each other, and only if we remember this will the confusions of many recent clinical studies and recommendations of treatment be resolved. I will not consider localised post-traumatic, inflammatory or such types of bone atrophy but only generalised bone atrophy and the profound change in the structural equilibrium of bone that underlies it, including various morbid states. Some of the latter are rare and of a malignant character, whereas others are common though often presenting difficulty in diagnosis. When the orthopaedic surgeon sees a patient with bone atrophy he must at once try to decide its physiopathological significance, because usually the skeletal disorder is only a reflection of the general constitutional disorder.

In considering the life of bone, and the perfect balance maintained in mature years between bone construction and bone destruction, we must recognise the interdependence and delicate balance of enzyme, endocrine, vitamin and dietary factors, coordinated by strict biophysical principles. Bone is not an inert matter with only a mechanical function. Bone is a living tissue which has a complex system, in which apart from other things the protein and phosphorus-calcium metabolism is delicately poised.

The investigations that have been pursued over the last twenty years in my own research department and elsewhere have shown that ossification takes place in three phases of osteoblastic activity. In the first phase osteoblasts produce an organic matrix. In the second phase calcium is laid down in the basic substance, and the fibrils then develop a new potentiality. In the third phase calcium salts are incorporated into the protein matrix, thus turning it into bone. Disturbance of these phases of osteogenesis, arising from many different origins, can nevertheless be grouped by histophysiological and histopathological findings, together with study of the hormonal balance and changes in blood chemistry, into the three groups of bone atrophy that have been identified—osteoporosis, osteomalacia, and generalised fibrous osteodystrophy.

CLINICAL AND EXPERIMENTAL CAUSES OF OSTEOPOROSIS

The list of causes of osteoporosis is long, and the length of it will show how complex is this subject and how difficult it may be for surgeons to establish the cause of bone atrophy. *Endocrine disorders* include disturbance of pituitary secretion such as pituitary dwarfism, acromegaly and Cushing's disease; hyperthyroidism and thyrotoxicosis; disturbance of the adrenal glands with hypersecretion of glucocorticoids by the cortex; gonadal disorders such as agenesis, castration, ovarian insufficiency, androgen deficiency, male and female menopause, post-pregnancy syndrome; and pancreatic deficiency with diabetes mellitus. *Vitamin deficiency*—We must consider deficiency of vitamin C and deficiency or lack of vitamin B₁₂. *Disturbances in the digestive tract*—These changes may arise from achlorhydria, gastrectomy, steatorrhoea, coeliac disease, or disease of the liver and bile ducts such as hepatitis, hepatosis, obstructive tumours and fistulae of the bile ducts. *Dietary deficiency*—Bone atrophy may arise from starvation, intestinal obstruction, or from deficiency of protein, calcium or copper metabolism. *Diseases of the blood*—We must consider pernicious anaemia, congenital haemolytic icterus and Cooley's disease. *Chemical agents*—Lathyrism and potassium thiocyanate or ammonium chloride poisoning are possible factors in causing osteoporosis. *Physical and mechanical factors*—Osteoporosis arising after immobility of limbs, especially when there have been multiple injuries or burns, must be related to other causes. *Congenital disorders and age factors*—These include osteogenesis imperfecta and senile involution.

OSTEOPOROSIS FROM ENDOCRINE DISTURBANCE

Cushing's disease is the most important example of osteoporosis from endocrine disturbance, this being the consequence of excessive production of anti-anabolic cortical hormones. Histologically osteoporosis due to Cushing's disease is indistinguishable from senile osteoporosis and some research workers believe it is present in 75 per cent of all the cases with such syndrome.

Treatment with cortisone and its derivatives—It is of great practical importance to remember that prolonged treatment with cortisone or adrenocorticotrophic hormone (A.C.T.H.) gives rise to a true Cushing's syndrome. Even although it may be on a smaller scale, the effect of prolonged treatment with cortisone is the same as Cushing's disease.

