A STUDY OF THE INCIDENCE, AETIOLOGY, AND PATHOLOGY OF SENILE OSTEOPOROSIS

VOLUME I

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF MEDICINE OF THE UNIVERSITY OF GLASGOW

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SUMMARY OF THE THESIS

This Thesis is about generalised osteoporosis, a condition in which there is reduction of calcified bone mass per unit volume of bone without distinctive biochemical changes in the serum. The incidence, aetiology, and pathology of senile osteoporosis, the most common variety of generalised osteoporosis, is studied.

The Introduction defines generalised osteoporosis and describes the conditions in which it may occur, thus providing the background of knowledge for this study. Because the hormones produced by the adrenal glands, ovaries and testes are obviously implicated in the production of osteoporosis the relevant articles describing the effects of cortisone, oestrogens, and androgens on bone are reviewed and summarised. The problems involved in radiological evaluation of the degree of bone mineralisation and previously reported methods are also briefly described.

The work is divided into three parts.

Part I describes a simple but accurate radiographic method of estimating the degree of mineralisation of lumbar vertebral bone slabs. This method is correlated with chemical analysis of the calcium content of the vertebral slabs and bone

histological examinations, undertaken with a view to determining the range of variation of calcium content and radiographic density of normal and osteoporotic bone. A series of 300 necropsy cases was examined in this manner.

Part II is a study of the histological and histochemical patterns of adrenal glands obtained within six hours of death from 31 of these 300 cases. By reference to the vertebral bone density results the adrenal cortical histochemical patterns in osteoporotic cases are compared with those in cases having a high normal bone density.

Part III describes how osteoporosis was produced in male albino rats (150 g.) by ablation of adrenal glands and testes and daily cortisone injections. The osteoporosis was treated either by oily solutions or microcrystalline suspensions of the sex hormones in various combinations and the results were assessed by radiographic, chemical, and histological examinations of the long bones.

The General Discussion which follows deals with the additional points raised by the combination of these three studies as a result of which a clear cut picture of the condition called senile osteoporosis is presented.

The text and tables are presented in Volume I and the illustrations in Volume II. An Appendix including some of the technical methods and an account of the work personally performed is also presented in Volume I.

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PREFACE

PREFACE

This thesis is a study of the incidence, aetiology and pathology of generalised osteoporosis, a bone disease in which the trabeculae of cancellous bone become unduly slender and ultimately vanish, while those that remain are widely separated. Compact cortical bone also undergoes cancellous transformation, by the progressive enlargement of resorption cavities which become filled with marrow tissues.

Although the brunt of this condition falls on the spine, and to a lesser extent on the pelvis, other bones, such as the peripheral limb bones, the long bones, and even the skull, are also affected but to a lesser degree. This type of osteoporosis frequently leads to severe deformity due to loss of height and kyphosis as a result of atrophy of vertebral bodies, to disability owing to bone pains, and to a greatly increased tendency to fractures of lumbar vertebrae and long bones.

The histological picture indicates an atrophy of the bony framework unaccompanied by osseous regeneration, by fibrous substitution, or by notable osteoclastic reaction, as Hadfield observed in reporting on the histological sections in the cases of Burrows and Graham (1945).

Such changes usually occur without the appearance of abnormalities in the serum chemistry, but in a few instances where there has been extremely rapid demineralisation in the early stages of the disease, the serum calcium has been elevated (Albright, et al. 1941).

Osteoporosis can and must be distinguished histologically from other bone rarefying diseases, such as osteomalacia where excessive bone osteoid tissue is formed and the blood serum has a low mineral and a high alkaline phosphatase content, from osteitis fibrosa due to hyperparathyroidism in which there is excessive osteoclastic breakdown of bone trabeculae and also distinctive biochemical changes in the serum, and from the osteolytic phase of Paget's disease of bone.

In view of the difficulty in obtaining a quantitative measurement of osteoporosis both from the clinical radiograph and in the laboratory, Part I of this thesis describes an accurate radiographic method of determining the bone density of samples of vertebral spine obtained from cases in the post-mortem room, with a view to determining the prevalence of osteoporosis among hospital patients. The spines of 300 unselected autopsy cases were examined by this method.

It is stated by Aegerter and Kirkpatrick (1958) that the cause of the most common varieties of generalised osteo-

porosis i.e. the post-menopausal and senile types, is often complex but that the endocrine deficiency is the initiating and often the most important factor. Albright (1947b) has suggested by analogy to the menopause in the female, that there is a Leydig-pause in the male, and that both sexes may undergo an adrenopause. I have been unable to trace any systematic study of the adrenal glands or of the testes in cases of osteoporosis, and because of this Part II of this thesis is a comparison of the histological and histochemical patterns of fresh adrenal glands obtained at autopsy from osteoporotic and non-osteoporotic cases.

Most authors are agreed that in post-menopausal and senile osteoporosis, and in certain other varieties associated with endocrine disorders such as Cushing's syndrome, treatment with one or other of the sex hormones is required. It is seldom however that radiological improvement of the bone condition is demonstrated even after prolonged treatment, and Urist (1958) has pointed out that the experimental basis of sex-hormone therapy in osteoporosis was founded, not on the treatment of animals with osteoporosis but on the treatment of normal animals with normal bones. Accordingly Part III of this thesis is a study of sex-hormone treatment of osteoporosis which was produced in male rats

by adrenalectomy, orchidectomy, and daily administration of cortisone acetate. The bone changes which took place during this experiment are evaluated by radiological, chemical, and histological methods.

INTRODUCTION

INTRODUCTION

Osteoporosis defined

The subject of generalised osteoporosis has recently been well reviewed by Cooke (1955), who stated as a generalisation that it is a much commoner condition than was generally realised. However, the assessment of its incidence has until very recently been made either by means of clinical radiography of the spine which is notoriously inaccurate, or by more accurate methods of radiographic examination of the less affected peripheral limb bones. Beck and Nordin (1960), have now described a biopsy method of examination of the iliac crest which appears to offer a much more accurate assessment of bone density during life, than has hitherto been possible. This method may prove to be of considerable value from the point of view of early diagnosis of the condition.

Osteoporosis has been defined as "a decrease in the hard portions of bone substance in favour of a relative increase in the soft portions" by McLean and Urist (1955), as "a metabolic bone disease in which the total body mass of bone is less than that of a normally active subject of comparable size" by Bartter (1957), and as "a bone condition which results in a reduction of calcified bone mass per unit

volume of bone" by Caldwell and Collins (1961). All of these definitions are more or less correct, but the former two lack precision and therefore it is the last mentioned definition which is adhered to throughout this Thesis.

It was first suggested by Albright et al. (1940), and later more definitely stated (Albright, 1947b), that osteoporosis resulted from a decreased production of osteoid, due, either, to an inadequacy of osteoblastic activity or, to a deficiency of supply or conservation of nitrogenous materials necessary for bone formation, but that the bone tissue remaining was normally calcified. More recently doubt has arisen as to whether there is in fact decreased production of bone in osteoporosis, because, although from the histological viewpoint there is a paucity of osteoblasts lining the bone trabeculae, and also of osteoclasts in both normal adult and osteoporotic bone, Urist (1958) has pointed out that in response to the stimulus of injury following fractures, osteoblasts do appear and produce new bone matrix in osteoporotic bones just as in normal bones.

It is now well recognised that bone resorption can occur in the absence of osteoclasts (Ham, 1952), and studies by Fraser (1959) using a method of strontium uptake have suggested that in cases of osteoporosis, bone resorption may in fact be more prominent than inhibition of formation.

Exactly what constitutes the normal calcium content of bone is not quite clear. Edelman et.al. (1954) have stated that although bone calcium content shows surprising consistency in mammals, cancellous bone has more than double the water content of cortical bone, and Nicolaysen, et.al. (1953) stated that the calcium content of primary periosteal bone is always higher than that of secondary Haversian bone. Attempts to show differences between the calcium content of the normal bones of aged and osteoporotic subjects have so far been unsuccessful (Kelly, et al. 1959), but as yet, no studies using the more specific technique of microradiography of osteoporotic bones have been published. It appears that this question will remain unresolved until some such specifically directed study has been made.

Generalised osteoporosis has been described in senility, as a result, of the menopause, of severe limitation of activity, of simple starvation, scurvy, calcium deprivation in animals, uncontrolled diabetes, hyperthyroidism, Cushing's syndrome, cortisone and ACTH therapy, acromegaly, and hypogonadism. A form has also been described which is not related to any of these conditions and which is referred to by most authors as idiopathic osteoporosis.

The occurrence of generalised osteoporosis in so many different conditions, is no doubt the reason why so many different theories of causation have been advanced. Before briefly reviewing these various theories, it is necessary to exclude a number of irrelevant conditions which some authors are prone to discuss with osteoporosis but which are obviously not related to that condition as it has been defined.

For instance diseases in which there is inadequate bone structure such as osteogenesis imperfecta, where there is an hereditarily transmitted generalised disorder of tissue of mesenchymal origin, involving the sclerae, tendons, and blood vessels, as well as the ossification of the cartilage model of the bone shaft. Localised conditions also occasionally included, are Sudeck's atrophy where there is usually an associated vasomotor disturbance, and osteoporosis cranii circumscripta, which has been shown to be a result of Paget's disease of bone where bone production fails to follow the stage of osteolysis (Collins and Winn, 1955). These forms are not considered relevant to the thesis and are omitted from further discussion but the conditions in which true generalised osteoporosis may occur are now reviewed as follows.

Osteoporosis in senility

Senile osteoporosis is becoming recognised as a fairly common condition (Cooke, 1955), and it is frequently considered to be merely one of the many manifestations of generalised tissue atrophy which occurs in the older age groups. Albright (1947b), postulated that it might be the result of failure of the anabolic steroid hormones in the face of the continued production of what he termed as the "anti-anabolic" adrenal cortical hormones. Diminished mobility he stated, was an additional factor which led to the reduction of the stresses and strains which are so necessary for normal osteoblastic activity. Reifenstein (1957) modified Albright's hypothesis to include the possibility that senile and other forms of osteoporosis might be due to imbalance between gonadal anabolic and adrenal "anti-anabolic" hormones.

Osteoporosis in the post-menopausal state

Like the senile variety with which it is reputed to be metabolically identical (Albright and Reifenstein, 1948), post-menopausal osteoporosis is also recognised to be a fairly common condition. The productive effect of oestrogens on bone formation in birds and certain laboratory animals is well known, and has been described by Riddle and

Reinhart (1926), and Gardner and Pfeiffer (1938). This fact, together with the subsequently reported occurrence of diffuse osteoporosis in the syndrome of ovarian insufficiency (Albright et al. 1942), led to the supposition that oestrogens were specifically stimulating to osteoblasts (Albright, 1947b) and that oestrogen withdrawal would lead to the development of osteoporosis; a view which was supported by McLean and Urist (1955).

Osteoporosis due to limitation of activity

Earlier studies on bone structure (Murray, 1936) had shown conclusively that osteoblastic activity was in some measure governed by the mechanical factor of stress. In a study of 85 patients immobilised for periods of five years or more Stevenson (1952), noted the development of severe radiological osteoporosis within 3 to 6 months of immobilisation, and this was not confined to the bones of the diseased limbs. A later study by Wyse and Pattee (1954) on paraplegic patients, suggested that the absence of muscular contractions was the principal factor in the production of this type of osteoporosis.

Osteoporosis in starvation

Alwens (1919) reported an epidemic of osteoporosis in Austria which he related to the malnutrition of World War I.

Eliot and Jackson (1933) described a high incidence of osteoporosis in undernourished children, and postulated that the nutritional disturbance resulted in slow bone growth with decreased lime salt deposition. Albright (1947b) considered that the defect here lay in deficiency of nitrogen building blocks, and that any condition in which the serum albumin level was low would lead to a similar kind of osteoporosis. Cameron (1958) is in general agreement with these views, and states, with particular reference to the healing of fractures, that osteoblasts when hard at work require generous supplies of - among other things - amino acids and protein, and that deficiencies in the supply of any of these will lead to serious pathological disturbances in the skeletal tissues. On the other hand, Armstrong (1944) states that it is difficult to produce osteoporosis in adults by dietary deficiency.

Osteoporosis in scurvy

The bone condition which results from prolonged hypovitaminosis C is widely described as an osteoporosis (Cooke, 1955; Bartter, 1957), owing to the fact that the bone matrix is defective. Angevine (1959), states that although the precise mode of action of vitamin C is still unknown, deficiency leads to failure of synthesis of collagen and intercellular matrix. From the absence of reported cases in which

vitamin C deficiency was the undisputed cause of osteoporosis, this must remain, at least in the meantime, a theoretical cause of the disease.

Osteoporosis due to calcium deprivation

That osteoporosis in the human subject can be so produced remains to be demonstrated, although Korenchevsky (1922) has shown in rats, that diets deficient only in calcium lead to osteoporosis, and Urist and McLean (1957) claim to have produced a limited form of osteoporosis in rats reared on calcium deficient diets. Malm (1958) who studied 44 male prisoners in the Oslo State Jail, of age range from 20 to 76 years, continuously over a period of 4 years, stated that a small proportion, (8 per cent) were in continuous negative calcium balance on a daily intake of calcium 20 per cent greater than that considered necessary in 1953, for the maintenance of calcium equilibrium in adult life by the Food and Nutrition Board of the United States National Research Council. When this level of calcium intake was halved, approximately 50 per cent of his subjects (aged 20 to 64 years) went into negative calcium balance, but although their balance states subsequently improved on this intake they never became satisfactory.

A persistent drain of calcium from the body will

lead in time to the development of osteoporosis whatever the state of the endocrine balance.

Osteoporosis in uncontrolled diabetes

It was predicted by Albright and Reifenstein (1948) that osteoporosis might occur in this condition because of deamination and degradation of nitrogenous material essential for bone matrix formation.

Hernberg (1952) claims to have demonstrated a slightly increased incidence of osteoporosis in diabetics under the age of 65, but this appears never to have been satisfactorily confirmed.

Osteoporosis in hyperthyroidism

Plummer et al. (1928) and Aub et al. (1929) have shown that the osteoporosis which may be seen when this condition is prolonged, or untreated, is associated with extremely high levels of urinary calcium excretion, and they concluded that thyroid hormone has a direct stimulating catabolic effect on bone. Their results showed that the rate of urinary calcium excretion was constantly proportional to the degree of increase of the B.M.R., but that other causes of increased B.M.R. (e.g. when produced by infection or leukaemia) did not lead, either to excessive calcium excretion, or to osteoporosis.

Osteoporosis in Cushing's syndrome

Osteoporosis of mainly vertebral distribution was first described in this condition by Mooser (1921), although he mis-diagnosed the case as the adipose-genitalis syndrome. Eisenhardt and Thompson (1939) noted a high incidence of osteoporosis in Cushing's syndrome, i.e. in 53 out of 67 cases but neither sex showed a greater tendency to develop bone rarefaction. Rib fractures which are not a feature of other varieties of osteoporosis (Sussman and Copleman, 1942) were first described in this variety by Lescher and Robb-Smith (1935). Albright (1947b) postulated that the osteoporosis here is due to excess production of the adrenal 'S' (sugar) hormone over the adrenal production of the 'N' hormone with the consequent predominance of "anti-anabolism".

Ostoeporosis in cortisone and ACTH therapy

In conditions such as rheumatoid arthritis where osteoporosis is a not uncommon complication, a greater incidence of vertebral fractures is seen among cases treated with cortisone and ACTH (Demartini et al. 1952; Curtis et al., 1954). Osteoporosis is also frequently reported in patients receiving similar treatment for other conditions (Teicher and Nelson, 1952; Urist, 1958).

Osteoporosis in acromegaly

Osteoporosis is not important from the clinical point of view in acromegaly (Albright and Reifenstein, 1948), and it is more difficult to account for the occurrence of osteoporosis in this than in other conditions. Bauer and Aub (1941) showed increased calcium excretion in 4 out of 5 cases and they considered the gonadal deficiency to be an important aetiological factor, but Albright and Reifenstein (1948) who describe two cases where improvement occurred under oestrogen therapy, state that in a number of cases which show osteoporosis there is no evidence of gonadal deficiency. These authors did however note increased urinary output of 17 ketosteroids in these cases, and postulated that this was due to increased production of ACTH leading to increased output of adrenal 'S' hormone. They also postulate that there is an increased general tissue requirement of nitrogenous substances in acromegaly which creates a relative starvation of the bone, and acts as a contributory factor.

Osteoporosis in hypogonadism

Following the demonstration of osteoporosis in the syndrome of ovarian insufficiency (Albright et al. 1942), Labhart and Courvoisier (1950) reported the development of osteoporosis in an eunuch. They found however that not all

castrates develop osteoporosis and explained this by postulating the presence of an additional factor, possibly a metabolic antagonist, in some cases, which may lead to the development of osteoporosis. The state of adrenal function does not appear to have been adequately investigated in any of these types of endocrine disorder.

Osteoporosis of unknown aetiology

When osteoporosis occurs in the absence of any of the foregoing conditions it is frequently termed idiopathic. The age at onset of symptoms in this group appears to be rather earlier than in the senile and post-menopausal cases, and Bartter (1957) states in his review of the literature, that in males the disorder frequently follows trauma, and that in both sexes the serum protein concentrations are low in a surprisingly large number of cases. It has been thought to be one of the rarer forms of osteoporosis, but recently, Jackson (1958) has described a personal series of 38 cases in which the average age at onset of symptoms was 41 for men and 27 for women. Eleven of his cases were female, and in 4 of them pregnancy was possibly a contributory factor. This is of special interest because cases have previously been reported in young women in association with pregnancy and childbirth (Albright and Reifenstein,

1948; Jones, 1953; and Nordin and Roper, 1955) and it has been attributed to the increased production of adrenal corticoids known to occur in pregnancy. Authors are unanimous in stating that cases of idiopathic osteoporosis fail to benefit from sex hormone therapy.

From all these descriptions it will be apparent that in many cases osteoporosis is merely the end-result of the interplay of many different factors. From the endocrine viewpoint six glands are known to exert an influence on deposition or resorption of bone, namely, the adrenal, ovary, testis, parathyroid, thyroid, and pituitary, but only three, the adrenal, the ovary and the testis are obviously implicated in generalised osteoporosis as it is usually seen and much attention has been given to the role they play. Accordingly the influences which their hormones exert on bone are briefly reviewed.

THE INFLUENCE OF CERTAIN ENDOCRINE FACTORS ON BONE

The adrenal cortex forms three main groups of steroid substances in health, but the gland is concerned essentially with the production of the C_{21} group of which

cortisone (Compound E) is the only one known to exert a pronounced influence on bone metabolism. Of the C_{19} group, which is produced in lesser amounts, only testosterone is thought to exert a skeletal effect. It is not certain at present if members of the C_{18} group (oestrogens) are produced in significant amounts by the normal gland (Symington, 1959).

The effect of cortisone on bone

It has been demonstrated that adrenal cortical hormone is necessary for the normal body growth of rats (Ingle and Prestrud, 1949), and Pearson (1956) has shown that bone repair cannot proceed in the absence of cortisone.

Excessive doses of cortisone however lead to negative nitrogen balances in animals and man (Ingle et al. 1946; Ingle and Meeks, 1952), which suggests a catabolic action, and Engel (1951) stated that the main site of this action is at the level of whole protein.

Previously, Albright and Reifenstein (1948) had introduced the term "anti-anabolic" to describe the action of cortisone, and this purported to correlate lack of cellular proliferation with the metabolic changes responsible for diversion of amino acids and fatty acids to the formation of sugar, and prevention of the synthesis

of new protein. This hypothesis was developed mainly from the interpretation of metabolic balance studies on patients with Cushing's syndrome, which was regarded at that time as a 'pure' state and it remained unchallenged for several years.

However, more recent investigations by Astwood (1957) and Jones (1957) on animals treated with cortisone, have revealed that there is retention of sodium and excretion of large amounts of potassium, liberated presumably by breakdown of cell cytoplasm, which suggests that the chief action of glucocorticoid hormones is in fact catabolic. Astwood, is quoted as having stated during a conference, that the major action of the adrenal corticoids is the lysis of protein (Urist, 1958). In respect of bone this would entail a direct action on the collagen of the bone matrix. Urist (1958) considers that the terms "anti-anabolism" and "catabolism" are not precise enough to comprehend the fact that the metabolic processes concerned may proceed in several directions simultaneously, particularly in a complex tissue such as bone.

The reported experimental actions of ACTH and cortisone on bone are not always in keeping. For instance, overdosage with ACTH in rats has been shown to impede the

growth of cartilage, and to inhibit the formation of osteoblasts so that new bone is laid down at a sub-normal rate (Becks et al. 1944). Baker and Ingle (1948) and Asling et al. (1951) confirmed that rats treated with ACTH developed osteoporosis. It was thought that this was due to suppression of bone deposition in association with a normal rate of resorption.

On the other hand, however, Follis (1951) has shown that cortisone administered in amounts sufficient to suppress bone growth, appeared to retard the resorption rather than the deposition of bone, because he observed an increase in the density of subepiphyseal bony trabeculae of long bones in cortisone-treated rats; a change which has since been observed by others. For instance, Nicolaysen et al. (1953), while not appreciating the cessation of growth, reported similar results in their own experiments on rats and interpreted these appearances as due to an increase in bone trabecular formation. They failed, however, to produce similar changes in rabbits or guinea pigs.

Sissons and Hadfield (1955) did succeed in producing similar changes in cortisone-treated rabbits, but found that in two separate groups of otherwise identical rats, only one group showed an increase in density of metaphyseal bone.

As there was no feeding or species difference they explained this by postulating that under some circumstances cortisone may favour either osteoblastic or osteoclastic activity. In this experiment it would appear that cortisone depressed both functions.

As the increase in density of metaphyseal bone is not a result regularly obtained by cortisone, one explanation of this anomaly may be that under certain circumstances cortisone is metabolised in part to androgens (Dorfman, 1956) which in turn can be partly converted to oestrogens. Oestrogens have been shown to increase the density of subepiphyseal bone in rats (Baker and Leek, 1946). Urist (1958) has since further complicated matters by stating that rats of all ages are resistant to cortisone.

It is not easy to explain how cortisone can produce diametrically opposite results on the bones of experimental animals of the same species under identical conditions. However, no such anomaly has been described in connection with ACTH administration and one may conclude that cortisone in less excessive amounts such as would be produced in animals given even fairly large amounts of ACTH does in fact produce the bone changes one would expect (on theoretical grounds) from a protein catabolist.

In the adult human subject, Urist (1958) has shown that prednisone therapy may lead to the appearance of osteoporosis on routine clinical radiography in less than 9 months, which as will be shown implies a loss of at least 30 per cent of the bone mass during this period. This finding, in conjunction with unpublished observations which he made on birds, led him to conclude that hydrocortisone has a specific role in haversian remodelling.

Support for this hypothesis is found in that, pathological fractures of bone are reported more commonly as a result of therapeutic overdosage with cortisone than are seen for instance in Cushing's syndrome (Strickland, 1954) where anabolic steroid hormone production may also be raised, and also in the fact that patients with rheumatoid arthritis who are already in negative calcium balance probably have their calcium loss augmented by cortisone (Demartini, et al. 1952).

Summary

Cortisone

In humans

(1) Is necessary in physiological amounts for bone repair and normal bone growth.

(2) Produces osteoporosis and pathological fractures.

In animals

(1) In moderate excess inhibits the formation of osteoblasts and leads to the development of osteoporosis.

(2) In excess under certain conditions retards the rate of resorption of subepiphyseal bone, although an alternative explanation for this apparent action may be its conversion to oestrogen in the body.

The effect of oestrogens on bone

These are perhaps the most widely investigated group of hormones in so far as bone changes are concerned.

The production of medullary endosteal bone formation in birds and certain mammals by oestrogens, has been shown by Riddle and Reinhart (1926), and Gardner and Pfeiffer (1938), and subsequently confirmed by many authors (Riddle and McDonald, 1945; Riddle et al. 1945; and Baker and Leek, 1946). It has also been shown that in hens, in addition to the bone changes, oestrogens control to some extent the amount of calcium absorbed from the intestine (Gardner and Pfeiffer, 1943), and hypercalcaemia has also been produced in guinea pigs (Poumeau-Delille and Fabiani, 1944). That they also produce an accelerated rate of turnover of bone mineral in

pigeons was demonstrated by Govaerts and Dallemagne (1948).

Albright (1947b) postulated that oestrogens exert a stimulating effect on osteoblasts, and this hypothesis received further apparent confirmation from Gillespie (1954) who demonstrated that oestrogens significantly increased the bone mineral content in rats. Unfortunately no histological studies were published with this report, and his findings might easily have resulted from delayed resorption of subepiphyseal bone, which had previously been noted in rats by Baker and Leek (1946).

The only notable effect of oestrogens to be demonstrated in dog bones, has been the early closure of epiphyses of the long bones (Sutro and Pomerantz, 1942).

Scowen (1948) discovered that in birds oestrogens could produce the simultaneous proliferation of one bone, and the dissolution of another, and two years later Duckworth and Ellinger (1950) demonstrated that oestrogens were unnecessary for the adequate repair of rats' bones.

Whether or not the female sex hormones exert any perceptible influence on normal or abnormal human adult bone is not yet clear. Hyperostosis frontalis has been described in a few cases during pregnancy and the puerperium (Henschen, 1949) at which time the corpus luteum hormone is thought to predominate, but no satisfactory evidence yet exists that

oestrogens actively influence either this or any other bone condition. There is, however, some indirect evidence that they may have an effect on post-menopausal osteoporosis in that the bone pains disappear, and the resorptive process is apparently arrested. For instance, Sherman (1948) has stated that oestrogen administration to a female patient aged 58 with osteoporosis and Paget's disease gave rise to a significant reduction in urinary calcium excretion, and to an increase in the radiological density of the bone within a short period, and Polishuk and Kleinhause (1952) also reported radiological improvement in a case of post-menopausal osteoporosis within 8 weeks of commencing oestrogen therapy. Cooke (1955) has also described radiological improvement in two treated cases, but states that demonstrable improvement cannot be expected in less than two to three years.

These results however, are from clinical radiography with its inherent errors, and must be regarded with some reserve because several workers using more precise methods - two involving chemical analysis of the bone concerned - have shown that from 25 to 50 per cent of the bone substance must be lost before osteoporosis is apparent in the clinical radiograph (Ardran, 1951; Cobb, 1952; Fusi, 1953). Furthermore, Howard (1950) has stated that a 70 kg. osteoporotic adult whose normal total skeletal calcium would be approx-

-imately

1750 g. retaining 0·2 to 0·4 g. of calcium per day (a good retention) would not show a normal bone radiographic picture in less than eight years. This argument assumes all the bones to be equally affected in osteoporosis, which is not the case, but it seems most unlikely that radiological improvement could be apparent in less than 18 months even when only the spine and pelvis are affected, and therefore from the point of view of time alone only the two cases reported by Cooke (1955) would be acceptable. Hennemann and Wallach (1957) have, however, reviewed radiological findings during the prolonged use of oestrogens and androgens in osteoporosis and from a large series of 200 cases of post-menopausal osteoporosis, state, that even after 20 years of therapy no evidence of bone remineralisation was recorded.

The great bulk of evidence is therefore against any demonstrable improvement of bone density in osteoporosis as a result of sex-hormone therapy.

Direct positive evidence of the beneficial effects of oestrogen therapy apart from the rapid alleviation of pain, and the apparent arrest of the radiographic lesion in patients with overt osteoporosis, is thus lacking. Such other indirect evidence as exists concerns mainly observations on calcium and phosphorus uptake and retention, and in

this respect Albright and Reifenstein (1948) have demonstrated in oestrogen-treated cases of post-menopausal and senile osteoporosis, a diminished faecal excretion of calcium and phosphorus, but only in the post-menopausal variety was there a significant retention of calcium.

In normal elderly females, Ackerman et al. (1954) observed that the greatest reduction of urinary calcium excretion with oestrogen therapy occurred when they were in positive nitrogen balance and negative calcium balance, but, in elderly males a significant net urinary calcium retention only occurred where frank osteoporosis was also present (Bogdonoff, et al., 1954).

The specificity of oestrogens in stimulating actual osteoid production and storage of calcium has been questioned by Anderson (1950), who showed that the negative calcium balances of patients with post-menopausal osteoporosis, could, under the influence of oestrogens only become positive when twice the normal intake of calcium and phosphorus was given. Shorr (1950) produced positive calcium balances in a similar group of patients without oestrogens by giving them approximately four times the normal daily calcium intake (i.e. 4·2 g.) and when oestrogens were added no additional calcium retention was achieved.

These results could be explained by accepting that oestrogens merely facilitate in some way the intestinal absorption of calcium in the human subject as they have already been shown to do in hens, and that they act possibly in a similar manner to vitamin D, the action of which in this respect is well known and with which they have a considerable basic similarity of molecular structure.

Support for this hypothesis can be found for instance in the work of Whedon (1956), who demonstrated that improvement could result in the weakly positive calcium balances of osteoporotic patients who were already in strongly positive nitrogen balance by mere administration of calcium salts. Greater calcium balance improvements occurred in his cases when oestrogens were given in addition, but unfortunately the oestrogen therapy was not controlled against simple vitamin D administration.

Summary

Oestrogens:-

(1) Stimulate medullary endosteal bone formation in birds and certain mammals.

(2) Increase the percentage of mineral ash content in rat bones.

(3) Accelerate the rate of turnover of bone mineral in pigeons.

(4) Diminish the rate of subepiphyseal bone resorption in rats.

(5) Lead to early epiphyseal closure of the long bones of dogs.

(6) Facilitate the intestinal absorption of calcium in hens and possibly also in humans, perhaps in a manner analogous to vitamin D to which they are chemically similar.

(7) Arrest the progress of bone demineralisation in human cases of osteoporosis.

The effect of androgens on bone

Probably the most convincing demonstration of the effect of testosterone on bone comes from Armstrong et al. (1945), who showed that it could prevent the bone atrophy which followed orchidectomy in rats. Previously, however, it had been shown (Kenyon et al. 1940) that testosterone reduced the urinary excretion of nitrogen, sodium, potassium and chloride very considerably, in eunuchoid male patients who were presumably markedly androgen deficient. Curiously enough, elderly male subjects who, one might reasonably suppose, were also androgen-deficient, were stated to respond only to the same degree as younger males, and even female patients had a similar though less marked response. No

action on bone was demonstrated in either sex. In spite of the anomaly in the male results, they postulated that the greatest response occurred in the patient with the greatest need. Further to this, Kenyon et al. (1944) observed the simultaneous retention of nitrogen and calcium in cases of Cushing's syndrome, and in senile osteoporosis under the influence of testosterone. In one similar group of cases of Cushing's syndrome previously reported by Albright et al. (1941) there also occurred a rise in the serum alkaline phosphatase with testosterone which they felt suggested that actual bone formation had occurred, and Albright (1947a) postulated that testosterone stimulated endochondral and endosteal bone formation. It is interesting to note that endogenous testosterone did not appear to exert any protective influence against the development of osteoporosis in the male cases of Cushing's syndrome, when their incidence is compared with that of the female cases described by Eisenhardt and Thompson (1939). This is not in keeping with Albright's suggestion (1942, 1947b) that in Cushing's syndrome the underlying defect is an excess of the adrenal cortical glycogenic hormone over the nitrogen-retaining corticoids and that the balance between these two groups of hormones might be restored by testosterone.

It has been claimed (Gardner and Pfeiffer, 1938 and 1943; Sussman and Copleman, 1942) that androgens enhance the actions of oestrogens on pigeon bones but inhibit such changes in rats, mice, chickens, and mammals. However, Urist et al.(1950) could not demonstrate any evidence of inhibition of oestrogenic changes in bones of mice even when androgens were given in such a high ratio as 60:1.

All authors are agreed that androgens, like oestrogens, have no effect on idiopathic osteoporosis. Unlike oestrogens, however, it is stated by Henneman and Wallach (1957) that androgens have no effect on the negative calcium balances of patients with post-menopausal osteoporosis. Unfortunately no accurate balance data are available to support this contention. Although the balancing action which testosterone is alleged to exert in Cushing's syndrome (Albright, 1947b) encouraged hope that it might exert a similar action in cases of osteoporosis produced by cortisone and ACTH therapy, a patient with pemphigus reported by Teicher and Nelson (1952) developed pathological fractures of the vertebrae presumably as a consequence of osteoporosis produced by treatment with cortisone and ACTH. The negative nitrogen balance induced in this patient by the cortisone therapy remained uncorrected by

androgen administration.

On the other hand, in the cases reported by Whedon (1956) already mentioned, the administration of androgens in combination with oestrogens in those patients already in strongly positive nitrogen balance, caused 8 out of 12 cases to store more calcium daily than did either hormone alone.

It remains a possibility that such a result may be due to the partial conversion of androgens to oestrogens in the body.

Finally, the effect of androgens in producing early epiphyseal closure in human long bones, for instance in cases of adrenal virilism is well recognised (Cappell, 1958).

Summary

Androgens:-

(1) Prevent the bone atrophy which may follow orchidectomy in rats.

(2) Inhibit the action of oestrogens on the skeletons of rats, chickens, and certain mammals.

(3) Enhance the action of oestrogens on the bones of pigeons but not on the bones of mice.

(4) Enhance the action of oestrogens in cases of human senile osteoporosis but not in cases where Paget's disease of bone co-exists.

(5) Produce early epiphyseal closure in human bones.

THE ASSESSMENT OF BONE MINERAL CONTENT BY RADIOLOGICAL AND RADIOGRAPHIC METHODS

The need for an accurate method

Owing to the increasing age of the population a need for a quantitative estimate of the degree of calcification of bones has arisen during the last two decades, and because radiography in ordinary practice reveals only advanced degrees of osteoporosis, it became necessary to develop an accurate method of measuring the changes in bone density of the spine - the region mainly affected in osteoporosis - in order to assess the frequency and degree of osteoporosis and the changes produced by sex-hormone therapy.

It will be shown that Mack et al.(1949) have most nearly approached this ideal but although they have stated as a generality that calcium is responsible for about 80 per cent of X-ray absorption by bone, none of the workers in this field have as yet related radiographic bone density to its calcium content.

Assessment of the degree of mineralisation of the skeleton by radiography tends to be inaccurate principally

because of variations in kilo voltage, in thickness of the bone and soft tissue layers, in scatter, in film quality and in processing conditions. In spite of the fact that certain of these disabilities have been overcome in radiographing peripheral limb bones it was necessary for me to review all the previous efforts in this direction in order to discover a satisfactory means of accurate measurement of changes in bone density of the spine.

A brief review of the methods previously employed

In 1935 Pauline Sanders (quoted by Mack et al. 1939) devised a photometric method of bone density estimation by which the amount of light passing through a bone X-ray image was compared with that passed through a reference system consisting of a series of steps of a density ladder exposed simultaneously on the same plate. Stein (1937) advocated that the reference system should be a wedge composed of materials which could be directly interpreted in relation to actual amounts of calcium and phosphorus. He suggested ivory as a suitable substance.

Mack et al. (1939) used a density ladder of known mineral composition, and adapted the light source by appropriate resistances to emit a constant beam focussed

through the bone image onto bromide paper on a revolving cylindrical drum, and compared tracings from the areas of bone to be examined with those from areas of the density ladder. Bywaters (1948), with a similar method, used small ivory cylinders as absorption standard to measure the bone densities of the finger phalanges, metacarpals, and wrist bones.

Engström et al. (1948) described a new type of densitometer, consisting of a small metal tube, housing a complex electronic computer but this was only capable of measuring a very small field at any given instant. Engström and Welin (1949) used this densitometer to analyse the density of the finger phalanges in a number of rheumatic subjects, and this was the first study in which bone calcium was expressed as per unit volume of bone and not per unit of weight, which, as will be seen from my results, is an important distinction in osteoporosis. Unfortunately, the amount of calcium in the bones was never accurately estimated by them. Their method was to perform radiography in two directions at right angles and the results were calculated as absorption per c.mm. of the bone thickness. To counteract the density variation of different films they used three aluminium wedges, and the density of the measured

bone region was expressed by the thickness of the aluminium giving the same density.

The most complex of all methods was introduced, and is still used, by the workers of the "Bone Density Research and Evaluation Centre" of the Pennsylvanian State University (Mack, et al. 1949) and in view of the value of this contribution it is described here rather more fully than those of other workers. They stated that accuracy depended on two assumptions:

(a) That the photographic density of the X-ray image is related in a known manner to the density of the mineral of the bone radiographed, and (b) by using a step wedge of homogeneous density, and similar in chemical composition to the bone itself corrections can be made for variations in exposure and development technique.

They employed a standardised exposure and development technique, and the bone examined - usually the os calcis - was exposed on two non-screen X-ray films at right angles, with a shadow tape wrapped around the foot on a level with the tracing path. Thus the processed film showed an outline of the outer edge of the soft tissue which lay over the bone.

At first an ivory, but in later work an aluminium alloy wedge was used as a reference. A contact positive was made from another film for locating tracing paths without causing damage to the X-ray film itself.

First a calibration curve was made by measuring the densities of a tracing path along the standard wedge. Then the density of the bone and soft tissue X-ray image was measured along the predetermined tracing path and each

bone tracing was divided into segments in accordance with a standardised procedure. The area under each segment of the trace and the heights of the steps in the wedge tracing were measured.

An interpolating formula was used to correct the average trace height of each segment of the bone tracing to take account of deviation in exposure and development in terms of observed height of trace and measured physical thickness of the adjacent steps on the ivory standardisation wedge. The corrected segment trace heights were then averaged to obtain a corrected average trace height value for the entire trace and expressed as centimetres of equivalent ivory thickness.

The cross-sectioned area of bone corresponding to the tracing path was estimated through measurements on both the exposed films, and based on studies of cadaver bones. The corrected equivalent ivory thickness of the trace was divided by the estimated average physical thickness of bone corresponding to the microphotometer tracing path and multiplied by the density of ivory in grams per c.c. of bone. A factor giving the ratio of bone density to the density of the specific area of the ivory wedge enabled the results to be reported as bone density in grams of bone ash per unit of volume. The effect of the soft parts was eliminated by subtracting the mass of tissue from the mass of bone plus soft tissue.

This method employed a complex bone density computing machine, and a photo-electric area measuring device. It will be appreciated that it is extremely elaborate.

Henny (1950) measured the density of lower femoral diaphyses where the cortex was very thin and there was a high proportion of cancellous bone. His method of measuring the effect of the soft tissues was to flatten them along the bone to be examined with a plexiglass plate on which was placed the reference system of two bone ladders and occasionally an aluminium ladder. These were then

radiographed along with the same soft parts as the bone although their relative positions were different.

Jackson (1951) also concerned with minimising the error produced by the scattering effect of the soft tissues, stated that he overcame this effect by radiographing the part (usually a hand) in a Perspex tray with added water to make a constant volume.

Brown and Birtley (1951) further developed the apparatus devised by Mack et al. (1949) and incorporated an instrument which made use of the Hurter and Driffield curve determined for each film, and their densitometer recorded results directly in terms of emulsion exposure.

A somewhat different technique was used by Evans et al. (1951) who passed the rays from a known quantity of Sr^{90} through a defined area of bone on specially prepared bone samples, and measured the successfully transmitted radiation with a Geiger counter, thus determining the degree of absorption by difference. Aluminium strips of varying thickness were used to calibrate this apparatus.

McFarland (1954) was the first to report the results of a survey of the bone density of a substantial number of unselected human subjects. Using the technique of Brown and Birtley (1951) he estimated the bone density of finger phalanges, ulna, radius, and os calcis in 1200 cases. The

density of a large group of normal adults fell within a two to one range of variation and multiple measurements on any one subject were reproducible to 5 per cent or better. It was found possible to place a subject to within one of about ten classes of bone density.

A more obscure method was used by Gershon-Cohen et al. (1955) in a similar but smaller survey, on 149 elderly subjects. They stated that the bone density measured on the middle phalanges of the little fingers, and the os calcis, was computed by physicist colleagues as a coefficient which accorded well with the visual clinical appraisal. They found the bone density of elderly women to be higher than that of elderly men and thought there was some reason to assume that bone density increased with age.

They tried but failed to compute a density coefficient for the dorso-lumbar spine.

Virtama (1957) aware of the lack of published data relating radiological bone density with the actual mineral content of the bones, X-rayed the basal phalanges of the fingers of 86 autopsy subjects. To standardise the effect of the soft tissues - water having been tried and found inadequate - he prepared wedge-shaped plates of a paste having approximately the same absorption qualities as the

soft tissues of the human fingers, and used these plates to maintain the upper surfaces of the fingers parallel to the film during exposure. The silver content of the excised radiographic image of the phalanges was estimated by an accurate titration method, and compared with the bone mineral content expressed as grams of total ashed bone.

These results accorded well and the bone mineral content could be estimated by combining the silver determination results with a statistical regression analysis.

Keane et al. (1959) have also related bone mineral content (but not calcium) to radiographic density. As a preliminary procedure they measured the densities of a bone slab and various thicknesses of aluminium. After ashing the bone slab they could relate aluminium thickness to effective mineral thickness expressed as g./c.cm. of bone ash.

By radiographing the forearm bones in two directions at right angles with the arm immersed in a tank of water, they stated that they could relate the density of the radial bones with the bone mineral content. This method assumed that the mineral content of the bones examined was identical with that of the bone slab originally estimated.

Summary

The methods used to estimate quantitatively the

degree of mineralisation of bone by radiography are highly complex, and are mainly concerned with bones which are only involved to a minor extent in osteoporosis which is probably the commonest primary bone rarefying condition. None of the methods so far described relates bone density to its calcium content, but the method which is now to be described in Part I of this Thesis was designed to rectify this deficiency.

PART I

THE EVALUATION OF VERTEBRAL OSTEOPOROSIS BY RADIOGRAPHIC AND CHEMICAL METHODS POST MORTEM

THE EVALUATION OF VERTEBRAL OSTEOPOROSIS BY RADIOGRAPHIC AND CHEMICAL METHODS POST MORTEM.

INTRODUCTION

The measurement of the changes brought about by disease is an important step in understanding it, and enabling one to determine its incidence and severity under various conditions.

A quantitative measurement of osteoporosis is however not easy to obtain. In a recently described method of bone biopsy of the iliac crest (Beck and Nordin, 1960), the trabecular structure of the cancellous bone was studied and compared with a standard series of photographs of graded histological sections covering a wide range of bone density.

This method offers a much more accurate assessment of bone density during life than has hitherto been possible. I was concerned however with obtaining a measurement of calcified bone mass, since the essence of osteoporosis is a reduction of calcified bone mass in a unit volume of anatomically normal bone. This measurement, in the case of spongy bone with only a thin rim of bone cortex, such as obtained in vertebral bodies, can be suitably expressed in terms of calcified bone in a certain volume of the skeletal structure, i.e. the calcium/volume ratio, if one accepts the

statement by Neuman and Neuman (1958) that the proportion of calcium in bone salt is virtually constant at around 36 per cent.

Before accepting the calcium/volume index as a measure of osteoporosis, however, it is necessary to be satisfied by histological or other examinations, that cases of osteomalacia, osteitis fibrosa, Paget's disease of bone, or other bone disease have not been included in the investigation.

Chemical analysis is the direct way of estimating the calcium content of bone, but there are also two indirect methods, (a) the radiographic density, and (b), the specific gravity. Although some of the difficulties in radiography of the peripheral limb bones have been overcome (Jackson, 1951; Virtama, 1957; Keane, et al. 1959) the radiographic methods as described in the general Introduction have inherent difficulties in dealing with the vertebrae, as it is frequently impossible to allow for the varying thicknesses of bone and soft tissue which have been radiographed.

The specific gravity method was used in the post-mortem room for some years by Collins (1959), who cut small cubes of cancellous bone from vertebral centra and dropped them into copper sulphate solutions covering a range of

specific gravities. This method, however was considered too crude for an accurate assessment of osteoporosis.

In the post-mortem room a uniformly thick slab of bone can be procured, and radiographed under standard conditions which eliminate intervening soft tissues and variation of bone thickness.

It is my purpose to describe the results of a study in which the radiographic density, and the calcium content of lumbar vertebrae obtained at hospital autopsies have been measured, to indicate the range of calcium contained in these bones, and to show that this can be predicted from the radiograph of the bone slab.

MATERIALS AND METHODS

In an initial pilot trial in which 39 cases were examined but are not further reported here, 1 cm. cubes from the vertebral centra were used for calcium estimation. These tended to crumble away during the subsequent procedures, thus giving variable results. To overcome such crumbling, an outer coating of 1 per cent gelatin was used to entrap the soft portions of the specimen, and it was decided that in future this must consist of the whole of a single vertebral body slab.

Thereafter samples of vertebral bone were obtained in a random series of 300 necropsies on persons dying of

various diseases at the Sheffield Royal Infirmary.

A 1 cm. thick parallel-sided slab of lumbar vertebral bodies and intervening discs, from L2 to S1, was obtained in each case by sawing from the front of the spinal column through to the spinal canal using two hand tenon saws coupled together with the blades 1 cm. apart. The saw cuts were made in such a direction as to include the mid-sagittal plane of the vertebral bodies. After light brushing under running water to remove bone dust, each specimen was briefly immersed in water containing 1 per cent gelatin, and then suspended in neutral 10 per cent formol-saline for 10 days. Specimens showing infiltration by tumour or gross Paget's disease were excluded from the series. The bones were kept under fluid until a few moments before radiography, and when satisfactory X-ray pictures had been obtained chemical and histological methods were carried out.