Acromegaly—The osteoporosis sometimes seen in patients with acromegaly is probably due to alteration in pituitary secretion with increased output of A.C.T.H. and reduced secretion of gonadotrophins.

Thyroid disease and diabetes—Osteoporosis arising from disturbance of thyroid secretion should be related to the increase of protein catabolism and the osteoporosis of diabetic origin with its well known metabolic imbalance.

Gonadal disorders—When gonadal activity is reduced or absent, the resulting osteoporosis should be ascribed to reduced trophic stimulus to the osteoblast or reduced influence on protein anabolism.

OSTEOPOROSIS FROM VITAMIN AND DIETARY DEFICIENCY

Vitamin C deficiency—When there is vitamin C deficiency there is generalised failure in the formation of binding matrix and the maturation of collagen. In consequence there is scanty formation of bone matrix with osteoporosis.

Achlorhydria—After gastrectomy, or even simple atrophic gastritis with achlorhydria, there is deficiency of calcium absorption causing osteoporosis, though I think that the well recognised lack of absorption of vitamin B₁₂ which occurs in these patients may be an important factor in disturbing protein anabolism.

Chronic pancreatitis—Osteoporosis develops in chronic pancreatitis by reason of changes in the digestive process and reduced absorption of proteins. Similarly bone atrophy developing after disease of the liver and bile ducts is not so much an example of osteoporosis as of osteomalacia or osteitis fibrosa cystica, according to whether the biliary disturbance leads only to deficient absorption of calcium or also to lack of absorption of vitamin D.

Dietary deficiency—Deficiency of diet, especially protein deficiency which also implies deficiency in the intake of phosphorus-calcium and vitamins, causes osteoporosis though usually with general deficiency of calcification of the matrix simulating osteomalacia. It must also be remembered that the general defence reaction to a deficient diet stimulates increased secretion of cortico-steroids which with prevalence of anti-anabolic glucocorticoid hormone greatly increases protein catabolism.

OSTEOPOROSIS FROM PHYSICAL FACTORS AND CONGENITAL DISORDERS

Immobility—It is well known that immobility of a limb from paralysis, or fixation in a plaster, causes osteoporosis from inactivity probably from the direct effect on the activity of osteoblasts through the differing effects of the stimuli of traction and compression.

Osteogenesis imperfecta—The bone atrophy of osteogenesis imperfecta is a true example of osteoporosis arising from primary congenital and often hereditary insufficiency of osteoblasts in their task of producing bone matrix.

OSTEOPOROSIS FROM SENILE INVOLUTION

From a practical point of view the most important of the osteoporotic disorders is that arising from senile involution. During the last few years considerable progress has been made in diagnosis and treatment. The subjective symptoms of senile osteoporosis are general tiredness, pain in the bones, especially the vertebral column and pelvis, and pain referred

round the loins and down the lower limbs. The pain is more severe when the patient first starts to move after a period of rest and when first getting out of bed in the morning. It is aggravated by bending movement or by climbing stairs. Sometimes it seems to be aggravated by changes in the weather and it is often interpreted as "rheumatic." There is usually a rounded kyphosis of the vertebral column with the ribs lying close to the iliac crests. Mobility of the spine is reduced. There is a loss of height up to ten centimetres or even more, giving rise to the typical appearance of a patient with short trunk, rounded back and an apparent disproportion in the relatively long limbs.

Radiographic features—The most characteristic signs in radiographs are in the vertebral column and pelvis, changes in long bones being minimal except in advanced cases. Indeed it has often been said that osteoporosis is not demonstrable radiographically until there is a mineral deficiency in the bone of about 50 per cent (Babiantz 1947, Turano 1953, 1954, Forni 1951). Thus when the patient first complains of pain there may be little radiographic evidence even in the spine, so that often a simple diagnosis of arthritis is made. In more advanced stages the vertebrae become translucent and there is loss of the normal appearance of trabeculation. One can see only the outlines of the vertebral bodies, and the impression arises that the radiographs have been taken with poor technique because there is such little contrast between the shadow of bones and that of soft parts. Later the thoracic vertebral bodies become wedge-shaped, and the lumbar vertebral bodies biconcave from pressure of the intervertebral discs on the softened bone. There may be evidence of spontaneous compression fractures. The skull is normal, and this is most significant in the differential diagnosis of osteoporosis from osteitis fibrosa cystica and von Recklinghausen's disease in which there is nearly always evidence of involvement of the skull.

THE POSSIBLE CAUSES OF SENILE OSTEOPOROSIS

As early as 1885 Pommer distinguished osteoporosis from osteomalacia. In 1900 Sudeck described the radiographic features of senile bone atrophy, attributing them simply to inactivity but distinguishing them from acute post-traumatic bone atrophy. In 1925 Kienböck attributed osteoporosis to deficient nutrition of bone. In 1939 Meulengracht thought that porosis of bone arose from dietary insufficiency and chronic disturbance of the digestive system. In 1940 Kienböck reviewed the problem again and postulated a defect in the endocrine glands governing skeletal growth and nutrition.

Post-menopausal osteoporosis—It was only in 1940 that Albright, Bloomberg and Smith gave the first nosological and etiological description of the osteoporotic syndrome which gave impetus to further research. After reaffirming the distinction from osteomalacia they ascribed osteoporosis to reduced osteoblastic activity from such causes as functional inactivity, protein insufficiency, dysfunction of the endocrine glands as in acromegaly, Cushing's syndrome and thyrotoxicosis, and congenital meiopragia of the osteoblasts. But none of these causes explained the osteoporosis found in women between the ages of forty and fifty years who had reached the menopause, whether physiological or surgical. Albright postulated a connection between this type of bone atrophy and the cessation of ovarian activity and he postulated "post-menopausal osteoporosis."

The views of Albright—In reaching these conclusions Albright had studied the relationship in birds between ovarian secretion and osteoblastic activity, and he knew that the administration of sex hormones could influence the structure of bone. He thought that post-menopausal osteoporosis occurred about nine years after the natural cessation of menstruation, and that when amenorrhoea had been induced surgically there was an even longer interval. He regarded the age of about sixty-five years as marking the distinction between post-menopausal osteoporosis and what he termed senile osteoporosis. He believed that, just as in women a deficiency of oestrogen was an important factor, so in men deficiency of androgen played an important part in this disorder.

The observations of Reifenstein—Albright's conclusions soon gained general approval. His collaborator, Reifenstein (1957), was able to measure the daily excretion of sex hormones in individuals of pre-senile and senile age, and showed that this diminished rapidly after the male and female menopause. At the same time, however, the output of adrenal glucocorticoids (anti-anabolic hormones) also diminished, but much less so. Reifenstein postulated that osteoporosis was due more to comparative increase in the output of adrenal anti-anabolic hormone than to decrease in the secretion of anabolic sex hormones. It seemed, in short, that osteoporosis was the result of adrenal gonadal imbalance.

The work of Urist—In 1958 Urist asserted that osteoporosis was no more common in women than in men, and that few of those suffering from osteoporosis had endocrine disturbance. (Urist 1960). He said that hypoproteinaemia, blood concentration and urine secretion of oestrogens, 17-ketosteroids (17-KS), 17-hydroxy-corticosteroids (17-OHCS) and their derivatives did not differ between subjects suffering from osteoporosis and those not suffering from it. Finally he affirmed that most patients suffering from endocrine changes such as ovarian deficiency, castration and senility did not suffer from osteoporosis. On the other hand, because none of the previous hypotheses excluded an endocrine change, Urist thought that there must be a common denominator among them, and that this common denominator could not be anything but the 17-OHCS which might be acting on bone even when the blood levels were normal. According to Urist, even osteoporosis from calcium deficiency (Nordin 1960) and osteoporosis from protein deficiency and starvation were caused at least partly by hypersecretion of 17-OHCS. In subjects living on a diet deficient in calcium or protein, or suffering from prolonged starvation, hypertrophy of the adrenal cortex has indeed been observed. On the other hand, because not all these individuals developed osteoporosis it was suggested that as in gastroduodenal ulceration, diabetes and hypertension there could be predisposition to osteoporosis, and that in elderly subjects there is an anti-osteoporotic factor that protects bone from the action of 17-OHCS.