Radiographic methods

Wet bone slabs were radiographed alongside an aluminium step-wedge. This was built of one to ten laminae of aluminium sheet 0·3 mm. thick, stepped in such a way as to give a thickness range of 0·3 to 3·0 mm., numbered from 1 to 10 step-wedge units (Figs. 1, 10 and 11). Constant

exposures of 10 secs. at 10 Kv. and 10 m.a. were made at a tube distance of 63·5 cm. X-ray films (Ilfex 6½ x 8½) were exposed in plastic cassettes and developed for 5 min. without agitation at 20°C. (Kodak 19B developer).

This technique was arrived at by a simple process of trial and error to ensure that the films were processed within the range of their maximum sensitivity (Hurter-Driffield, see Appendix).

Densitometric methods

Light from an electric lamp (Philips Photocrescenta) 240 v. 75 w.) was directed through a neutral grey gelatin filter, and thence through a 2 x 1 cm. slot to each step image of the step wedge, and the intensity of the light transmitted in each position was measured by a 6 cm. photocell (E.E.L.) connected to a galvanometer (Pye, Scalamp) (Fig. 1).

The density of the central region of the image of each 4th lumbar vertebra (1 cm. thick) was also estimated photometrically in terms of the step-wedge units, and where the vertebral slab images showed patchy variation of density the average of several readings was recorded. Care was also taken to observe that each step of the wedge gave a proportionate galvanometer deflection (Fig. 2), thus ensuring that each film had been exposed and processed

correctly (see Appendix).

Chemical methods and calcium analysis

After radiography, the 4th lumbar vertebral body was dissected from its neighbours, and with a scalpel stripped of adherent cartilage, blood vessels, and connective tissue. Marrow tissues still protected by a film of hardened gelatin remained undisturbed. The block of vertebra was then blotted to remove surface moisture and suspended on one arm of a balance by a fine nylon thread, where it was quickly weighed in air and then in tap water at 20°C. The difference in the two weights in grams recorded the volume of the specimen in cubic centimetres.

The bone block was then ashed in a silica crucible in a muffle furnace at 600°C. to constant weight (usually for 24 hours). The calcium content of the ash was estimated at first by the standard chemical titration method of Vogel (1951) but, as the whole procedure for individual calcium estimations with this method could not be performed in less than one week, from case 134 onwards results were obtained with a flame photometer, (see Appendix) at first in parallel with the chemical estimations, (20 cases) and then alone.

Histological methods

Following fixation, the 3rd lumbar vertebrae were processed by standard methods, double embedded (celloidin-paraffin) by Peterfi's methyl benzoate method (Carleton and

Drury, 1957) and sections were cut at 6μ. In every case a section was stained by haematoxylin and eosin and in a number of cases other more specific staining methods were also employed. These methods are all well-recognised and are therefore not described in detail but the source of the method and where applicable, the modification used are appended in brackets as follows.

1. Alcian Blue (Pearse, 1953)
2. Dialysed iron method for acid mucopolysaccharides (Hale in Pearse, 1953).
3. Haematoxylin (Harris's) and Eosin (Carleton and Drury, 1957).
4. Millon reaction (Baker's modification, in Pearse, 1960).
5. Periodic acid-Schiff reaction (Gomori, 1952).
6. Reticulin (Laidlaw, 1929).

RESULTS

The results are summarised in Tables 1 to 5 and illustrated in Figures 3 to 24.

Chemical results

Bone calcium estimations were made on 150 cases in which there were 87 men and 63 women whose ages ranged from sixteen months to eighty eight years (Table 1). The overall average calcium content of the 4th lumbar vertebral body was 67·9 mg. Ca. per c.c. volume of bone (range 38 to

Table 1

The radiographic density (step wedge units), calcium content (mg. per cc.), degree of biconcavity, and the presence of pink material in lumbar vertebrae in 150 consecutive post-mortem cases. Age, sex and duration of confinement to bed during the final illness are included.

Case No.	Age	Sex	Calcium content of 4th lumbar vertebra (mg. per cc.)	Step Wedge (units)	Bi-concave Index (per-ent)	Pink material in verte-bral bodies	Duration of confine-ment to bed (days)
40	11	M	81	8	95	-	0
41	62	F	65	6	86	+	4
43	69	M	67	5	77	+	2
44	29	F	83	8	85	-	3
45	61	M	66	6	77	++	33
46	56	M	76	7	79	-	8
48	56	M	89	8	84	+	22
50	48	M	56	6	83	+	4
51	56	M	73	8	84	+	0
52	54	M	67	7	80	-	11
53	67	F	59	6	74	+	31
54	61	F	55	5	85	+	9
55	20	M	80	8	80	-	7
56	63	M	59	6	87	-	0
57	59	M	68	7	87	+	7
58	67	M	54	6	83	-	3
59	71	F	73	6	65	+	4
60	81	F	60	6	97	+	0
61	69	F	49	5	78	+	16
62	66	M	57	6	88	-	14
63	67	M	49	6	75	-	31
64	43	F	83	7	82	-	1
65	61	F	74	7	75	+	2
66	79	F	64	7	81	++	1
67	54	M	91	8	84	+	3
68	37	M	88	7	89	-	13
69	70	F	77	7	96	+	3
70	69	F	60	5	87	-	53

Table 1 (contd.)

Case No.	Age	Sex	Bone Calcium	Step Wedge	Biconcave Index	Pink material	Confinement to bed
71	88	F	48	4	67	++	62
72	64	M	47	5	83	-	2
73	57	F	71	7	87	+	11
74	60	M	69	6	85	-	0
75	64	F	50	5	80	+	17
76	57	F	63	7	86	-	2
77	69	M	76	6	87	+	41
78	63	M	88	7	86	+	18
79	57	F	76	7	81	-	1
80	29	F	99	9	89	-	14
81	58	F	60	6	78	-	6
82	70	M	67	6	79	+	18
83	75	M	65	5	73	-	5
84	71	F	61	6	81	+	27
85	61	M	64	5	89	+	7
86	79	M	65	6	83	+	36
87	77	F	64	7	82	-	11
88	72	F	51	5	79	-	1
89	43	M	92	10	83	-	5
90	53	F	71	7	83	+	29
91	67	M	68	7	88	+	1
92	53	M	53	6	81	-	15
93	68	F	45	5	85	+	4
94	49	M	79	8	83	-	40
96	61	M	75	7	78	+	4
97	59	M	66	6	93	-	8
98	60	F	67	6	93	-	3
99	76	F	62	5	85	-	0
100	64	M	75	6	86	-	0
101	63	F	45	5	81	-	2
102	66	F	75	7	86	-	12
103	78	F	53	6	86	++	22
104	33	M	102	9	84	-	0
105	52	F	50	6	78	-	6
106	16	M	69	8	87	-	5
107	70	M	71	7	85	-	17
108	84	M	48	5	77	+	0
109	49	M	78	7	82	-	0
110	70	M	94	7	80	-	26
111	70	M	57	6	82	+	30
112	17	M	87	7	84	+	0

Table 1 (contd.)

Case No.	Age	Sex	Bone Calcium	Step Wedge	Biconcave Index	Pink Material	Confinement to bed
113	58	M	80	7	86	+	29
114	72	M	71	6	80	-	6
115	16	M	97	10	85	-	2
116	69	F	54	6	90	+	25
117	70	F	88	8	89	-	86
118	66	M	55	5	93	-	8
119	60	M	81	7	90	++	4
120	17	M	81	7	83	-	1
121	33	M	75	6	89	-	43
122	25	M	101	8	86	++	4
123	39	F	62	7	96	-	34
124	83	M	51	5	72	+	30
125	66	M	59	6	79	-	12
126	76	M	84	9	89	-	1
127	72	F	56	6	83	-	1
128	65	M	60	7	80	++	3
129	39	M	72	7	86	-	37
130	31	F	70	7	81	-	94
131	48	M	81	8	79	-	7
132	66	F	89	7	74	-	2
133	46	M	85	7	90	++	1
134	65	M	60	6	93	-	0
135	64	F	62	6	90	++	30
136	55	M	38	5	81	-	4
137	66	M	74	7	79	-	11
138	76	F	49	5	79	+	0
139	59	M	46	5	82	-	2
140	58	F	84	8	86	-	3
141	22	M	58	6	90	-	29
142	42	F	71	9	83	-	36
143	70	F	47	6	83	-	20
144	67	M	71	7	97	+	32
145	68	M	43	5	86	-	28
147	39	M	72	7	80	+	2
148	32	M	77	7	91	-	0
149	51	F	60	6	79	+	1
150	20	M	65	7	83	-	80
151	64	M	66	6	90	-	26
152	52	F	62	6	80	+	11
153	72	F	77	8	84	-	4
155	29	F	68	7	90	-	29
156	52	M	69	7	83	-	3

Table 1 (contd.)

Case No.	Age	Sex	Bone calcium	Step Wedge	Biconcave Index	Pink material	Confinement to bed
157	59	M	60	6	76	-	1
158	53	M	61	6	85	+	3
159	64	M	55	5	87	+	12
160	59	F	63	7	88	-	3
161	52	M	63	6	81	-	16
162	64	M	53	5	77	-	2
163	52	M	67	6	78	-	18
164	67	F	48	5	76	-	26
166	56	F	55	6	81	-	6
167	50	M	78	7	90	++	28
168	31	F	72	7	89	+	31
169	59	F	72	8	83	-	30
170	68	M	65	6	85	-	27
171	23	F	89	9	86	+	0
172	73	F	57	6	81	++	17
173	52	M	81	7	83	+	10
174	54	M	67	6	93	+	50
175	10	M	75	7	86	-	15
176	38	F	63	6	83	+	11
177	71	F	45	5	84	++	50
178	47	F	58	6	86	-	1
179	74	M	66	6	90	+	17
180	55	F	73	7	89	+	60
181	72	M	56	5	85	++	10
182	1	M	110	9	113	-	35
183	83	F	58	5	84	+	1
184	56	F	91	9	82	-	0
185	83	M	77	7	86	-	1
186	66	M	71	7	86	-	74
187	54	F	59	6	87	+	137
188	56	M	76	8	80	+	2
189	58	M	75	7	86	-	1
190	59	M	84	8	82	+	27
191	34	M	88	8	80	-	0
192	61	F	72	7	77	+	20
200	59	F	78	8	82	-	11
217	44	M	71	7	83	-	2
219	42	F	84	8	93	-	3
222	23	F	75	8	87	-	48

Table 2

The radiographic density (step wedge units), degree of biconcavity, and the presence of pink material in lumbar vertebrae in a further 150 consecutive post-mortem cases. Age, sex, and duration of confinement to bed during the final illness are included.

Case No.	Age	Sex	Step Wedge (units)	Biconcave Index (percent)	Pink material in vertebral bodies	Duration of confinement to bed (days)
193	54	M	6	83	+ +	5
194	5	M	7	83	-	37
195	39	M	7	93	+	7
196	59	M	6	93	+	1
197	40	M	8	90	-	1
198	71	M	6	88	+	17
199	35	M	10	73	-	7
201	69	M	6	90	+	52
202	52	M	7	80	+	76
203	60	M	7	93	-	49
204	66	F	7	83	-	12
205	68	M	5	87	+	2
206	61	F	6	83	+	3
207	48	F	10	87	-	1
208	79	F	8	81	+	4
209	63	F	7	83	-	1
210	64	F	5	73	+	1
211	70	F	5	81	+	1
212	62	F	8	83	-	2
213	67	F	5	81	+	15
214	63	M	4	87	+ +	2
215	51	F	8	84	-	12
216	79	F	6	77	+ +	1
218	74	M	5	88	-	20
220	58	M	7	83	-	0
221	59	M	7	91	-	4

Table 2 contd.

Case No.	Age	Sex	Step Wedge	Biconcave Index	Pink material	Confinement to bed
223	56	F	7	77	+	1
224	75	M	6	77	+	2
225	61	M	6	84	-	26
226	25	M	10	82	-	29
227	57	M	6	90	-	1
228	43	F	7	81	-	21
229	76	F	5	83	+ +	7
230	61	M	5	81	-	1
231	76	F	5	85	-	1
232	72	M	6	78	-	9
233	76	M	7	90	-	14
234	74	F	6	92	+	6
235	81	M	5	86	+	21
236	69	M	4	78	+ +	5
237	54	M	8	84	-	1
238	38	M	8	71	-	1
239	53	M	6	73	+	29
240	36	M	8	90	-	26
241	46	F	8	87	-	1
242	69	M	6	83	+	5
243	30	F	8	84	-	11
244	66	M	6	77	+	15
245	34	F	9	90	-	64
246	79	F	5	78	-	25
247	81	M	5	89	+ +	14
248	14	F	8	83	+ +	1
249	68	M	6	77	-	18
250	43	M	7	87	-	13
251	49	M	8	78	-	1
252	73	M	6	76	-	1
253	19	M	10	82	+ +	10
254	50	F	8	83	-	22
255	45	F	8	82	+	1
256	57	M	8	81	+ +	4
257	73	M	8	80	+ +	6
258	82	M	6	80	-	42
259	60	M	5	86	+	2
260	75	F	6	80	+ +	5
261	39	M	8	78	-	16
262	72	F	6	77	-	32

Table 2 contd.

Case No.	Age	Sex	Step Wedge	Biconcave Index	Pink Material	Confinement to bed
263	57	F	9	89	-	1
264	45	M	7	90	-	5
265	58	F	7	85	-	64
266	52	M	9	76	-	9
267	65	F	6	83	-	8
268	26	M	7	90	-	3
269	50	M	6	80	-	9
270	49	F	9	79	-	1
271	67	M	7	90	-	10
272	68	F	7	90	-	1
273	83	M	6	83	-	2
274	60	M	7	82	-	7
275	66	F	7	85	+	12
276	60	M	6	81	-	9
277	76	M	6	81	-	2
278	81	F	6	82	+	17
279	60	M	6	84	-	1
280	45	F	7	89	-	37
281	67	F	8	83	-	3
282	75	M	6	86	-	9
283	58	M	8	77	-	1
284	19	M	8	82	-	4
285	71	F	6	86	+	29
286	53	M	6	83	-	2
287	60	M	9	73	+	22
288	39	F	9	85	+	8
289	50	F	10	86	-	36
290	57	M	6	89	-	15
291	51	M	7	79	+	6
292	39	M	9	86	-	1
293	41	M	7	87	-	0
294	46	F	8	90	-	0
295	77	M	6	92	-	10
296	68	F	6	86	-	22
297	34	F	9	90	-	19
298	4	M	9	94	+	0
299	36	F	6	83	+	3
300	60	M	8	97	-	22
301	83	F	6	83	-	6
302	53	M	6	80	-	0

Table 2 contd.

Case No.	Age	Sex	Step Wedge	Biconcave Index	Pink Material	Confinement to bed
303	37	F	8	82	-	8
304	76	M	5	83	+	10
305	62	M	7	90	-	13
306	74	M	5	89	-	2
307	72	M	4	55	+	17
308	80	M	5	81	-	10
309	58	M	7	91	-	16
310	22	M	9	94	-	0
311	58	F	5	80	+	43
312	34	M	7	82	-	16
313	66	M	6	83	+	17
314	66	M	3	79	-	15
315	74	M	6	65	-	30
316	61	M	6	81	+ +	1
317	68	F	6	80	+	6
318	58	F	5	65	+ +	60
319	83	M	5	77	+	16
320	56	F	7	82	+	0
321	60	M	8	90	-	10
322	17	M	9	91	-	210
323	26	M	7	76	+	12
324	64	F	6	86	+ +	3
325	60	F	6	90	+ +	0
326	57	M	6	72	+ +	22
327	63	F	6	86	-	2
328	66	M	5	76	+	16
329	53	M	8	83	+ +	3
330	79	F	6	82	+ +	14
331	58	F	7	86	-	1
332	64	M	7	86	-	3
333	48	M	9	83	-	1
334	61	F	7	79	-	21
335	70	M	5	79	-	5
336	33	F	9	86	-	11
337	53	F	8	82	-	4
338	58	M	8	83	+	6
339	57	M	9	81	+ +	15
340	58	M	9	83	-	2
341	82	M	5	73	+ +	7
342	70	M	7	74	-	0
343	56	F	8	87	+	11
344	57	M	6	84	+	1
345	45	F	7	72	-	1
346	52	M	7	69	+	0

Table 3

Calcium content (mg. per c.c.) of male and female lumbar vertebral bodies; average in each age group; number of cases in each group in brackets.

Age group (years)	0 - 34	35 - 44	45 - 54	55 - 64	65 - 74	75 - 84	85 -94
Males	76·4 (15)	76 (6)	70·7 (13)	67·9 (22)	64·9 (21)	66·6 (7)	-
Females	79·4 (7)	75·3 (4)	64·6 (8)	67·3 (20)	62 (19)	58·6 (7)	48 (1)

Table 4

Calcium content (mg. per c.c.) of lumbar vertebral bodies grouped according to their radiographic density in step-wedge units

Radiographic density (step-wedge units)	Males				Females				Average Calcium for males and females (mg.per cc.)
	No.of cases	Calcium mg.per c.c.		Average Age	No.of cases	Calcium mg.per c.c.		Average Age	
		Average	Range			Average	Range		
4	-	-	-	-	1	48	-	88	
5	13	53	36-67	68	13	51•4	45-62	69•9	52•2
6	28	63	49-76	60•6	19	59•5	47-73	62•0	61•3
7	29	75•8	60-83	51•2	18	71•5	62-89	55•1	73•7
8	12	75•2	69-101	40•3	8	80•1	72-88	51•5	77•7
9	3	95•3	84-110	36•8	4	87•7	71-99	37•5	91•5
10	2	94•5	92-97	28•5	-	-	-	-	

Table 5

The average radiographic density of lumbar vertebrae of male and female subjects arranged in 10 year age groups.

Age	Males		Females	
	No. of Cases	Average step wedge reading	No. of Cases	Average step wedge reading
0-9	3	8	-	-
10-19	9	8	1	8
20-29	7	8	6	8
30-39	14	7	11	7•7
40-49	14	8•6	11	8•0
50-59	45	6•9	29	7•2
60-69	53	6•0	30	6•1
70-79	25	6•0	27	6•0
80-89	10	5•4	5	5•4
90 and over	-	-	-	-
	180	7•1	120	7•0

110 mg. Ca. per c.c.). In seventy five per cent of cases calcium values fell within the narrower range of 52 to 83 mg. per c.c. Higher calcium contents were observed in younger persons. Thus the three most heavily calcified vertebrae with 110, 102, and 101 mg. Ca. per c.c. respectively, were from males aged sixteen months, 33, and 25 years of age. One with 99 mg. Ca. per c.c. was from a woman aged 29 years. Males tended to have more calcium in a unit volume of vertebral bone than females. The average in 87 male cases was 69·5 mg. Ca. per c.c. (range 38 to 110) and in 63 female cases the average was 65·6 mg. Ca. per c.c. (range 45 to 99).

Even when allowance is made for the greater proportion of young men than of young women, as in any general hospital necropsy series, the lower calcium content of female bones is still evident at all ages (Table 3). This table also indicates the decline of calcium in the vertebrae with increasing age, when the figures are expressed as averages. A wide range of calcium values was however still encountered in the older age groups but with the number of results available it was not possible to calculate a statistically significant regression of calcium on age. A better correlation was achieved between age and radiographic density measured on a ten-degree scale.

The correlation between radiographic bone density and bone calcium content

Table 4 shows the average and range of calcium content of vertebrae manifesting radiographic densities of 4 to 10 step-wedge units. A relationship between calcium quantities and X-ray densities is apparent as may be expected *a priori* but I was concerned with the possibility of predicing the calcium content from the step-wedge reading, and of seeing whether in doing so it was necessary to take into account the factors of sex, age, and number of days in bed for which information was in each case available.

Miss Hilda M. Davies of the Department of Statistics kindly undertook the analysis and has reported as follows:

> Multiple regression analyses of calcium on step-wedge reading, age and number of days in bed during the final illness were carried out for males and females separately, and standard errors of regression coefficients calculated. In both cases the regression of calcium on age and on days in bed proved to be non-significant while that of calcium on step-wedge reading was very highly significant and it can therefore be assumed that some estimate of the amount of calcium can be obtained from the step-wedge reading, and that in doing so there is no need to take either age, or number of days in bed into account. This hypothesis was further supported when the product-moment correlation, and partial correlation coefficients were computed and it was found that the highly significant correlations between calcium and step-wedge reading of about 0·8, both for males and for females was very little altered by correcting for either, the age factor, or the days in bed.

To investigate the possibility of a sex differ-

ence in such a prediction a multivariate test of significance (Bartlett, 1947) was applied, to test the difference between the bivariate mean values of step-wedge reading and calcium for males and females. This gave an F criterion which was significant at the 5 per cent. level, suggesting that there is a sex difference. An analysis of variance on calcium and step-wedge data for both sexes combined testing the effects due to common regression, to difference in location of sex means, and to the very small difference in slopes of separate sex regressions gave no significant difference between the separate slopes but gave an F criterion for difference in location of means which was significant at the 1 per cent level (N = 150), thus bearing out the conclusions of the previous test.

Hence it would appear that the regressions of calcium on step-wedge reading are parallel for the two sexes but slightly different in position. The separate regression lines of calcium on step-wedge reading for males and females were fitted by the usual method of least squares. The difference between the two regression coefficients was tested against its standard error by the usual students' t-test and proved not significant, the value of t, in fact, being 0·56 with ninety six degrees of freedom, which accorded well with the appropriate value of F in the analysis of variance and bore out the hypothesis that the data are samples from two populations, which, though having a different mean value, have the same regression slope.

It would seem therefore that the step-wedge reading can be used to give a prediction of amount of calcium present provided that the sex is taken into account and the appropriate regression used. The 95 per cent confidence limits for such a prediction have been calculated in each case.

Figure 3 (males), and Figure 4 (females), show the distribution of calcium quantities in each step-wedge group. The regression of calcium on step-wedge readings and the

calculated 95 per cent confidence limits for predicting calcium content from radiographic density in the step-wedge scale here used are also shown.

Figure 5 compares the regression of calcium on density readings in the two sexes. The statistical analysis revealed that the difference in the location of the means was significant, but the implication of this result is not clear, and the matter will be referred to in the discussion.

Radiographic bone density

It was now realised that because the degree of correlation between the radiographic bone density and the bone calcium content was so satisfactory, step-wedge comparisons were sufficient, and no more calcium estimations were performed. A further 150 cases were examined by the radiographic method (Table 2) thus bringing the total number of cases examined by this method to 300. Of these, 180 were males and 120 were females whose ages ranged from sixteen months to eighty eight years, and their age and sex composition is shown in Figure 6. The age and sex composition of these cases which were adjudged to be osteo-porotic because they had a radiographic bone density which corresponded to less than 6 steps of the step wedge (see discussion) is shown in Figure 7.

Table 5 shows the average step-wedge readings of vertebrae in 10-year age groups of men and women and Figure 8 the scatter diagram of radiographic density readings on age.

Figure 9 shows the regression of radiographic density on age. Miss Davies's statistical report was as follows:

> It was noted that the negative product-moment correlations between step-wedge and age were significant and not much influenced by the presence or absence of the other effects while that of calcium on age with step-wedge held constant was in both cases very small and well below the border-line of significance implying some relationship between step-wedge reading and age, but not between calcium and age. In view of this, an analysis of variance of similar type as before was carried out for the regression of step-wedge on age. This gave a very highly significant value for the combined regression ($F\ 1,146 = 61{\cdot}64$ $N = 150$) while there was no significant difference for the two sexes between, either, the separate slopes, or the separate locations (F slightly<1 in both cases) implying that there is no sex difference in the effect of age on step-wedge reading but that there is a marked overall regression effect. The data for the relationship of step-wedge and age was grouped in decades and means for each decade were plotted on a graph distinguishing between male and female. These suggested that the relationship was curvilinear rather than linear, and a parabola was fitted to the original data separately for each sex and for the group as a whole. The parabolic regression was found to be a significantly better fit than the straight line (giving $F\ 1,297 = 5{\cdot}49$ which is about equal to the 2 per cent criterion level of significance). It is clear from the graph that even the curve is not ideal, as there appears to be a sudden drop in the step-wedge reading between the 40- and 50- decades, more than is accounted for by the

fitted curve and more so for the female group than the male. It is hoped to investigate this further when time permits as no adequate statistical tests for these differences have yet been made.

The technique of radiographing parallel-sided sagittal slices of the vertebral bodies of uniform (1 cm.) thickness, laid flat on the film cassette, made it possible to obtain very clear pictures of the trabecular structure, cortical thickness, and contour of the bones. These features are well shown in Figures 10 and 11. In particular, the highly rarefied bones of case 88 as shown in Figure 10, show how in osteoporosis the strong system of little bony plates of the normal vertebral spongiosa are reduced to a delicate web of thin struts, as Collins (1959) demonstrated in macerated specimens. Cortical atrophy is not the most important feature of vertebral osteoporosis; the circumferential cortex of a vertebral body is thin in any case and may become more prominent in osteoporosis, so-called stencilling of outline, by virtue of the greater translucency of the centrum. The bones illustrated in Figure 11 show an average radiographic density and texture. The radiograph of the bone slab gives much more information about cancellous texture than does the thin histological section where many trabeculae are incomplete and cut across obliquely (Figs. 12 and 13).

Bone histology

Histological sections were prepared in all cases. This was usually carried out on the 3rd lumbar vertebra and served to relate the histological pattern to the radiographic density and also to exclude other conditions such as osteomalacia, osteitis fibrosa, Paget's disease, tumour infiltration, or other causes of bone rarefaction or sclerosis.

Where such conditions were absent and there was marked reduction in trabecular thickness with cancellisation of the cortex, and loss of many of the transverse trabeculae, it was possible to conclude that any vertebral slab having a step-wedge reading of 5 or less with this method, showed unequivocal osteoporosis.

Cases up to the age of 21 years showed areas where osteoblasts lined the bone trabeculae, but, after this age the trabeculae were devoid of cells lining their free borders of either osteoblastic or osteoclastic type.

Pink material

In a proportion of cases sections showed the presence of an eosinophilic structureless material resembling a fluid coagulum (Figs. 14 and 15). This pink material was found mainly in elongated masses close to and parallel with trabecular surfaces, but it was also present in various

spaces and gaps created by section cutting, and occasionally it occupied an area similar in shape to a trabeculum. Pink-staining material is not infrequently seen in histological sections stained by eosin and is caused by local accumulations of the adhesive (albumin) used in mounting the section on the glass slide. However in view of the much greater frequency of this material in sections of osteoporotic bones (Fig. 16), and the theory that osteoporosis is due to an increased rate of bone resorption (see Introduction), pink material was further investigated. An estimation of the frequency of the material is recorded in Tables 1 and 2. The symbol + designates two to four foci per histological section, and ++ five or more foci. The relationship between the presence of two or more foci per section and the radiographic density of the bone (step-wedge units) is shown in Figure 16. The curve demonstrates that osteoporotic bones are much more liable to contain pink material.

The material was insoluble in absolute alcohol and xylol thus eliminating the possibility of its being celloidin or paraffin wax, and it was noted that streaks or collections of the material were nearly always on the same side of contiguous trabeculae throughout each section, and that these trabeculae showed slight displacement in one

direction, probably due to pressure from the microtome knife.

Sections containing pink material and slides smeared with the adhesive (glycerine albumen) were stained for acid mucopolysaccharides by the Alcian blue and Hale methods, for glycogen and mucins, by the Periodic acid-Schiff method, and for tyrosine-containing protein, by the Millon reaction.

Both materials gave a positive result with the P.A.S. method and negative results with the other methods. It was thus established that this pink material was the adhesive and that its greater frequency in osteoporotic bones was due to their liability to sustain trabecular damage or displacement during section cutting, which permitted the adhesive to enter the gaps so produced.

Brush borders

As interest had been aroused in the subject of bone lysis, sections from 30 cases whose ages ranged from 5 to 84 years were stained for reticulin fibres (see discussion). Many of the trabeculae so treated showed a ciliated margin or brush border (Figs. 17 and 18). These were not demonstrated in the four cases aged between 5 and 21 years (Fig. 19), but in all the cases over 21 years of age brush borders could be demonstrated, although it was not found possible to equate the frequency of their appearance with

the bone density.

Vertebral biconcavity

The presence of vertebral biconcavity is frequently regarded as a diagnostic sign of osteoporosis (see discussion), and it can be measured from the radiographic profile of the vertebral body. The ratio of the least to the greatest vertebral depth is similar to that used by Barnett and Nordin (1960), and is expressed as an index in Tables 1 and 2. An index of 80 per cent or less indicates severe biconcavity (Figs. 20 and 22). The 4th lumbar vertebra was used in all cases.

In 51 cases which had a step-wedge value of 5 or less the average biconcavity index was 80·1 per cent (range 55 to 93), whereas in 29 cases with a step-wedge value of 8 or more, the average index was 85·5 per cent (range 72·6 to 113). The incidence of biconcavity in vertebræ of various step-wedge densities is plotted as a curve in Figure 23. The incidence is high in osteoporotic bones, and falls sharply when the range of normal bone density is entered, but 14 per cent of vertebrae of very high radiographic density (step wedge 10 units) show biconcavity according to these criteria (Fig. 22).

The incidence of osteoporosis

The incidence of osteoporosis in this series of 300

cases was 17 per cent (Fig. 7). Of the 51 cases with a step-wedge value of 5 or less, there were 30 males and 21 females. The incidence of osteoporosis in male subjects was 16·7 per cent and in female subjects 17·5 per cent. Among patients aged 50 years and over the incidence in males was 22·5 per cent, and in females 23·1 per cent i.e. approximately one case in every 4 to 5 patients. The youngest female to display spinal osteoporosis was aged 51 years and the youngest male 55 years.

DISCUSSION

The results show that the methods used in this investigation are appropriate to the study of post-mortem material. They confirm that the radiographic density of cancellous bone is statistically related to its calcium content (Table 4 and Figs. 3 and 4), a fact which was implicit in Virtama's (1957) radiological investigation of the phalanges post mortem, and was to be expected on *a priori* grounds. By using parallel-sided bone slabs of standard thickness devoid of superimposed soft tissues the radiographic measurement by means of a step-wedge, and a photo-electric cell, becomes much simplified. To counteract the variables of soft-tissue shadows, and distance between bone and film in clinical radiography, complex methods must be used such as those devised by Mack, *et al*. (1949).

For present purposes one was satisfied that the simple radiographic procedures as outlined in the description of methods and in Figure 1, gave a sufficiently accurate prediction of the mineral content of the bone specimens for use as a method of surveying post-mortem material that is much quicker than chemical analysis. In individual cases some discrepancies between radiological and chemical measurements were encountered (Figs. 3 and 4), and the peculiar fact emerged that though falling into the same step-wedge group of density, female bones tended to have slightly lesser amounts of calcium than males (Fig. 5). At the step-wedge value of 7, female bones contained on the average 6·8 per cent less calcium than did male bones.

The reason for this discrepancy probably lies in the fact that in a specimen of cancellous bone one is radiographing a system of calcified trabeculae dispersed to a varying degree in soft marrow tissues that also absorb some rays.

Carstairs (1959) stated as a result of X-ray diffraction studies that calcium salts vary in composition in the bones of different individuals, and Mack et al. (1949) stated as a generalisation that calcium is responsible for about 80 per cent of X-ray absorption by bone, but this proportion obviously varies between individuals and also

with the ratio of calcified structure to soft tissue mass. The radiographic scale based on a 10-step aluminium wedge gave in effect only 8 degrees of bone density, since no bone was encountered so rarefied as to match with the first 2 steps of the wedge. Two hundred and sixty seven of the 300 cases showed densities corresponding with the four step-wedge units 5 to 8. The 29 bones of greater density showed no pathological variation of either radiographic texture (Fig. 10), or of histological structure. Of the 4 examples of bones showing a step-wedge reading of 4 or less, one was from an old lady aged 88 years, who on account of "pemphigoid" had been treated for several weeks before her death with ACTH and prednisolene which may have accentuated the osteoporotic process. In no other case were there diseases, or treatments, or other circumstances, which might have caused or accentuated osteoporosis. It is interesting also that none of the cases in whom osteoporosis was disclosed, complained of symptoms referable to this condition and it was clinically unsuspected.

Radiographs of the bone slabs gave a very clear picture of the cancellum of the vertebral bodies (Figs. 10 and 11), and they also showed to what a small extent cortical compact bone entered into the specimen taken in this manner. Both radiographic and chemical methods

mainly measured variations in the size, number, and degree of separation of trabeculae, and differences in the degree of calcification of the osseous rami. It seems highly probable that osseous rami do differ in the extent to which they are calcified and although osteoporotic bone has not yet been specially investigated, Amprino and Engström (1952), have shown by microradiography not only that the osteones of cortical bone vary in their degree of mineralisation, but that more imperfectly calcified structures are present in spongy than in compact bone. These structures with a low content of mineral salts represent bone in the process of being mineralised (Engström, 1956).

It would be important, therefore, with a view to establishing the direction of metabolic activity of osteoporotic bone, that the vertebral cancellum should sometime be studied either by microradiography or by interference microscopy (Davies and Engström, 1954). In every case as in the present series, ordinary histological preparations must be examined since this is the only way of excluding gross variations in the calcium/osteoid relationship such as pertains in osteomalacia, and of recognising hyperparathyroidism, or other pathological causes of bone resorption. In 10 of the 300 cases the radiograph revealed a patchy unevenness of the shadow of the lumbar vertebral body similar

to that described by Carstairs (1959). This was unrelated to kyphosis, other deformity, or apparent disease. It was not explained, but as it did not interfere with obtaining an average photometric reading of density for the whole bone it was not further investigated.

Loss of the transverse trabecular markings, and accentuation of the vertical markings is a valuable and constant sign of osteoporosis (Fig. 10), and this characteristic was found to correspond with a density reading of 5 step-wedge units or less. I was confident in recording as osteoporotic the 51 cases (30 male, 21 female) showing this degree of rarefaction.

Biconcavity

The finding of biconcavity in the radiographs of both normal, and osteoporotic bones in this series (Figs.20 and 22) calls for some discussion of other reported findings. It has come to be regarded by many authors as indicative of osteoporosis but in my experience it was of little value in assessing the presence or absence of this condition (Fig.21). Black et al. (1941) in a radiological study of 208 cases of spinal osteoporosis, noted vertebral biconcavity in all of 19 cases in whom the condition was severe. Cooke (1955) tacitly concurred with this, and stated without qualification that in osteoporosis the vertebral centra became

biconcave, and Nassim (1959) also, gave no indication that severe osteoporosis might be present without biconcavity. On the other hand Bohatirchuk (1955) who performed clinical radiological examination of 75 subjects, of whom 69 were over 60 years old, stated that despite the presence of osteoporosis, the vertebrae in subjects who were otherwise normal retained their shape (Fig. 21). Collins (1949, 1959), formed the opinion that biconcavity resulted from pressure of the still turgid intervertebral disc and that if osteoporosis developed after the discs had degenerated or collapsed, the vertebral body did not become deformed in this way. He suggested that biconcavity was a reflection of mutual stresses between vertebral body and adjoining discs, and, that if biconcavity was regarded as certain evidence of deformation of a softened vertebral body, then the finding of some biconcave vertebrae of normal density could only be explained by assuming that such bones had become remineralised after a period of osteoporosis.

In view of the fact, that with the exception perhaps of one reported case of osteoporosis in a growing subject with Cushing's syndrome (Wang and Robbins, 1956), no authenticated cases of redensification of bone have been reported in cases of generalised osteoporosis, the presence

of at least one biconcave vertebra in 14 per cent of the cases (Figs. 22 and 23) which showed the highest grade of radiographic bone density in this series (step-wedge 10 units), merits further investigation of this problem.

Carstairs (1959) has postulated other possible factors which might lead to biconcavity such as changes in the composition of calcium salts in the trabeculae, alterations in the physical arrangements of these salts, and alterations in the nature and quantity of trabecular cellular elements. The results of my investigation did not shed any further light on this matter.

Calcium analysis results

The chemical results giving the amount in mg. of calcium per unit volume (1 c.c.) of anatomical bone are of special interest, because, so far as I know, calcium assays have not previously been made of human vertebra and this method of expressing the results has not been generally adopted in other bone analyses. It seems however to be the only way in which the results can be expressed in order to measure the changes in the bones which I have, somewhat arbitrarily, called osteoporotic, and to tally with readings of radiographic density. Ingalls (1931) weighed individual bones from 100 cadaver skeletons after a standard drying procedure, but he did

not estimate their volumes and therefore his method did not allow for arthritic changes. His curves of individual total bone weights of vertebral bodies in 10 year age groups showed as a result a gradual increase in the older age groups, and his results are consequently not comparable with my own.

The range of calcium contents encountered, was quite surprisingly wide, from as little as 38 to as much as 110 mg. Ca. per c.c. of bone, but 75 per cent of cases fell within the narrower range of 52 to 83 mg. per c.c. The average for the series of 87 males was 69·5 mg. per c.c. (range 38 to 110), and for the 63 females was 65·6 mg. per c.c. (range 45 to 99). These figures cannot be taken as averages of general application; much depends on the ages of the subjects comprised in any sample, since it is clear that there is a steady decline with age both of calcium content (Table 3) and of radiographic density (Table 5 and Fig. 9). Even in age-groups the range of calcium results is still wide, and the occasional old person may be found with vertebrae that are highly calcified though histologically normal. It was not possible to establish an exact level of calcium content below which a vertebra may be held to be osteoporotic, but on general grounds the figure of 60 mg. Ca. per c.c. seemed to be about the lower

limit of normal in the adult subject. It was more practicable, if less precise, to name a bone as osteoporotic on radiographic density and texture, other causes of rarefaction having been excluded. Nevertheless, although there was some overlap of individual results, the generally low calcium of the osteoporotic bones of step-wedge values 4 or 5, is apparent in Table 4. The average calcium for the 13 osteoporotic males was 56·9 mg. per c.c. as compared with an average of 72·6 for the 74 male cases judged to show no osteoporosis. In the 13 osteoporotic female cases, the average calcium was 51·1 mg. per c.c., as compared with an average of 69·2 for the remaining 50 female cases judged to show no osteoporosis.

When the results of calcium analysis are arranged in groups of 10 mg. per c.c., and shown against the percentage of cases in the series whose vertebra contain this amount, the plotted curve for both sexes combined is a fairly smooth one, but when the sexes are shown separately there is a peak for females which is to the left of the peak for males (Fig. 24). The skewness of the curves may well be due to the inclusion in the series of a majority of older subjects who tend to show lower calcium values. The main fact which emerges from a study of these curves

and from the distribution of the density readings shown in Table 4, is that there is no separate peak which might indicate that the sample contained two populations of "normal", and "pathological" bones.

The results indicate the numerical range of mineral disturbances to which the vertebrae are subject. In round figures it may be stated that the lumbar vertebrae, among a mainly elderly hospital population, are calcified to the average extent of 70 mg. Ca. per c.c., with extreme divergences of 30 mg. on either side, bones containing 40 mg. Ca. per c.c. being severely osteoporotic, and bones containing 100 mg. Ca. per c.c. being mainly among the strong bones of young men. Statements have often been made that a bone must lose at least half of its calcified bone mass before it can be recognised as osteoporotic in the clinical radiograph (e.g. Fraser, 1959). Fusi (1953), who largely excluded the soft tissue interference by using vertebral body slabs of similar thickness to my own, reported radiological evidence of demineralisation when only 3 per cent of the original calcium content was extracted, and with an extraction rate of 30 per cent the radiological changes were strongly evident; but when the body soft tissues were interposed an extraction of 60 per cent of the calcium was necessary to show a notable abnormality. My

analyses partly confirm these findings since on the average vertebrae judged to be osteoporotic contained 15·7 per cent less calcium in the male and 18·1 per cent less in the female. There is of course no way of estimating the loss of calcium in any individual bone affected by osteoporosis, since its original calcium content remains unknown. Indeed it seems that the vertebrae in any individual are most heavily calcified in youth, and that in the vast majority of subjects they then proceed to lose calcium (and bone substance) progressively throughout life. Amprino and Bairati (1936) who examined histologically, femoral, phalangeal, and occipital bones, but not vertebral bodies, from a large number of subjects, whose ages ranged from foetal life to 94 years, reported that between the ages of 35 to 55 years resorption was not so well compensated as in younger age groups, and that from 55 years onward, erosion overtook apposition and was more intense in females. My own results to a large extent confirm these findings but in my series the loss of calcified bone substance commenced earlier in adult life and the period of more intense loss of bone occurred approximately a decade earlier, at least in female subjects. Gershon-Cohen et al. (1955) used a

radiographic method to study the density of the middle phalanges of the little fingers, and the os calcis of 149 elderly subjects. It is difficult to relate their finding of greater bone density in females than in males, and their conclusion that bone density increased with advancing age, with my own, and with other reported results. However they did not reveal how their bone density coefficients were calculated except to say that this was done by physicists. It may be, that as in the case of Ingall's (1931) results, the hypertrophic changes of arthritis influenced the findings.

It seems that in osteoporosis, as in osteoarthritis (Collins, 1949) and, perhaps, as in hypertension (Pickering, 1955) one may be confronted once again with the problem of determining how, and in what manner changes that are so frequently the accompaniment of age are to be distinguished as pathological.

Brush borders

Junghanns (1931) was probably the first to suggest that the porotic changes which occur in the vertebral column of the elderly, are part of a normal and therefore physiological ageing process, but in this series, the presence of structurally normal bones of high normal density

in elderly subjects suggests that this is not universal. Two main theories have arisen which could explain the mechanism of production of osteoporosis at bone level. The theory of Pommer (1914) frequently referred to as the hormone-cellular hypothesis, stated that osteoblastic activity diminished, but osteoclastic activity continued at its normal rate. This theory was supported by no less an authority than Albright (1947a) who suggested that osteoblastic activity depended on an adequate production of adrenal 'N' hormone, and the appropriate sex hormone for the individual concerned. The other theory is that of humoral bone resorption, and it was first proposed by Kilian (quoted by Beck, 1925). This envisaged an atrophic absorption by a chemical process without obvious cellular action, and it was supported by Ham (1952), who stated that there were good reasons for doubting that osteoclasts were necessary for bone resorption. He also stated that the mineral containing cement and the reticulin fibres of bone do not dissolve simultaneously but that the latter remain just a shade behind and look like cilia.

The finding of pink material (Figs. 14 and 15) in my histological sections, drew my attention to the problem of bone resorption in osteoporosis, and the subsequent demonstration of numerous brush borders or 'cilia' (Figs.

17 and 18) in the absence of osteoblasts and osteoclasts is considered to be evidence in favour of the humoral theory of resorption. Brush borders were found in cases over 21 years of age in bones of normal density as well as in osteoporotic bones. It would thus appear that steady bone resorption occurs throughout adult life and its rate is greater than the rate of formation (Fig. 9). The failure to find osteoblasts in any of the sections from adult material, suggested that until the age of 40 to 45 years the rate of resorption was slow although it accelerated thereafter. It is perhaps significant that the period of accelerated bone resorption coincides with the decline of sex-gland activity particularly in the female but also to some extent in the male. This is of special interest, because although many authors (e.g. Cooke, 1955; Bartter, 1957) accept Albright's (1947b) statement that osteoporosis is due to decreased production of osteoid, and therefore to diminished formation and activity of osteoblasts, others (e.g. Shorr, 1950; Urist, 1958; Fraser, 1959) on the basis of metabolic balance studies, believe that an increased rate of bone resorption is the more important factor. Ham (1952) however, has stated that resorption of bone will always take place when its surface is unclothed by a layer of osteoblasts.

The evidence of more rapid progression of osteoporosis at the time of the menopause in my cases may, perhaps, be the result of oestrogen withdrawal leading to even further diminution of formation and activity of these cells. If this is the case then both the theories of causation of osteoporosis at bone level are partly correct.

The incidence of osteoporosis

The case has now been presented to show that by taking into account the radiographic, and histological findings, supported by the results of calcium analysis, of lumbar vertebral slabs, a reasonably accurate assessment of the calcified bone mass per unit volume of bone, can be made. A consecutive series of unselected post-mortem cases is more reliable than a picked series, but even they were selected from a special hospital population and this itself varies between hospitals.

All studies of incidence are based on the somewhat arbitrary and self-imposed standards of the investigators, but by my own standards I have called osteoporotic the lumbar vertebrae of 30 out of 180 males (16·7 per cent) and 21 out of 120 females (17·5 per cent).

From histological examination of 40 osteoporotic vertebral columns Pañella-Casas and Monteys-Orta (1950)

reported that females predominated in the ratio of 5 : 1. Cooke (1955) and Buhr and Cooke (1959) reported that in clinical radiography, osteoporosis is six times commoner in women than in men, but Jackson (1958) found 27 male to 11 female patients with osteoporosis of unknown cause, developing in men before the age of 55, and in women before the menopause. Using entirely different though equally arbitrary standards, namely the flotation of cubes cut from the vertebral centra in copper sulphate solutions of specific gravity 1·050, Collins (1959) studied a large series of hospital necropsies, and calculated the incidence of spinal osteoporosis as 8 per cent among 189 men, and 18 per cent among 147 women aged 40 years or more. In samples of iliac crest bone from 275 consecutive necropsies, Beck and Nordin (1960) reported a 12 per cent incidence of osteoporosis in males, and a 23 per cent incidence in females, by histological assessment.

There is no information of the age at which porosis first developed in my series of cases but only four patients. two males aged 55, and 59, and two females, aged 51 and 58, were younger than 60 years at the time of death.

The period of confinement to bed during the last illness was noted but could not be correlated with the state of the vertebrae. It was considered that confinement to bed as it occurs in the ordinary hospital case had

no bearing on the development of osteoporosis. This was in keeping with the findings of Stevenson (1952) who found that immobilisation must be rigid to produce osteoporosis, and of Wyse and Pattee (1954) who showed that muscular contractions prevented its appearance even in such cases.

The nature of the fatal illness was too varied in this series of cases to have any apparent bearing on the condition of the bones.

SUMMARY

Radiological, and histological examinations have been made of the lumbar vertebral bodies in 300 consecutive necropsies on patients dying in a general hospital, and calcium estimations were also made on the first 150 cases of the series, with a view to determining the range of variation of calcium content and radiographic density in normal and osteoporotic bone.

Radiographs were made of sagittal mid-line vertebral body slabs uniformly 1 cm. in thickness, and the radiographic density of these specimens was measured in relation to an aluminium step wedge of 1 to 10 units. Radioopacity of different vertebrae ranged from 3 to 10 units. The specimen radiographs also clearly revealed the trabecular structure and the lateral profile of the bones.

Calcium was chemically estimated and expressed as weight of the element per unit volume of the whole bone mass (i.e. of anatomical bone including soft marrow tissue). It ranged from 38 to 110 mg. per c.c. of bone. In 75 per cent of the cases the range was 52 to 83 mg. per c.c. of bone. High calcium values were mostly encountered in young adults, and the calcium per unit volume tended to diminish with age, but a wide range of calcium was still encountered in the older age groups; a better correlation with age was achieved by radiographic density. Both calcium content, and radiographic density, tended to be higher in the male than in the female bones at all ages.

The calcium results showed a fairly smooth distribution curve but it tended to be rather skewed due to the inclusion in the series of more older people with less well mineralised bones; the absence of a double peak in this curve suggests that the examination was made on a homogeneous population and does not indicate a separate pathological group of osteoporotic subjects.

Arbitrary standards must be used to distinguish osteoporotic from normal bones, since neither radiological measurements, chemical assay, nor histological assessment reveal a point at which the two groups can be

separated.

In the present series, it seemed satisfactory to regard as abnormal, all bones showing a radiographic density of 5 or less step-wedge units, and by this standard 51, or 17 per cent of the 300 cases (30 M., 21 F.) were deemed to be osteoporotic. Of patients over the age of 49, 22·5 per cent of the males and 23·1 per cent of the females showed osteoporosis.

Histological examination excluded other forms of bone rarefaction and revealed changes consistent with a degree of acellular, or humoral bone resorption in both normal and osteoporotic bones of many of the adult subjects. No evidence of bone formation was seen in any of the adult cases. Vertebral biconcavity was noted in a small number of cases showing normal bone density, and was not infrequently absent in cases of osteoporosis. Vertebral biconcavity is therefore not a reliable sign of osteoporosis.

The regression of calcium on the density measurements proved to be statistically significant, and was not affected either by age, or by the number of days in bed during the last illness.

A small difference between the sexes was apparent, there being slightly less calcium in the female than in

male bones of equal radiographic density. Provided this is taken into account, the radiographic density scale can be used to predict the calcium content of vertebral bone specimens, and should prove a rapid and accurate method in a survey of osteoporosis in post-mortem room material.

The relationship between step wedge density and age for the series gave a very highly significant overall regression effect, but there was no significant difference between the curves for the different sexes, either between the separate slopes, or the separate locations. There was an accelerated falling away of the curve between the 40- and 50- age groups, which was rather more pronounced in the female. This more rapid period of diminution of radiographic bone density corresponds to the period of sex-gland involution, particularly in the female.

PART II

A STUDY OF THE HISTOLOGICAL AND HISTOCHEMICAL PATTERNS OF HUMAN ADRENAL GLANDS IN OSTEOPOROTIC AND NON-OSTEOPOROTIC SUBJECTS

A STUDY OF THE HISTOLOGICAL AND HISTOCHEMICAL PATTERNS OF HUMAN ADRENAL GLANDS IN OSTEOPOROTIC AND NON-OSTEOPOROTIC SUBJECTS

INTRODUCTION

Many advances in our knowledge of the function of the adrenal cortex have taken place during the last twenty five years, for it was in 1936 that Hans Selye first described his concept of a "General Adaptation Syndrome" and the essential part which he considered that the adrenal cortical hormones played in this reaction. In 1946, he extended this concept to include the possibility that hormonal imbalance could produce certain specific diseases, for instance hypertension, polyarteritis, rheumatic fever, rheumatoid arthritis, and diabetes mellitus. Later, however, as a result of many experiments it was shown by Ingle (1953), that the adrenal corticoids in fact only rendered conditions suitable for the normal metabolic response of the body to trauma, or stress, to proceed, and it became apparent that the role of the adrenal corticoids was only a secondary one. This has now come to be described as a "permissive action" and its general acceptance has deprived Selye's theories of much of their significance.

The history of the growth of knowledge of adrenal cortical enzymes, and their relationship to function has been reviewed by Symington (1958), but only such aspects of this complex subject as appear relevant to a possible association between adrenal cortical activity and osteoporosis, will be mentioned.

Albright (1947b) postulated that a form of adrenal failure in elderly subjects may be associated with the development of senile osteoporosis, and this adrenal failure which he postulated, was termed by him as an "adrenopause", by analogy with the menopause, which is due to the cessation of ovarian hormonal activity. The very aptness of the simile lent substance to his theory, so much so, that other writers on the subject of osteoporosis who took up the theme (e.g. Cooke, 1955), have enhanced its plausibility by mere repetition. Albright (1947b) also suggested that osteoporosis of endocrine origin, for instance in Cushing's syndrome, might be due to an excess production of adrenal 'S' hormone over adrenal 'N' hormone, but so far as I can ascertain from the literature, no study of adrenal cortical function has ever been related to bone density.

Histochemical and histological studies of the adrenal

glands have frequently been correlated with their state of activity however, and these will now be reviewed briefly, so that criteria may be established in order to determine a possible failure of activity which could be described as an "adrenopause", and applied to a series of osteoporotic and non-osteoporotic cases. Descriptions of the histochemical and histological appearances of adrenal glands under normal conditions, following stress, and after administration of corticotrophin, have recently been published (Symington, et al., 1956; Symington and Davidson, 1956).

Furthermore it has been stated (Grant, et al., 1957) that certain of the changes which were described by these authors, can be interpreted in terms of adrenal functional activity. These histochemical changes are as follows:

Ribonucleic acid (RNA) is present in granular form in the cytoplasm of cells of the zona reticularis, and zona glomerulosa of normal adrenal glands, but the zona fasciculata of these glands is composed entirely of clear lipoid-containing cells which are devoid of RNA granules. However, as a result of stress, or administered ACTH the clear cells of the zona fasciculata become compact in appearance, with intensely eosinophilic cytoplasm rich in RNA, and devoid of lipoid. The result of this is a

merging of the zona fasciculata with the underlying zona reticularis. Simultaneously, there is an increase in the activity of phosphatase and dehydrogenase enzyme systems which closely parallels the altered RNA pattern. Symington (1959) has stated that such alterations in the morphology and histochemistry of the cells of the zona fasciculata are associated with an increase in 11β hydroxylation, and an increased gland output of cortisol, and that the significance of these changes can only be appreciated in the light of corticosteroid biosynthesis. He has however also emphasised the difficulty of assessing the degree of adrenal cortical function from a static histological picture, as for instance might be the case where a lipoid depleted gland, having ceased its period of hyperfunction, might yet be mistaken for a highly active gland.

Bearing in mind the possibility of such misinterpretations, it would appear that in general a good impression of the degree of adrenal cortical activity can be gained by consideration of these features, and if there does exist a state of "adrenopause" as envisaged by Albright, then it should be reasonable to expect glands so affected to show reduction in weight and in signs of functional activity.

It therefore seemed appropriate, that, having at my disposal a method of classification of lumbar vertebral

spines obtained at autopsy into osteoporotic, and normal bone density groups, the appearances of the adrenal glands should, where possible, also be studied in relation to the bone density.

MATERIALS AND METHODS

Adrenal glands were obtained from 31 cases of the larger series of 300 post-mortem cases in which lumbar vertebral bone density was estimated. Both glands were removed from the body in every case within six hours after death, and immediately dissected free of fat, and weighed.

Histochemical procedures

Lipoids. After fixation in neutral 10 per cent formol-saline, frozen sections were cut at 12μ on a freezing microtome, and stained for lipoids by Oil Red 4B (Gurr, Lillie 1944) and counterstained by haematoxylin.

Succinic dehydrogenase. To demonstrate locations of succinic dehydrogenase activity, thin slices (2 mm.) of each gland were treated by the triphenyl tetrazolium chloride (T.T.C.) method as described by Pearse (1953), and frozen sections were then cut at 12μ on a freezing microtome. This examination was performed immediately after the glands were weighed and, as the red reaction colour which denoted the reduction of triphenyl tetrazolium chloride to formazan faded within several hours, visual examination and photographic recording of the result was

carried out without delay. To ensure specificity, sodium malonate was used to inhibit the reaction at a concentration of M/10 and was incorporated in a control solution, but only an incomplete degree of inhibition was achieved, even at this relatively high concentration of malonate.

Ribonucleic acid. Formalin-fixed, paraffin embedded sections were cut at 5μ on a Cambridge rotary microtome and stained by pyronin-methyl green for RNA according to the technique described by Brachet (1953). Test and control sections were employed with and without ribonuclease. RNA appeared as pink or red granules in the cytoplasm of cortical cells. To ensure specificity, one control section from each gland was treated with a solution of crystalline ribonuclease (Kunitz), containing 0·1 mg. of enzyme per ml. aqua dest. at pH 6·0 for one hour at 37°C., and another with aqua dest. only, for one hour at 37°C. Loss of granules in the ribonuclease treated sections indicated specificity.

Histology

Paraffin embedded sections were cut at 5μ and stained by haematoxylin and eosin.

Bone density

Radiographic and chemical estimation of the density

of the 4th lumbar vertebrae and histological examination of adjacent vertebral bodies were performed by methods described in Part I and the Appendix.

RESULTS

The results of the examinations of the adrenal glands, and lumbar vertebral bodies, on 31 cases (19 male, and 12 female) are here presented. The gland weights, histochemical patterns, and the density of the fourth lumbar vertebral bodies expressed as mg. of calcium per c.c. are shown in Table 6.

The cortical contents of lipoid, and RNA were assessed visually from sections stained by Oil Red 4B and pyronin methyl green respectively, and each is expressed as a percentage of the total possible cortical content of the substance. Cortical lipoid varied in amount from 5 to 90 per cent (Figs. 25 and 26), and RNA from 10 to 95 per cent (Figs. 27, 28 and 29). These two results varied inversely in every case, and were so closely in agreement that only RNA is subsequently considered as it is the more direct index of activity.

The succinic dehydrogenase reaction results are recorded in layers to indicate how each of the three cortical zones reacted. The uppermost symbol represents the amount of reaction present in the zona glomerulosa,

Table 6

The adrenal weights, histochemical patterns and the degree of bone density of the 4th lumbar vertebrae as mg. per c.c. of bone, in a series of 31 autopsied cases.

Case No.	Sex	Age	Vertebral Body Calcium (mg. per c.c.)	Adrenal Weights (g.)	Succinic dehydrogenase (see text)	Cortical lipoid (per-cent)	Cortical RNA (per-cent)
112	M	17	87	9•0	-	50	55
117	F	70	88	11•0	-	90 (reversion)	15
125	M	66	59	16•0	+ + 0 0	80 (reversion)	10
126	M	76	84	10•5	+++ 0 +	25	80
131	M	48	81	12•8	-	60 (reversion)	50
136	M	55	38	15•0	+ + 0 0	75	30
140	F	58	84	20•0	+ + 0 0	80	25
156	M	52	69	14•0	+ + 0 0	30	80
172	F	73	57	11•0	+ + 0 + +	75	20

Table 6 (contd.)

Case No.	Sex	Age	Vertebral Body Calcium (mg.per c.c.)	Adrenal Weights (g.)	Succinic dehydrogenase	Cortical lipoid (per-cent)	Cortical RNA (per-cent)
179	M	74	66	14·0	+ + 0 + +	5	95
185	M	83	77	9·0	+ + 0 +	70	40
187	F	54	59	13·0	+ + 0 +	75	30
188	M	56	76	14·5	+ + + +	20	75
190	M	59	84	11·0	+ + 0 +	15	85
191	M	34	88	16·0	+ + + + +	60	45
192	F	61	72	13·5	+ + 0 +++	30	80
200	F	59	78	16·5	+ + 0 +	10	90
253	M	19	106	15·5	+++ 0 +	25	80
257	M	73	82	12·5	+++ 0 +	75	30
259	M	60	44	14·5	+ + 0 +	80	30

Table 6 (contd.)

Case No.	Sex	Age	Vertebral Body Calcium (mg. per c.c.)	Adrenal Weights (g.)	Succinic dehydrogenase	Cortical lipoid (per-cent)	Cortical RNA (per-cent)
269	M	50	59	13·8	+++ + +	80	20
275	F	66	60	11·0	+++ 0 + +	75	30
278	F	81	48	12·0	+++ + + + +	30	80
280	F	45	67	15·5	+ + 0 +	5	90
281	F	67	77	15·5	+ + 0 +	70	40
285	F	71	53	10·0	+++ + + +	20	90
291	M	51	81	13·5	-	20	80
296	F	68	55	16·5	+++ 0 0	10	95
304	M	76	47	13·5	+++ 0 + +	45	60
313	M	66	53	20·0	+ + 0 0	5	95
316	M	61	47	14·5	+++ 0 +	80	25

Table 7

The adrenal weight and RNA content, the cause of death and the duration of stress in eight cases of vertebral osteoporosis

Case No.	Principal conditions leading to death	Age	Sex	Duration of stress (days)	Gland weight (g.)	Cortical RNA (per-cent)	Verte-bral bone calcium (mg. per c.c.)
136	Cor pulmonale	55	M	4	15·0	30	38
259	Coronary thrombosis	60	M	2	14·5	30	44
278	Cholecystitis (operated)	81	F	17	12·0	80	48
285	Portal cirr-hosis Oesophageal haemorrhage Broncho-pneumonia	71	F	29	10·0	90	53
296	Carcinoma of rectum (operated) Peritonitis	68	F	22	16·5	95	55
304	Uraemia and Broncho-pneumonia	76	M	10	13·5	60	47
313	Carcinoma of stomach Bronchopneu-monia	66	M	17	20·0	95	53
316	Hypertension Cerebral haemorrhage	61	M	1	14·5	25	47
	Average	67			14·5	63	48

Table 8

The adrenal weight and RNA content, the cause of death and the duration of stress in eight cases having a high normal vertebral bone density

Case No.	Principal conditions leading to death	Age	Sex	Duration of stress (days)	Gland Weight (g.)	Cortical RNA (percent)	Vertebral bone calcium (mg./c.c.)
112	Accidental instantaneous death	17	M	-	9·0	55	87
117	Aortic thrombosis Cerebral softening	70	F	86	11·0	15	88
126	Coronary thrombosis	76	M	1	10·5	80	84
140	Malignant cerebral tumour	58	F	3	20·0	25	84
191	Accidental instantaneous death	34	M	-	16·0	45	88
253	Chronic nephritis Uraemia	19	M	10	15·5	80	106
257	Cerebral haemorrhage	73	M	6	12·5	30	82
291	Carcinoma of pancreas Biliary cirrhosis	51	M	6	13·5	80	87
	Average	50			13·5	51	88

the intermediate symbol the result in the zona fasciculata, and the lowermost symbol the result in the zona reticularis. The symbol 0 designates a negative reaction, + a weak patchy reaction, ++ a weak uniform reaction, and +++ a strong uniform reaction. These results did not show such a wide range of activity as might have been expected from results recorded by Symington et al. (1956). For instance, although in 15 cases there was a cortical RNA content of 60 per cent or over (25 to 30 per cent was considered normal, i.e. when only the zona glomerulosa, and the zona reticularis were positive) the zona fasciculata showed a ++ degree of dehydrogenase activity in only one case (Figs. 30 and 31): a further four cases showed only a + degree of activity (Fig. 32). The degree of inhibition of the reaction achieved by M/10 sodium malonate, though never quite complete, was sufficient to indicate the specificity of the reaction (Fig. 33).

There was however a wide range of bone density results which varied from 38 to 106 mg. of calcium per c.c. Eight cases had a vertebral calcium content of 55 mg. per c.c. or less, and were considered to be within the osteoporotic range. A further eight cases showed vertebral calcium contents which ranged from 82 to 106 mg. per c.c. and they were considered to be within the range of high normal bone density. For contrast, the results

in these two groups of cases are more closely compared in Tables 7 and 8. where, in addition to gland weights, RNA content, and vertebral calcium content, the condition or conditions leading to death, and the approximate duration of the period of stress, assessed from the clinical report, are also shown.

The osteoporotic group (Table 7) have an average vertebral calcium content of 48 mg. per c.c. and the high normal bone density group (Table 8) an average of 88 mg. per c.c. Other bone rarefying conditions were excluded by histological examination.

The average age of the osteoporotic group of 67 years is 17 years greater than that of the control group of 50, and the osteoporotic group tend on the whole to have been subjected to a greater degree of stress than the cases with normal bone density.

The average gland weight of the osteoporotic group of 14·5 g. is slightly greater than that of the non-osteoporotic group of 13·5 g. but there is still greater difference between the average percentage RNA contents; the osteoporotic group having an average of 63 per cent against the 51 per cent for the non-osteoporotic group, which as explained above is still well above the normal. Two otherwise normal young adult males,

included in the non-osteoporotic group, who suffered accidental instantaneous death showed 45 and 55 per cent of cortical RNA respectively, which is considerably in excess of the normal amount.

Histological examination of the glands revealed differences of compactness of cells, which was related to differences in their RNA content as described above, but differences in other features, such as tubular formation by groups of cortical cells, nuclear size and mitotic activity, areas of cellular necrosis, and capillary congestion, did not conform to any distinct pattern. Thus no point would be gained by discussing these differences further.

DISCUSSION

The incidence of osteoporosis in this group of 31 cases cannot be compared with that of the series as a whole because special efforts were made to obtain early permission for autopsies in potentially osteoporotic (senile) cases in this part of the work, so that the material could be obtained within six hours of death. In other respects however the examination of cases was carried out as in the main series of 300 cases.

The reason why the results of the succinic dehydrogenase reaction did not closely follow the pattern

of the RNA results (Table 6), as was shown by Symington et al. (1956) is not clear, but these workers used a modification of the method described by Pearse (1953) whereas in this study Pearse's method has been closely followed. Although more recently described methods for demonstrating succinic dehydrogenase (Dawson, 1960) are superior, in that the occurrence of lipoid diffusion is prevented, the results of my experiment did not suggest that this was an important source of difference.

Four of the five males in the osteoporotic group were over 60, and two of the three females over 70 years of age, thus it may fairly be said that these cases were true examples of senile osteoporosis and therefore worthy of consideration in connection with adrenal activity. With regard to the possibility that lipoid reversal might lead to a false inference of increased adrenal activity, as described by Currie and Symington (1955), it must be stated that this appearance was only present in three of the whole series of cases (Table 6). Of the two contrasted groups, lipoid reversal was seen only in one case (Fig.34) No. 117 which had a high normal bone density, so that there was no danger of the result being prejudiced against the hypothesis under examination. The higher average age of the osteoporotic group is consistent with results

already described in Part I.

The finding that the average gland content of RNA in the osteoporotic group was 12 per cent greater than that for the non-osteoporotic (Tables 7 and 8) group, was unexpected and requires consideration. As the average gland weight was also somewhat higher in the osteoporotic group, then the figure of 12 per cent is probably a rather low estimate in terms of total adrenal cortical hormone output. It appears that the greater degree of stress suffered by the patients in the osteoporotic group (Table 7) will explain at least a large proportion of this difference, and the essential feature which emerges is that the greater activity of the glands in those cases of senile osteoporosis is in no way suggestive of an adrenal failure. In fact the possibility that there is excess adrenal activity in osteoporosis cannot be excluded, on these results.

The other explanation which Albright (1947b) put forward for the occurrence of certain varieties of senile osteoporosis, namely the theory of excess production of adrenal 'S' hormone over 'N' hormone, must also be considered. The methods employed in this investigation do not distinguish between glucocorticoid and sex-hormone production, but if it be assumed that the entire glandular

activity demonstrated, was concerned with glucocorticoid synthesis to the exclusion of androgens, then it is a possibility that the osteoporosis could have been so produced if the imbalance had been present for some time. The minimum period which must elapse for osteoporosis to become manifest as a result of Cushing's syndrome, or during cortisone administration, is not yet known, but the duration of the stress, and therefore one assumes of the glandular hyperactivity it stimulated, varied from only two to twenty-nine days among the osteoporotic group of cases. If the lumbar vertebrae, the pelvic and certain other bones had indeed lost from between 30 and 50 per cent of their calcium during this period, as would be implied by the terminal low calcium contents of the lumbar vertebrae, then an exceptionally high degree of calcium excretion must have taken place during the stress period. Evidence of pathological calcification - e.g. renal calculi or renal calcinosis - was lacking at autopsy, and it seems most unlikely that such a rapid loss had occurred. There was no clinical or pathological evidence to suggest a major difference in glandular function between the two groups of cases although the possibility, that slight excess production of glucocorticoids over androgens, had been going on over a more prolonged

period cannot be entirely excluded. It is however concluded that neither of the hypotheses put forward by Albright can adequately account for the degree of osteoporosis which was present.

The occurrence of a higher than normal cortical RNA content in the two cases of accidental instantaneous death, where the factor of stress would normally have been considered to be absent, is not easily explained. No pathological features other than traumatic, which might have set the stress mechanism in motion, were discovered at autopsy, but each individual was an active young male subject who was involved in a motor cycling accident. Perhaps the indulgence in motor cycling under present day road conditions in such subjects is a stressor in its own right, and can produce such changes, but it has been suggested by Dawson (1960) that the adrenal cortex may undergo periods of cyclical activity, and this may explain the finding of greater activity than would be considered normal in an unstressed individual.

SUMMARY

Albright has stated that senile osteoporosis may be associated, either, with adrenal failure which he termed an "adrenopause", or with an excess production of

adrenal 'S' hormone over 'N' hormone.

To investigate the accuracy of these theories, fresh adrenal glands within six hours of death, were obtained at autopsy from a series of 31 cases (19 male and 12 female) which formed a part of the larger series of 300 cases in which bone density measurements were made. The degree of glandular cortical activity was assessed by histochemical tests for lipoid, RNA, and succinic dehydrogenase, and these results were compared with the degree of vertebral bone density as calcium per c.c. Histological examination showed no significant differences between the glands of different cases.

Eight elderly cases were adjudged to be osteoporotic, and their gland weights and RNA contents were compared with those of a further eight cases who were adjudged to have a high normal bone density. The cases of senile osteoporosis, had on the average 12 per cent more adrenal cortical activity than those with normal bone density, and this was held to be largely due to the greater degree of stress which they underwent.

The period of stress in the osteoporotic group varied from 2 to 29 days, and this is considered too short a period in which to have induced a bone calcium loss of the order of 50 per cent, merely as a result of

excess production of glucocorticoids. Therefore the osteoporosis described is unlikely to be due to any recent adrenal imbalance between 'S' and 'N' hormone production.

The evidence of greater adrenal cortical activity in cases of senile osteoporosis than in cases having normal bone density is against the theory of an "adrenopause".

PART III

A STUDY OF THE EFFECT OF SEX HORMONES IN EXPERIMENTALLY PRODUCED OSTEOPOROSIS IN RATS

A STUDY OF THE EFFECT OF SEX HORMONES IN EXPERIMENTALLY PRODUCED OSTEOPOROSIS IN RATS

INTRODUCTION

In cases of post-menopausal and senile osteoporosis, the administration of the deficient endocrine factor is considered an essential part of the therapeutic regime (Aegerter and Kirkpatrick, 1958), and this involves the giving, either of an oestrogen, or an androgen, or in certain cases of a combination of both substances. Although such treatment is effective in so far as the progress of these conditions is apparently arrested, and there almost invariably follows relief of certain subjective symptoms such as bone pains, there is as yet, despite those cases reported by Sherman (1948), Polishuk and Kleinhouse (1952), and Cooke (1955), no valid evidence that reversal of the disease progress towards normal bone density takes place. Patients receiving sex-hormone therapy have now been followed for periods of up to twenty years without any demonstrable radiographic improvement (Hennemann and Wallach, 1957).

Urist (1958) has pointed out that the experimental basis of sex-hormone therapy in osteoporosis is based on the treatment of normal animals with normal bones. This

seems far from ideal, and therefore the possibility that an unknown factor, or perhaps factors, remain to be demonstrated, must be borne in mind. In view of the fact that methods for rendering animals osteoporotic are well known, it was felt that a further assay of sex hormone administration using osteoporotic animals in place of normal animals was required, and to avoid further confusion of results in this field, it was also decided that this assay should be performed on animals deprived of their sources of endogenous sex hormone supply.

It has been previously mentioned (see general Introduction) that cortisone administration can produce osteoporosis in man and animals, and Albright (1947b) has stated, that in the osteoporosis of Cushing's syndrome, there is a specific antagonism between therapeutically administered testosterone, and endogenous adrenal glucocorticoids. The daily administration of cortisone to adrenalectomised and orchidectomised male animals should simulate the balance of hormones in Cushing's syndrome, and the administration of sex hormones, particularly testosterone, to such animals should therefore be a valuable method of testing the validity of Albright's statement.

Oestradiol and testosterone may be administered in several ways. Subcutaneous implantation of tablets is a

popular method, as it dispenses with the necessity for repeated injections, but the rate of absorption is extremely variable, at least in the experimental animal (Ghadially, 1959), so this method was not employed. Injections of oily solutions, or microcrystalline suspensions of the hormones are also used, and it has been suggested by Polishuk and Kleinhouse (1952) that the latter method is superior from the point of view of absorption. It was decided to use both methods in parallel in this experiment in order to assess the merit of this claim.

The possibility that many of the conflicting statements which have been reviewed in the general Introduction, regarding the site and mode of action of cortisone, and the sex hormones on bone, may be due to the fact that the majority of the workers in this field have used only one, and occasionally two, of three methods of study - i.e. radiological, chemical, and histological - suggested the use of all three methods in parallel. This study was designed as an attempt to resolve some of the discrepancies.

Despite the drawbacks of species variability, the rat was selected as a suitable experimental animal because much of the previous experimental work in this field has

been done on rats.

MATERIALS AND METHODS

Animals, operation, and treatment

Sixty, male, white, albino rats of approximate weight 150g. were used. Forty-eight rats survived bilateral adrenalectomy and orchidectomy, having been operated on in two main batches, consisting of thirty in the first, and eighteen in the second. This fact is important, because by the time of operation of the second batch, additional knowledge of the technique of adrenalectomy and its shortcomings had been gained. In an attempt to preclude any possibility of regeneration of outlying islands of adrenal tissue, the whole adrenal fatty bed was also excised in these animals. At the time, this additional procedure only resulted in a much higher operative mortality rate, but subsequently other differences between the two batches developed, as will be shown.

All the operated animals were then divided into eight groups with six rats in each, and the groups numbered 1, 1A, 2, 2A, 3, 3A, 4 and 5 respectively. The "A" groups were composed of the animals which had undergone additional excision of the adrenal bed. Twelve unoperated rats remained and these were divided into groups labelled 6 and 7 respectively. All animals were fed ad lib. on a complete diet of

rat cake (see Appendix), and were also given abundant water which contained 25 per cent milk by volume to augment their already adequate daily calcium intake.

Under ether anaesthesia each animal was radiographed immediately after operation using a standard exposure (10 Kv. 10 ma. 10 secs.) at a constant tube distance of 63·5 cms. The animals were individually laid ventral surface down on a film cassette with the rear limbs widely abducted, and the knee joint flexed to form a right angle. This ensured that the hind limbs lay close to and parallel with the film to be exposed, so that the bone image obtained bore a constant relationship to the actual bone length. The unoperated rats were also radiographed and subsequent examinations were made on all animals at intervals of 100, 130, 160, and 175 days after operation. The bone lengths were measured from the radiographs using a ruler.

All groups, save group 5, which was maintained on 1 per cent saline throughout, and group 7 which was chosen to act as the non-operated untreated control group, received 1 mg. of cortisone acetate daily (Merck, Sharp & Dohme Ltd.) - approximately 7 mg./kg. of body weight by intra-muscular injection for 100 days; these same groups received thereafter 1·5 mg. daily (7 mg./kg.) because they

had all grown during the 100 days. Group 6, the other non-operated group, acted as the cortisone-treated control group.

One hundred days after operation, all the operated groups showed radiographic evidence of osteoporosis, and thus there were eight groups available to receive sex-hormone injections, but one of these was kept as the saline-maintained control group. Of the remaining seven groups, the weekly weighings revealed that group 4 showed a higher average weight than the others, and to ensure the most stringent test of any beneficial action of the sex hormones, this group was chosen to act as the operated, cortisone maintained, but untreated control group.

Thus only the six groups 1, 1A, 2, 2A, 3, and 3A, subsequently received intra-muscular injections of sex hormone as follows.

Group 1. 20 µg. oestradiol monobenzoate (Ovocyclin Ciba Ltd), oily suspension (O.S.) on alternate days for 60 days.

Group 1A. 20 µg. oestradiol monobenzoate (Ovocyclin 'M' Ciba Ltd.), microcrystalline suspension (M.S.) on alternate days for 40 days and thereafter on every 4th day for 20 days.

Group 2. 20 µg. oestradiol monobenzoate (O.S.) and 2·5 mg. testosterone proprionate (Perandren Ciba Ltd.) (O.S.),

on alternate days for 60 days.

Group 2A. 20 µg. oestradiol monobenzoate (M.S.) and 2·5 mg. testosterone isobutyrate, (Perandren 'M' Ciba Ltd.) (M.S.) on alternate days for 20 days.

Group 3. 2·5 mg. testosterone propionate (O.S.) on alternate days for 60 days.

Group 3A. 2·5 mg. testosterone isobutyrate (M.S.) on alternate days for 40 days and thereafter on every 4th day for 20 days.

The sex-hormone injections were given over a period of only 60 days because it has been shown both in animals (Poumeau-Delille and Fabiani, 1944), and man (Albright et al, 1940), that the effects of injected sex hormones persist for at least forty days after administration, and it therefore seemed reasonable to expect that the effect of the sex hormones used in this experiment, would persist for at least a further fifteen days, i.e. up to the completion of the experiment. Because of their failure to gain weight, the "A" groups had their doses of hormone reduced after a period of 40 days.

Group 4. Operated, and given daily cortisone injections, but no sex-hormone treatment. The operated cortisone treated control group.

Group 5. Operated and maintained on 1 per cent saline in place of cortisone. No sex-hormone treatment. The operated saline treated control group.

Group 6. Not operated, but given daily cortisone injections. No sex-hormone treatment. The unoperated cortisone treated control group.

Group 7. Not operated, no cortisone and no sex-hormone treatment. The unoperated normal control group.

Post-mortem examinations

All surviving animals were killed after receiving their final radiograph from 174 to 176 days after operation, and their femora dissected free and thoroughly cleaned with scissors and scalpel. The right femora were then immediately wrapped in polythene to prevent drying. The right tibiae from the groups 1A, 2A, and 3A were also removed for examination.

All right femora were individually suspended by thin nylon thread, weighed quickly in air, and then in water at 20°C. to ascertain their volume and subsequently ashed to constant weight in a muffle furnace at 700°C. The calcium content of the ash was estimated by flame photometry (Appendix), and the calcium/volume and calcium/wet weight ratios calculated for each individual bone.

Bone histology

The left femora of all animals, and the right tibiae of the A groups, were preserved for histological examination by fixation in 10 per cent. neutral formol-saline for seven days and were subsequently decalcified in an aqueous solution of citric acid (15 per cent) and formic acid (29 per cent).

Because it is frequently stated that osteoporosis affects cancellous bone earlier, and to a greater degree than compact bone, the ends of the long bones of these animals were selected for study. The decalcified bones were processed by standard laboratory methods and double embedded (celloidin-paraffin) after which sections were cut at 5μ in the plane connecting the pit for attachment of the ligament to the femoral head, and the uppermost point of the greater trochanter. This gave comparable sections which were stained by haematoxylin and eosin.

RESULTS

The results are summarised in Tables 9 to 22 and illustrated in Figures 35 to 144. The changes in the group average body weights throughout the experiment are shown in Figures 35 and 36 and the individual and averaged results of the femoral calcium analysis for the animals

Table 9

Group 1. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·6710	0·4550	79	0·2147	3·0
2	0·5560	0·3263	94	0·2037	2·95
3	0·5021	0·3416	56	0·1666	2·90
4	0·7497	0·4649	113	0·2717	3·10
5	0·5529	0·3304	83	0·2160	3·05
Average	0·6063	0·3836	84·8	0·2415	3·15

Table 10

Group 1A. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·6989	0·4836	78	0·2110	3·0
2	0·6388	0·4287	81	0·2176	3·0
3	0·7860	0·5216	95	0·2630	3·25
4	0·6871	0·4572	85	0·2237	3·05
Average	0·7027	0·4727	85	0·2288	3·05

Table 11

Group 2. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·6775	0·4590	98	0·2103	3·05
2	0·7161	0·4359	90	0·2548	3·15
3	0·8450	0·5864	119·0	0·2871	3·25
Average	0·7462	0·4937	103·5	0·2507	3·15

Table 12

Group 2A. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final length of Right Femur (cm.)
1	0·6216	0·4104	79	0·2127	2·95
2	0·5760	0·3539	78	0·1991	2·90
3	0·5988	0·3962	78	0·2030	2·90
Average	0·5988	0·3868	78	0·2049	2·90

Table 13

Group 3. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·6946	0·4568	71	0·1980	2·90
2	0·5949	0·4300	58	0·1654	2·90
3	0·6619	0·4563	82	0·2270	3·10
4	0·6950	0·4819	81	0·2212	3·0
5	0·8453	0·5054	105	0·2860	3·20
6	0·8330	0·4875	101	0·2868	3·10
Average	0·7208	0·4696	83	0·2307	3·0

Table 14

Group 3A. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·8207	0·5665	98	0·2844	2·90
2	0·6482	0·4052	63	0·1817	2·80
3	0·7146	0·5040	74	0·2070	2·80
4	0·6795	0·4788	83	0·2282	2·90
5	0·6580	0·4464	75	0·2127	2·80
6	0·6057	0·4901	78	0·2103	2·80
Average	0·6878	0·4818	78·5	0·2207	2·80

Table 15

Group 4. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·7475	0·4505	98	0·2683	3·15
2	0·7743	0·4896	113·5	0·2844	3·15
3	0·6425	0·4100	75	0·2078	3·10
4	0·6914	0·4726	79	0·2127	3·10
5	0·7329	0·4359	84	0·2385	3·10
6	0·7585	0·4910	97·5	0·2693	3·10
Average	0·7245	0·4583	91	0·2468	3·10

Table 16

Group 5. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·7535	0·4996	105	0·3036	3·15
2	0·7135	0·4226	90	0·2563	3·10
3	0·8810	0·5462	136	0·3252	3·15
4	0·7373	0·4390	82	0·2383	3·10
5	0·7572	0·4838	101	0·2770	3·10
Average	0·7685	0·4782	103	0·2801	3·10

Table 17

Group 6. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·8583	0·5241	133	0·3515	3·15
2	0·8403	0·5766	116	0·3220	3·15
3	0·6019	0·3821	82	0·2315	3·15
4	0·8225	0·5613	110	0·3094	3·15
5	0·9557	0·5845	139	0·4092	3·30
Average	0·8157	0·5257	116	0·3247	3·20

Table 18

Group 7. The results of femoral chemical analysis of individual animals

Rat No.	Wet Weight of Right Femur (g.)	Volume of Right Femur (c.c.)	Calcium Content of Right Femur (mg.)	Ash Content of Right Femur (g.)	Final Length of Right Femur (cm.)
1	0·7534	0·4906	138	0·3615	3·20
2	0·8670	0·5337	140	0·3764	3·25
3	0·8438	0·5838	125	0·3427	3·20
4	0·7900	0·5156	124	0·3290	3·20
5	0·8277	0·5648	132	0·3807	3·20
6	0·9098	0·5815	159	0·4326	3·25
Average	0·8320	0·5350	136	0·3705	3·20

Table 19

The various rates of bone growth in the groups, and a comparison between the amount of growth occurring during sex-hormone treatment and the total amount of bone growth of each group.

Group No.	The group averaged femoral and tibial bone length					The group averaged bone length increase	
	Pre-operation	100 days post-operation	130 days post-operation	160 days post-operation	175 days post-operation	During the whole experiment	During sex-hormone treatment
1	2·90	3·23	3·33	3·35	3·38	0·48	0·15
1A	2·98	3·0	3·13	3·25	3·33	0·35	0·35
2	2·90	3·10	3·28	3·35	3·45	0·55	0·45
2A	2·98	3·08	3·13	3·15	3·18	0·20	0·10
3	2·90	3·13	3·23	3·25	3·35	0·45	0·22
3A	2·98	3·05	3·08	3·13	3·13	0·15	0·08
4	2·90	3·33	3·40	3·40	3·40	0·50	0·07
5	2·90	3·23	3·45	3·46	3·48	0·58	0·25
6	2·90	3·23	3·28	3·48	3·53	0·63	0·40
7	2·90	3·45	3·53	3·53	3·53	0·63	0·08

Table 20

The averaged results of the femoral chemical analysis in each group

Group	Final Averaged Femoral volume (c.c.)	Final Averaged Femoral length (cms.)	Femoral Averaged Ash Weight (g.)	Femoral Averaged Calcium content (g.)	Femoral Averaged Calcium/ Volume ratio	Femoral Averaged Calcium/ wet wt. ratio
1	0·3836	3·15	0·2415	0·0848	0·2228	0·1402
1A	0·4727	3·05	0·2288	0·0850	0·1794	0·1240
2	0·4937	3·15	0·2507	0·1055	0·2117	0·1380
2A	0·3868	2·90	0·2049	0·0780	0·2061	0·1305
3	0·4696	3·0	0·2307	0·0830	0·1800	0·1153
3A	0·4818	2·80	0·2207	0·0780	0·1655	0·1142
4	0·4583	3·10	0·2468	0·0911	0·1982	0·1226
5	0·4782	3·10	0·2801	0·1020	0·2170	0·1340
6	0·5257	3·20	0·3247	0·1160	0·2207	0·1420
7	0·5450	3·20	0·3705	0·1360	0·2505	0·1638

Table 21

The visual radiological assessment of the degree of osteoporosis in the various groups at intervals during the experiment

Group No.	Before sex-hormone treatment		During sex-hormone treatment		
	Pre-operation	100 days post-operation	130 days post-operation	160 days post-operation	175 days post-operation
1	0	+ +	+ +	+	0
1A	0	+ + + +	+ + +	+ +	0
2	0	+ +	+ + +	+ +	+
2A	0	+ + + +	+ + +	+ +	+
3	0	+ + + +	+ + +	+ + +	+ +
3A	0	+ + + +	+ + +	+ + +	+ + +
4	0	+ + + +	+ + + +	+ + + +	+ + + +
5	0	+ + +	+ + +	+ + +	+ +
6	0	0	0	0	0
7	0	0	0	0	0

Table 22

The effect of sex-hormone administration on body weight and bone length

Group	The average percentage body wt. increase during		Ratio of sex-hormone period wt. increase to total wt. increase	The average percentage increase in bone length during		Ratio of sex-hormone bone length increase to total length increase	Final number of rats in each group
	the experiment	Sex-hormone treatment		the experiment (Femur & Tibia)	Sex-hormone treatment (Femur & Tibia)		
1	59·7	27·2	0·456	14·2	4·4	0·314	5
1A	54·1	6·7	0·128	10·5	10·5	1·0	4
2	86·3	70·0	0·812	15·9	13·0	0·820	3
2A	20·4	1·9	0·089	6·3	3·2	0·50	3
3	72·5	40·3	0·556	13·4	6·6	0·491	6
3A	43·7	5·4	0·129	4·7	2·8	0·534	6
4	66·6	20·8	0·312	14·7	2·3	0·139	6
5	63·3	6·1	0·096	16·7	7·2	0·432	5
6	87·3	14·25	0·164	17·9	11·3	0·636	5
7	126·0	13·8	0·109	17·9	2·3	0·127	6

in each group are presented in Tables 9 to 18. The averaged serial bone length results for each group are given in Table 19 and in Figures 37 to 39. The data concerning the various ratios which were calculated, e.g. the calcium/volume, and the calcium/wet weight ratios of the bones are presented in Tables 20 and 22. The results of the serial radiographic examinations are summarised in Table 21, and also illustrated along with the bone histological appearances in Figures 68 to 144. For the convenience of the reader, a number of the illustrations (Figs. 40 to 67) are arranged so that comparison of examples of the extreme changes in radiographic bone density and histology can easily be made. This was done because in the subsequently presented main body of illustrations (Figs. 68 to 144) intermediate changes, which are less readily distinguished, are also shown. The illustrations of this preliminary short set are, however, repeated in their proper context along with the main block.

Body weights

The curves which represent the averaged group weekly body weights both before, and during the period of sex-hormone administration are shown in the Figures 35 and 36.

At autopsy the rats which were given hormone injections in oily solution were found to have large amounts of unabsorbed oil pooled in the tissues. In several instances this was equivalent to about 40 per cent of the total amount given, and was most pronounced in the groups given testosterone, because the dose of this substance was dissolved in 0·5 ml. of oil, whereas in the case of oestradiol the amount of oil, was only 0·2 ml. As would be expected the animals with the largest amounts of unabsorbed oil in the tissues were in the group given combined oestradiol and testosterone. The weight gains shown by animals which received O.S. hormone injections were therefore of doubtful significance, firstly because of the influence of the weight of the pooled oil in the tissues on the total body weight, and secondly because of the difficulty of assessing how much of the hormone itself had been effectively absorbed from the pool.

However, to facilitate comparison between the groups, a ratio was calculated between the averaged percentage weight increase of each group, during the period of sex-hormone administration, and the percentage weight increase which occurred during the whole experiment (Table 22). For example, if a 150 g. rat eventually

reached 300 g. then it underwent a 100 per cent weight increase and if this total increase occurred solely during the period of sex-hormone injections then the ratio was $\frac{100}{100}$ or 1·0

For the operated untreated control group No.4 this ratio was 0·312, whereas for the groups given oestradiol, oestradiol and testosterone, and testosterone alone, the ratios were 0·456, 0·812, and 0·556 respectively. Thus the group which received the combined therapy and therefore the greatest amount of oily vehicle showed the greatest rate of weight increase during the injection period.

In contrast the groups given the M.S. hormone, i.e. groups 1A, 2A, and 3A, showed the much smaller ratios of 0·128, 0·089, and 0·129 respectively. In their case the group which received oestradiol and testosterone combined, showed the smallest rate of weight increase during therapy, but all the ratios were low, and indicated the virtual failure of these three groups to gain weight during the treatment period.

Bone growth

Of perhaps greater significance are the various rates of growth shown by the different groups. These

rates were assessed by averaging the lengths of right femur and tibia - obtained from the radiograph - for each animal (Table 19). A group average measurement was then calculated for each period of assessment and these data are presented in Table 19 and in Figures 37 to 39.

The group average percentage increase of final bone length (a) during the period of sex-hormone administration and (b) throughout the experiment was calculated (Table 22).

The ratio of a/b gives some measure of the efficacy of the sex hormones in stimulating bone growth, for if growth took place only during the period of their administration, the ratio was 1·0. The operated but untreated control group 4 showed a low ratio of 0·139, which is in contrast to those of the hormone treated groups in which, with only one exception (group 1) the ratios range from 0·49 to 1·0. The omitted group, group 1, showed the rather lower ratio of 0·314 but this group received O.S. oestradiol and absorption of hormone as well as oil may have been defective.

With the exception of this single anomalous result, the averaged group ratios were, for the group given M.S. oestradiol 1·0, for both groups given combined oestradiol

and testosterone 0·660, and for the groups given testosterone alone, 0·512. The higher ratios in the sex-hormone treated groups were only paralleled by one other group - the unoperated but cortisone treated control group 6 in which the ratio was 0·636. The intact adrenal glands of this group render close comparison between it and the sex-hormone treated groups impossible, because of the compensatory production of sex hormones. It is interesting to note, however, that in this group alone the final bone length equalled that of the normal control group 7, despite a growth delay in the early weeks of the experiment.

Radiographic bone density

The development of pathological changes within the bone was recognised in the radiographs by a fine bubble pattern at the ends of the long bones (Fig. 76). This appearance was due to the combination of small areas of radiographic translucency with fine intersecting lines of opacity. The radiographic appearances of the subepiphyseal areas closely corresponded to their histological patterns, which were characteristic of osteoporosis; and were unaffected by minor variations in film exposure and development.