The observations of Nordin—Contrary to general opinion Nordin (1960) ascribed osteoporosis to chronic calcium deficiency arising from low mineral content of the diet, scanty resorption of calcium, or excessive elimination of calcium in the urine. Experiments on animals seemed to prove that deficiency of calcium produced osteoporosis, whereas deficiency of vitamin D produced osteomalacia. Under these conditions, if the blood calcium level remained normal, the activity of the parathyroid glands was not accentuated. Further evidence in support of Nordin's view was that the subjects of osteoporosis who were treated with calcium salts showed a positive calcium balance and they were relieved of pain at least partly if not wholly. Because the calcium balance in the osteoporotic subject is negative the organism draws slowly but progressively on skeletal deposits. Progressive elimination of the matrix seems to follow quickly on that of the blood calcium. Like all other workers Nordin has been obliged to fall back on the idea of individual predisposition to osteoporosis, because only some and not all patients with a negative calcium balance show coincident bone atrophy.

IS THE HORMONAL CAUSE OF OSTEOPOROSIS TO BE ACCEPTED?

The theory of reduced sex hormone activity put forward by Albright and his fellow workers is now treated with reserve. Why should atrophy of the skeletal system necessarily be conditioned by functional atrophy of the gonads? It would seem that we are continually trying to explain atrophy of one tissue, or one organ, as being conditioned by atrophy of another tissue or organ. It may be insisted that the influence of sex hormones on skeletal growth and on the organism as a whole has now been well documented; but it should not be forgotten that not only sex hormones but also the pituitary, thyroid and pancreas play a part in the complex and carefully integrated phenomenon of body growth. There is not sufficient evidence as to whether or not post-menopausal osteoporosis accompanies atrophy of

other tissues with mechanical function such as cartilage, tendon, muscle and perhaps also parenchymatous tissue and organs.

The entire validity of the theory of hormonal causation of post-menopausal osteoporosis is shaken by day-to-day clinical observation. No more than about 20 per cent of women show unmistakable clinical and radiographic signs of osteoporosis at the menopause. Why do not the other 80 per cent suffer from this state of hormone deficiency? And if the male sex hormone also exerts an anabolic action upon bone tissue, why should the percentage of men suffering from osteoporosis after the menopause be even lower than the percentage of women? Do oestrogens and androgens really and unquestionably have a bone-forming action? Whatever the answer, a deficiency in their production cannot alone be responsible for senile osteoporosis. As part of the prelude to old age one can point not only to reduction in gonadal activity but also to deficiency of thyroid activity, of the activity of pancreatic islet cells, and of the pituitary. It may be concluded that Albright's hormonal theory does not fully explain the etiology of osteoporosis.

But even Reifstein's theory which attributed osteoporosis to a menopausal imbalance between anabolic sex hormones and anti-anabolic glucocorticoid hormones loses all its force in consequence of Urist's demonstration that there is no difference between the adrenal-gonadal imbalance in subjects suffering from senile osteoporosis and in those not so suffering. Even Reifstein concluded that the adrenal-gonadal imbalance was not so much a factor determining osteoporosis as a factor predisposing to it; and that the cause was to be sought not so much in the endocrine glands and their hormones as in bone itself. Though Urist accepts the broad lines of Reifstein's theory he is obliged, since it explains no more than a small percentage of the cases of osteoporosis, to maintain that in elderly people not suffering from osteoporosis there must be some factor that he defines as "anti-osteoporotic" which protects bone from the anti-anabolic adrenal hormones.

With regard to the importance that Nordin attached to a protracted negative calcium balance, it may well be that the patients he reported had a slight degree of generalised osteitis fibrosa cystica. The bone atrophy observed in dietary calcium deficiency arises not so much from deficiency in protein matrix formation, as it does in osteoporosis, as to destruction of the matrix brought about by osteoclasts. On the other hand Nordin himself found that slight but prolonged calcium deficiency causes osteoporosis in adult animals, whereas it produces generalised osteitis fibrosa cystica in growing animals, especially when there is a marked deficiency in calcium.