In the absence of a reliable densitometric method for rat bones the densities had to be assessed visually

from the radiographs using a 6 X hand lens. The anatomical area selected for comparing these changes in different animals was the right knee region, which is rich in cancellous bone, and the same animal from each group was used for comparison throughout the experiment. The results of the serial radiographic examinations of all groups are summarised in Table 21, where the changes in bone density which occurred, are shown in terms of the degree of osteoporosis. The symbol 0 designates normal bone density, + a minimal degree of osteoporosis, ++ a mild degree, +++ a moderate degree and ++++ a severe degree. All operated animals had developed osteoporosis by one hundred days post operation, but this was usually most pronounced in the eighteen animals which had the whole fatty adrenal bed resected, in addition to the adrenal glands and their capsules (groups 1A, 2A, and 3A).

The unoperated animals of groups 6 and 7 did not develop osteoporosis, despite the daily administration of cortisone to the animals of group 6. All animals which received oestradiol (groups 1 and 1A) had recovered normal bone density by the end of the experiment, and a lesser degree of recovery was seen in the groups which received combined oestradiol and testosterone (groups 2 and 2A), but only slight recovery was seen in the groups which received

testosterone alone (groups 3 and 3A). The operated but untreated control group 4 showed no evidence of recovery of bone density at the end of the experiment. Ant-91 mg Ca.

Preliminary radiographs had shown that all animals had bones of normal density prior to operation. These appearances, and the serial radiographic bone changes which occurred, are illustrated as photographic negative enlargements as follows.

Group 1 (Figs. 68 to 72). The O.S. oestradiol treated group. The illustrations show development of a mild degree of osteoporosis by 100 days after operation, but by 175 days normal bone density has been restored.

Group 1A (Figs. 75 to 79). The M.S. oestradiol treated group. The illustrations show development of severe osteoporosis by 100 days after operation but by 175 days normal bone density has been restored.

Group 2 (Figs. 85 to 88). The O.S. oestradiol and testosterone treated group. In this group the relevant radiographic negative taken at 100 days after operation was unfortunately damaged before reproduction could be made and it is not shown, but the remaining illustrations show development of a moderate degree osteoporosis by 130 days after operation. By 175 days there was only a minimal degree of residual osteoporosis.

Group 2A. (Figs. 91 to 95). The M.S. oestradiol and testosterone treated group. The illustrations show development of a severe degree of osteoporosis by 100 days after operation, but by 175 days there was only a minimal degree of residual osteoporosis.

Group 3 (Figs. 101 to 105). The O.S. testosterone treated group. The illustrations show the development of a severe degree of osteoporosis by 100 days after operation, but by 175 days there was only a mild degree of residual osteoporosis.

Group 3A (Figs. 108 to 112). The M.S. testosterone treated group. The illustrations show development of a severe degree of osteoporosis by 100 days after operation, but by 175 days there was a moderate degree of osteoporosis.

Group 4 (Figs.117 to 121). The operated untreated control group. The illustrations show development of a severe degree of osteoporosis by 100 days after operation, which persisted throughout the experiment. Yet had 91 mg Ca in femur c/f see Tables 9, 10 & 15.

Group 5 (Figs. 124 to 128). The operated saline maintained control group. The illustrations show development of a moderate degree of osteoporosis by 100 days after operation, and by 175 days there was still a residual mild degree of osteoporosis.

Group 6 (Figs. 131 to 135). The unoperated cortisone-treated control group. The illustrations show that radiographic bone density was normal throughout the experiment.

Group 7. (Figs. 138 to 142). The unoperated normal control group. The illustrations show that radiographic bone density was normal throughout the experiment.

Bone calcium analysis

The results of the individual femoral calcium analysis for each animal in its group are given in Tables 9 to 18, but the averaged results for each group are presented in Table 20.

Osteoporosis occurs in both spongiosa and cortical bone, but in humans the cortical bone is complicated by arthritic changes and the measure of osteoporosis used in the case of human vertebrae (see Part I), i.e. the calcium/volume ratio, which takes greater account of the volume of the spongiosa is more suitable. In small rat bones, however, mixture of the two components is unavoidable and for this reason the calcium/volume ratio was found to be an unsuitable index (Table 20), and is not further mentioned, but the calcium/wet weight ratio which was also calculated for each animal and averaged for each

group was found to accord fairly well with the visual radiographic assessment of bone density.

As expected, higher ratios were found in denser normal bones, and the highest group average ratio, 0·1638, was found in the intact control group 7. This contrasted with the low average ratio of 0·1226 for the operated but untreated control group 4. These ratios are expressed to four decimal places because they were calculated with the aid of logarithmic tables. If one takes into consideration the error of the experimental method it would probably be more correct to claim accuracy only to two decimal places.

Of the operated sex-hormone treated animals the highest average ratio of 0·1402 was found in one of the groups which received oestradiol (group 1). The lowest average ratios 0·1153 and 0·1142 were found in the groups which received testosterone alone (groups 3 and 3A), and intermediate ratios 0·1380 and 0·1305 were found in the groups which received combined oestradiol and testosterone injections (groups 2, and 2A respectively). There was however a 50 per cent mortality rate in groups 2 and 2A during the course of the experiment, and as only 3 rats remained in each group, the value of their results was

correspondingly diminished.

Bone histology

Marked group variability was noted between the numbers and calibre of the subepiphyseal bone trabeculae of femoral heads, and there were also group differences in the regularity of the patterns formed by these trabeculae, and in the frequency, and regularity, and calibre, of the smaller interconnecting transverse bone trabeculae. Occasionally, failure of osseous transformation of the cartilaginous caps was seen in sections from all groups.

Osteoblasts which are usually seen as a single continuous layer of cuboidal mononuclear cells lining bone trabeculae, were only present in a few instances in the femoral head sections. This may have been due to the marked retraction of cellular constituents away from the bone trabeculae in most of the sections, thus making cell identification very difficult. Osteoclasts, which are larger cells than osteoblasts, are usually multinucleated and are characteristically found in small depressions on the surfaces of bone trabeculae (Howship's lacunae). No osteoclasts were seen in femoral head sections. Group differences in the appearances of the epiphyseal cartilages of the femoral heads were not striking, but are described in terms of cartilage width, and the appearances of the cells

of the zones of proliferation and maturation. The zone of proliferation is in the upper part of the cartilage, and its cells are small, compact, closely packed, in columns, and usually ovoid but occasionally reniform in appearance. Nuclear mitoses are not infrequent.

In the zone of maturation, the cells still in regular columns, are larger, and more widely separated by intervening ground substance. They are frequently polygonal, and their cytoplasm is often vacuolated. This is the classification of epiphyseal cartilage cells usually adopted by workers in this field (e.g. Ramamurti and Taylor, 1959).

Sections of the tibial heads showed that the subepiphyseal trabeculae, where present, were lined by a continuous sheet of osteoblastic cells (Fig. 83) but no evidence of osteoclastic activity was seen in these sections either. The following are descriptions of typical examples of the bone histology for each group, and reference is also made to the illustrations. The groups are dealt with in the reverse order so that the normal appearances of groups 6 and 7 may first be described, and illustrated. Since diminution in numbers and calibre of bone trabeculae is a diagnostic feature of osteoporosis, the average number of longitudinal subepiphyseal trabeculae in sections of

femoral heads is appended in brackets along with the representative descriptions, but only in those groups where they were clearly enough defined in sufficient sections for an average result to be of value.

Femoral head sections

Group 7 (Figs. 143 and 144). The unoperated untreated normal control group. This showed the normal regular trabecular pattern. There were numerous subepiphyseal trabeculae of sturdy calibre (average 14) and frequent smaller transverse interconnecting bars of bone were also present. The epiphyseal cartilages showed the normal appearance of the zones of proliferating cartilage, and maturing cartilage, for rats of this age according to Ramamurti and Taylor (1958).

Group 6 (Figs. 136 and 137). The unoperated cortisone treated control group. The appearances were similar to those of group 7, but subepiphyseal trabeculae were slightly less frequent (average 13). In the epiphyseal plates, the zones of proliferating cartilage were similar to those of group 7, but the cells of the zones of maturing cartilage were rather plumper and more prominent, although not more frequent.

Group 5. (Figs. 129 and 130). The operated saline maintained control group. There was another slight reduction

in numbers (average 12) and now also in calibre of longitudinal subepiphyseal trabeculae but the general pattern was regular. The epiphyseal plates showed slight diminution in numbers of cells of the proliferating zones, and almost complete disappearance of cells in the zones of maturing cartilage.

Group 4 (Figs. 122 and 123). The operated cortisone-treated control group. There was marked reduction in numbers (average 7), and calibre of longitudinal and interconnecting subepiphyseal bony trabeculae, and also considerable irregularity of their pattern. There was narrowing of the epiphyseal plates and marked atrophy of cells of the proliferating and maturing zones.

Group 3A (Figs. 113 and 114). The M.S. testosterone treated group. There was fairly marked reduction in numbers (average 9) of subepiphyseal trabeculae, and their slender calibre was consistent with osteoporosis. The epiphyseal plates showed a normal zone of proliferating cartilage cells, but there was moderate cellular disorganisation of the zones of maturing cartilage cells.

Group 3 (Figs. 106 and 107). The O.S. testosterone treated group. The appearances were in general, similar to those of group 3A, but in several animals there was marked irregularity of trabecular pattern, in one instance amounting

to severe disorganisation of trabecular pattern, in addition to the reduction of trabecular numbers due to osteoporosis (Fig. 106). The changes in the epiphyseal plates were also similar to those of group 3A, but there was less disorganisation of the cells in the zone of maturing cartilage.

Group 2A (Figs. 96 and 97). The M.S. oestradiol and testosterone treated group. This group also showed marked reduction in numbers of longitudinal subepiphyseal trabeculae (average 8), the pattern of which showed considerable irregularity. The epiphyseal plates showed only slight atrophy of the cells in the zones of proliferating cartilage, but there was almost complete absence of the cells of the maturing cartilage zones.

Group 2 (Figs. 89 and 90). The O.S. oestradiol and testosterone treated group. The appearances were similar to those of group 2A, but subepiphyseal trabeculae were slightly more frequent, and the pattern too was rather more regular although individual trabeculae were more slender than normal. There was much less atrophy of the cells of the proliferating and maturing zones of the epiphyseal plates, but slight cellular disorganisation of cells of the maturing zones was seen.

Group 1A. (Figs. 80 and 81). The M.S. oestradiol treated group. The general pattern was regular and subepiphyseal trabeculae were only slightly reduced in number (average 12) and calibre. The proliferating and maturing zones of the epiphyseal plates showed normal cell populations, and there was no disorder of the maturing zones.

Group 1 (Figs. 73 and 74). The O.S. oestradiol treated group. The subepiphyseal trabeculae were of near normal frequency (average 13) and pattern, but individual trabeculae were more slender than normal. The epiphyseal plates showed evidence of closure in some areas, but in general the cells of the proliferating and maturing zones were regular and of normal appearance.

Tibial heads

Group 3A (Figs. 115 and 116). There was virtually-complete absence of subepiphyseal trabeculae. The cells of the epiphyseal cartilages were not reduced in number, but in the zones of maturation the regular columnar arrangement was completely lost.

Group 2A. (Figs. 98 to 100). Slender, recently formed, subepiphyseal trabeculae were present in moderate numbers and length. The epiphyseal cartilages were narrower than those of group 1A and the cell columns, although regular, were distinctly shorter. The cells themselves

did not stain so well and appeared less active.

Group 1A (Figs. 82 to 84). There were numerous elongated subepiphyseal trabeculae of regular pattern in every case. This group showed the broadest epiphyseal cartilages with numerous cells in the proliferating and maturing zones. These cells were present in long regular columns.

DISCUSSION

Differences have been demonstrated between the various groups by all the methods of investigation, and many points requiring discussion have arisen. The results more closely in agreement throughout were those achieved by radiographic and histological studies, but the findings of the calcium analyses were also broadly consistent with them. The only demonstrable inconsistency was the relative failure of bone growth in group 1 during the period of administration of O.S. oestradiol (Fig. 37). This contrasted with the considerable improvement in radiographic bone density of this group (Table 21), and will be discussed later in this context. In other respects the changes demonstrated can be related either to the operation, the cortisone, or sex-hormone administration, or to a combination of two or more of these factors.

Body weights

As might be expected, by analogy with the results of major surgical procedures in the human subject, the operated animals showed a diminished rate of weight gain during the immediate post-operative period (Figs. 35 and 36). This negative phase was most severe in those groups which also received cortisone injections and in them actual weight loss took place for several weeks. The subsequent rapid weight gain shown by the groups to which the O.S. sex-hormone injections were given, has been mentioned (Table 22, Fig. 35). Testosterone, which is known to stimulate the rate of increase of body weight in human eunuchoid males (Kenyon, et al. 1940) produced the greatest rate of weight increase during this experiment, but much of this effect was no doubt due to the accumulation of the poorly absorbed oily vehicle. It is interesting to speculate to what degree previously reported weight increases in experimental animals (e.g. Saunders, 1958) have been influenced by the same phenomenon. On the other hand the animals which were given M.S. hormone in equivalent amounts did not gain weight until the terminal few days of the experiment, by which time, on account of their failure to gain weight, the dose of hormone had been practically reduced by half (Fig. 36). Armstrong et al.

(1945) have shown that in rats, failure to gain weight during administration of oestradiol, resulted from depression of appetite, and was an effect of hormone overdosage. No clear decision on the degree of inhibition of weight gain of groups given O.S. hormone could be made from this experiment, as the individual effects on body weight, produced by pooled oil, and actual somatic tissue increase, could not be distinguished. Because the quantities of the hormones used in this experiment were of the same order as those used by other workers (e.g. Gillespie, 1954) and as failure of animals to gain weight on similar amounts has not previously been recorded, it seems probable that the failure of the groups which received M.S. hormone in this experiment, to gain weight until the dose had been substantially reduced, is an indication of greater potency of these preparations. This may be associated with a more rapid rate of absorption.

The effect of cortisone

In order to assess which bone changes were due solely to the action of the sex hormones, the effects produced by cortisone must first be defined. Continuous administration of cortisone has been shown to produce cessation of bone growth in rabbits and rats, probably due to cessation of endochondral bone formation (Sissons and

Hadfield, 1955), to lead to thinning and deformity of epiphyseal plates in rabbits, possibly due to interference with synthesis of chondroitin sulphate (McCluskey and Thomas, 1959), and to cause accumulation of subepiphyseal bone associated with cessation of growth in growing rats (Follis, 1951; Nicolaysen, et al., 1953). Given intermittently, cortisone has produced appositional bone growth on the periosteal surfaces of the cranium, the pre-maxilla, and the middle of the femoral shaft in rabbits (Storey, 1958).

In my experiment temporary cessation of bone growth was observed in the groups which received cortisone (Table 19). This was most pronounced in the groups where the more thorough resection of adrenal and associated tissue was carried out. Accumulation of subepiphyseal bone was also noted, particularly in the group which received M.S. oestradiol (Fig. 82), but the absence of this change, for instance, in the group which received M.S. testosterone (Fig. 115) is evidence against it being due to cortisone.

No particular differences were observed between the epiphyseal cartilaginous plates in cortisone treated and untreated groups (Figs. 137 and 114) (the unoperated groups 6 and 7), and no periosteal changes were found in any of the

bones. It would appear that apart from the initial inhibitory effect on bone growth (e.g. in group 6) (Fig. 39) and a slight catabolic effect (Fig. 35) which probably contributed to the production of osteoporosis, (osteoporosis occurred only in conjunction with the deprivation of the sex hormones) the effect of cortisone in the production of bone changes in this experiment was slight enough to be negligible. The delayed but eventually rapid bone growth which occurred in group 6 was probably due to the animals becoming resistant to cortisone - a finding which has been previously reported in rats (Urist, 1958).

The effect of sex hormones

The notable degree of bone growth which occurred in certain of the groups receiving sex-hormone therapy, and which took place shortly after commencing the injections (Fig. 38) requires careful consideration.

Baker and Leek (1946) have reported accumulation of subepiphyseal bone and epiphyseal cellular disorder in rats given oestradiol. They suggested that the appearances were due to the inhibition of the mechanism of bone resorption but no serial estimations of bone growth were performed in their experiments.

In my experiment, the progressive bone growth which was noted shortly after commencing injections in the group given M.S. oestradiol, the demonstration of osteoblastic activity around their tibial subepiphyseal trabeculae (Fig. 83), and the broadening of the epiphyseal cartilage by regular elongated columns of healthy cells (Figs. 83 and 84) are conclusive evidence of active bone formation. To pick out only one feature, e.g. the presence of elongated subepiphyseal trabeculae, as previous workers have done (e.g. Baker and Leek, 1946; Nicolaysen, et al., 1953) and invoke other mechanisms such as failure of resorption as the cause, appears to be quite unnecessary. The absence of epiphyseal cellular disorder in my experiment, is probably accounted for by the longer period over which oestradiol was allowed to act, thus permitting a greater degree of cellular organisation.

It has been shown by Wyman and Tum-Sudan (1945) that increased epiphyseal growth occurred in rat tibiae following adrenalectomy, and Storey (1958) has demonstrated bone growth in rabbits during intervals between periods of cortisone administration. These authors have suggested that this growth is a rebound phenomenon associated with an increased secretion of growth hormone. In my experi-

ment, evidence is presented that these changes are due to direct oestradiol stimulation and not to the action of growth hormone, in that the marked growth response to M.S. oestradiol was almost immediate, and also because lesser degrees of bone growth took place in animals receiving combined M.S. hormone therapy or M.S. testosterone alone. The finding, that during the period of rapid bone growth, the body weights of the animals remained more or less static (Figs. 36 and 38), is strong evidence that the growth hormone was not responsible for the increase in bone length. It appears that at a certain dose level, the effect of oestradiol on extraskeletal tissue is anti-anabolic, but that it also simultaneously exerts an anabolic effect on osteoblasts, and stimulates differentiation of their precursors. A similar dissociated effect on bones of birds has been demonstrated by Scowen (1948), who showed that oestrogens may simultaneously stimulate bone formation in one bone and dissolution in another.

The method by which oestradiol exerts its influence on bone, however, remains obscure. Oestrogens have been stated not to exert a "local" effect on bone (Urist, et. al. 1950), although arguments have been advanced that they may stimulate differentiation of marrow cells towards bone formation (Lacroix, 1951). This theory may

apply to the effect oestrogens have been shown to exert on medullary bone formation in mice (Urist, et al. 1950), but no such effect was demonstrated in this experiment.

It has also been suggested that oestrogens may exert an effect on bone by way of vasomotor control (Ravault, et al., 1939), and it is interesting in this connection to recall that certain localised forms of osteoporosis have been attributed to vasomotor disturbances (Lazani, et al., 1959). The conditions of this investigation did not provide any evidence for or against a vasomotor effect.

My findings, show that oestradiol stimulated endochondral bone formation, and thus bone growth, even although at a certain dose level body growth was simultaneously inhibited. In this respect they are in agreement with Albright's (1947b) statement, that oestrogens stimulate osteoblastic activity. Albright however was doubtful as to whether they exerted a stimulating effect on epiphyseal cartilage, but in my experiment this was their main site of action. My results have also shown that oestradiol is capable of curing osteoporosis produced mainly by sex-hormone deficiency. This action has not previously been demonstrated in experimental animals, and it has not been conclusively demonstrated in man.

The similarity of the effects of oestradiol on bone growth and bone density in this experiment, is not surprising when one remembers that the degree of osteoporosis was assessed from subepiphyseal regions where the majority of the large bone trabeculae stemmed directly from the epiphyseal cartilage. However, an increase also occurred in the numbers and size of the short intercommunicating trabeculae (Fig. 80) which did not have a direct epiphyseal origin. This difference in origin between the two types of bone trabeculae may explain the slight discrepancies between the results of bone growth and radiographic density. Although it has been stated (Albright and Reifenstein, 1948), that oestrogens are without effect in the osteoporosis of Cushing's syndrome, and may even be harmful in this condition, Scowen (1948) does not agree with this view, and has stated that oestrogens may in fact reverse the negative calcium balance in Cushing's syndrome. My findings are more consistent with the latter view. Urist's statement (1958), that the beneficial effects of gonadal hormones in endocrine osteoporosis, are due to the suppression of secretion of corticoid hormones of the adrenal cortex, is however not in accordance with my results. The osteoporosis in my experiment was cured by oestradiol <u>despite</u> the continued administration of cortisone, in amounts

which are usually considered to be close to the upper limits of tolerance of the rat. In view of the rapid return to normal bone density produced by oestradiol (Table 21, Figs. 76 and 79), and of other results shortly to be discussed, it seems reasonable to suggest that oestradiol and not testosterone should be the treatment of choice, at least in certain endocrine varieties of osteoporosis.

Testosterone, on the other hand, is here shown to exert only a slightly stimulating effect on endochondral bone formation and growth (Table 22, Fig. 38). Its effect was much less than that shown by oestradiol alone and also less than the effect of oestradiol and testosterone in combination, but the groups given testosterone did, however, show a rather greater rate of bone growth than did the operated untreated control group 4 during the same period (Table 19). The slightly broader epiphyseal cartilages with their more numerous cells in the testosterone treated groups, compared with group 4 (Figs. 107, 114, and 123) are confirmatory evidence of slight growth stimulation, but the irregularity of the cartilage cells in the zones of maturation of the testosterone treated groups (Fig. 116), suggests that an inhibiting factor was also present. From this evidence it is concluded that testosterone exerts a

slight stimulating effect on endochondral bone formation. These results only barely confirm Albright's (1947b) statement, that testosterone stimulates endochondral and endosteal bone formation, but they do not support his further statement that testosterone will rectify the hormonal imbalance which results when there is excess of 'S' hormone over 'N' hormone, thus making testosterone a specific therapuetic agent for the osteoporosis of Cushing's syndrome.

That testosterone inhibits the skeletal changes produced in mammals by oestrogens (Gardner and Pfeiffer, 1938, 1943), is confirmed in this study by comparison of the epiphyseal cartilages and the subepiphyseal regions of the bones. This is well demonstrated by comparing the histological sections of the tibial epiphyseal cartilages in the group given combined M.S. oestradiol and testosterone (group 2A, Figs. 98, 99, and 100) with those from the group given M.S. oestradiol alone (group 1A, Figs. 82, 83, and 84). In the former group the cartilage plates were narrower and the cell columns shorter. The length of the columns of the subepiphyseal bone trabeculae were also reduced. In the former group therefore the changes representing stimulation of bone growth were present in a considerably modified form. It is not clear why Urist et al. (1950) failed

to demonstrate this effect on mice.

Slight differences were observed between cell numbers and degree of organisation in the epiphyseal cartilages, between members of different groups given the same hormone (e.g. between groups 2, and 2A, and between groups 3 and 3A). However, similar degrees of variability were also seen between members of the same group given O.S. hormone. This may well have been due to a small amount of adrenal gland regeneration in several animals, because in these groups where the whole adrenal fatty bed was excised, the appearances tended to be more consistent. It would probably be unwise therefore to ascribe the differences to greater efficiency of one or other of the hormone preparations.

SUMMARY

Male albino rats of approximately 150 g. initial weight, maintained on adequate diet with high daily calcium intake, were rendered osteoporotic by a combination of cortisone administration, and surgical removal of both adrenal glands and testes. The osteoporosis was treated by injections of sex hormones in various combinations and the results of this regime were assessed by radiographic, chemical, and histological methods, using four

control groups. Two different preparations of oestradiol and testosterone - i.e. an oily solution, and a micro-crystalline suspension - were used and tested in parallel as follows.

Of sixty normal male rats bilateral adrenalectomy and orchidectomy was performed on forty-eight. Of these, thirty-six were divided into two main groups of eighteen rats per group, and each group received either one or the other type of sex-hormone preparation. Each main group was sub-divided into three groups of six rats per group, amd these groups received either oestradiol, or oestradiol and testosterone, or testosterone alone, in doses previously reported as effective and satisfactory for rats. One control group of six operated rats did not receive sex-hormone therapy and was maintained on 1 per c ent. saline without cortisone.

All the other operated groups and one unoperated control group of six rats received daily injections of cortisone acetate (7 mg./kg.) throughout the experiment. One of these operated groups of six rats which received cortisone, did not receive sex-hormone treatment and served as an operated but untreated control. A further control group of six unoperated rats received no treatment of any sort.

Weekly weights and serial radiographic examinations, gave information as to changes in body weight, bone length, and bone density. At the conclusion of the experiment, chemical and histological examination of the bones gave information concerning total calcium content, and structural abnormalities.

As a result of these investigations the following conclusions were drawn.

1. Oestradiol stimulates endochondral bone formation in rats.
2. Oestradiol antagonises the action of cortisone on bone and can reverse the osteoporosis produced by adrenalectomy, orchidectomy, and cortisone administration in rats.
3. Oestradiol can simultaneously stimulate bone growth and inhibit body growth.
4. Testosterone does not materially stimulate endochondral bone formation.
5. Testosterone does not materially antagonise the action of cortisone on bone.
6. Testosterone does inhibit the action of oestradiol on rat bones.
7. Cortisone does not of itself cause osteoporosis in doses of 7 mg./kg. of body weight, but it temporarily

inhibits bone growth in rats. Rat bones are therefore not entirely resistant to the action of cortisone.

8. The absorption of microcrystalline suspensions of sex hormones, is probably superior to the absorption of oily solutions.

GENERAL DISCUSSION

GENERAL DISCUSSION

This survey of necropsy material was planned to give a clearer and more accurate account of generalised osteoporosis than has previously been reported. Methods involving radiology, histology, and chemistry, have been correlated in a study of vertebrae, the bones affected earliest and most severely in this condition. Histological and histochemical studies have been made on the adrenal glands because they are frequently stated to play a major role in the production of osteoporosis. The results obtained were correlated with the density of lumbar vertebrae in each case, so that glands from osteoporotic cases could be compared with glands from cases with normal bone density. Osteoporosis has been produced in rats given abundant calcium, and the effect of injections of sex hormones on their bones has been assessed by radiological, histological, and chemical methods. It has been found that the combination of these three studies raised points requiring general discussion.

From the results of the necropsy series of cases it was found that there was a gradation from normal to abnormal bone density, and arbitrary standards had to be adopted to define the condition of osteoporosis. In the

following discussion are the main conclusions to be drawn, giving a clear cut picture of the condition with suggestions for diagnosis and possible treatment before serious effects occur. It must first be emphasised that none of the cases complained of symptoms referable to their osteoporosis, and the condition was unsuspected. Although it is difficult to be certain that their dietary intake was always adequate, no evidence was found which suggested that any of the other specific conditions described in the general Introduction as producing osteoporosis was present in these patients.

The incidence of osteoporosis.

It is frequently stated that osteoporosis is an inevitable accompaniment of advancing years, but the demonstration in this survey, that bones of normal and even of high normal density are present in elderly individuals of both sexes, is against this belief. For this reason the finding in the present series, among patients dying in hospital aged 50 or over, that one in four or five suffers from osteoporosis with its inherent tendency to fracture, and disability, constitutes a major diagnostic and therapeutic challenge to the physician, which is at present not widely recognised. It has been stated (Albright and Reifenstein, 1948) that post-menopausal and

senile osteoporosis are metabolically identical. Apart from surveys based on clinical radiography of the spine, on examination of peripheral limb bones, and the finding of earlier age of onset in females, which can no longer be accepted as definitive, no other differences have been found between the two conditions. The finding in the present series that the sexes are equally affected, makes it virtually certain that they are in fact the same condition - i.e. that of senile osteoporosis.

The role of calcium deficiency in senile osteoporosis

Nordin (1958) has claimed, with justification, that it remains to be proved that prolonged dietary calcium deficiency is not a factor in the production of senile osteoporosis, and Malm (1958) has shown that on a dietary intake of calcium, twenty per cent above that considered necessary for good health, a small proportion of healthy adult males remained in continuously negative calcium balance.

In my survey the regression of radiographic bone density with advancing age, revealed a slight but progressive decrease of bone density in both sexes commencing early in adult life and continuing up to about the age of fifty. The possibility of this being due to dietary calcium deficiency, cannot be entirely excluded, but there

was no significant difference between the regression curves of the two sexes, during this or any period. It appears therefore that the undoubted calcium drain produced in females by pregnancy, and lactation, was not an important factor in producing senile osteoporosis, because some degree of sex difference would have been demonstrated if calcium deficiency were the cause. It is also of interest in this connection that rats developed a severe degree of osteoporosis in spite of high calcium intake. + fully adrenal cortical atrophy.

Of greater interest was the more rapid decrease of bone density which took place in my survey about the age of fifty, and which was more pronounced in females in whom the decline of sex-gland activity at the menopause, is known to be sharper. The similarity of adrenal histochemical patterns in two groups of human cases, one with normal bone density and the other with osteoporosis, excluded purely adrenal factors as the cause of this condition. If it be taken into account that in rats, cortisone alone did not produce either osteoporosis, or other significant bone changes, it seems highly probable that in them, as in the human cases, the osteoporosis was due to gonadal sex-hormone deficiency. It is therefore concluded that gonadal sex-hormone deficiency is the major cause of senile osteo-

porosis.

Therapeutic implications

In view of this conclusion, the fact that prolonged sex-hormone therapy of osteoporosis, has so far failed to produce demonstrable bone redensification in cases of human osteoporosis, calls for further consideration of possible additional factors. It is difficult to know how far a parallel can be drawn between the artificially produced osteoporosis in the rat, and the naturally occurring condition in man. If a close comparison were possible, then a cure so readily effected by oestradiol in 75 days in the rat whose normal life span is 3 years (Farris, 1942) should also be demonstrable in man within a period of 5 years. The review by Hennemann and Wallach (1957) of more than 200 cases of post-menopausal osteoporosis who received sex hormone therapy, for periods of up to twenty years without demonstrable radiological improvement, suggests that the parallel is not a close one. One explanation for this may be that the principal sites of action of sex hormones in the rat were the epiphyseal cartilages at the ends of the long bones. In the case of vertebral bodies of elderly human subjects, however, the epiphyseal cartilages are no longer normally active sites of bone

production, and may no longer be capable of responding to sex-hormone stimulation.

Only one convincing instance of radiological improvement of spinal osteoporosis in humans has been reported (Wang and Robbins, 1956), and that was in a girl aged 11 with Cushing's syndrome stated to be due to adrenal cortical hyperfunction, in whom the only treatment consisted of irradiation of the pituitary gland. It seems likely that in such a young person the vertebral epiphyseal cartilages were still active and were thus able to restore the lost bone substance after the adrenal function had been returned to normal.

It is generally accepted however, that sex hormone therapy does arrest the progress of osteoporosis and the authors of a recently published text-book recommend the replacement of "the deficient hormone". (Aegerter and Kirkpatrick, 1958). By this they imply that oestrogens should be given to females, and androgens to males. In cases of osteoporosis due to Cushing's syndrome testosterone is recommended for both sexes (Albright, 1947b). Other authors (e.g. Cooke, 1955, Whedon, 1956) go further, and state that androgens, together with oestrogens (in small doses) should be given to both sexes. Urist (1958) considers that the senile state is in reality a mild form of

Cushing's syndrome. One presumes that he would therefore recommend only testosterone for all cases.

My results show that not only was oestradiol much more effective than testosterone in experimentally produced osteoporosis in male rats, but that the effect of testosterone alone was very slight, and this finding cannot be too strongly emphasised. In these animals the balance of hormones was in some measure comparable to that in Cushing's syndrome, and yet testosterone partly inhibited the curative effect of oestradiol. These results strongly suggest that oestradiol alone should be given to all cases of osteoporosis of endocrine origin, at least for a period of trial.

Prevention

Cooke (1955) has stated, that "no one could seriously advocate universal endocrine substitution therapy after the age of 50." He recommended that older people should have an adequate protein and mineral dietary intake, reasonable physical activity, an annual physical examination, and that caution should be exercised in giving cortisone and ACTH to patients in these age groups. In my survey there was no evidence to suggest that calcium deficiency, or other dietary factors, or reduced physical activity played an important role in producing osteoporosis. Although one

must agree with Cooke, that an annual physical examination paying particular attention to loss of height, the appearance of transverse abdominal skin folds, and aches and pains in the trunk for no good reason is highly desirable, I feel that these measures are inadequate. By the time such changes have appeared, a considerable degree of irreversible but perhaps preventable osteoporosis has developed. The frequency of the condition surely merits a more thorough attempt at earlier diagnosis, for instance by bone biopsy along the lines indicated by Beck and Nordin (1960) whenever people in the older age groups come under medical hospital care. Later, when better methods of estimating early bone changes by radiology have become standard, an even wider section of the community can be surveyed. Individuals thus found to be developing senile osteoporosis, should be given interrupted courses of "the appropriate" sex hormone which I suggest is probably oestradiol, so that the progress of the condition may be arrested.

Suggestions for further study

In any work of this size a number of possibilities for further investigations are bound to arise. It has previously been mentioned in Part I of this Thesis that more specific studies using either microradiography, or

interference microscopy should be made on the vertebral cancellum so that the direction of metabolic activity, and the degree of mineralisation of osteoporotic bone can be evaluated.

The successful treatment of experimental rat osteoporosis by oestradiol, suggested that Vitamin D which is chemically similar to oestradiol and also promotes absorption of calcium from the intestine, (oestradiol was reported to do this in hens by Gardner and Pfeiffer, 1943) might have been just as effective under similar conditions. This possibility requires investigation.

Nordin (1958) has drawn attention to the fact that all the reported evidence supplied by studies of nitrogen and calcium balance, concerning the effects of androgen and oestrogen therapy in human osteoporosis, is slender. In view of the success of oestradiol therapy in male rat osteoporosis, when the bulk of published evidence suggested that testosterone should be more specific than oestradiol, it is suggested that further careful balance studies on human cases incorporating the study of bone changes by biopsy, are required to establish whether my findings may not be applicable also to human subjects.

CONCLUSIONS

CONCLUSIONS

1. By the use of the densitometric method described, the calcium content of bone can be confidently predicted provided that the sex of the patient is taken into account.

2. Bone density reaches its maximum in early adult life and thereafter it tends to diminish with advancing age, although a wide range is still seen in older age groups.

3. In view of the homogeneity of the series of cases examined, the grading of cases as normal, or osteoporotic, must be an arbitrary procedure.

4. Osteoporosis is much more common than is generally realised. In this unselected general hospital series of necropsies the incidence was 22·7 per cent among cases aged 50 or over.

5. Although osteoporosis occurred several years earlier in females, post-menopausal osteoporosis and senile osteoporosis are considered to be the same condition - i.e. senile osteoporosis.

6. Vertebral biconcavity in the radiograph may be present in patients with normal bones and absent in cases of osteoporosis. It is therefore not a reliable sign of osteoporosis.

7. Senile osteoporosis results from diminished production and activity of osteoblasts. Thus bone trabeculae are left unprotected and an increased amount of bone resorption takes place.

8. Senile osteoporosis is fundamentally due to gonadal sex-hormone deficiency, and it is not the result of abnormal adrenal cortical function.

9. Oestradiol stimulated endochondral bone formation and bone growth in rats. Production of cancellous bone was also stimulated but to a lesser degree. At a certain dose level oestradiol stimulated bone formation and growth, although it simultaneously inhibited body growth.

10. Oestradiol cured osteoporosis produced by gonadal sex-hormone deficiency in rats.

11. When testosterone was given in combination with oestradiol, the above effects of oestradiol on bone were partly inhibited.

12. Testosterone alone did not materially stimulate either endochondral bone formation or bone growth in rats.

13. Testosterone alone was much less effective than oestradiol in curing osteoporosis produced by gonadal sex-hormone deficiency in rats.

14. A new approach is required, probably involving more widespread use of the techniques of bone biopsy, and improved radiological methods when these become available, if cases of senile osteoporosis are to be recognised sufficiently early.

15. Earlier diagnosis thus leading to the earlier administration of "the appropriate hormone" (oestradiol is suggested) is the only means of preventing substantial loss of bone substance with its inherent tendency to fracture, deformity, and disability.

APPENDIX

APPENDIX

1. Chemical procedures

Bone calcium estimations were at first performed by the volumetric titration method against N/10 potassium permanganate described by Vogel (1951), used routinely in many laboratories, and therefore not described in detail. Although reasonably accurate it is time consuming. Accordingly a more rapid method using a flame photometer was adopted, as described by Powell (1953) for estimating calcium in the blood, but since found satisfactory also for estimating calcium in bone. The method involves precipitating the calcium of bone ash as oxalate with an oxalic acid - ammonium oxalate mixture, redissolving the precipitate, and estimating the calcium on the photometer using a special filter.

In the first place it was necessary to prepare a stock calcium solution as follows. Five grams of calcium chloride were weighed and dissolved in 500 ml. distilled water. Ten ml. of this solution were titrated with decinormal silver nitrate solution using potassium chromate (5 per cent) as indicator. The end point was noted by observing the change of colour from yellow to turbid red persisting even after shaking. The calcium content of the

solution was calculated from the amount of silver nitrate solution used. Thus,

11·7 ml. of N/10 $AgNO_3$ was required to titrate 10 ml. of $CaCl_2$

$\therefore$ 1 ml. $CaCl_2$ = 1·17 ml. N/10 $AgNO_3$

but 1 ml. N/10 $AgNO_3$ = $\frac{0·0111}{2}$ g of $CaCl_2$

$\therefore$ 1·17 ml. N/10 $AgNO_3$ = $\frac{0·0111}{2}$ x 1·17 g. $CaCl_2$

111·0 g. of $CaCl_2$ contain 40·08 g. of calcium

$\therefore$ 1 ml. of stock solution will contain

$$\frac{40·08}{111} \times \frac{0·0111}{2} \times \frac{1·17}{1}$$

= 0·00234 g. or 2·34 mg./ml. of calcium

The solution was then rediluted so that the final concentration was 2·0 mg. calcium per ml., and this was confirmed by titrating it again in the same way.

In a preliminary experiment using a flame photometer (Evans Electroselenium Ltd.) with a coal gas flame and a supply of compressed air at a pressure of 10 lb. per sq. inch, standard solutions containing different concentrations of calcium were prepared from the stock calcium chloride solution. The galvanometer of the flame photometer was adjusted to give '0' reading with resin filtered water and

a full scale deflection - '100' with a standard solution containing 25 mg. of calcium/100 ml., using a calcium filter. The different standard solutions of calcium were then sprayed through the flame photometer. The results thus obtained were plotted on a graph, (Fig. 145) where it is shown that there is a straight line relationship between the calcium content of the solution, and the flame photometer readings.

Sastoskar (1957) using the same instrument estimated the calcium content of 45 different bone solutions and five known calcium standards in parallel with the chemical method.

He found that neither method gave a complete recovery of calcium from the known standard solutions but no significant difference($F < 0.25$) between the methods was demonstrated.

The technique of estimation of calcium in bone by the flame photometer

Solutions required

(a) 0·1 M oxalic acid (12·6 g./l.)

(b) 0·1 M ammonium oxalate (14·2 g./l.)

Oxalic acid - ammonium oxalate mixture was prepared by mixing 5 ml. of solution (a) and 95 ml. of solution (b).

(c) 0·05 N perchloric acid. This was prepared fresh on each occasion by diluting 1·66 ml. of 60 per cent commercial perchloric acid to 200 ml. with resin filtered water.

Procedure

In the first two stages there were slight differences of procedure for human bones and rat bones but thereafter the procedure was common to both.

(a) Human vertebrae

1. The ash from the vertebral slab was dissolved in 25 ml. of 20 per cent HCl and washed into a beaker to a volume of 70 to 100 ml. It was then boiled and filtered (Whatman No. 41 11") and made up to 250 ml. with resin filtered water which was used for all such operations.

2. 3 ml. of this solution was then made up to 200 ml.

(b) Rat femora

1. The femoral ash was dissolved in 5 ml. of 20 per cent HCl, and made up to 30 ml.

2. 5 ml. of this solution was made up to 250 ml.

Both 3. 5 ml. of diluted bone solution was placed in a conical centrifuge tube.

4. 3 ml. of the ammonium oxalate -oxalic acid mixture were added, gently shaken, and allowed to stand for one hour.
5. The tube was centrifuged for 10 min. at 2,500 r.p.m. and the supernatant fluid poured off. The tube was then inverted on filter paper for three minutes.
6. 5 ml. of 0·05 N perchloric acid were added to the precipitate and the tube shaken gently to dissolve it completely.
7. A standard calcium solution (10 mg./100 ml.) was then prepared in the 0·05 N perchloric acid from the stock calcium chloride solution.
8. The flame photometer with the calcium filter in position was adjusted for sensitivity so that resin filtered water gave no galvanometer deflection, and the standard calcium solution gave a deflection of 40 divisions.
9. The unknown was compared with the standard over five readings, resin filtered water being interposed each time.
10. From the mean of the five readings the amount of calcium (mg./100 ml. in the unknown) was calculated from the graph (Fig. 145) and

this was then corrected for dilution thus giving the total quantity of calcium in the original solution.

2. Diet

The composition of the standard rat food used in this study was as follows:

Wheat, whole ground	50 per cent.
Barley " "	25 " "
White fish meal	7 " "
Meat and bone meal	6 " "
Dried brewer's yeast	5 " "
Dried grass meal	5 " "
Cod liver oil	1 " "
Salt	1 " "
	100 " "

Theoretical composition:

Moisture	14·3 per cent.
Soluble carbohydrate	53·4 " "
Protein	20·0 " "
Fat	3·8 " "
Fibre	3·3 " "
Ash (Ca: 0·7, P: 0·8)	5·2 " "
	100 " "

3. Radiographic methods

All radiographs were made with a portable X-ray unit, Model K-5 (Newton Victor Ltd.). It was necessary to find a suitable exposure (Kv. and m.a.) so that the range of density of the step wedge fell within the linear area of the Hurter-Driffield curve of sensitivity for the emulsion of the X-ray plates.

This was done by the process of trial and error, employing a constant tube distance (63·5 cm.), holding the m.a. component constant and varying the Kv. component until a value was obtained whereby a uniform galvanometer deflection resulted with each consecutive step of the step wedge (Fig. 2). Subsequently, prior to taking bone density readings all plates were tested in this fashion to ensure that they were correctly exposed.

4 Surgical methods

(a) Adrenalectomy

Following removal of dorsal hair from the rats by clippers, a mid-line dorsal incision was made over the lower thoracic and upper lumbar spines. The skin was reflected for some distance on either flank, and the adrenal glands were in succession exposed by a small oblique incision through the muscle, just below and parallel with the rib margins. This was expanded by blunt scissor blades and the adrenal pedicle grasped with curved haemostat forceps, and held firmly for a few moments. The gland was then removed complete with its capsule, using a scalpel. In eighteen animals as much as possible of the periadrenal fat was also removed by scissors and forceps. Each muscle incision was then closed by two interrupted braided linen sutures and the dorsal incision was closed by Michel clips.

(b) Orchidectomy

The animals were laid on their backs and the testes expelled from the abdomen into the scrotum by digital pressure. A single vertical incision was made in the mid-line of the scrotum and the testes with their cords expressed on to the surface. Curved haemostat forceps were clamped on to each cord about 1 cm. from the testis, and the

cord on the distal side of the clamp snipped through with scissors, thus removing the distal part of the cord and the testis. The proximal parts of the cords were returned to the scrotum without ligature, and the incision sutured with two or occasionally three interrupted braided linen sutures.

Work apportionment

The work for this Thesis was done during the four and a half years of my tenure of the post of Lecturer in Pathology at the University of Sheffield, which continues. This work was performed at all times in conjunction with a full share of teaching and routine hospital duties at the Sheffield Royal Infirmary, and the majority of the work was carried out personally by me except as will now be stated.

Most of the lumbar vertebral slabs were collected by Mr G. Colgrave, mortuary attendant at the Sheffield Royal Infirmary. These were subsequently radiographed in my presence by Mr T.L. Platts, Senior technician in the University department, who also performed the photography for Parts I and III of the Thesis under my supervision. The photography for Part II of the Thesis was performed by me.

I also performed the many subsequent procedures on bone, for Parts I and III of the Thesis, which included cleaning, weighing in air and water, the calcium estimations and the measurements of radiographic density. The adrenal glands were also personally collected, dissected, and weighed. The technical procedures for the demonstration

of lipoids, and succinic dehydrogrenase activity, including the cutting and mounting of frozen sections, were however performed by Mr A.C. Welsford, Senior technician in the laboratory at the Sheffield Royal Infirmary.

All other histological procedures were carried out by Mrs S. Loomes, my technical assistant, who also undertook the care of the animals and their weekly weighings, but the cortisone and sex-hormone injections were given by me. Mr A.P. Foster, medical artist to the Sheffield Royal Infirmary drew the diagram and assisted with the presentation of a number of the graphs.

All other procedures were carried out personally by me.

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A STUDY OF THE INCIDENCE, AETIOLOGY, AND PATHOLOGY OF SENILE OSTEOPOROSIS

VOLUME II

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF MEDICINE OF THE UNIVERSITY OF GLASGOW

BY

R.A. CALDWELL M.B., Ch.B.

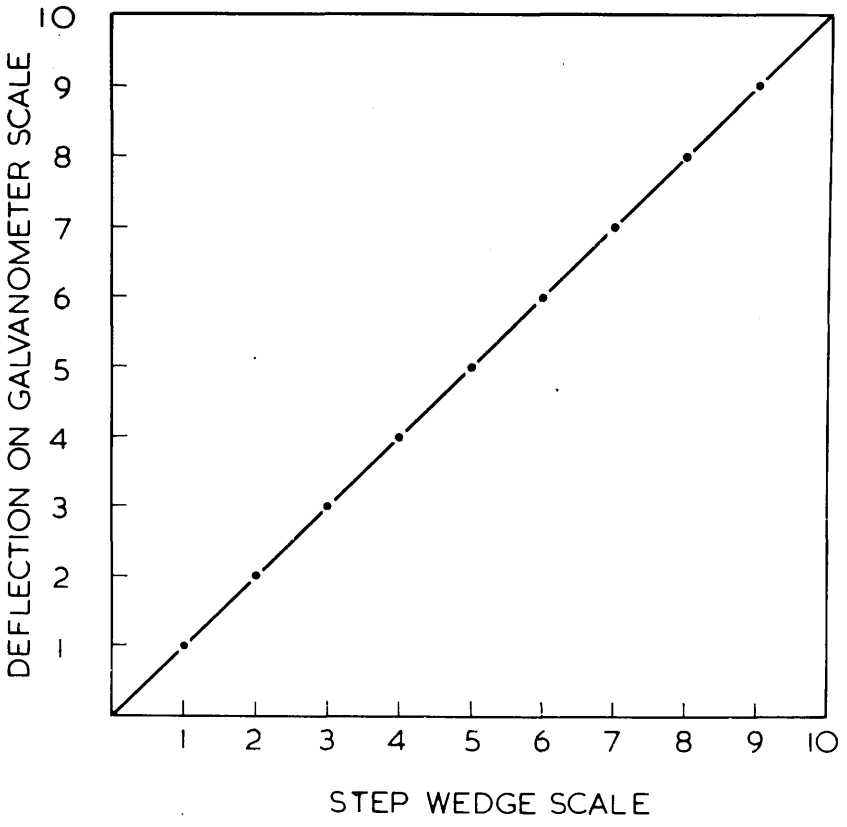

Fig. 2. A graph showing the straight line relationship between the different galvanometer readings obtained at the various steps of the step wedge from a correctly exposed and processed X-ray film. It indicates that the film is exposed within its range of maximum sensitivity (Hurter-Driffield).

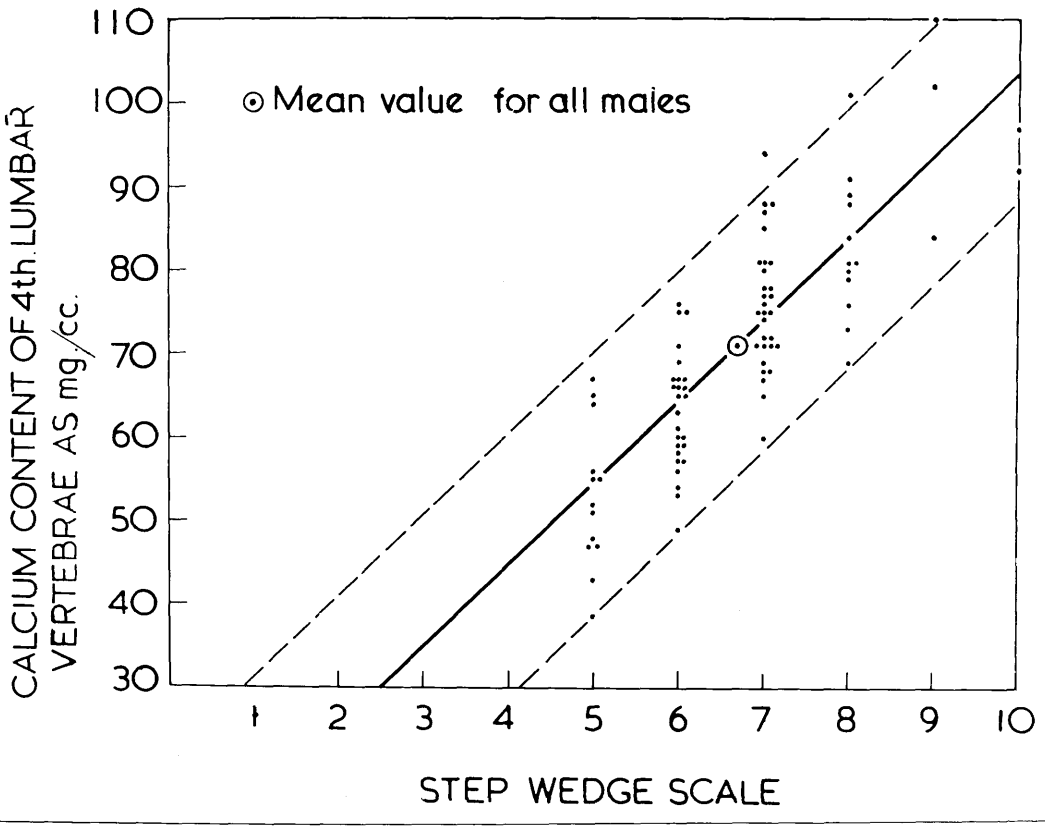

Fig. 3. A graph showing calcium (mg. per c.c.) plotted against radiographic density (step-wedge units) of lumbar vertebrae showing the regression and the 95 per cent confidence limits (87 male cases).

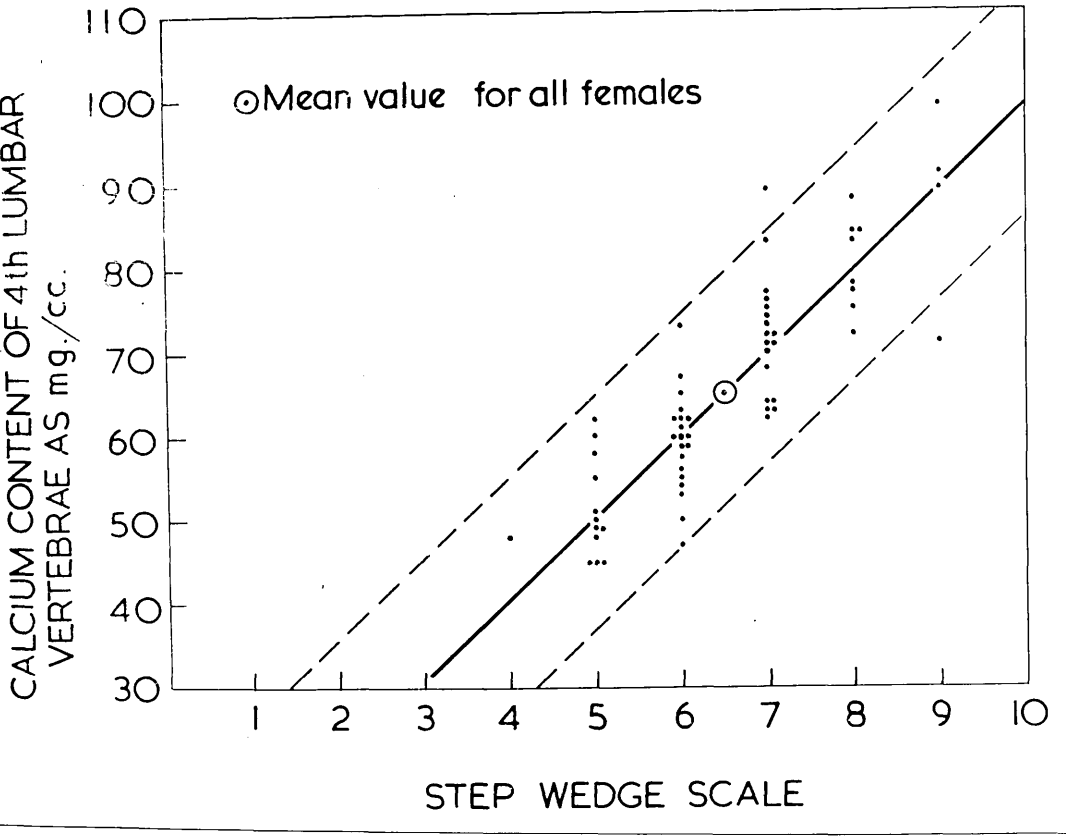

Fig. 4. A graph showing calcium (mg. per c.c.) plotted against radiographic density (step-wedge units) of lumbar vertebrae showing the regression and the 95 per cent confidence limits (63 female cases).

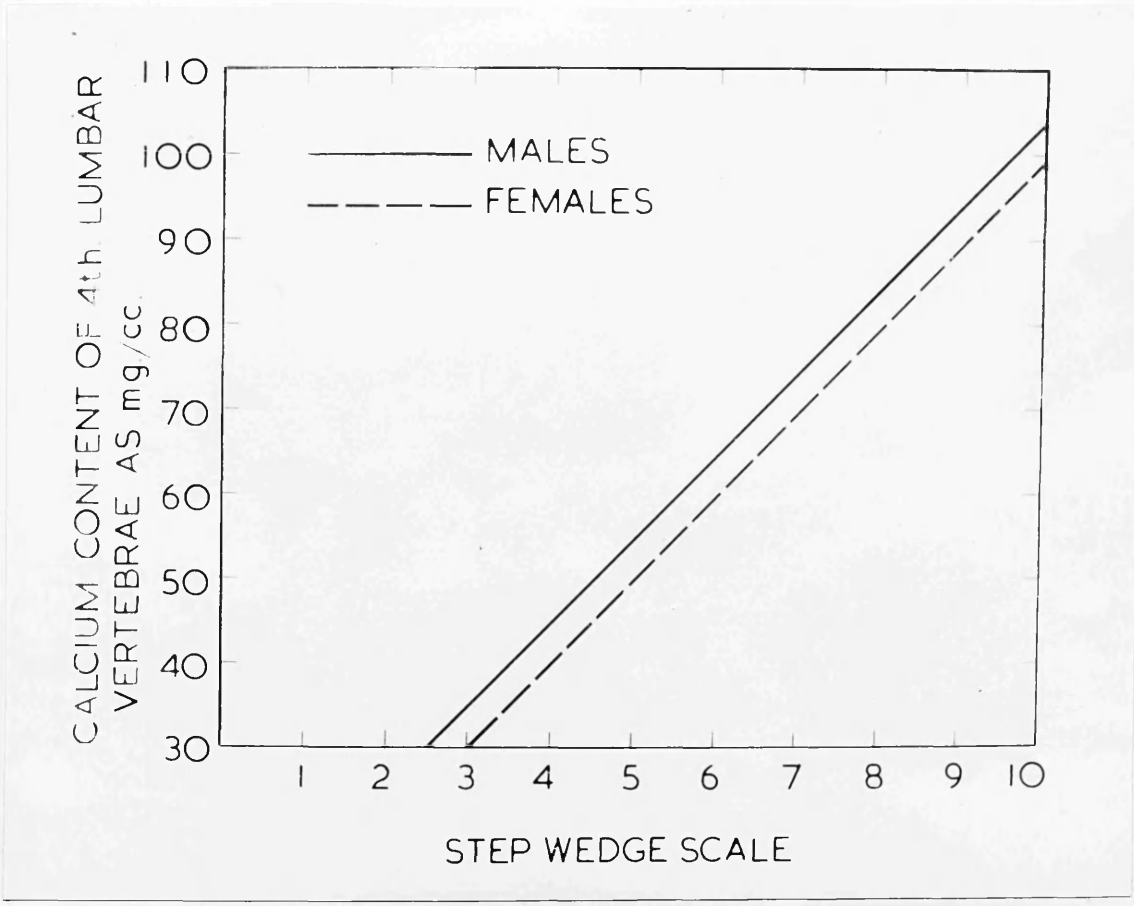

Fig. 5. A graph showing the regression of vertebral calcium content (mg. per c.c.) on step-wedge limits of radiographic density (150 cases).

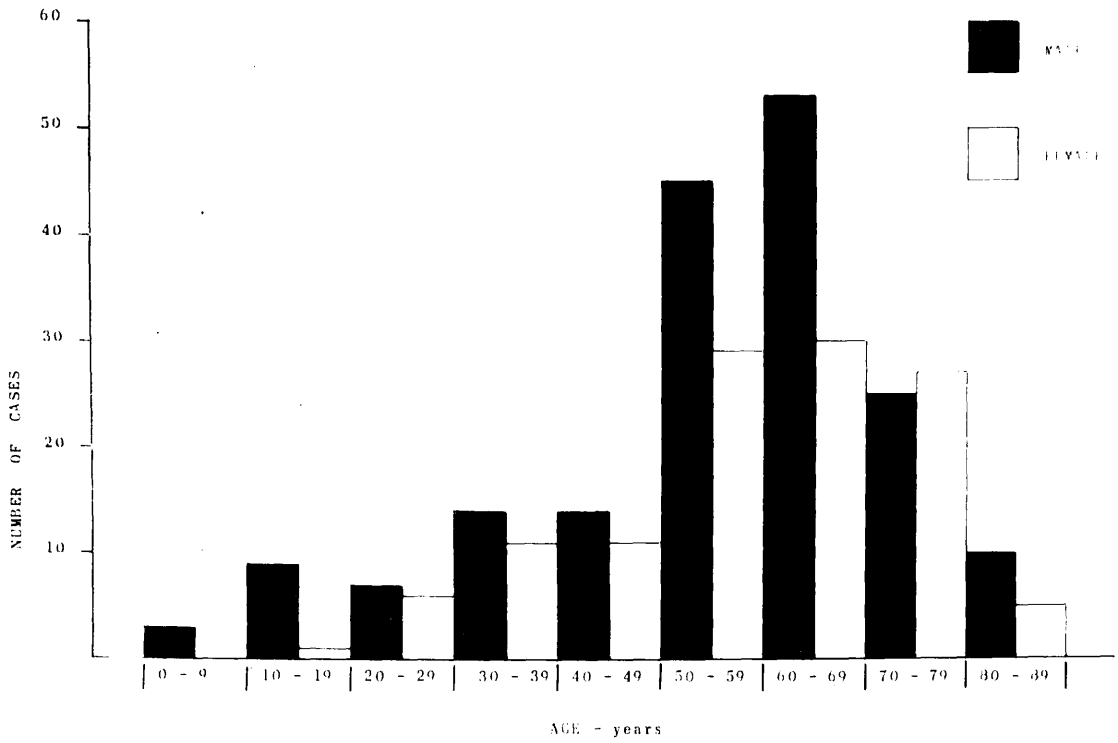

Fig. 6. A histogram showing the composition of the series of 300 necropsy cases by age and sex.

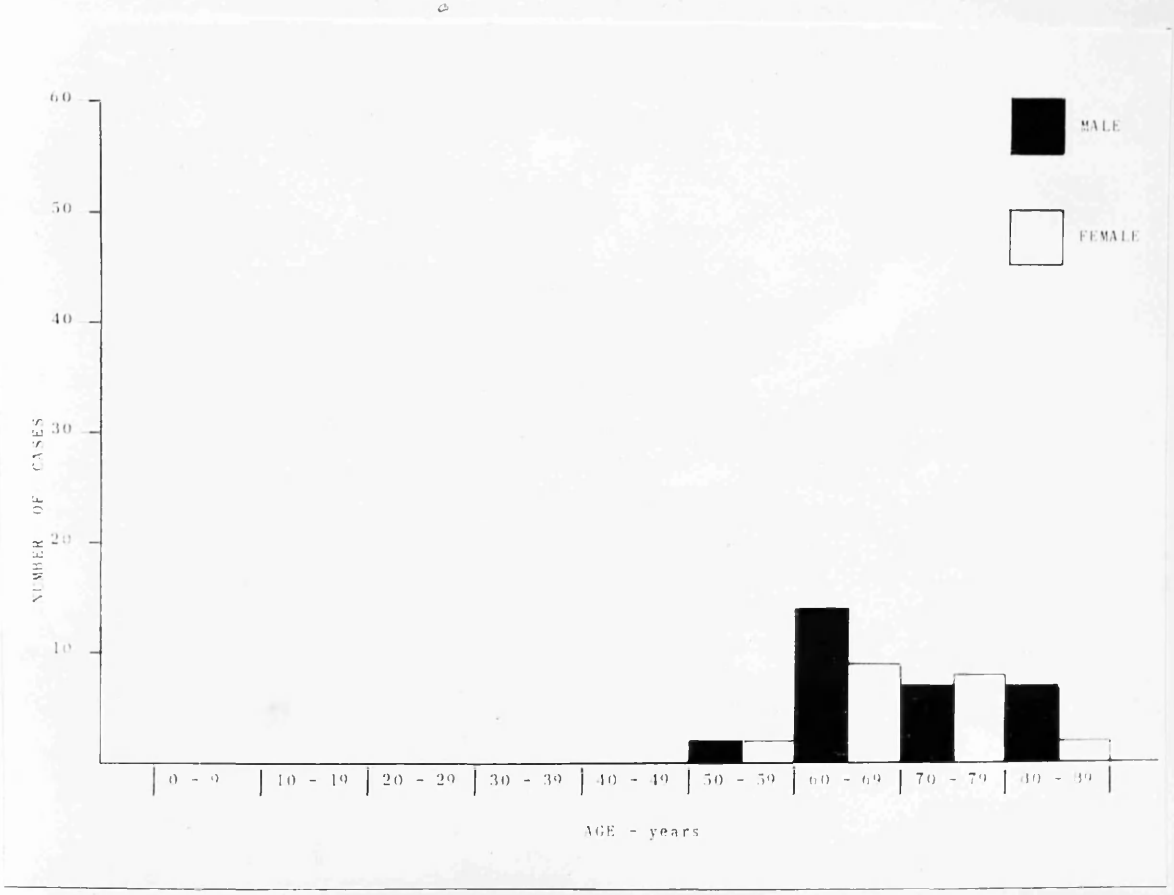

Fig. 7. A histogram showing the composition of the 51 osteoporotic cases by age and sex.

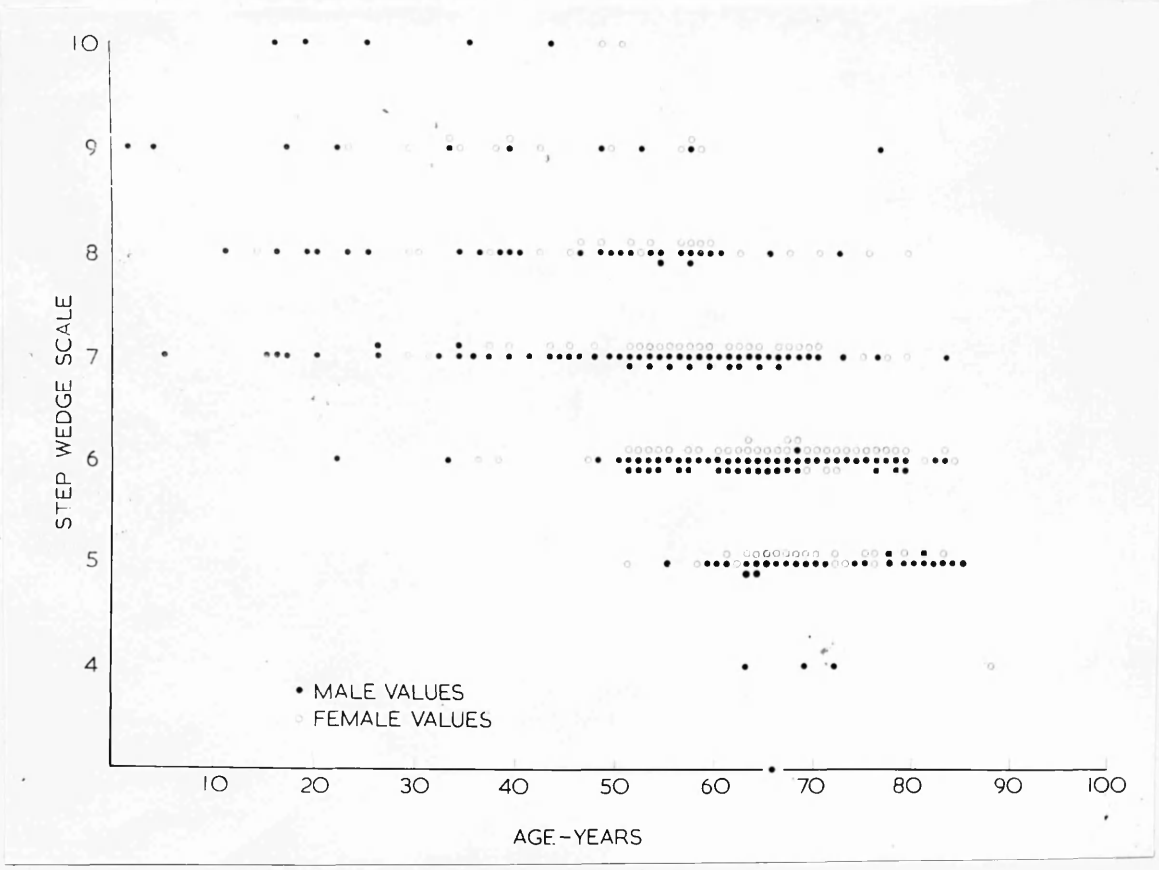

Fig. 8. The scatter diagram of radiographic density readings (step-wedge units) against age in years (300 cases).

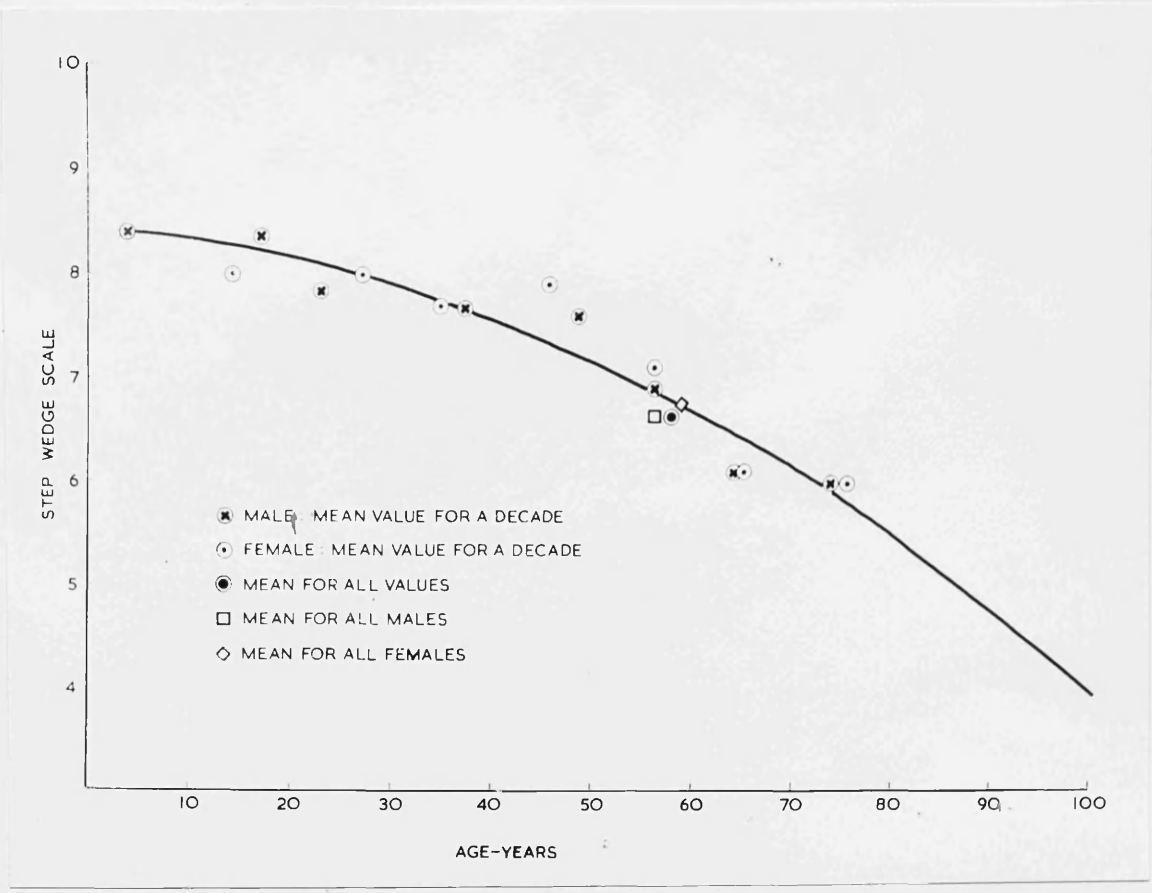

Fig. 9. The fitted curve of the regression of radiographic density (step-wedge units) on age in years (300 cases).

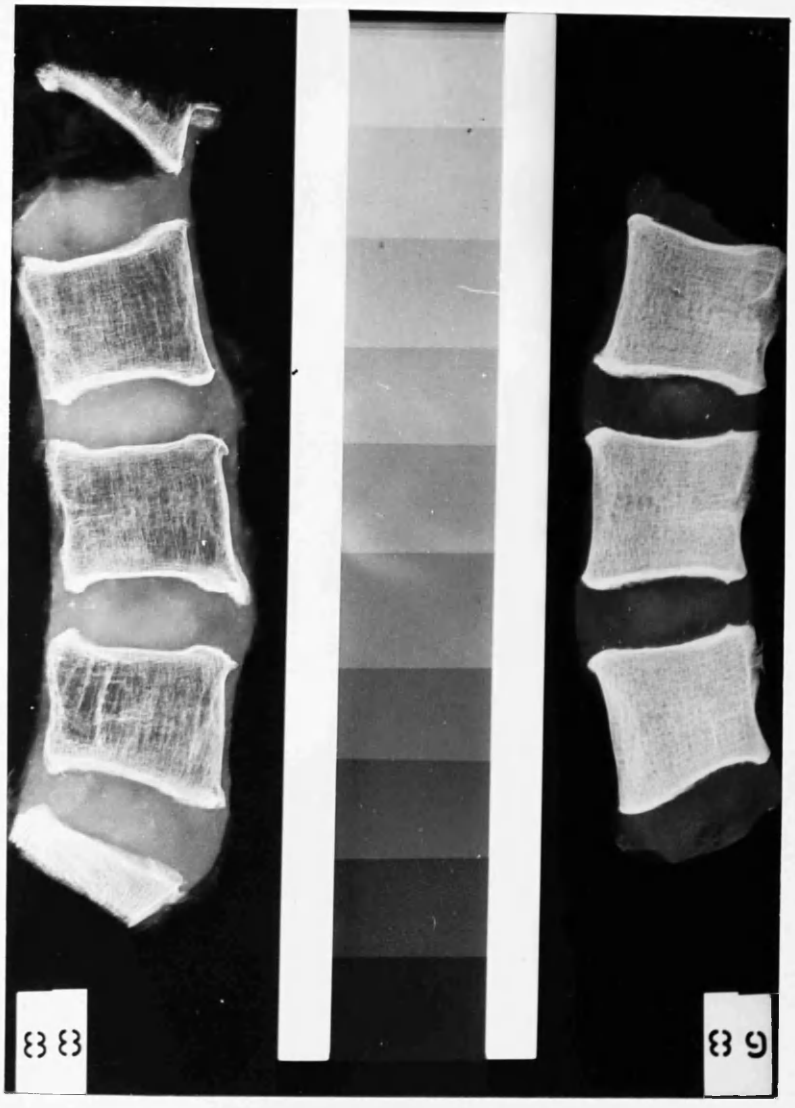

Fig. 10. Reduction of a radiograph of two vertebral slabs and the aluminium step-wedge. The bone slabs are of a uniform thickness of 1 cm.

Specimen 88 (on the left) shows advanced osteoporosis. The density of the fourth lumbar vertebra corresponded to 5 sheets of the aluminium wedge and its calcium content was 51 mg. per c.c.

Specimen 89 (on the right). The fourth lumbar vertebra is of high normal density and corresponded to 10 steps of the wedge. Its calcium content was 92 mg. per c.c.

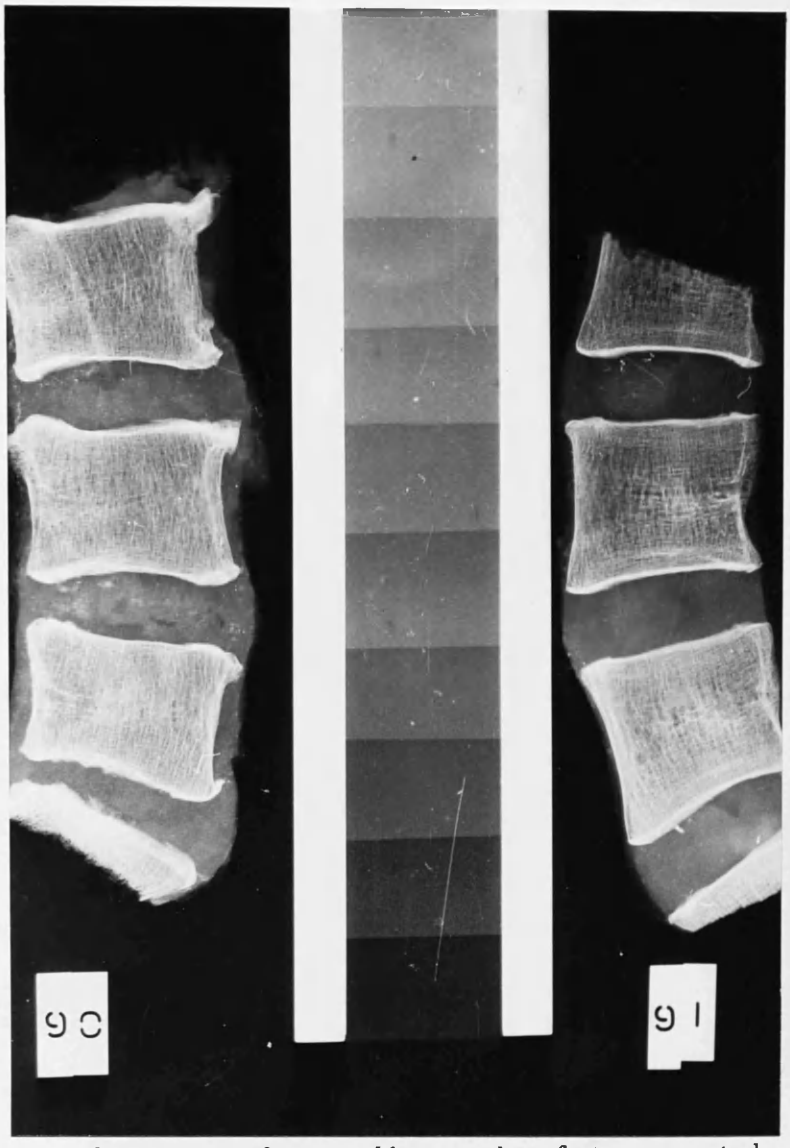

Fig. 11. Reduction of a radiograph of two vertebral slabs (1 cm. thick).

Specimen 90 (on the left). The fourth lumbar vertebra is of average normal density and corresponded to 7 steps of the wedge. Its calcium content was 71 mg. per c.c.

Specimen 91 (on the right). The fourth lumbar vertebra is of average normal density and corresponded to 7 steps of the wedge. Its calcium content was 68 mg. per c.c.

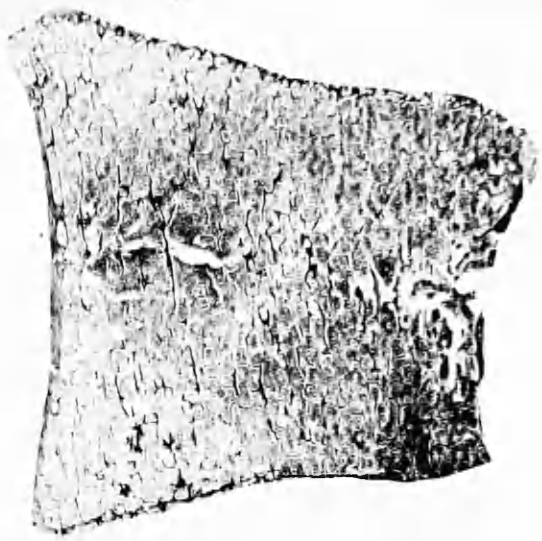

Fig. 12. A histological section of a lumbar vertebral body. It is of low normal density and its radiographic reading corresponded to 6 steps of the wedge. Its calcium content was 72 mg. per c.c.
Haematoxylin and eosin x 1·75

Fig. 13. A histological section of a vertebra of high normal density having a radiographic reading of 9 steps of the wedge. Its calcium content was 90 mg. per c.c.
Haematoxylin and eosin x 1·75

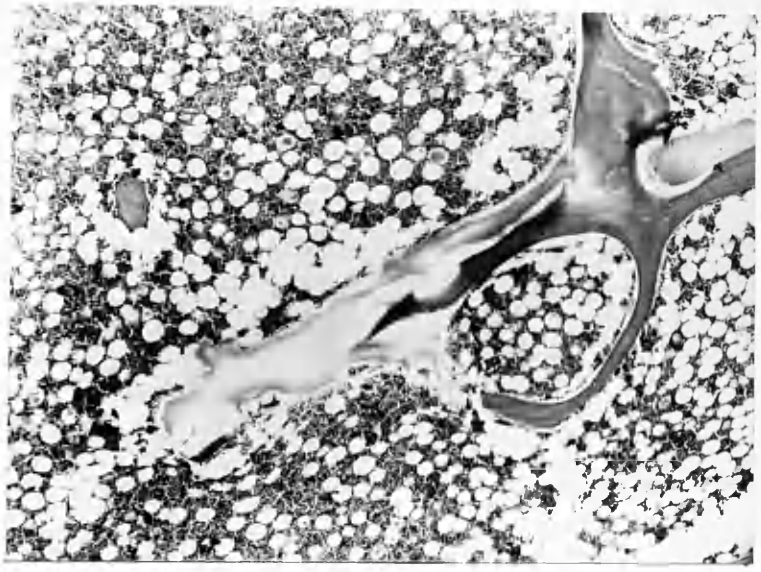

Fig. 14. A histological section of a lumbar vertebral body showing para-trabecular pink material. H. & E. x 75.

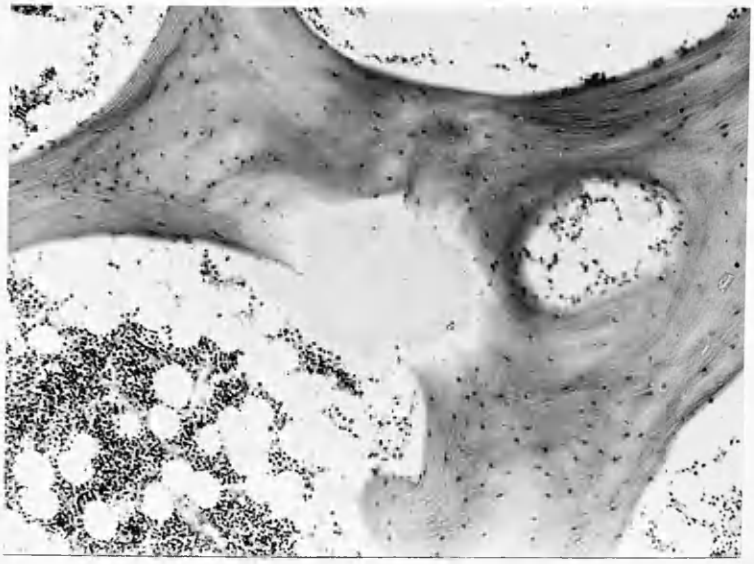

Fig. 15. A histological section of a lumbar vertebral body showing pink material in a space produced during section cutting. H. & E. x 190.

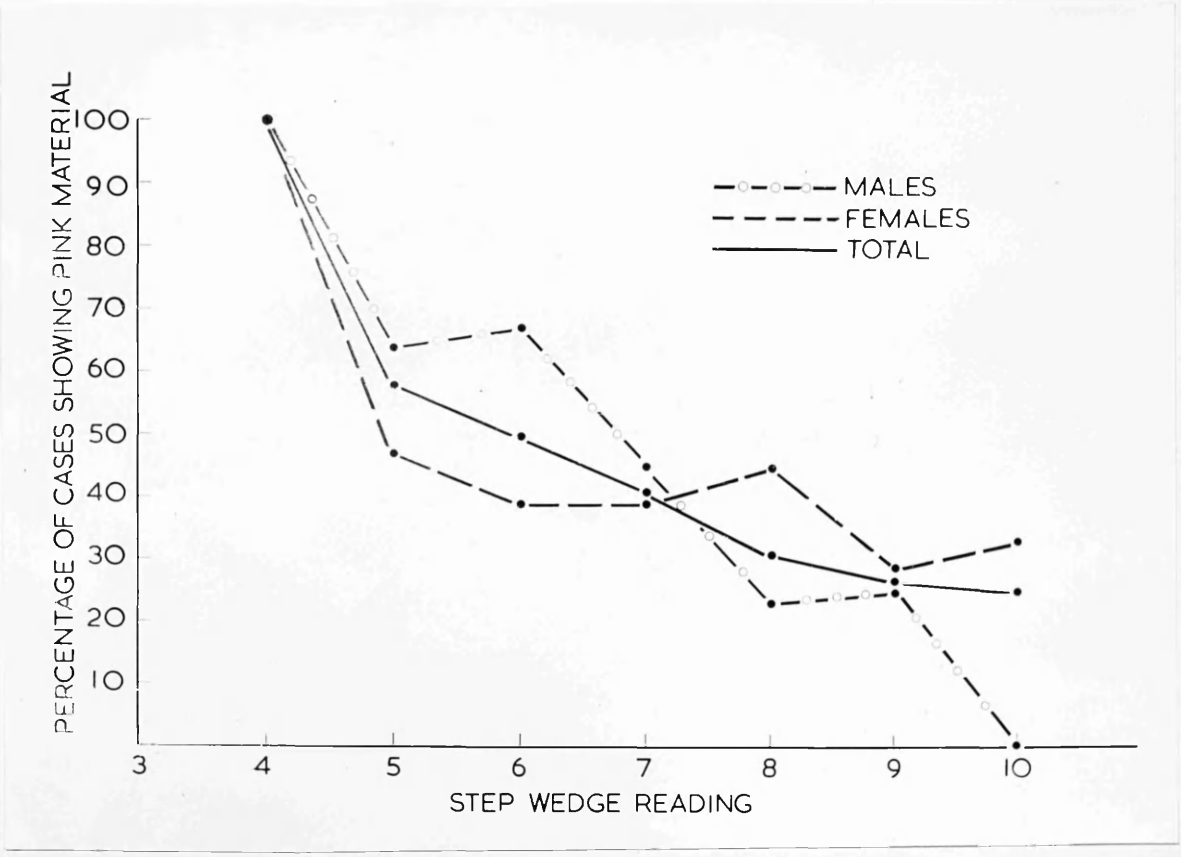

Fig. 16. To show the percentage incidence of cases having two or more foci of pink material per histological section of lumbar vertebra in the various grades of radiographic bone density (step-wedge units).

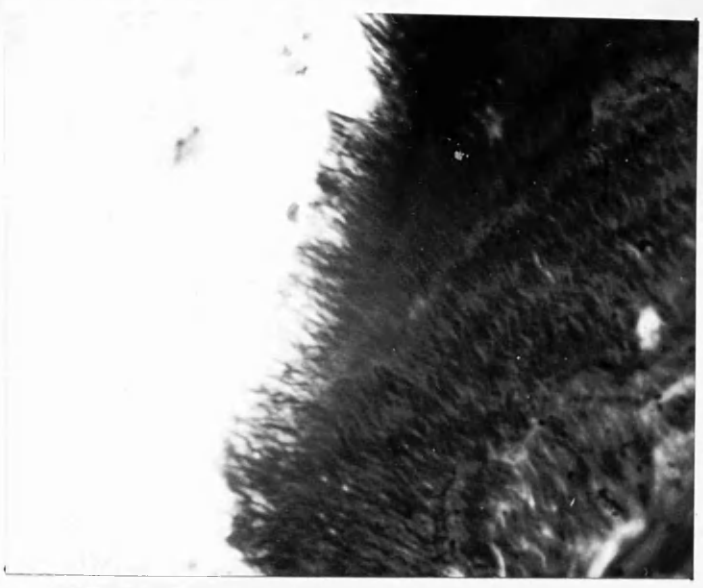

Fig. 17. A histological section of an adult lumbar vertebra showing a bone trabeculum with a ciliated margin.

Reticulin stain x 300.

Fig. 18. A histological section of an adult lumbar vertebra showing a bone trabeculum with a ciliated margin.

Reticulin stain x 300.

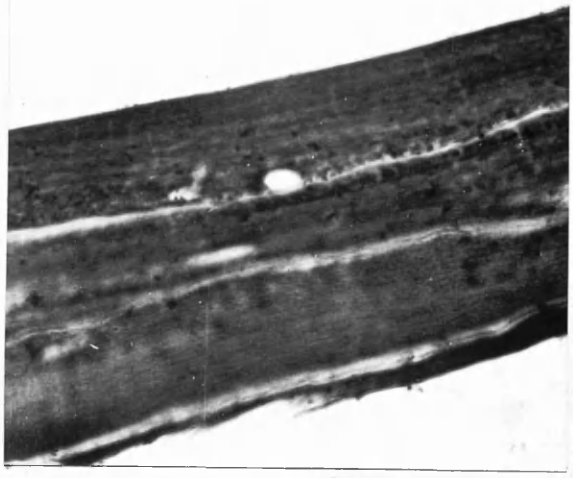

Fig. 19. A histological section of a lumbar vertebra from an adolescent subject aged 17 years) showing absence of a ciliated margin.
Reticulin stain x 300.

where ?

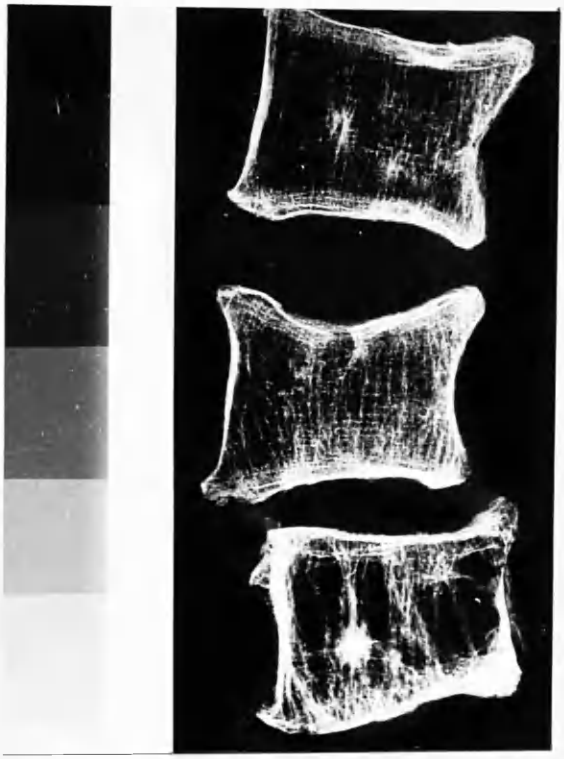

Fig. 20. Case 124. Reduction of a radiograph showing biconcave osteoporotic vertebrae, (index 72) per cent). The radiographic density was equivalent to 5 units of the step-wedge and the bone calcium content was 51 mg. per c.c.

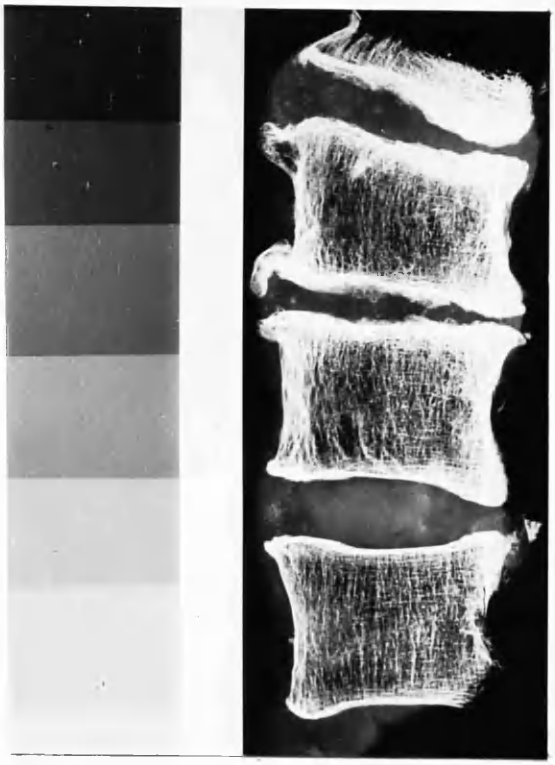

Fig. 21. Case 247. Reduction of a radiograph showing osteoporotic vertebra without biconcavity (index 89 per cent). The radiographic density was equivalent to 5 units of the step-wedge.

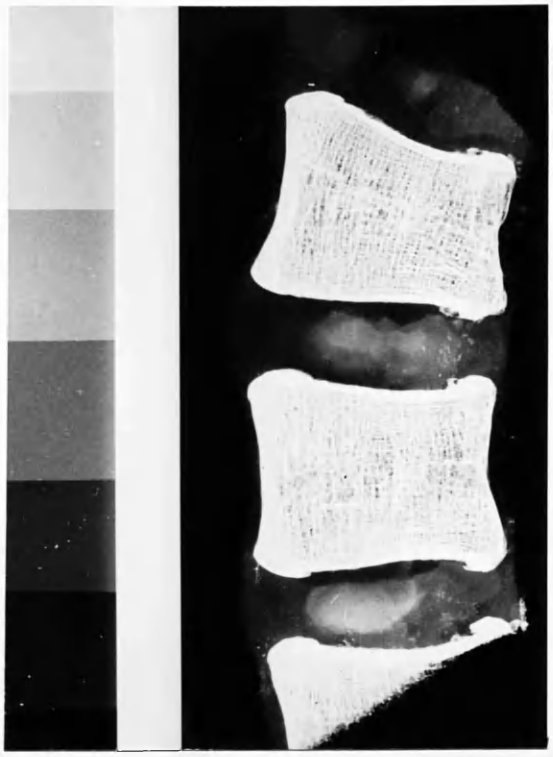

Fig. 22. Case 238. Reduction of a radiograph showing biconcavity of a lumbar vertebra (index 71 per cent). The radiographic density was equivalent to 8 units of the step-wedge.