None of the pathogenetic interpretations of senile osteoporosis suggested by the theories that have been quoted proves satisfactory. For some years in my own school we have been studying the changes in bone (Bertolin and Mattucci 1962), cartilage (Bertolin and Mattucci 1962), tendon (Bertolin 1961, Fonzone and Bertolin 1962) and ligament (Bertolin and Fonzone 1958) at different times of life, and with particular reference to old age. It is not out of place here to refer to the results of that part of our histochemical and analytical chemical research which has dealt with the study of the changes taking place in the protein bone matrix of a human subject suffering from senile osteoporosis and with the study of the functional activity of the cellular elements of such matrices (Casuccio, Bertolin and Falzi 1962).

RESEARCH STUDIES ON SENILE OSTEOPOROSIS

Our observations seem to us to add some help in finding a solution to the mysteries of senile osteoporosis. The findings were gathered from examination of the twelfth thoracic and first lumbar vertebrae from one hundred cadavers of varying ages and both sexes, all free from congenital or acquired skeletal disease.

Anatomical and radiographic investigations have made it clear that, in the elderly, spongy bone tissue is made up of fewer trabeculae which, however, seem to have undergone thickening, especially in the middle third of the vertebral body. These two morphological aspects seem

to contradict the usual definition whereby osteoporosis is characterised by thinning of the bone trabeculae. It would be more exact to say that in osteoporosis there are, alongside trabeculae which have undergone thinning, other trabeculae which are hypertrophied. The term "hypertrophic atrophy" would be appropriate.

Changes of bone structure in old age reflect a disturbance of the functional state of those cells believed to be responsible for the formation of the organic matrix. Our microscopic examinations have therefore been particularly directed towards one special aspect of the functional state of the osteocyte, namely the behaviour of its perilacunar sheath and the prolongations thereof. This histochemical investigation starts off from the previous investigations carried out for the same purpose by Leblond (1950), Lipp (1954), Rutishauser and Majno (1951) and others on human and animal bones. Our investigations have shown that, alongside the already mentioned resorption of the cellular elements, in the elderly subject one may often find trabeculae with the lacunae empty, or in which only Rouget-Neumann's perilacunar sheath is left, whose metachromasia, so marked in the young subject, appears rather weak and sometimes absent. The sheath is thickened, shrivelled, fragmented, and no longer applied to the walls, while the canaliculi, reduced in number, appear very short, with a consequent diminution in the anastomotic network, and therefore in their functional activity. Moreover, the bone lacunae in elderly subjects often appear dilated, containing osteocytes with pyknotic nuclei surrounded by basophilic halos. This appearance seems to indicate intralacunar resorption of the bone, particularly evident in osteoporosis. The changes often seem to be to the detriment not so much of the lacunar sheath as of the mucoprotein cylinders which fill out the bone canaliculi: these appear fewer, shorter, segmented, with terminal club-shaped thickenings, and sometimes convoluted rather than stellate. The different types of polysaccharides that constitute the Rouget-Neumann sheaths undergo structural transformation in senile involution which are made evident by changes in staining affinity for thiazine stains at a given pH. The interfibrillary bone matrix, moreover, presents considerable variations in staining affinities from one subject to another whereby, alongside some histological sections whose trabeculae stained well metachromatically, one encounters others, rather more numerous, where only the cementing lines react metachromatically. One can, however, often see trabecular segments with serrated edges and with indistinct surroundings lined only in some places by a light metachromatic border. This variation in staining affinities, this breaking up of the only slightly metachromatic cementing lines persuades us that the bony trabeculae in elderly subjects have undergone not only morphological changes but also, and more particularly, changes in their composition, especially of the mucoproteins of the bone matrix.