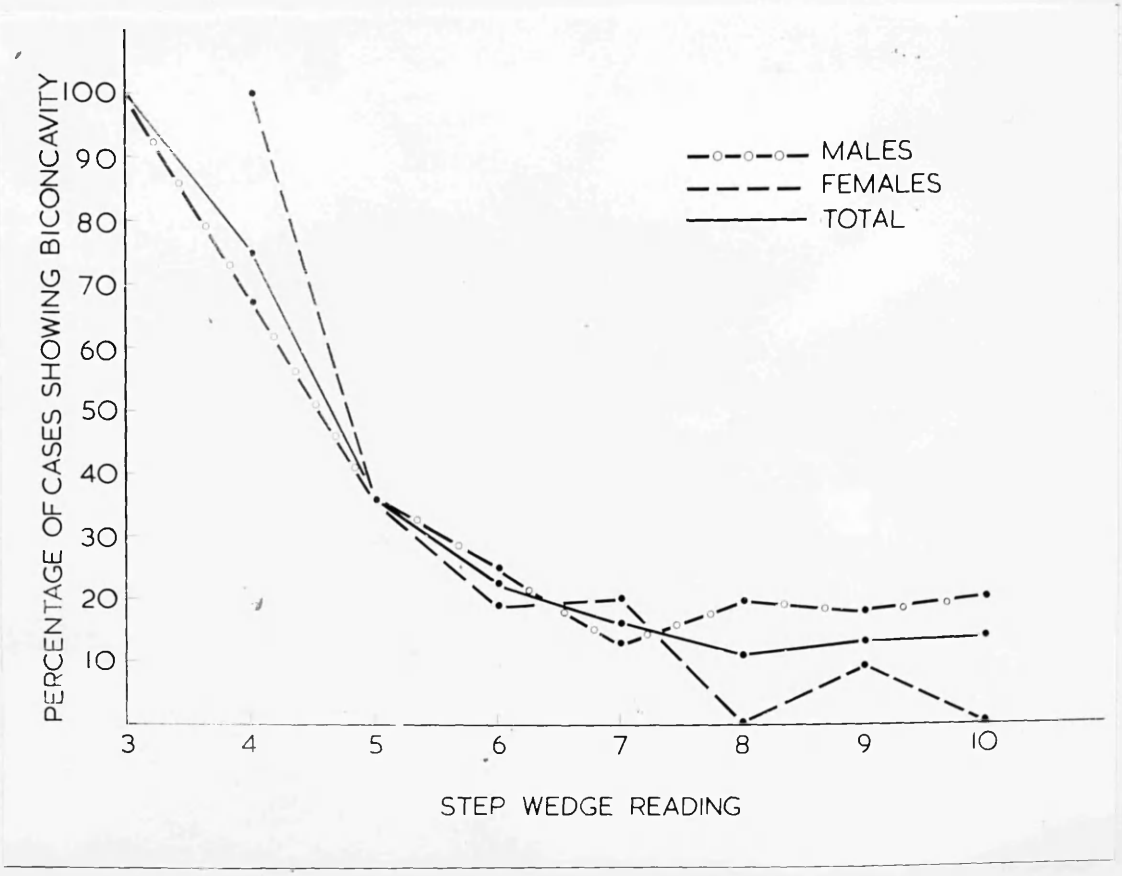

Fig. 23. A graph showing the percentage incidence of cases with vertebral biconcavity in the various grades of radiographic bone density (step-wedge units).

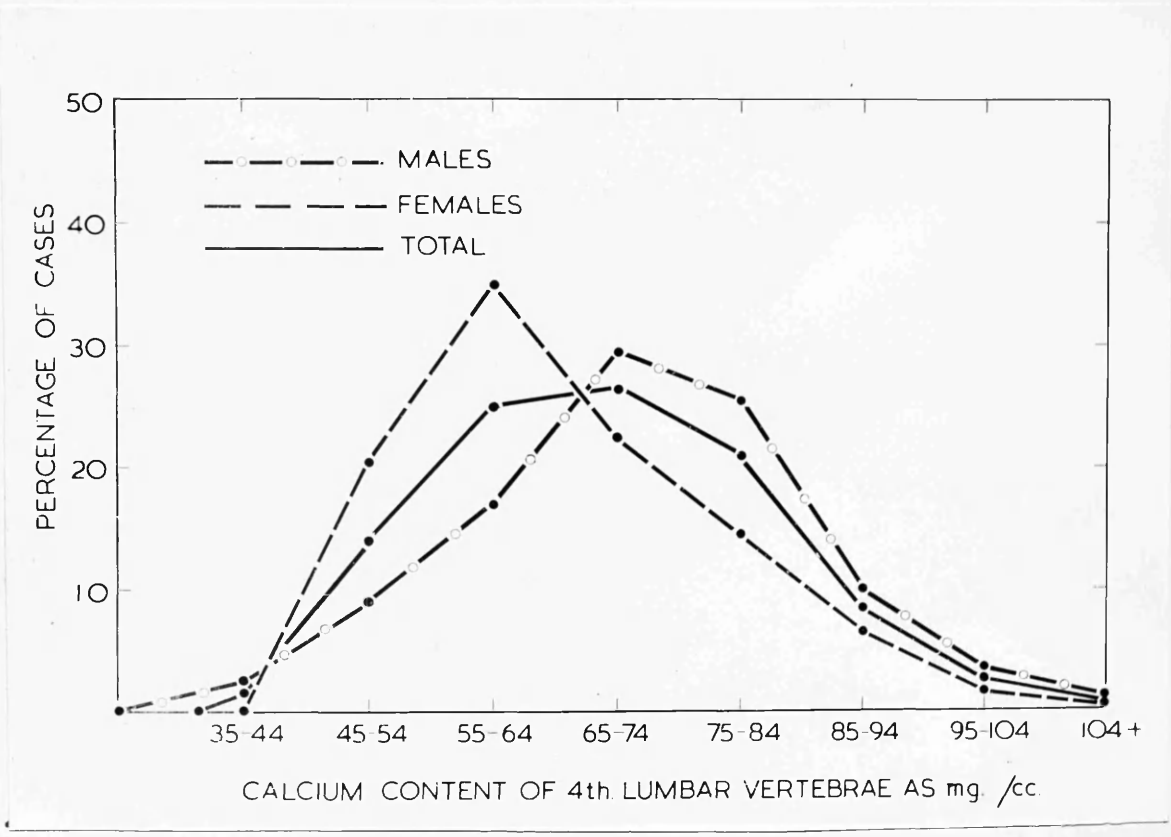

Fig. 24. A graph showing the percentage incidence of the results of calcium analysis of the lumbar vertebrae arranged in groups of 10 mg. per c.c. (150 cases).

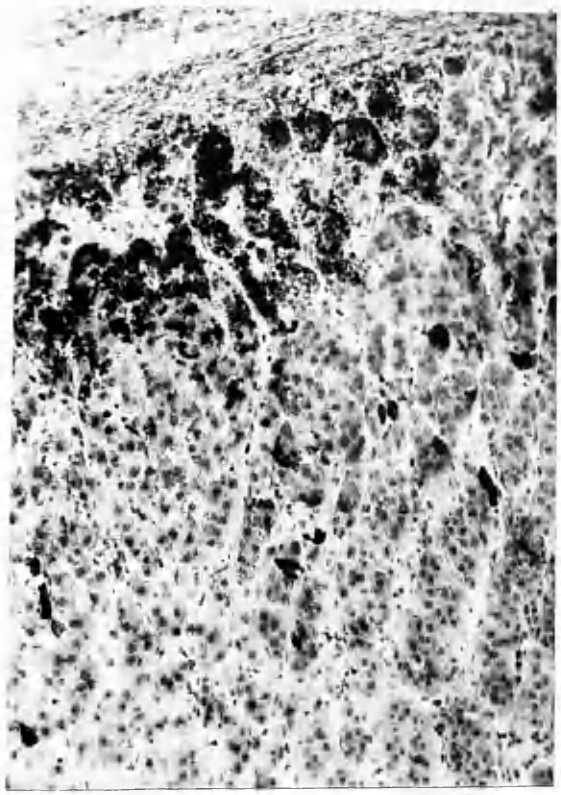

Fig. 25. Histological frozen section of adrenal gland, of which the cortex contained 5 per cent of lipoid.
Oil red 4B and haematoxylin x 95.

Fig. 26. Histological frozen section of an adrenal gland of which the cortex contained 90 per cent of lipoid.
Oil red 4B and haematoxylin x 95.

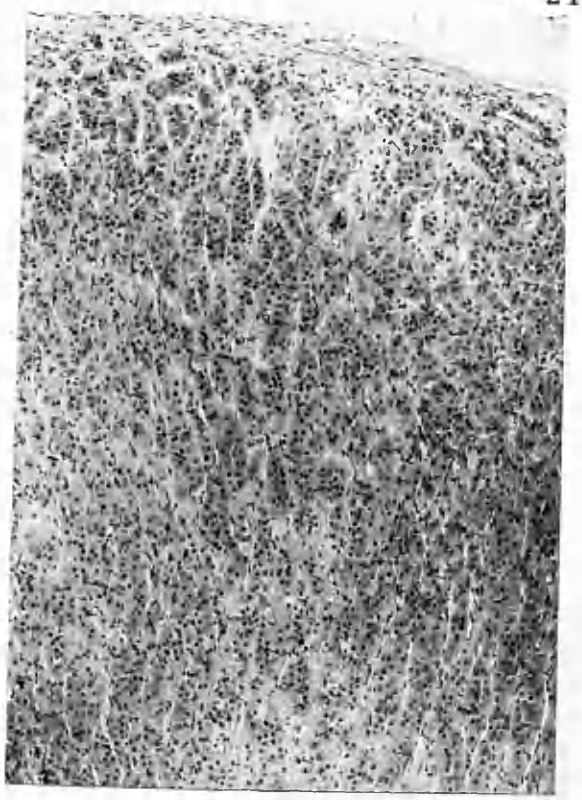

Fig. 27. Histological section of adrenal gland of which the cortex showed 95 per cent of RNA. Pyronin methyl green x 120.

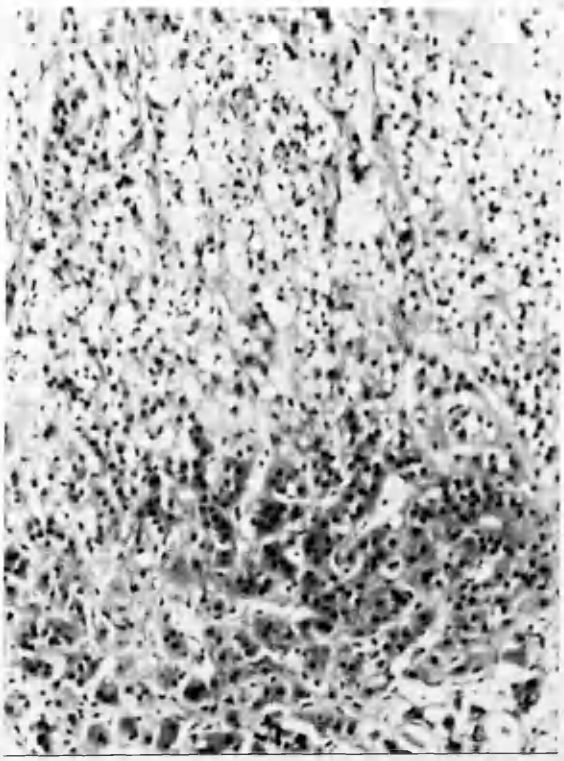

Fig. 28. Histological section of adrenal gland of which the cortex showed 10 per cent of RNA. Pyronin methyl green x 150.

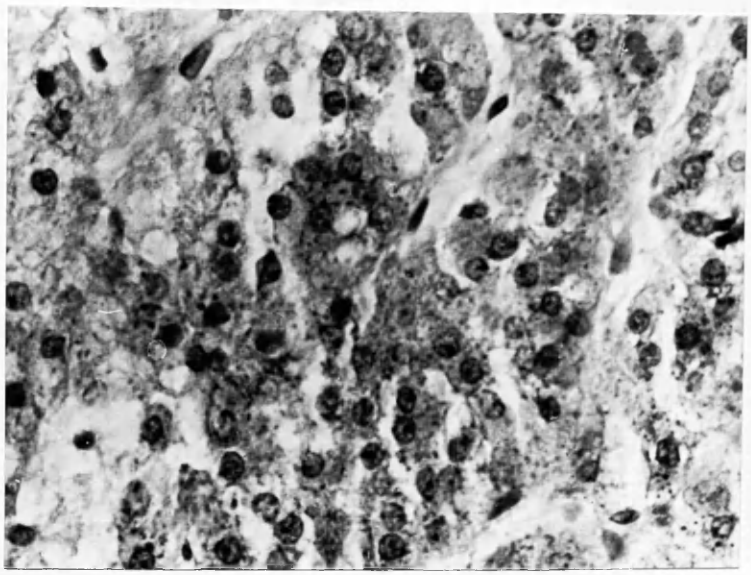

Fig. 29. As Fig. 28. Histological section showing the detail of the cytoplasmic RNA granules. Pyronin methyl green x 360.

Fig. 30. Photograph of thin slices of adrenal glands showing succinic dehydrogenase activity in all cortical layers. The central control slice was untreated. Triphenyl tetrazolium method.

Fig. 31. Histological frozen section of the gland shown in Fig. 30 showing a + + degree of succinic dehydrogenase activity in the zona fasciculata. Triphenyl tetrazolium method x 95

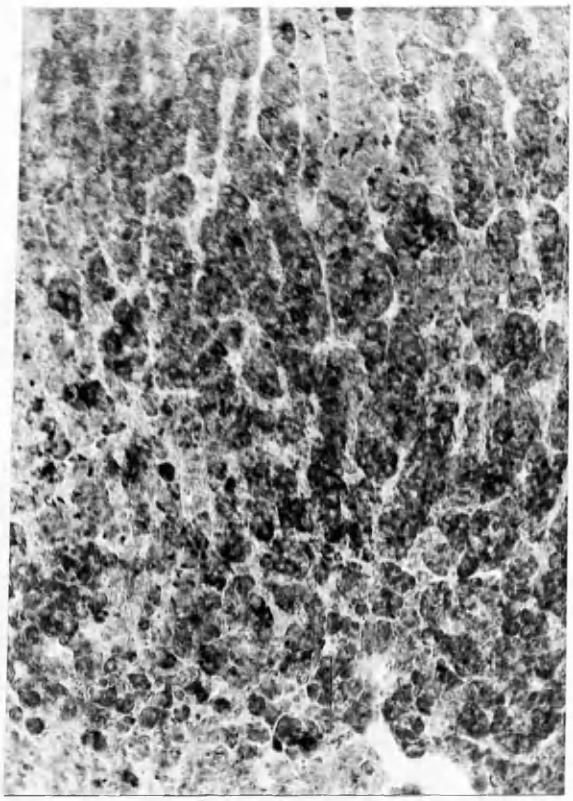

Fig. 32. Histological frozen section of an adrenal gland showing a + degree of succinic dehydrogenase activity in the zona fasciculata.
Triphenyl tetrazolium method x 95.

Fig. 33. Photograph of slices of adrenal glands to demonstrate succinic dehydrogenase activity which is restricted to the zona glomerulosa and the zona reticularis. The central control slice was untreated, and in the two slices on the right the reaction was partly inhibited by M/10 sodium malonate.
Triphenyl tetrazolium method

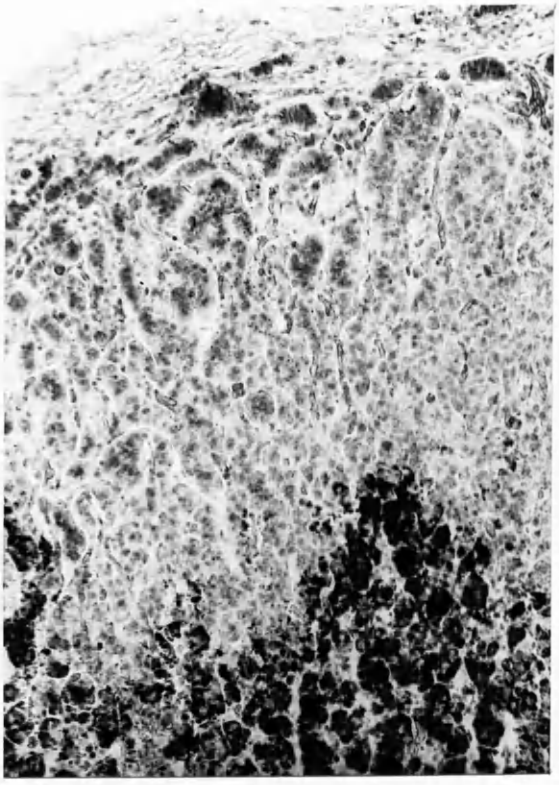

Fig. 34. Histological frozen section of a previously active adrenal gland showing reaccumulation of lipoid in the zona reticularis.
Oil red 4B and haematoxylin x 95.

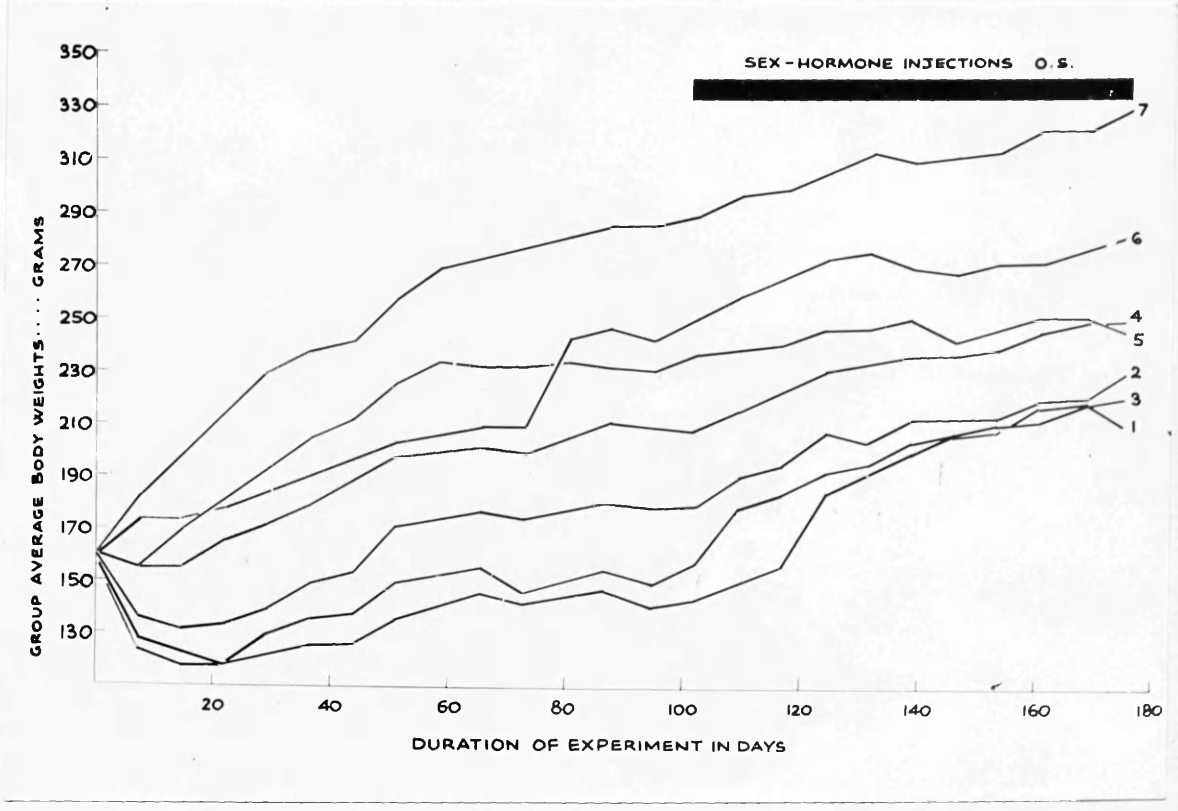

Fig. 35. Comparison of group average weekly body weights (grams) throughout the experiment (groups 1, 2, 3 and 4 to 7.

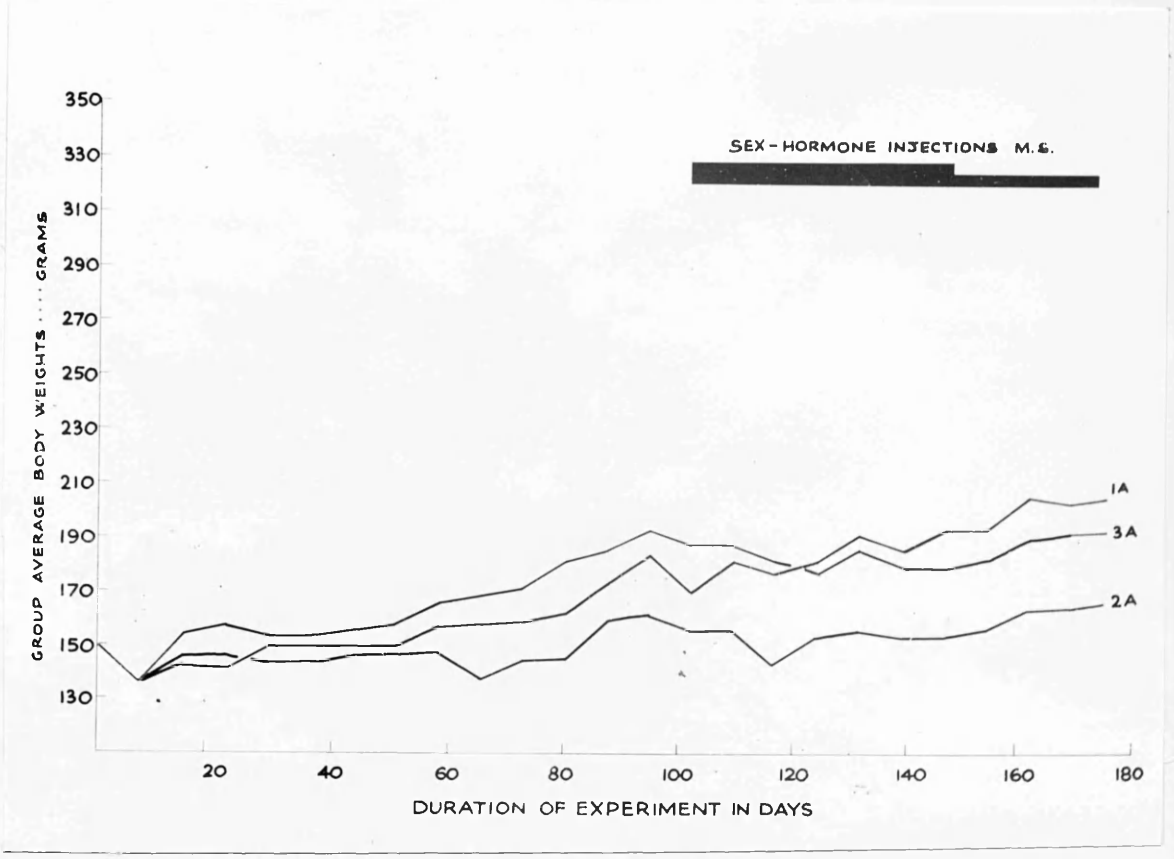

Fig. 36. Comparison of group average weekly body weights (grams) throughout the experiment (groups 1A, 2A, and 3A).

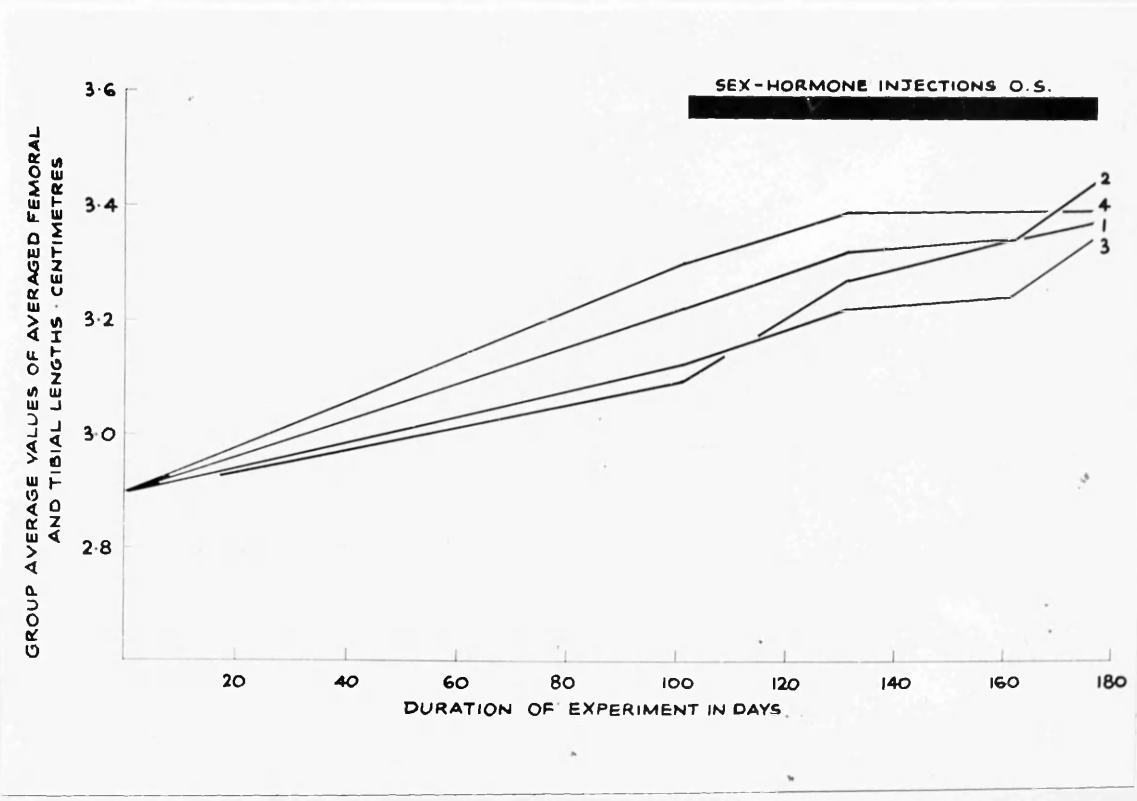

Fig. 37. Comparison of the rates of bone growth (cm.) of groups 1, 2, 3 and 4 throughout the experiment.

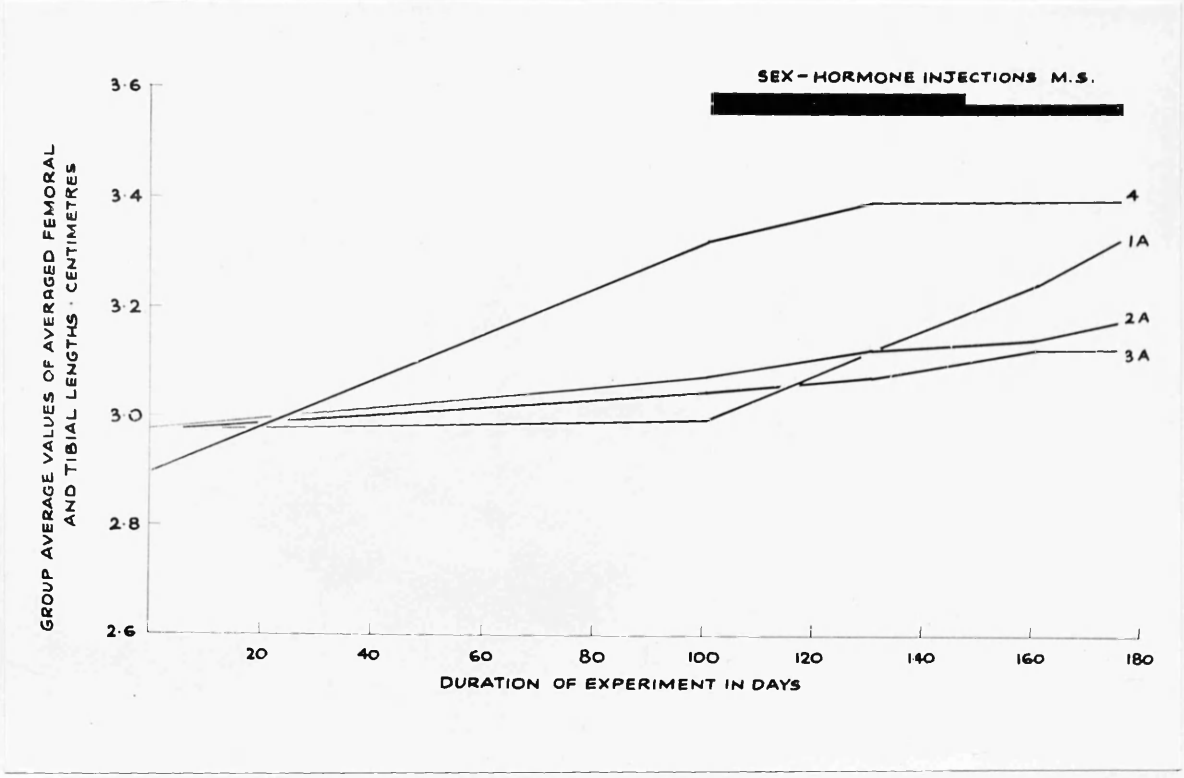

Fig. 38. Comparison of the rates of bone growth (cm.) of groups 1A, 2A, 3A, and 4 throughout the experiment.

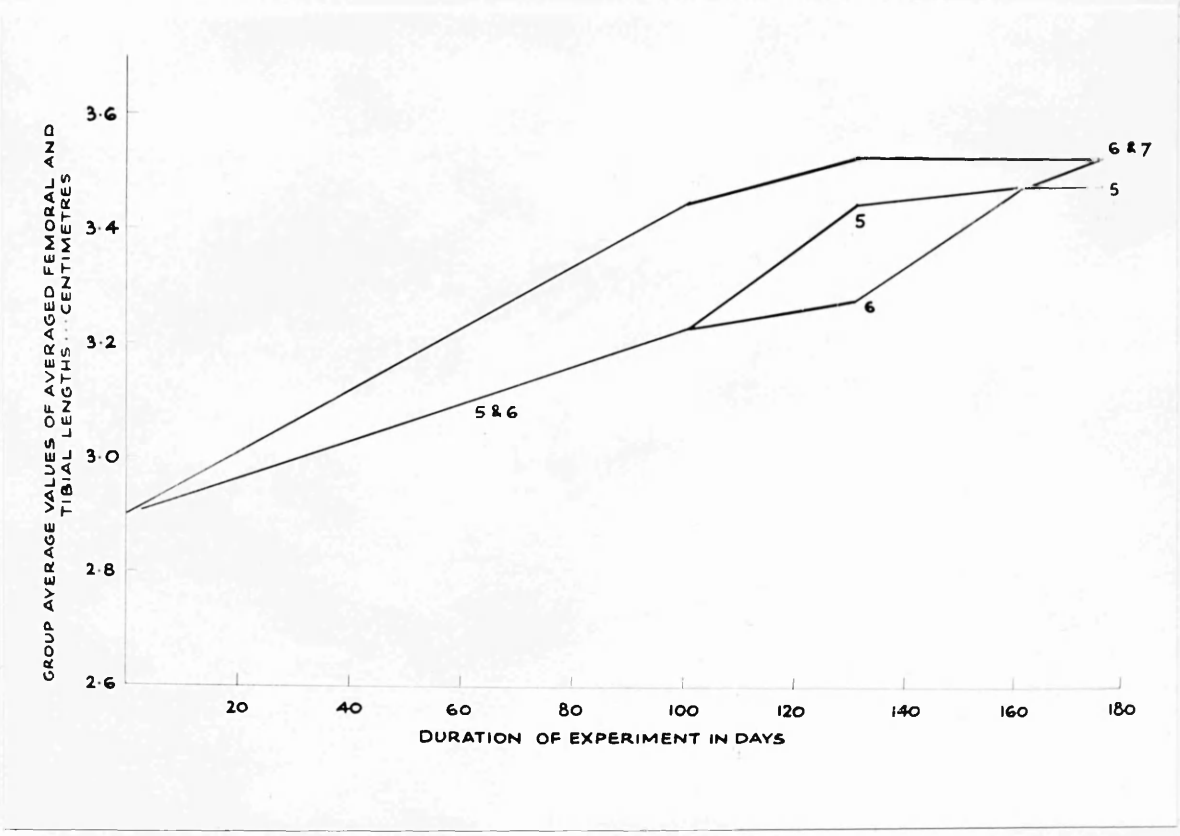

Fig. 39. Comparison of the rates of bone growth (cm.) of groups 5, 6, and 7 throughout the experiment.

FOR COMPARISON

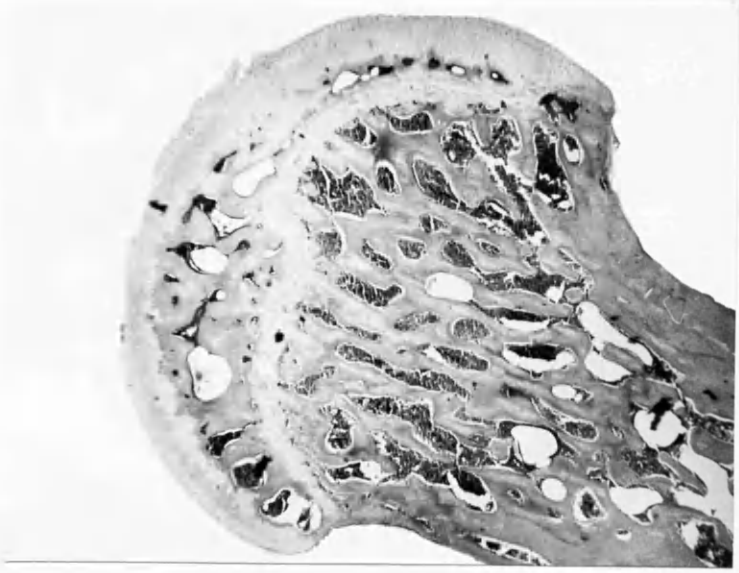

Fig. 40. Group 7 (normal control group). Histological section of femoral head showing the normal appearance. H. and E. x 25.

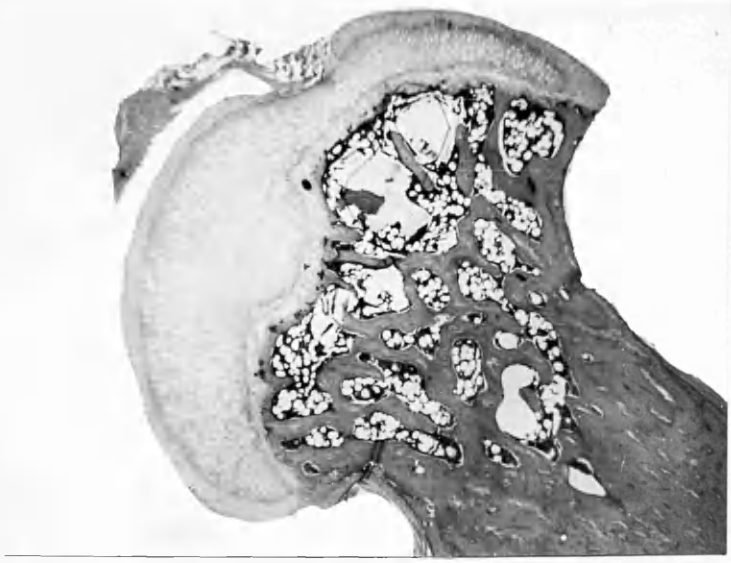

Fig. 41. Group 4 (operated untreated control group). Histological section of femoral head showing osteoporosis and a narrowed epiphyseal cartilage. H. and E. x 25.

FOR COMPARISON

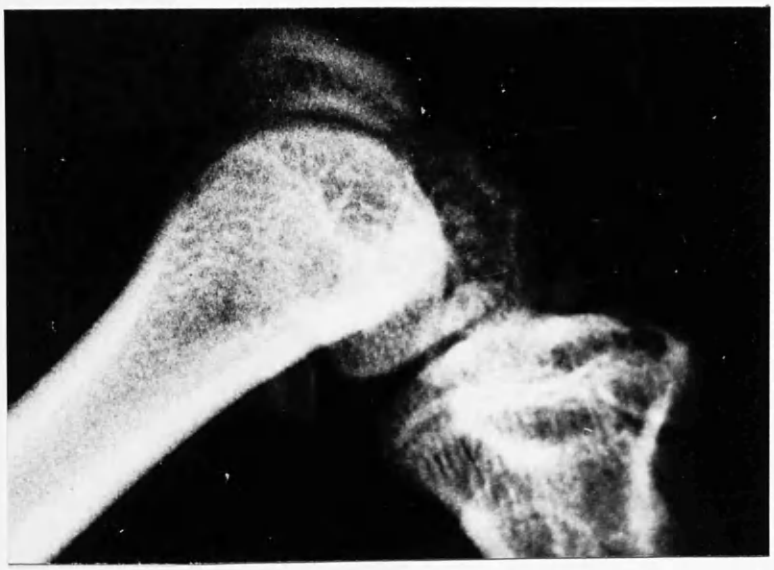

Fig . 42. Group 7 (normal control). Photographic enlargement of a radiograph of knee region, 175 days post-operation, showing normal bone density. x 7.

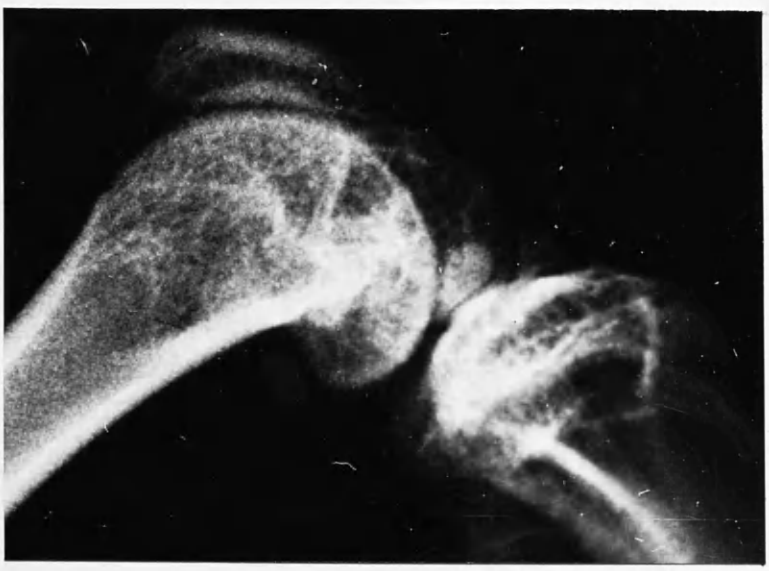

Fig. 43. Group 4. (operated untreated control group). Photographic enlargement of a radiograph of knee region 175 days post-operation, showing + + + + osteoporosis. x 7.

FOR COMPARISON

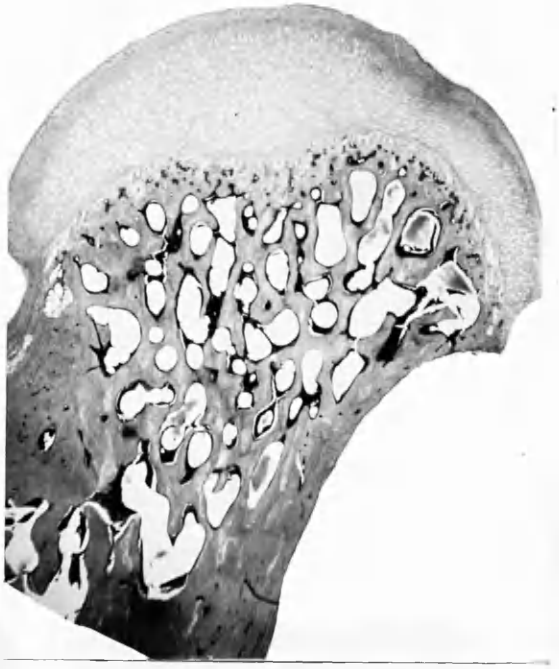

Fig. 44. Group 1 (operated O.S. oestradiol group). Histological section of femoral head showing rather slender trabeculae but normal bone structure. H. and E. x 25.

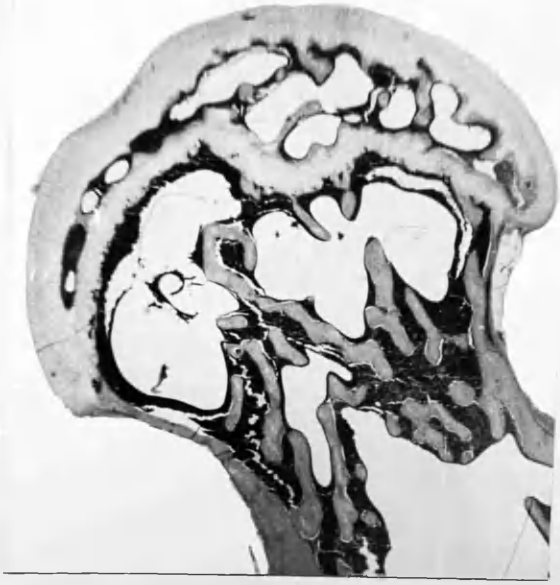

Fig. 45. Group 3 (operated O.S. testosterone group). Histological section of femoral head showing marked osteoporosis and considerable irregularity of subepiphyseal bone pattern. H. and E. x 25.

FOR COMPARISON

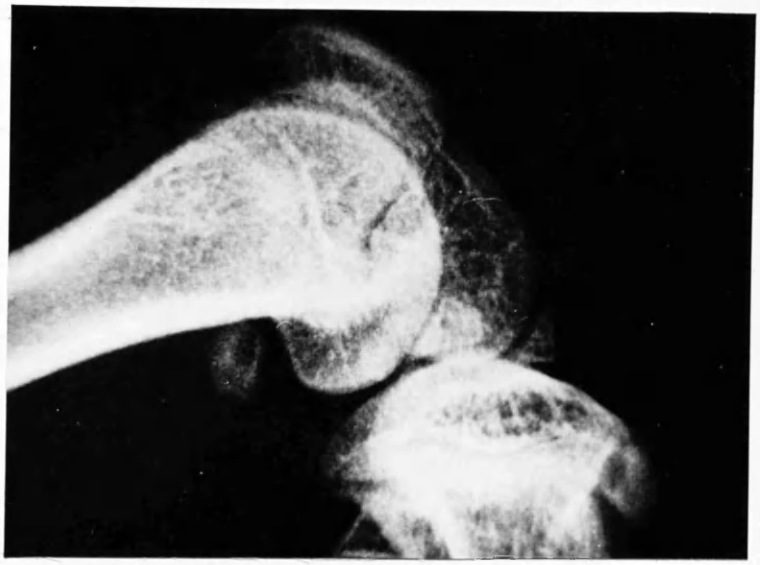

Fig. 46. Group 1A (operated M.S. oestradiol group). Photographic enlargement of a radiograph of knee region, 175 days post-operation showing normal bone density. x 7.

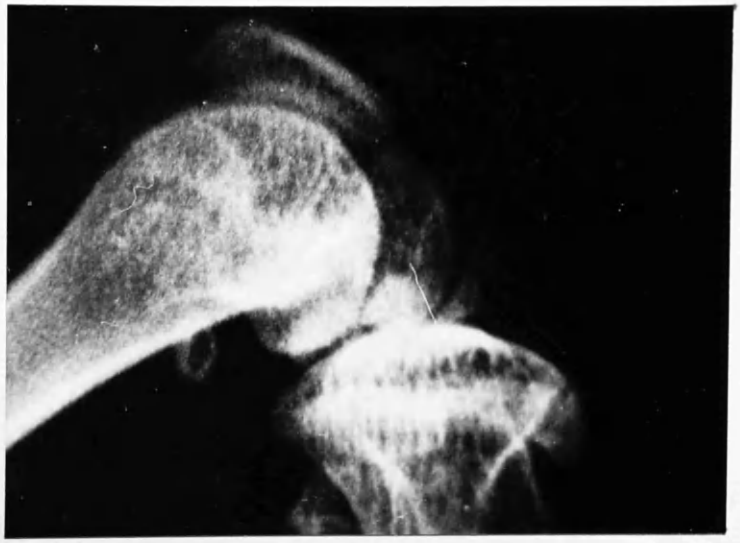

Fig. 47. Group 3A (operated M.S. testosterone group). Photographic enlargement of a radiograph of knee region, 175 days post-operation showing + + + osteoporosis. x 7.

FOR COMPARISON

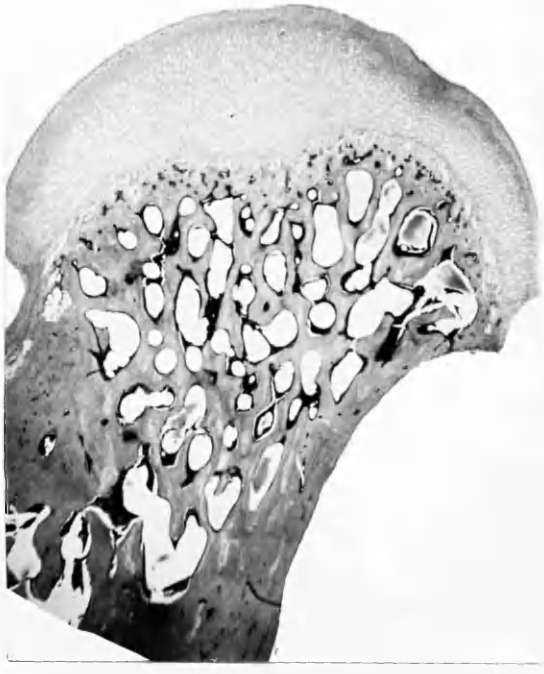

Fig. 48. Group 1 (operated O.S. oestradiol group). Histological section of femoral head showing slender trabeculae but normal bone structure.
H. & E. x 25.

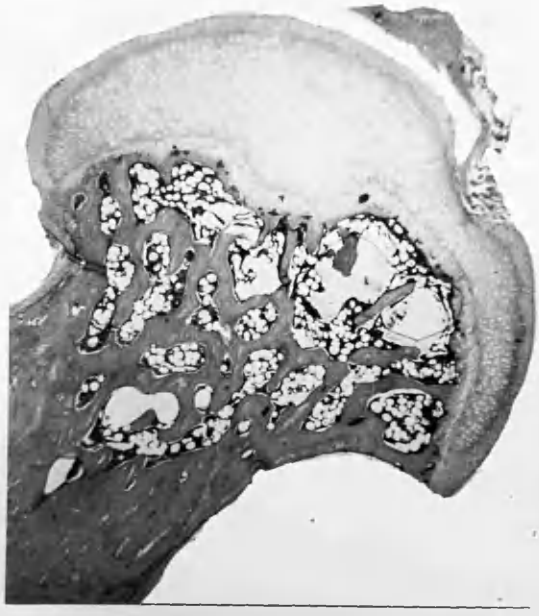

Fig. 49. Group 4 (operated untreated control group). Histological section of femoral head showing severe osteoporosis.
H. & E. x 25.

FOR COMPARISON

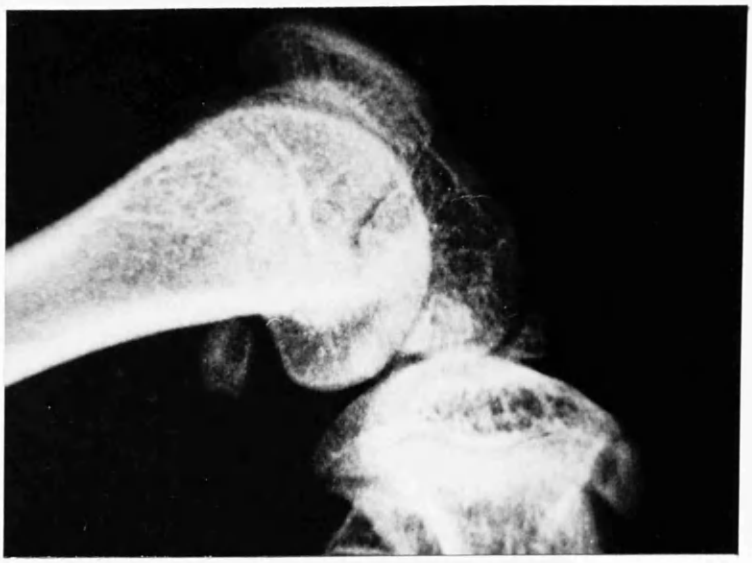

Fig. 50. Group 1A (operated M.S. oestradiol group). Photographic enlargement of a radiograph of knee region, 175 days post-operation, showing normal bone density. x 7.

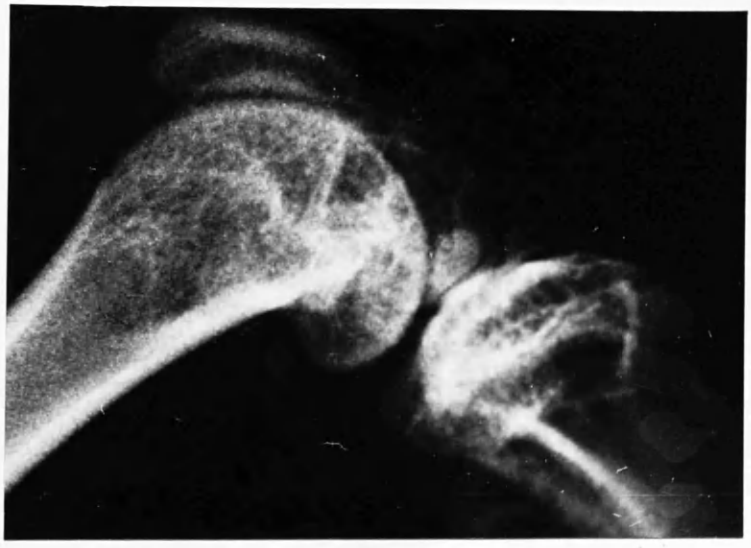

Fig. 51. Group 4 (operated untreated control group). Photographic enlargement of a radiograph of the knee region, 175 days post-operation, showing + + + + osteoporosis. x 7.

FOR COMPARISON

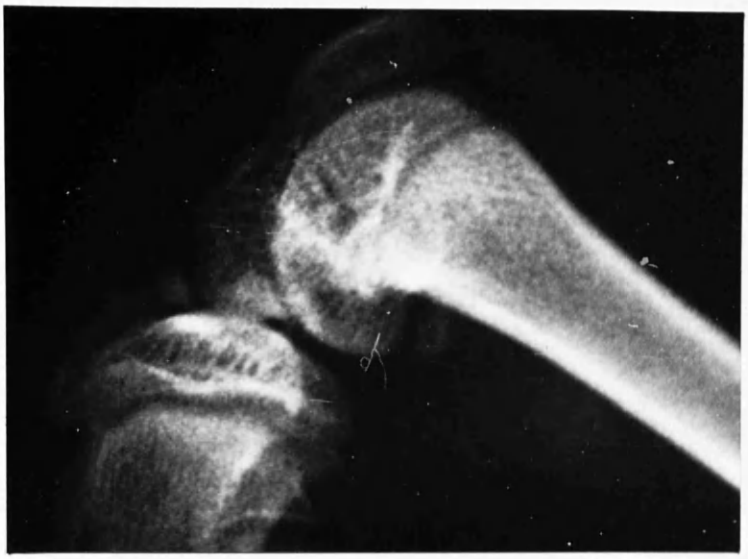

Fig. 52. Group 1A (operated M.S. oestradiol group). Photographic enlargement of a radiograph of knee region immediately prior to operation, showing normal bone density.

x 7.

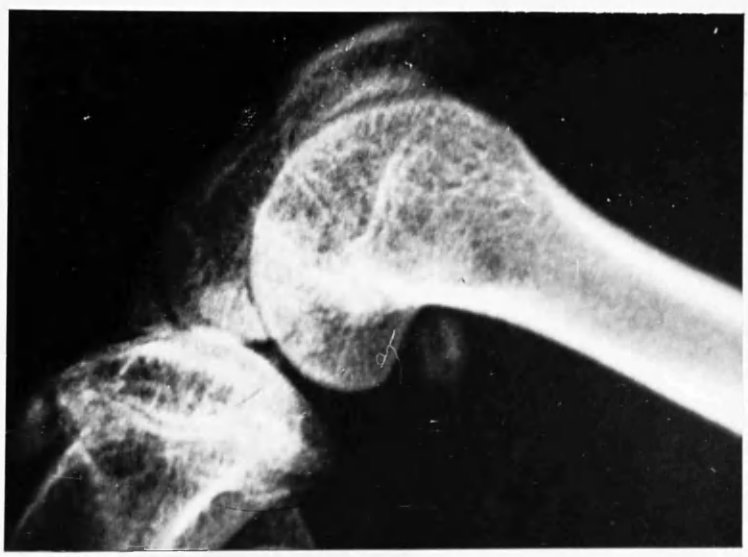

Fig. 53. As for Fig. 52, 100 days post-operation showing + + + + osteoporosis.

x 7.

FOR COMPARISON

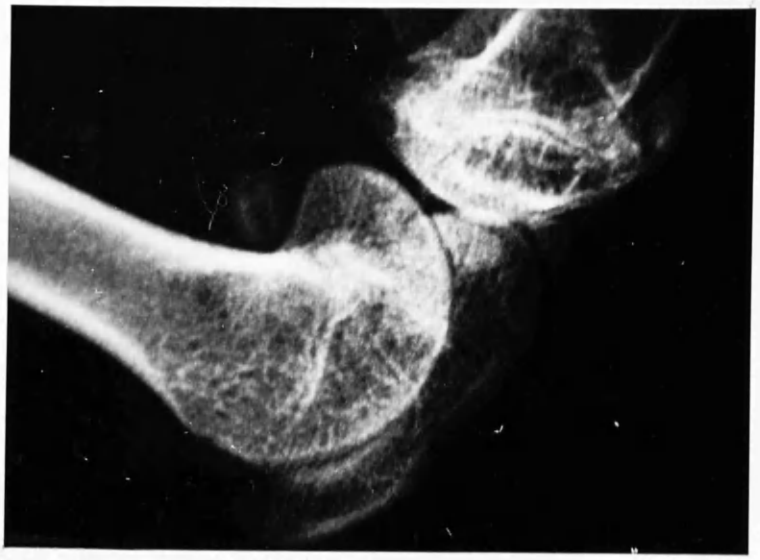

Fig. 54. Group 1A (operated M.S. oestradiol group). Photographic enlargement of a radiograph of knee region, 100 days post-operation, showing + + + + osteoporosis. x 7.

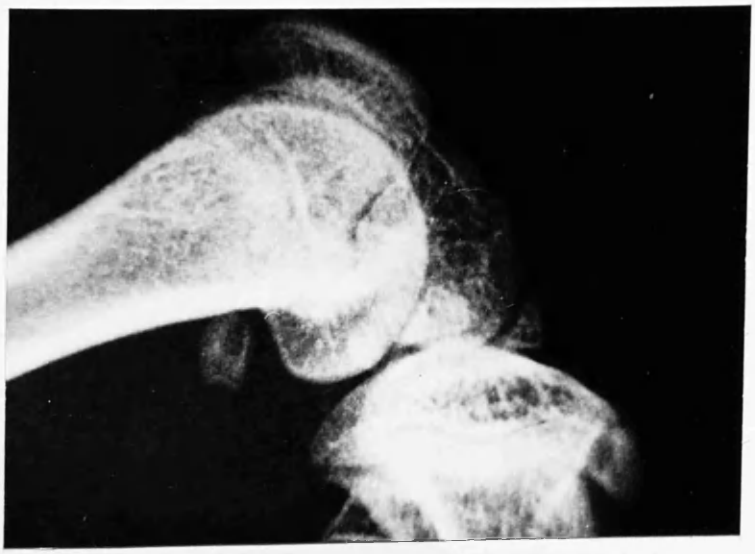

Fig. 55. As for Fig. 54, 175 days post-operation, showing normal bone density. x 7.

FOR COMPARISON

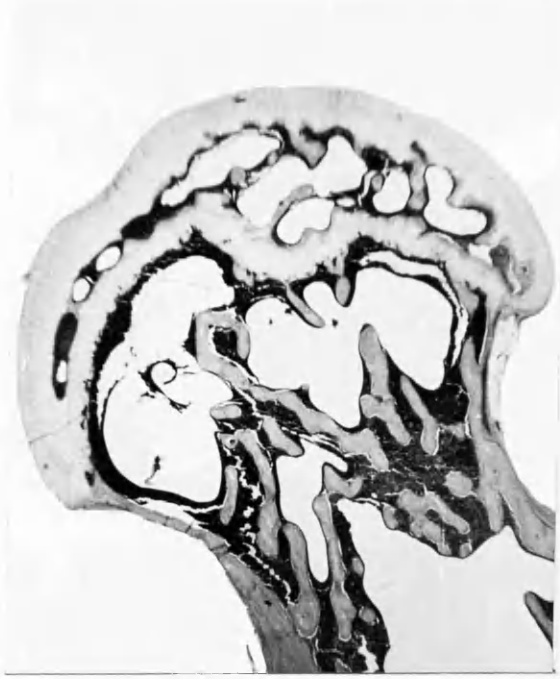

Fig. 56. Group 3 (operated O.S. testosterone group). Histological section of femoral head showing severe osteoporosis and marked disturbance of subepiphyseal bone pattern.
H. & E. x 25.

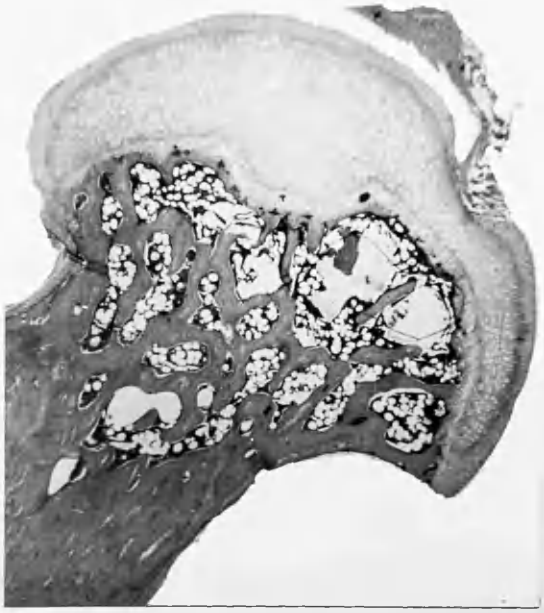

Fig. 57. Group 4 (operated untreated control group). Histological section of femoral head showing severe osteoporosis but regular subepiphyseal bone pattern.
H. & E. x 25.

FOR COMPARISON

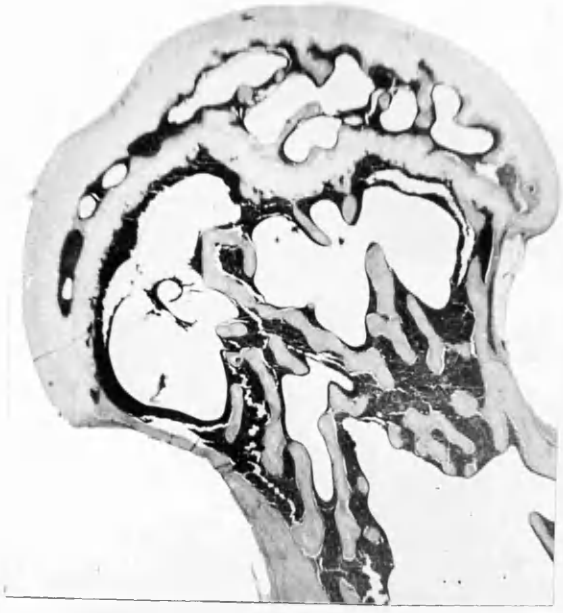

Fig. 58. Group 3 (operated O.S. testosterone group). Histological section of femoral head showing severe osteoporosis and marked disturbance of subepiphyseal bone pattern.
H. & E. x 25.

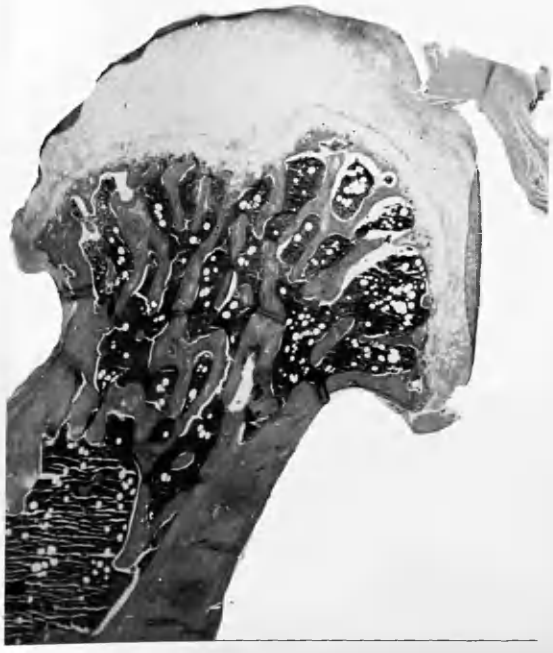

Fig. 59. Group 3A (operated M.S. testosterone group). Histological section of femoral head showing moderate osteoporosis with regular subepiphyseal bone pattern.
H. & E. x 25.

FOR COMPARISON

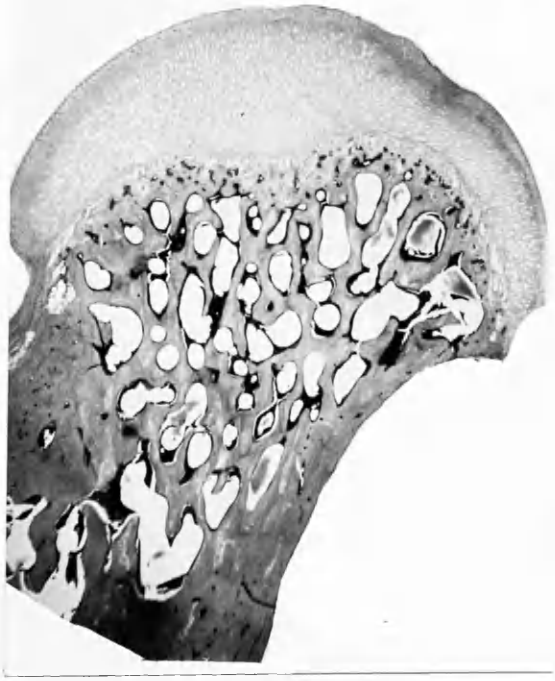

Fig. 60. Group 1 (operated O.S. oestradiol group). Histological section of femoral head showing rather slender trabeculae but normal trabecular pattern.
H. & E. x 25.

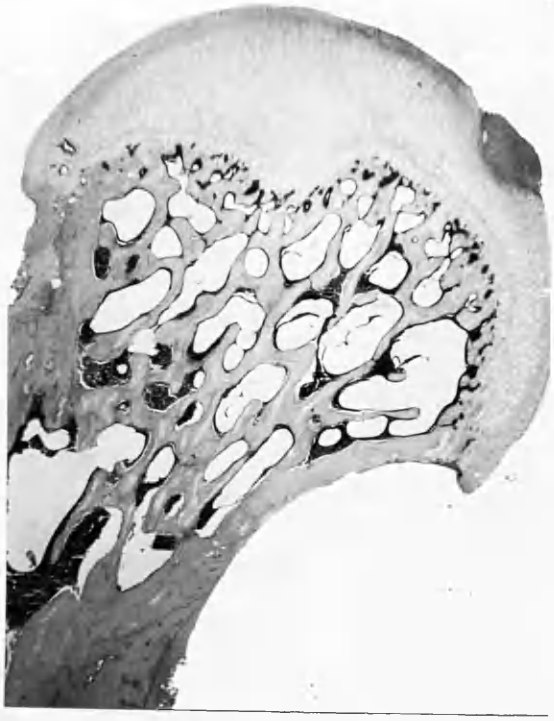

Fig. 61. Group 2A (operated M.S. oestradiol and testosterone group). Histological section of femoral head showing moderate osteoporosis.
H. & E. x 25.

FOR COMPARISON

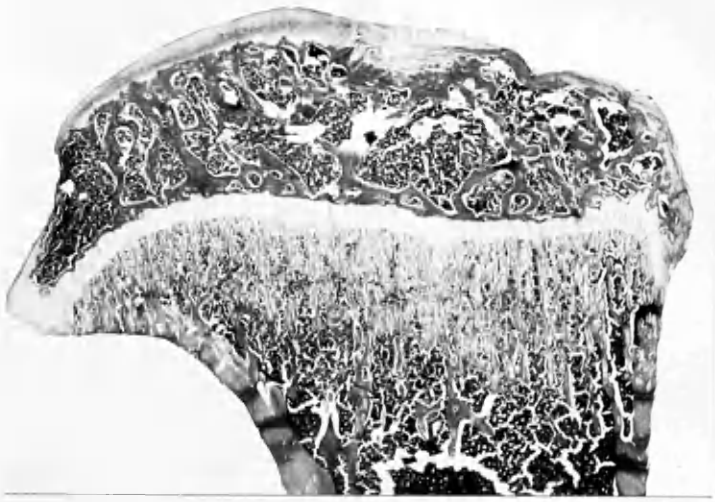

Fig. 62. Group 1A (operated M.S. oestradiol group). Histological section of tibial head showing active subepiphyseal bone formation.
H. & E. x 17.

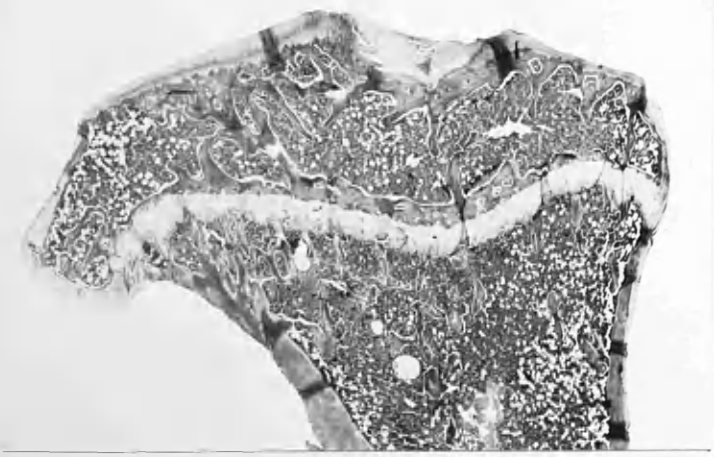

Fig. 63. Group 3A (operated M.S. testosterone group). Histological section of tibial head showing marked reduction of subepiphyseal bone formation.
H. & E. x 17.

FOR COMPARISON

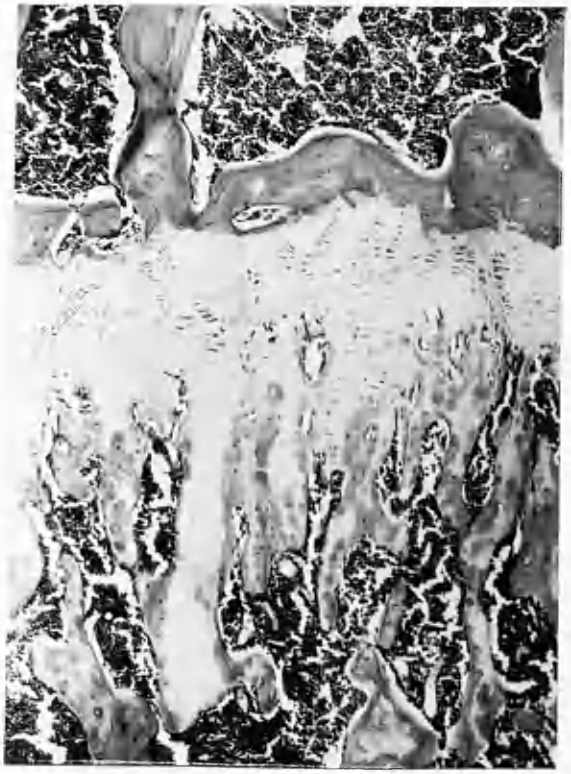

Fig. 64. Group 1A (operated M.S. oestradiol group). Histological section of upper tibial epiphyseal cartilage. A broad active cartilage showing long regular cell columns. Newly formed cartilaginous trabeculae are lined by osteoblasts laying down bone.
H. & E. x 75.

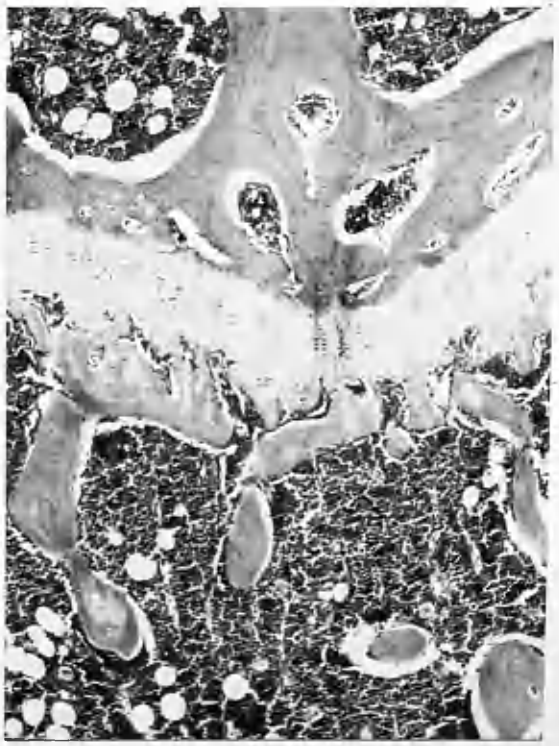

Fig. 65. Group 3A (operated M.S. testosterone group). Histological section of upper tibial epiphyseal cartilage. The cartilage is narrower than in Fig. 64 and there is slight irregularity of the cartilage cells in the zone of maturation. Bony trabeculae are scanty and irregular and osteoblastic activity is not conspicuous.
H. & E. x 75.

FOR COMPARISON

Fig. 66. Group 1A (operated M.S. oestradiol group). Histological section of upper tibial epiphyseal cartilage showing a broad cartilage with long regular cell columns.
H. & E. x 500.

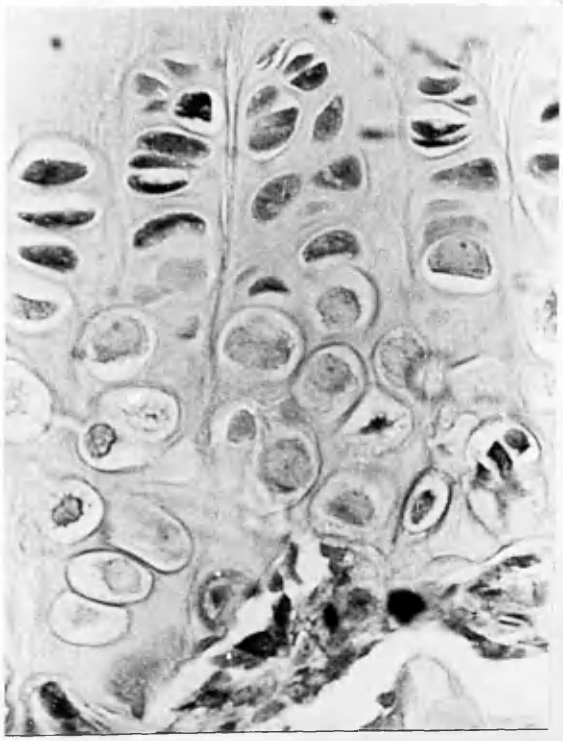

Fig. 67. Group 3A (operated M.S. testosterone group). Histological section of upper tibial epiphyseal cartilage. The cartilage is much narrower than that in Fig. 66, and the cells in the zone of maturation show slight disorganisation.
H. & E. x 500.

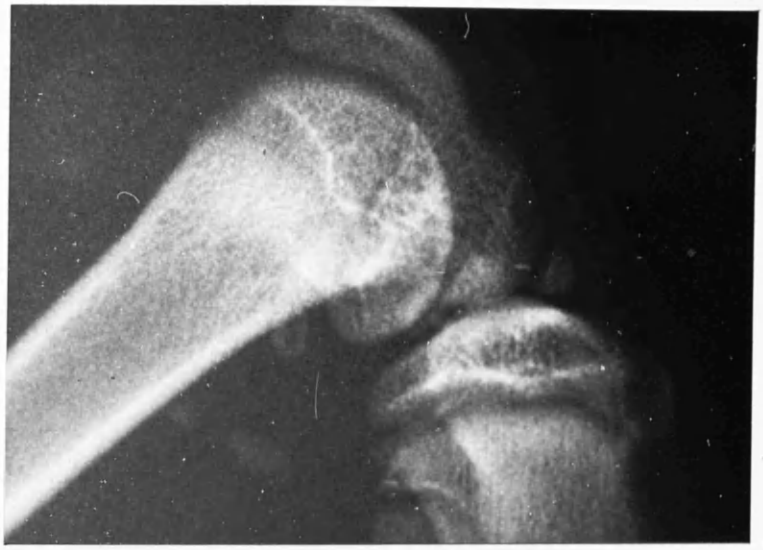

Fig. 68. Group 1 (O.S. oestradiol group). Photographic enlargement of a radiograph of knee region immediately prior to operation showing normal bone density. x 7.

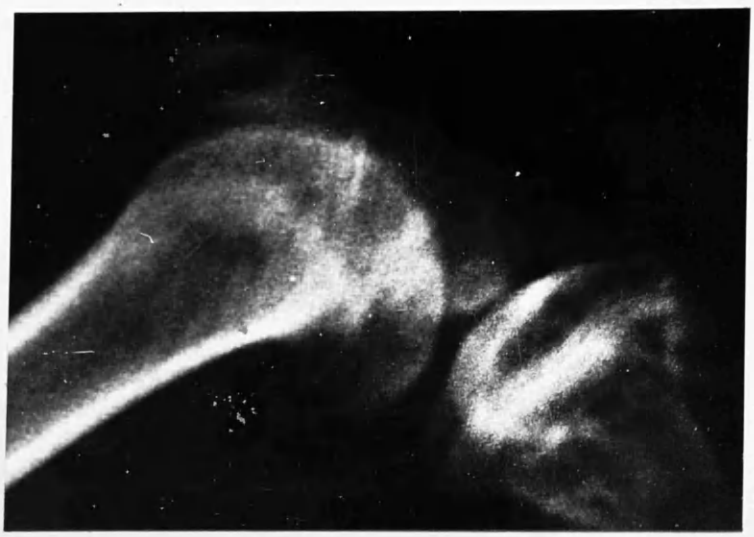

Fig. 69. Group 1. As for fig. 68, 100 days after operation showing + + osteoporosis. x 7.

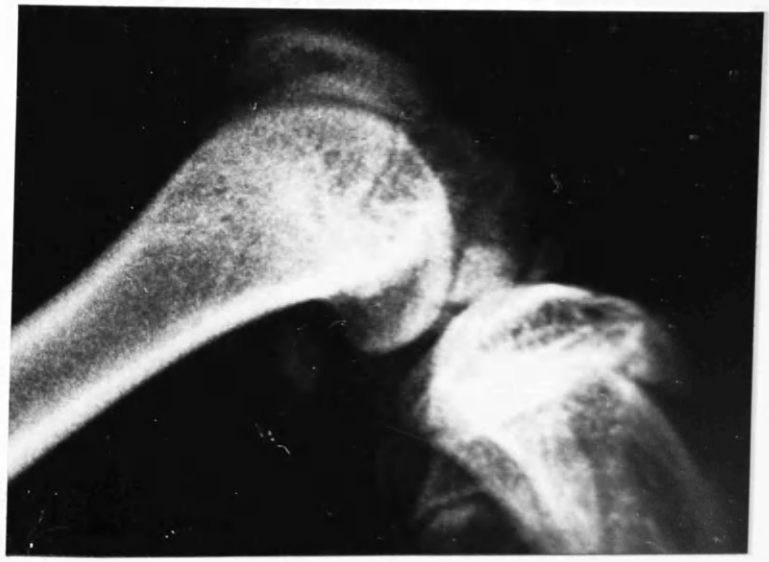

Fig. 70. As for Fig. 69, 130 days after operation showing + + osteoporosis.
x 7.

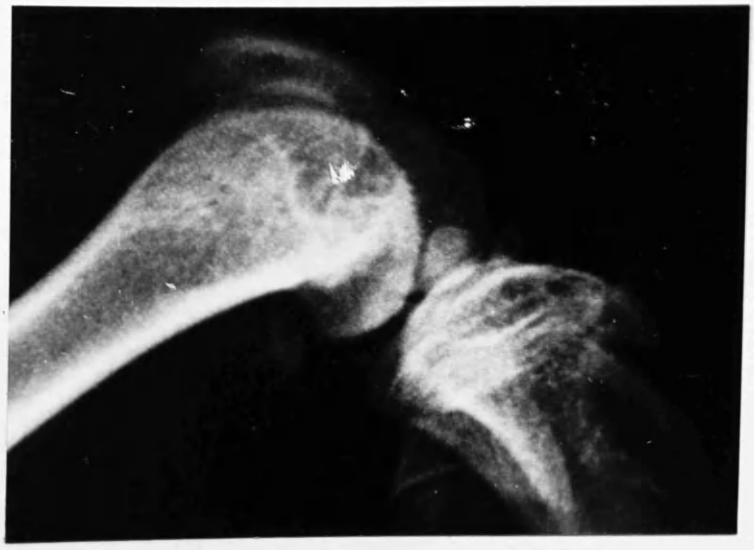

Fig. 71. As for Fig. 70, 160 days after operation showing + osteoporosis.
x 7.

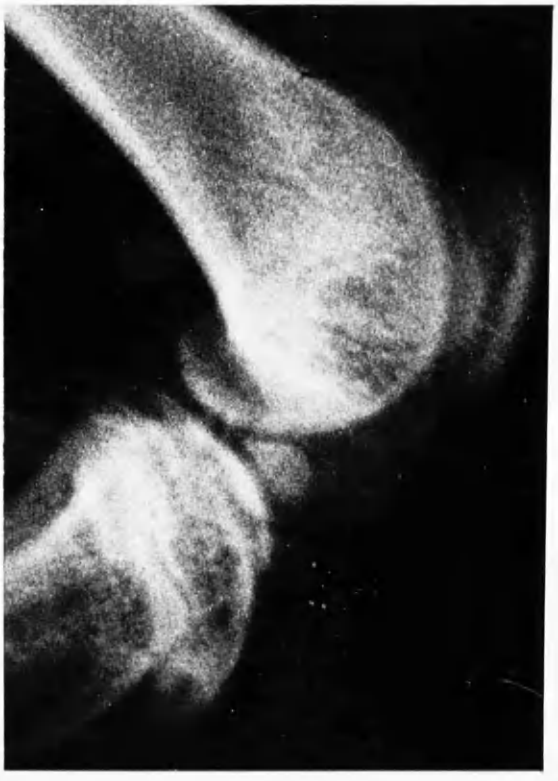

Fig. 72. As for Fig. 71, 175 days after operation showing normal bone density.
x 7.

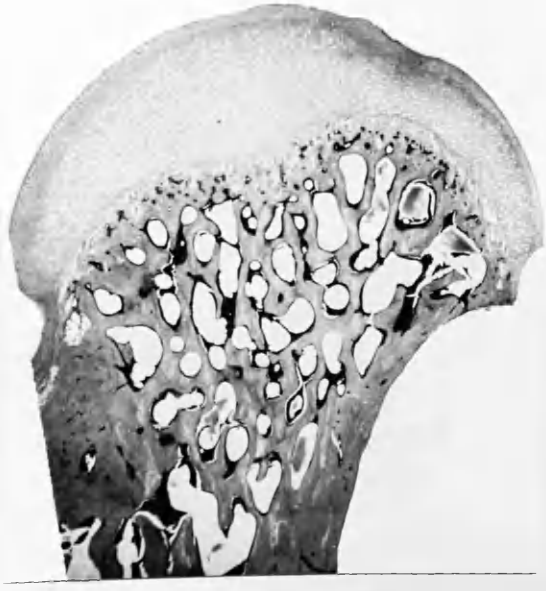

Fig. 73. Group 1. Histological section of femoral head region showing normal bone structure with numerous rather slender subepiphyseal and interconnecting trabeculae.
H. & E. x 25.

Fig. 74. As for Fig. 73. In the epiphyseal cartilage the cells of the zones of proliferation and maturation are regular and show the normal appearance for an adult rat.

H. & E. x 230.

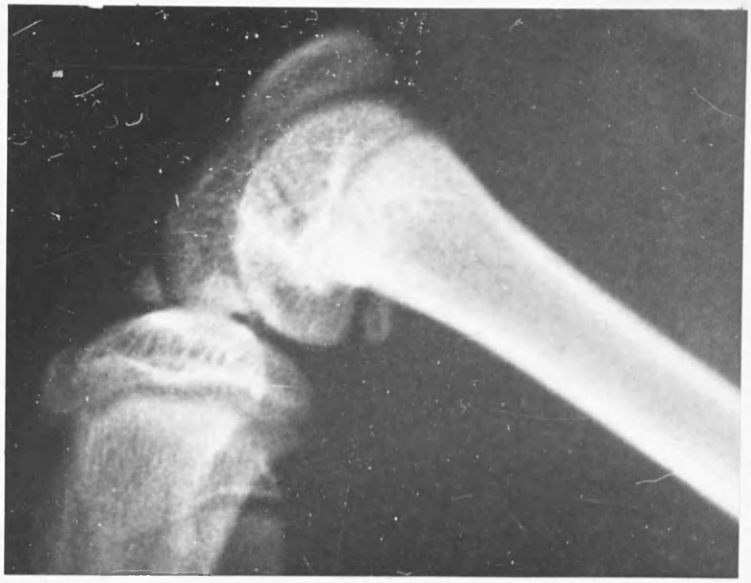

Fig. 75. Group 1A. (M.S. oestradiol group). Photographic enlargement of a radiograph of the knee region immediately prior to operation showing normal bone density.

x 7.

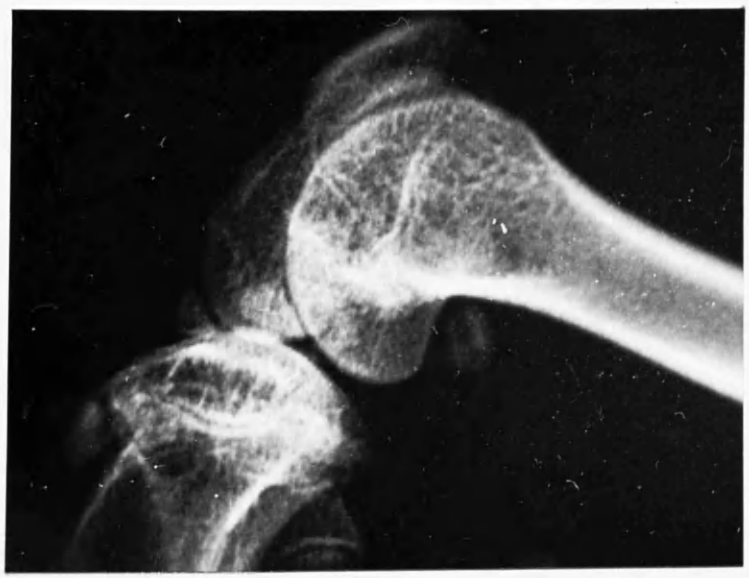

Fig. 76. As for Fig. 75, 100 days after operation showing + + + + osteoporosis.

x 7.

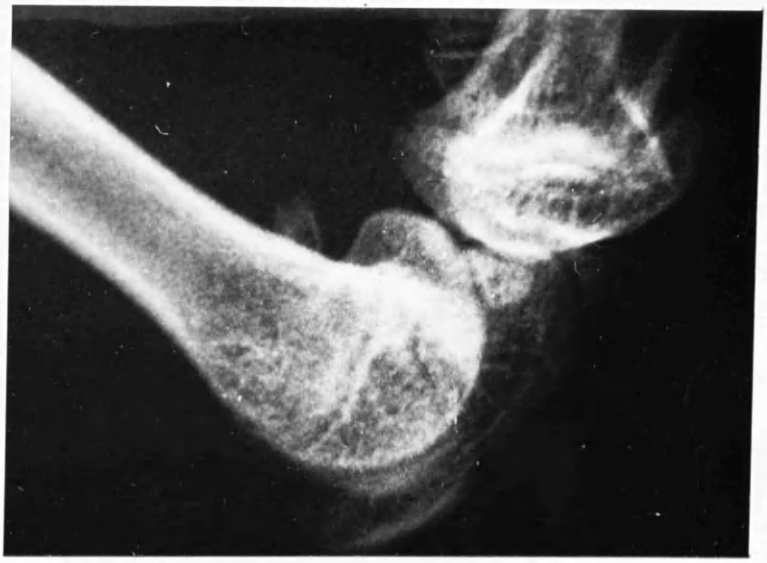

Fig. 77. As for Fig. 76, 130 days after operation showing + + + osteoporosis. x 7.

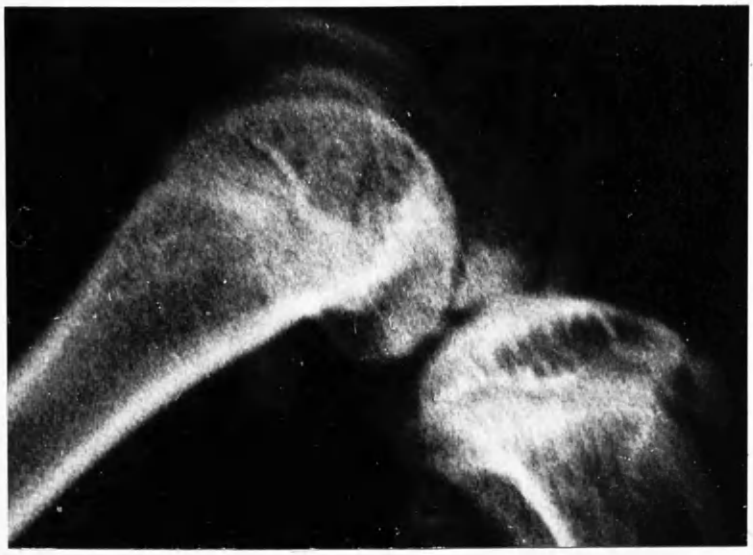

Fig. 78. As for Fig. 77, 160 days after operation showing + + osteoporosis. x 7.

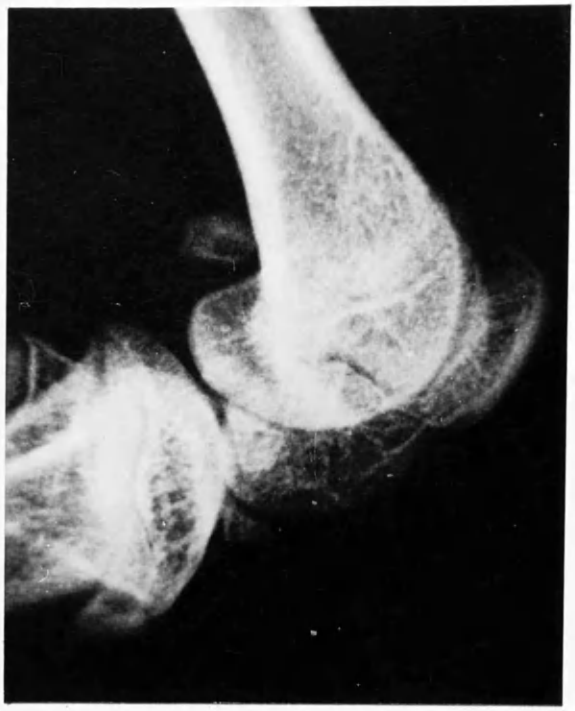

Fig. 79. As for Fig. 78, 175 days after operation showing normal bone density.
x 7.

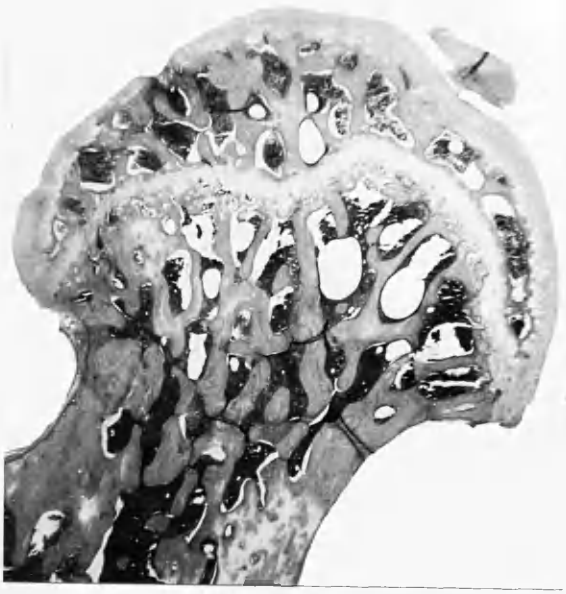

Fig. 80. Group 1A. Histological section of femoral head region showing a normal pattern of slender trabeculae which are only slightly reduced in numbers.
H. & E. x 25.

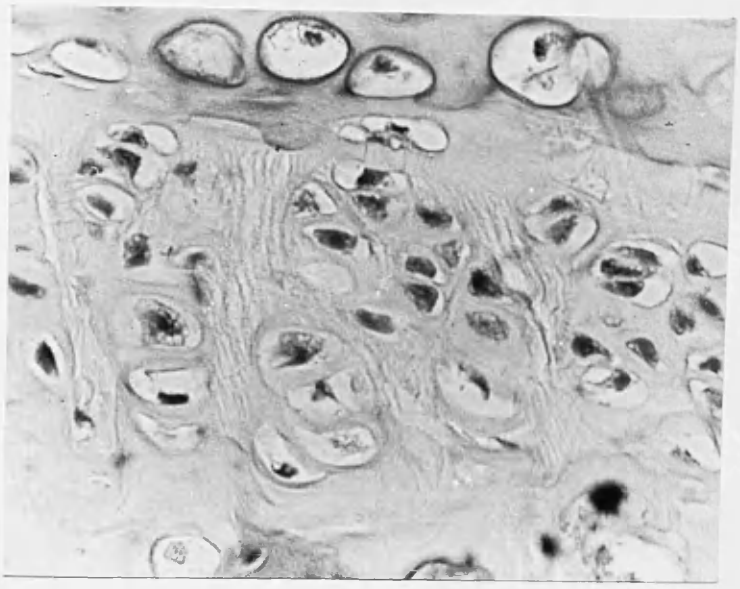

Fig. 81. As for Fig. 80. The epiphyseal cartilage is of good average width and shows a normal cell pattern.
H. & E. x 230.