Turning now to our analytical chemical investigations, also carried out on human spongy vertebral bone tissue from subjects of various ages, we determined the percentage content of total nitrogen, hydroxyproline, hexosamine, calcium and phosphorus. The chromatographic method was used to determine the percentage content of galactosamine and glucosamine, as well as the amino-acid composition of the non-collagenous proteins linked with the mucopolysaccharides.

The decrease in the protein nitrogen content of spongy vertebral bone is progressive and considerable, especially after the fiftieth year. It exceeds 50 per cent in osteoporotic subjects. The amino-sugar content of bone undergoes a similar change, but the content by weight of hydroxyproline increases. It was also found that a quantitative decrease of mineral salts takes place in old age, but the decrease is only of the order of 6 to 9 per cent. Hence we became convinced that the process of senile atrophy which is a feature of senile osteoporosis is a biological phenomenon which concerns only the constituents of the organic matrix. In order to broaden our knowledge on this point we carried out on the same material a fractionation of mucopolysaccharides using the method recently applied by Dische, Danilczenko and Zelmenis (1958). We obtained four fractions. *The first fraction* was the soluble sodium-ethylenediamine tetra-acetic acid (Na-EDTA) fraction, and it was made up of all the mineral

salts together with a certain amount of mucoproteins which came away with the mineral salts through the action of the Na-EDTA. There seems to be some connection between this small quantity of mucoproteins and the calcium salts, because no additional weight of the former could be obtained when the extractive action of the Na-EDTA was continued. The quantity of mucoproteins does vary, however, with the age of the subject, particularly in the amino-sugar content, which diminishes noticeably in old age, to the order of more than 50 per cent. In young subjects it makes up about 45 to 50 per cent of the whole of the mucoproteins present in the bone: in elderly subjects less than 25 per cent. An examination of the chromatography column showed that both of the amino-sugars in this fraction of the mucoproteins are always present. As the age of the subject increases, particularly if he or she is osteoporotic, the ratio by weight between galactosamine and glucosamine is found to change. The mucoprotein which absorbs the mineral salts in young subjects is rich in galactosamine: the ratio by weight between the two amino-sugars is more than three to one. In subjects suffering from senile osteoporosis the galactosamine content decreases and the galactosamine-glucosamine ratio works out at less than 1.8 : 1. But the amino-acids making up the non-collagenous protein which is combined with these mucopolysaccharides appear to go unchanged, whatever the age of the subject. *The second fraction* to be extracted was the insoluble organic Na-EDTA fraction. This consists of all the collagenous matter and the interfibrillary substance. As the age of the subject increases, the hydroxyproline content of this fraction increases, while the nitrogen and amino-sugar content decreases. If we now place the collagenous matter thus obtained in an alkaline-alcohol solution (5 per cent potassium hydroxide (KOH) in alcohol at 80 degrees Centigrade) we achieve a total solution of the collagenous matter and of almost all the residual bone matrix, thus arriving at *the third fraction*, the alkaline-alcohol-soluble fraction. *The fourth fraction*, the alkaline-alcohol-insoluble fraction, is an interesting one. It is made up of the insoluble residue of the above-mentioned alkaline-alcohol hydrolysis of the collagenous matter. It is actually always a very small residue, but is relatively rich in carbohydrate matter, containing 20 per cent of all the amino-sugars in the bone. In the composition of the amino-sugars making up the prosthetic group of this mucoprotein, the two hexosamines were always well in evidence with galactosamine constantly appearing in a higher ratio by weight. This ratio varies from 3.3:1 in young subjects to 2.3:1 in elderly subjects. When compared with the trend of general changes in the polysaccharides, this last mucoprotein fraction shows a relative increase. We believe that it has considerable importance, firstly in conditioning the formation of collagen, and secondly in creating the cross-links between the single polypeptide chains of the collagen. In this mucoprotein fraction there is not only a modest increase in weight of the amino-sugars but also, as the age of the subject increases, an increase in its nitrogen content. This corresponds in all probability to an increase of diamine amino-sugars with respect to monamine amino-sugars among the non-collagenous proteins.

To sum up, the whole of the bone matrix decreases by weight with old age, but not in a way that is uniform or parallel for the different mucoprotein fractions we have isolated and examined. The fraction which most reflects the biological phenomenon is the one believed to be linked to the mineral salts, because it was with them that it was extracted by means of the Na-EDTA solution. It undergoes a decrease in old age of more than 50 per cent. The mucopolysaccharide content of the interfibrillary metaplasia may be extracted without heating by means of the alkaline-alcohol solution together with the whole of the collagen. It makes up about 40 per cent of the total amino-sugars, and this amount decreases in elderly subjects, although the decrease does not run parallel with the decrease noted in the first fraction. On the other hand the amino-sugars in the mucoprotein fraction which resists the dissolving solution of alcoholic KOH, and which is therefore left behind as a residue, remain constant or increase slightly with old age. This is a fact which can be justified by considering the increase in the cross-links in the collagen found in elderly subjects. These links are in all probability supplied

to the polypeptide chains of the elementary helicoidal units of collagen by this mucoprotein fraction, and are believed to be responsible for the increased resistance of the collagenous fibres to hydrothermic contraction. It is necessary to admit that a different composition of the mucoprotein as regards the two amino-sugars (the presence of the prosthetic nucleus of a neutral or acid polysaccharide, either sulphated or not) necessarily implies both a different connection between the various components of the protein matrix and a different state of polymerisation in them. *It is just these quantitative and qualitative differences in the bone matrix which characterise the ageing of the tissues.* All the variations we encountered in the composition of the protein matrix of the bone and especially in that of the two principal protein components of the matrix (fraction 1, which contains the proteins connected with the mineral salts, and fraction 4, which contains the proteins which cement the collagenous protofibrils) serve to demonstrate clearly that human bone undergoes changes with advancing age, and particularly in old age, and that these changes bear witness to a reduced and altered metabolism in the bone matrix. The marked loss of formative material (mucoprotein) from the protein component of the bone matrix in an aged subject (which in serious cases of atrophy can reach a value as high as 70 per cent) persuades us that the productive activity of the bone cells is much reduced. As a confirmation of this the histochemical changes referred to above bear witness to a negative balance.

I would mention in this connection that the investigations carried out by my co-workers (Bertolin and Greco 1962) show that both in osteogenesis imperfecta and in osteoporosis there is a considerable daily elimination of polysaccharides in the urine, and that the ratio by weight of galactosamine to glucosamine is higher than in normal subjects of the same age.

Cellular activity of the bone tissue in elderly subjects, and more markedly in osteoporotic subjects, not only prevents the production of a suitable and adequate quantity of formative material but also changes the composition of the material actually produced. This is proved by the fact that the mucopolysaccharides in the atrophic matrix of elderly subjects differ in composition from the mucopolysaccharides found in young subjects. In fact, hyaluronic acid and kerato-sulphate and the neutral polysaccharides replace chondroitin-sulphuric acid in many mucoproteins as age advances. This is demonstrated by the altered ratio between the two amino-sugars. This substitution of components may influence on the one hand the connection between the organic matrix and the mineral salts, and on the other the reactivity of the collagen itself, thus making the activity of the organic framework unstable.

All this is a clear indication that the true cause of the senile involution of the protein-containing bone matrix is to be imputed in some particular way to this altered and diminished functional state of the bone cell. *We therefore believe that it is erroneous to define the composition of the protein matrix in osteoporosis as "normal."*

CONCLUSIONS

Relating the results of our investigations to the knowledge hitherto acquired about the etiology of osteoporosis (which I have already referred to), I am inclined to interpret the pathogenesis of osteoporosis in the following way: 1) *Primary osteoblastic deficiency*: congenital (Lobstein); involutive (senile osteoporosis?); 2) *Reduced osteoblastic activity from absence of trophic stimuli*: (inactivity, ovarian agenesis, eunuchoidism, menopause); 3) *Reduced osteoblastic activity from inhibitory stimuli*: (cortisone, adrenocorticotrophic hormone (A.C.T.H.), stress, Cushing's disease, thyrotoxicosis); 4) *Normal osteoblastic activity but insufficiency of constructive material*: (malnutrition, disturbances of the digestive system, insufficiency of vitamin C, diabetes, thyrotoxicosis, cortisone, A.C.T.H., stress, Cushing's disease).

Osteoporosis may therefore be the consequence either of a congenital osteoblastic deficiency, such as that found in cases of osteogenesis imperfecta, or of reduced osteoblastic

activity due to absence of trophic stimuli such as mechanical stress and the sex hormones, or of reduced activity of the bone cells due to anti-anabolic substances which inhibit them, such as cortisone and its derivatives and the thyroid hormone in strong doses, or lastly of reduced availability of construction material due to its introduction in reduced quantities (starvation, dysfunction of the digestive system) or due to hindering of synthesis (deficiency of vitamin C, diabetes, cortisone and its derivatives) or due to an excessive degree of destruction (thyrotoxicosis). In the case of anti-anabolic hormones from the adrenal cortex, the mechanism may thus be twofold: inhibition of the osteoblasts and deprivation of the osteoblasts of glucoprotein material due to a general anomaly of metabolism. This may perhaps explain the most serious forms of bone atrophy which are usually observable in cases of hyperfunction of the adrenal cortex.

Senile osteoporosis should, in my opinion, be included in the first of our groups because it cannot be said to be brought about by any of the causes usually cited for osteoporosis—such as deficiency of sex hormones, excess of hormones from the adrenal cortex, deficiency of calcium, etc.—and in all probability it will depend on a progressive involution of the osteoblasts brought about by old age.

Senile involution is an expression of the descending phase of life's parabola and it involves all the organs and all the parenchymatous tissues in the human body, but it does not cause a parallel reduction of functions and activities on all of them equally. The skeletal system is one of the first to feel these reductions, because in old age life necessarily becomes less intense. Consequently in the economy of the ageing subject the generally reduced level of metabolism brings about a sort of selection in the nourishment of the different organs and systems, and sometimes almost a dismantling of some of these in an attempt to fall in with the new and reduced level of activities of some of the parenchymatous tissues, activities which may be incomplete or even transferred elsewhere. We believe that the moment which originally determines the beginning of senile osteoporosis coincides with the involutional process of cellular metabolism that strikes at all parenchymatous tissue during old age—striking, in the case of osteoporosis, hardest of all at the bony tissues.

There is, indeed, no doubt that certain essential processes of cellular metabolism do alter with age, and that the reduction in the activity of the gonads does have considerable importance. In any case, just as adolescence and old age cannot be explained only in terms of gonadal activity, so the involution of the skeleton cannot be due merely to the involution of the gonads. How should one then interpret the well known benefit afforded by administration of sex hormones in cases of osteoporosis? Probably the action of oestrogens and androgens is, in this case, of a pharmacological nature, and comparable, for instance, to the action of digitalis on the cardiac muscle. It will be remembered how digitalis acts almost exclusively on myofibrils which have become inadequate, and has little or no effect on a normal myocardium. Similarly, the sex hormones would seem to exert a stimulating action on osteoblasts that are on the way to involution, while they exert little or no action on normal osteoblasts. In support of this we have the findings of Urist and other workers, who demonstrated that the administration of sex hormones produces calcium and nitrogen retention only in osteoporotics, while in non-osteoporotic subjects of the same age it produces no effect. On the other hand, the action of the sex hormones might act in cases of senile osteoporosis by returning the changed level of protein metabolism to normal.

From the data in the literature and from the results of our own investigations, I conclude that osteoporosis in general, and senile osteoporosis in particular, are first and foremost the result of a disturbance in the metabolism of bone, and that the metabolic disturbance is closely and exclusively related to the degree of activity and the state of activity of the cells in the bone. Lastly, I believe that senile osteoporosis should not be considered an actual disease but rather as one limited aspect of the normal descending parabola which affects to a greater or less degree all the tissues of the body.

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