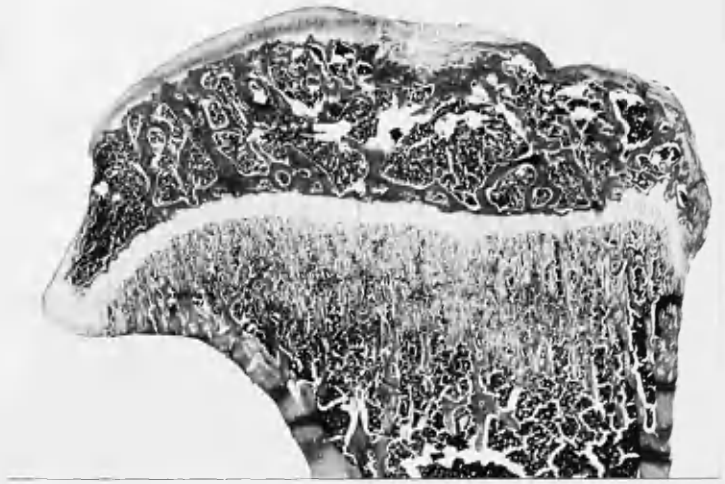

Fig. 82. Group 1A. Histological section of upper end of tibia showing a broad epiphyseal cartilage with numerous elongated subepiphyseal bone trabeculae of regular pattern.
H. & E. x 17.

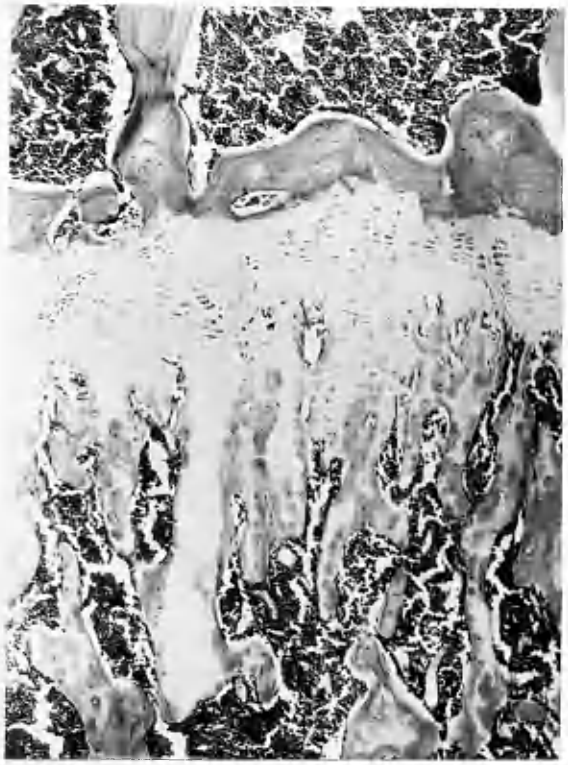

Fig. 83. As for Fig. 82. The epiphyseal cartilage shows long regular cell columns. The subepiphyseal trabeculae are lined by sheets of osteoblasts which are forming bone at the peripheral margins of cartilaginous downgrowths. H. & E. x 75.

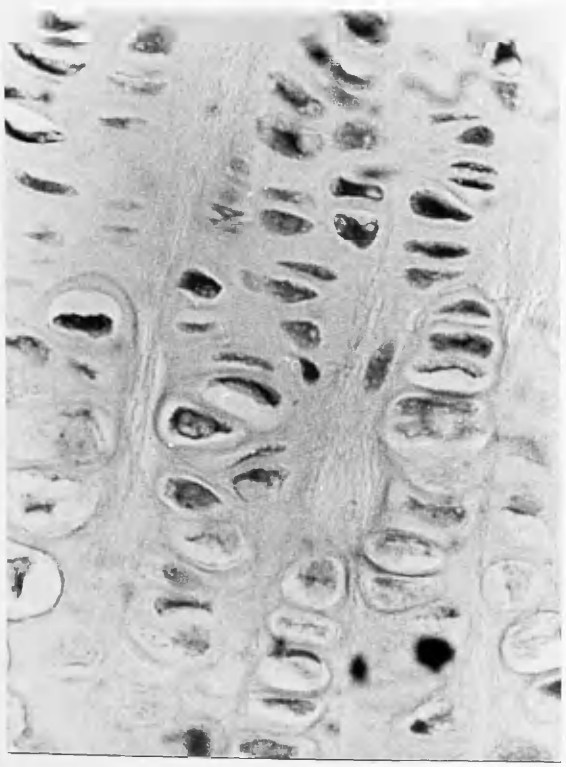

Fig. 84. As for Fig. 83. showing detail of the cartilage cell columns. H. & E. x 500.

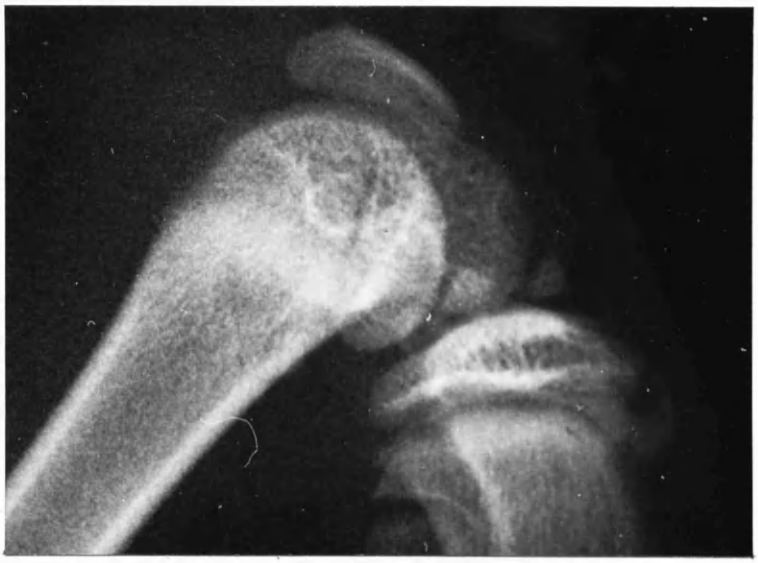

Fig. 85. Group 2 (O.S. oestradiol and testosterone group). Photographic enlargement of a radiograph of knee region immediately prior to operation showing normal bone density.

x 7.

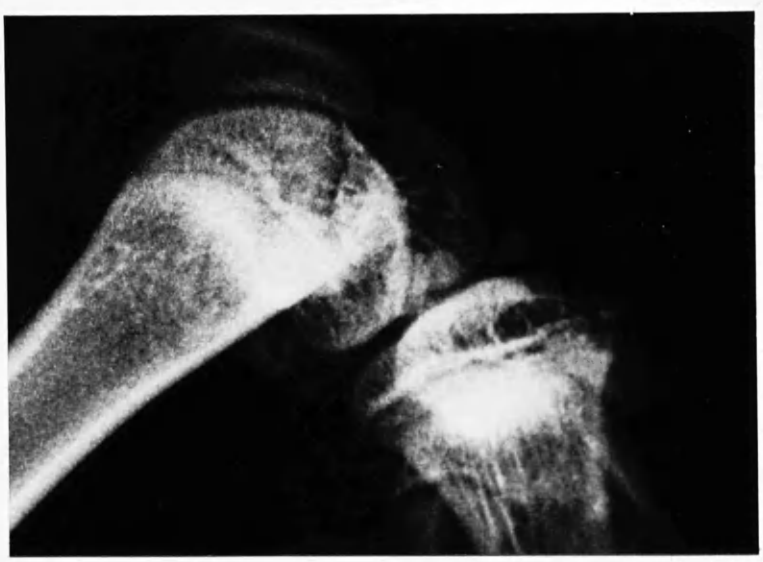

Fig. 86. As for Fig. 85, 130 days after operation showing + + + osteoporosis.

x 7.

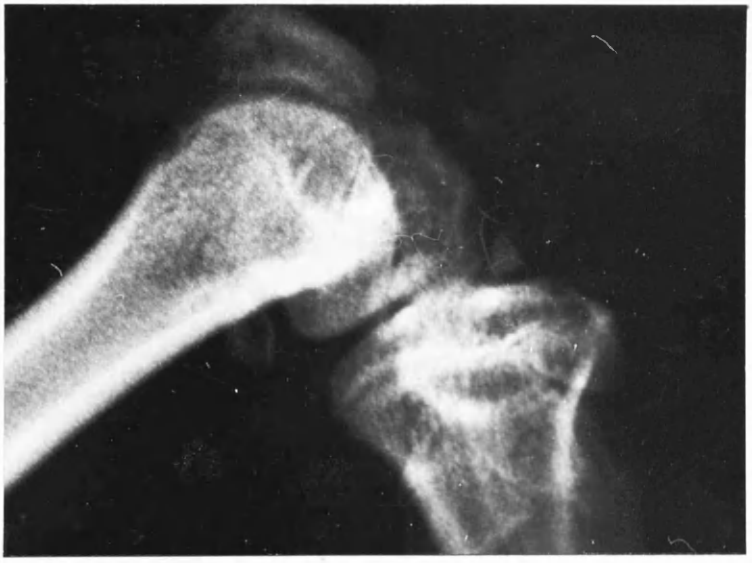

Fig. 87. As for Fig. 86, 160 days after operation showing + + osteoporosis. x 7.

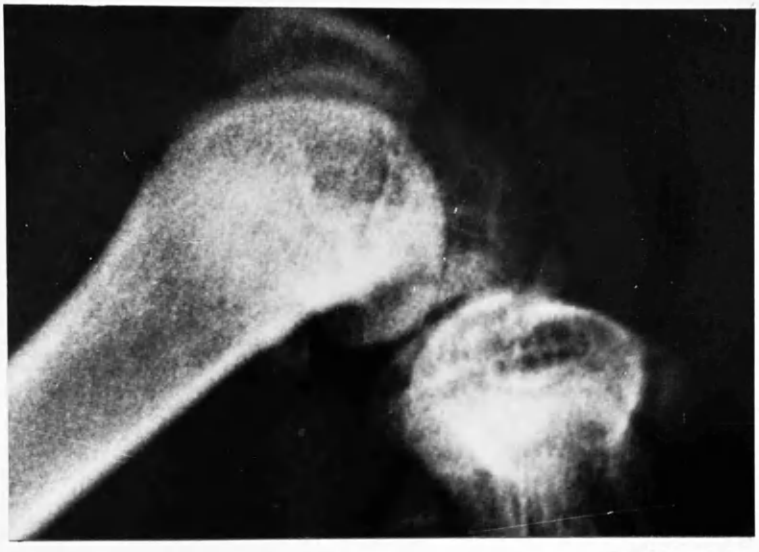

Fig. 88. As for Fig. 87, 175 days after operation showing + osteoporosis. x 7.

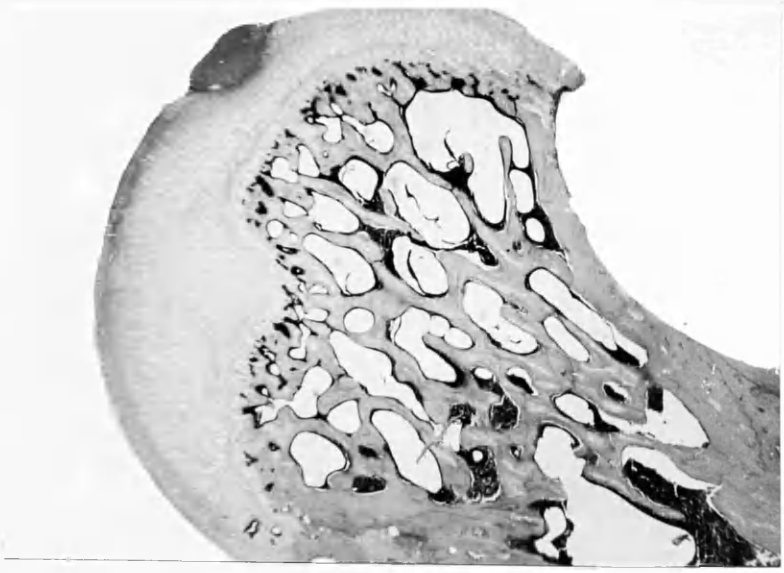

Fig. 89. Group 2. Histological section of femoral head region showing moderate reduction in numbers and calibre of subepiphyseal and interconnecting trabeculae.
H. & E. x 25.

Fig. 90. As for Fig. 89. The epiphyseal cartilage shows slight disorganisation of the cells of the zone of maturation.
H. & E. x 230.

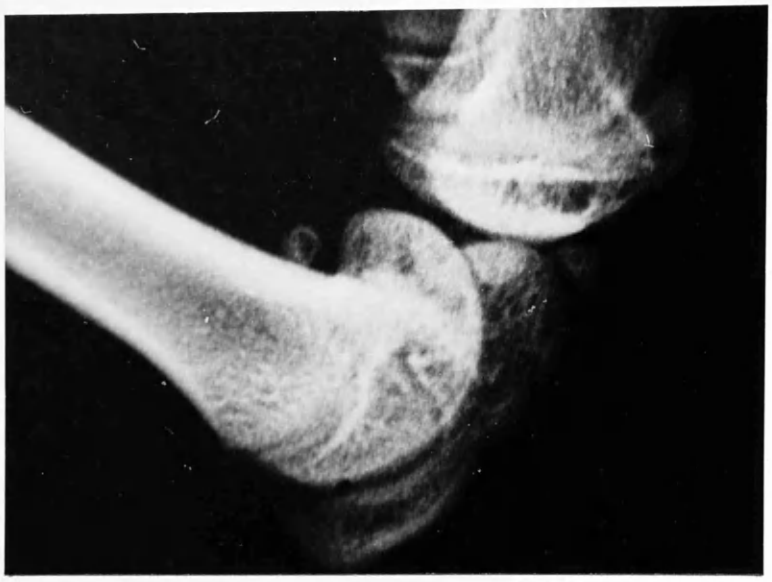

Fig. 91. Group 2A (M.S. oestradiol and testosterone group). Photographic enlargement of a radiograph of knee region immediately prior to operation showing normal bone density.
x 7.

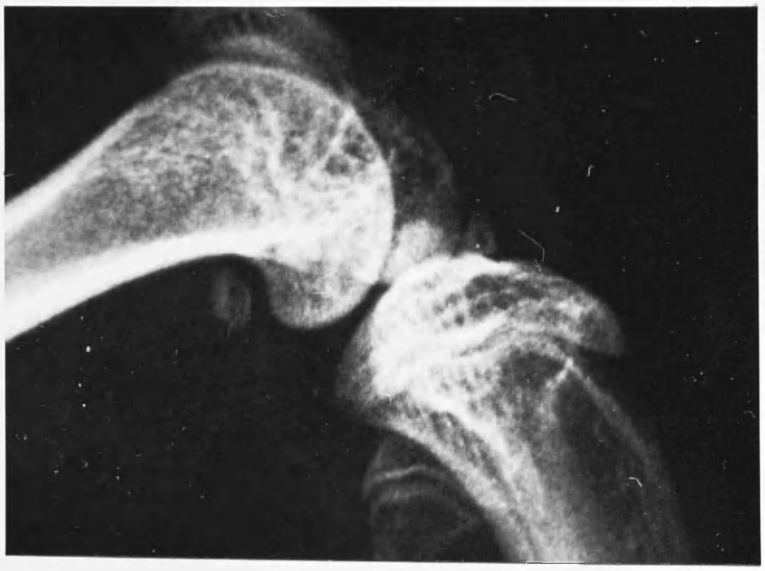

Fig. 92. As for Fig. 91, 100 days after operation showing + + + + osteoporosis.
x 7.

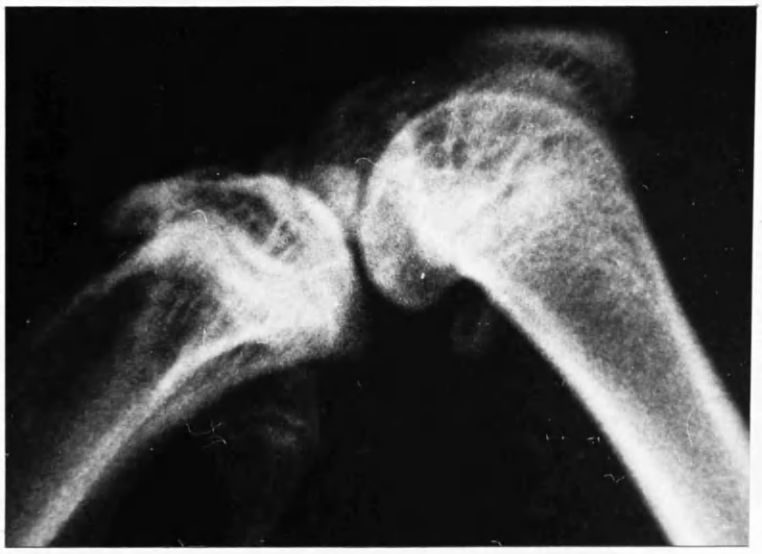

Fig. 93. As for Fig. 92, 130 days after operation showing + + + osteoporosis. x 7.

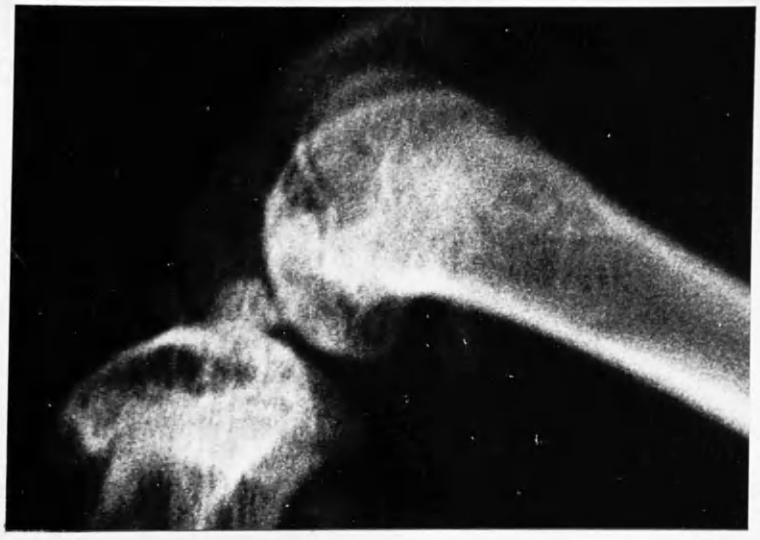

Fig. 94. As for Fig. 93, 160 days after operation showing + + osteoporosis. x 7.

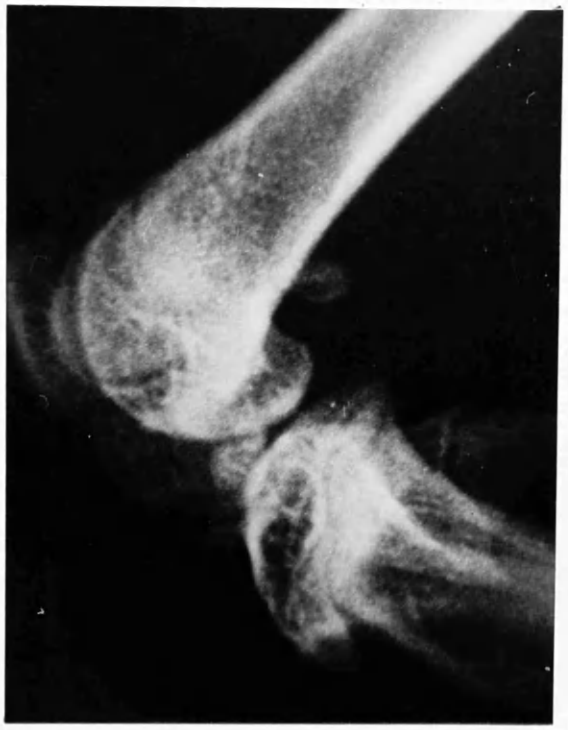

Fig. 95. As for Fig. 94, 175 days after operation showing + osteoporosis. x 7.

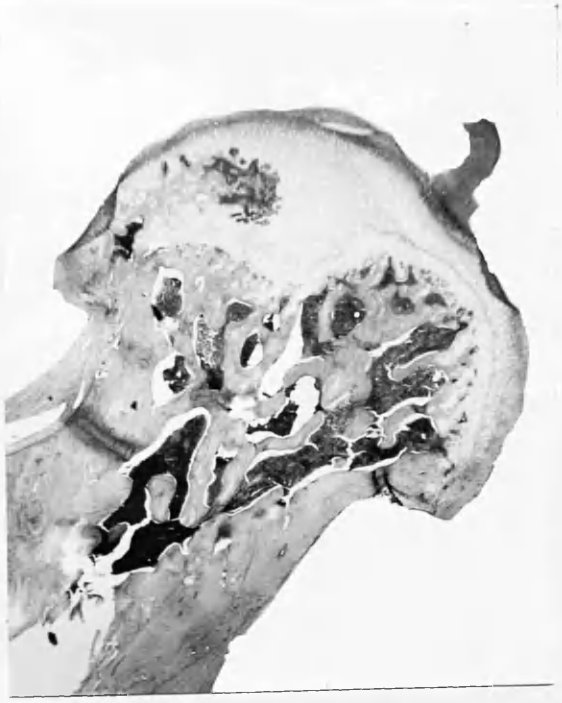

Fig. 96. Group 2A. Histological section of femoral head region showing irregularity of pattern and reduction in numbers of sub-epiphyseal trabeculae. H. & E. x 25.

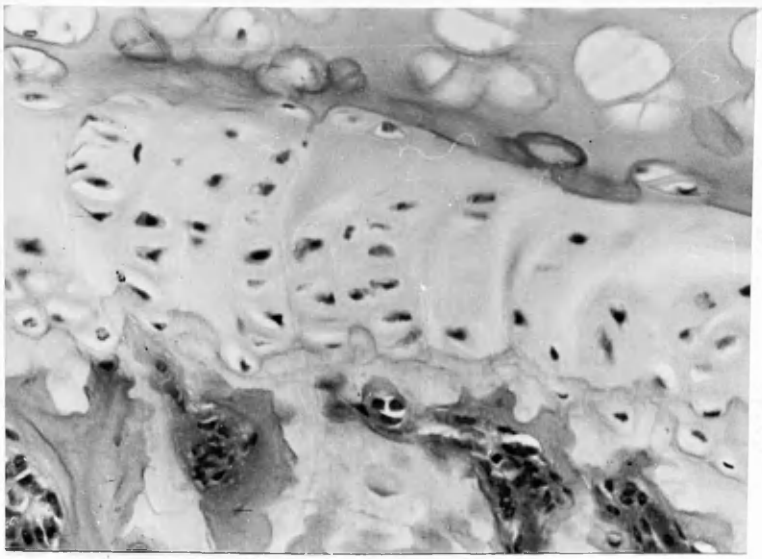

Fig. 97. As for Fig. 96. The epiphyseal cartilage shows marked reduction in numbers and moderate disorder of the cells of the zone of maturation.
H. & E. x 230

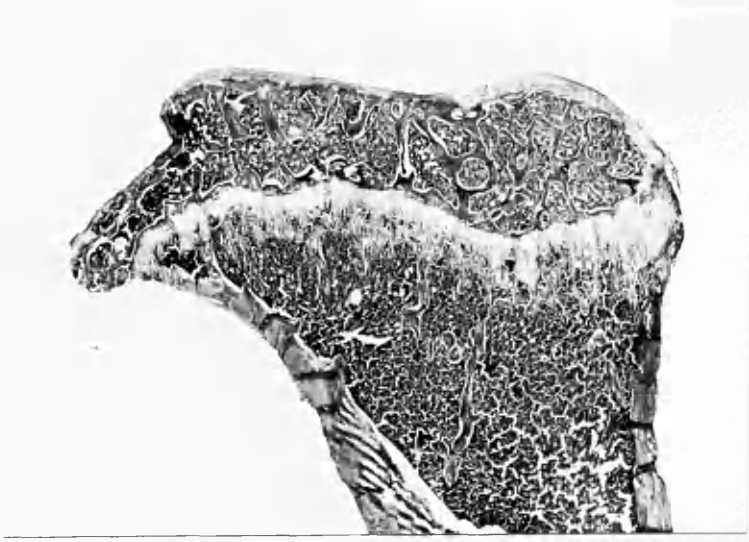

Fig. 98. Group 2A. Histological section of upper end of tibia showing an epiphyseal cartilage of average width and subepiphyseal trabeculae of moderate length.
H. & E. x 17.

Fig. 99. As for Fig. 98. The epiphyseal cartilage shows fairly regular cell columns. H. & E. x 75.

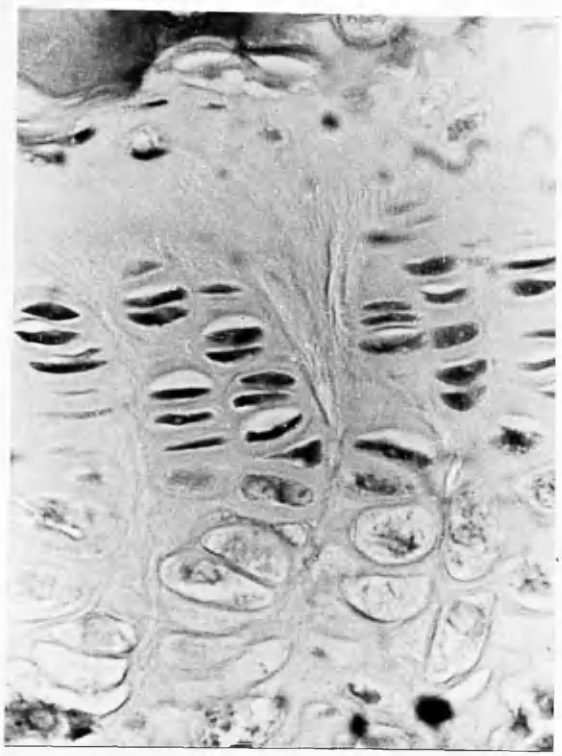

Fig. 100. As for Fig. 99. The cells of the zone of maturation of the epiphyseal cartilage show slight disorder. H. & E. x 500.

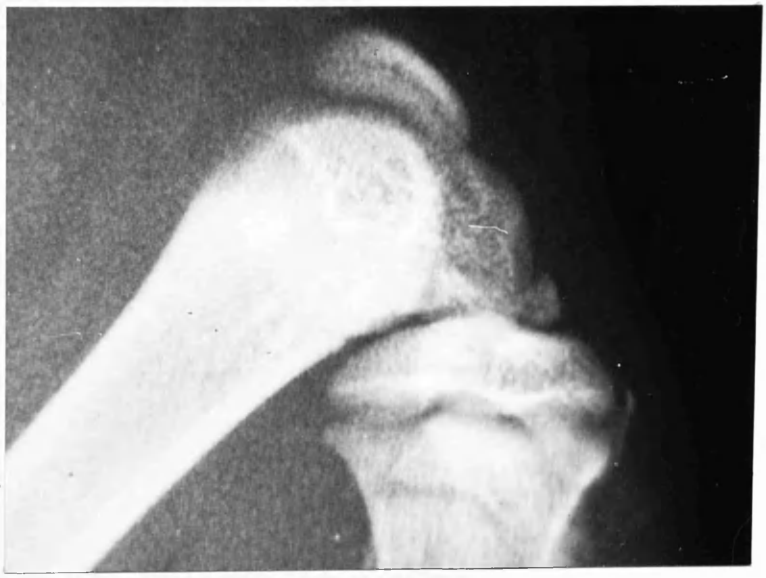

Fig. 101. Group 3. (O.S. testosterone group). Photographic enlargement of a radiograph of knee region immediately prior to operation showing normal bone density. x 7.

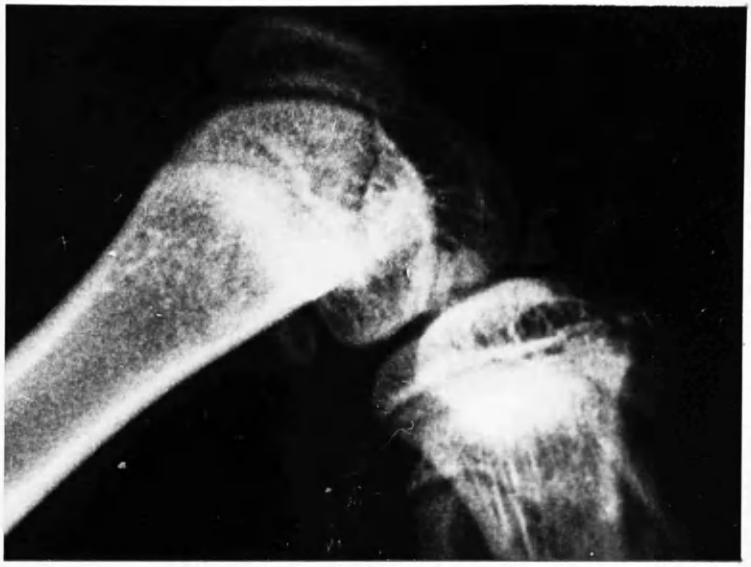

Fig. 102. As for Fig. 101, 100 days after operation showing + + + + osteoporosis. x 7.

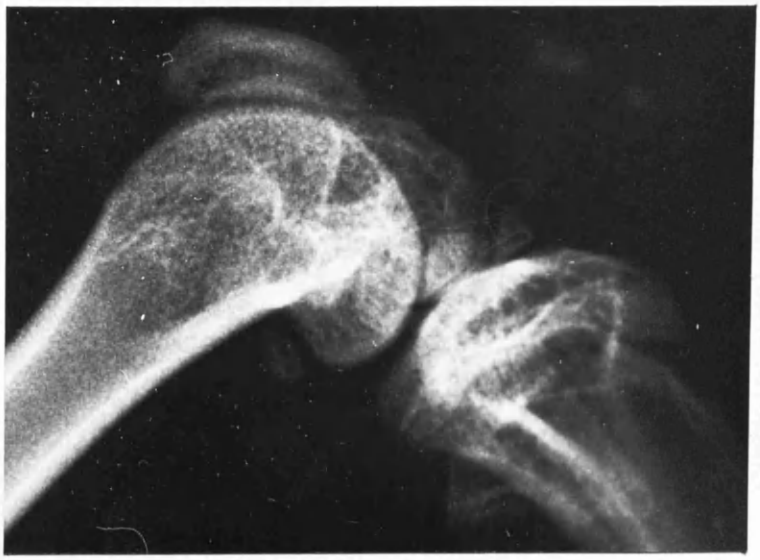

Fig. 103. As for Fig. 102, 130 days after operation showing + + + osteoporosis.

x 7.

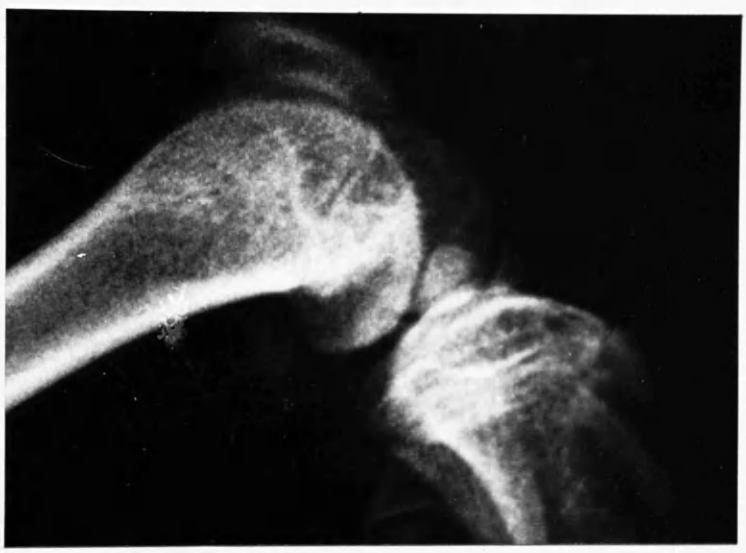

Fig. 104. As for Fig. 103, 160 days after operation showing + + + osteoporosis.

x 7.

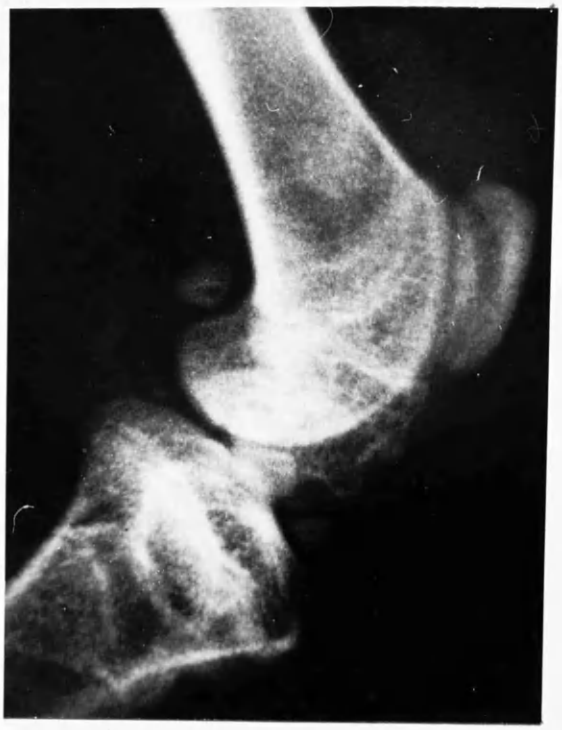

Fig. 105. As for Fig. 104, 175 days after operation showing + + osteoporosis. x 7.

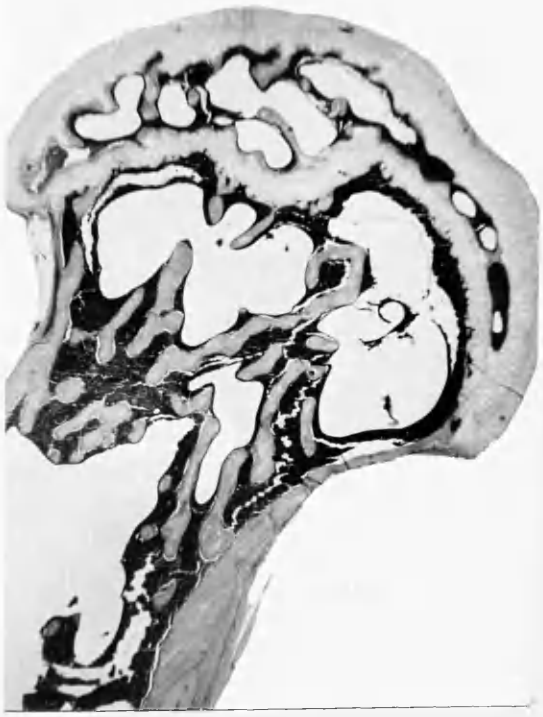

Fig. 106. Group 3. Histological section of femoral head region showing marked osteoporosis and an extreme degree of trabecular disorganisation. H. & E. x 25.

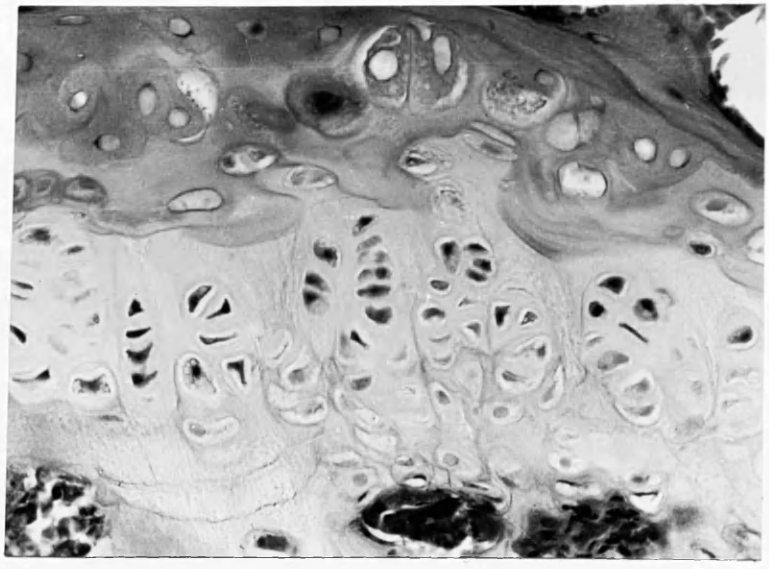

Fig. 107. As for Fig. 106. In the epiphyseal cartilage the cells of the zone of maturation show marked disorganisation.
H. & E. x 230.

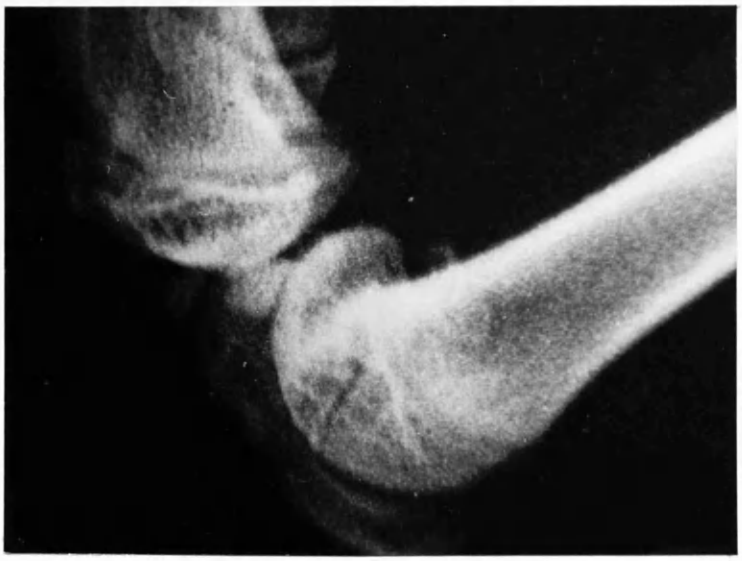

Fig. 108. Group 3A. (M.S. testosterone group). Photographic enlargement of a radiograph of knee region immediately prior to operation showing normal bone density.
x 7.

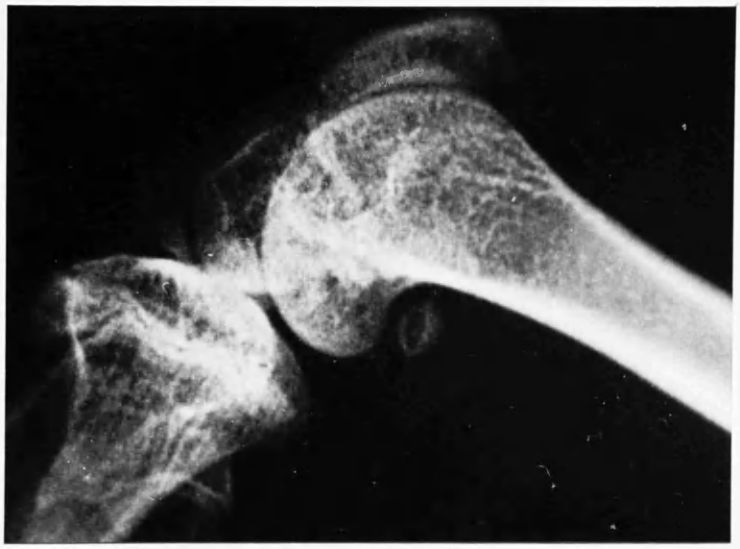

Fig. 109. As for Fig. 108, 100 days after operation showing + + + + osteoporosis.
x 7.

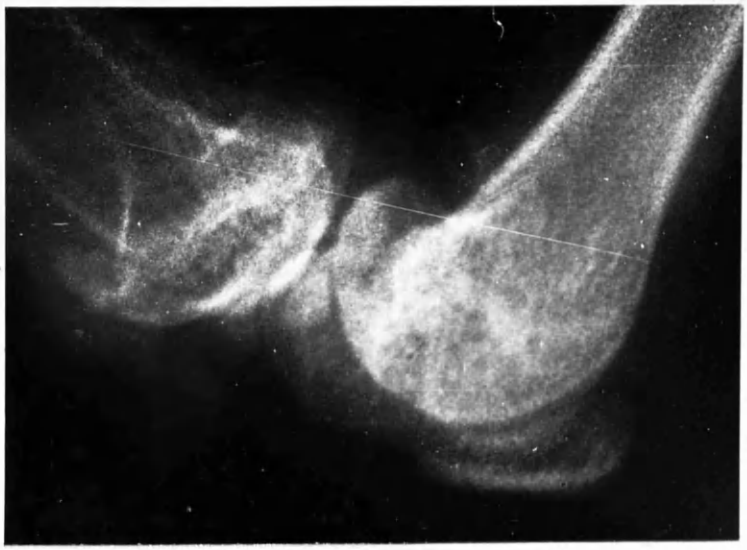

Fig. 110. As for Fig. 109, 130 days after operation showing + + + osteoporosis.

x 7.

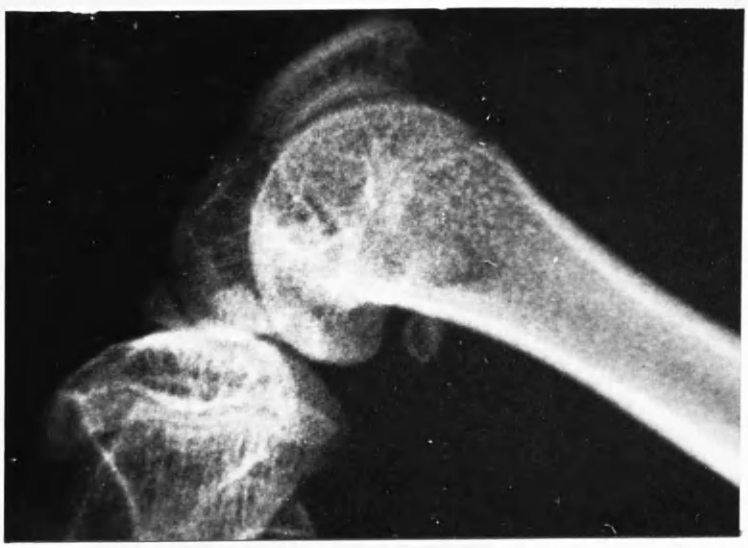

Fig. 111. As for Fig. 110, 160 days after operation showing + + + osteoporosis.

x 7.

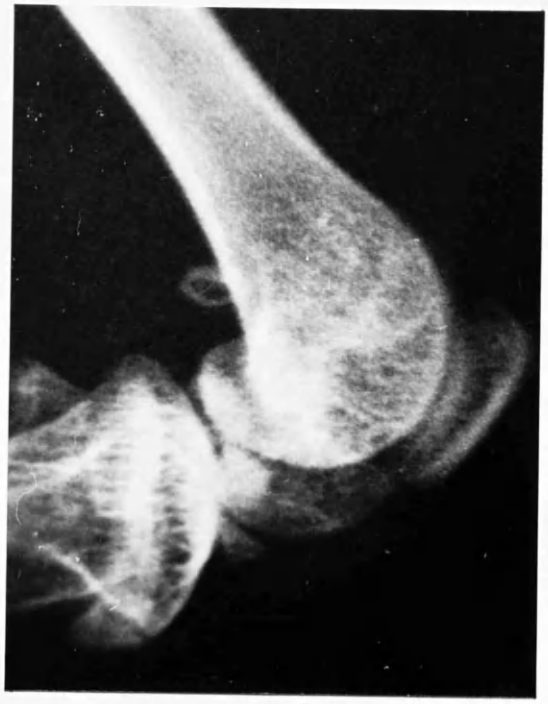

Fig. 112. As for Fig. 111, 175 days after operation showing + + + osteoporosis.
x 7.

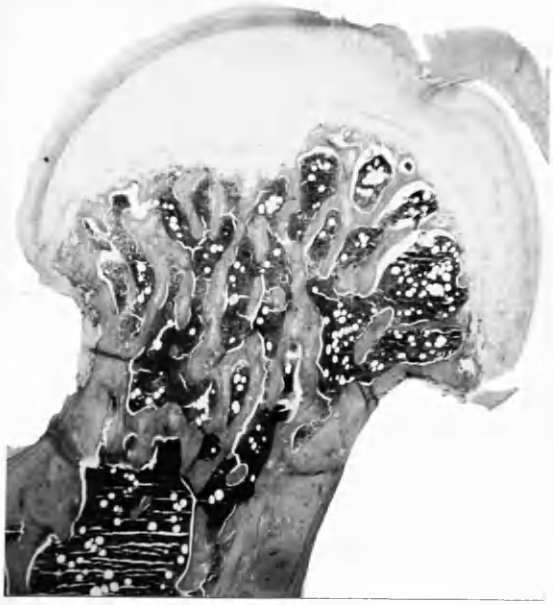

Fig. 113. Group 3A. Histological section of femoral head region showing a regular bone pattern but fairly marked osteoporosis.
H. & E. x 25.

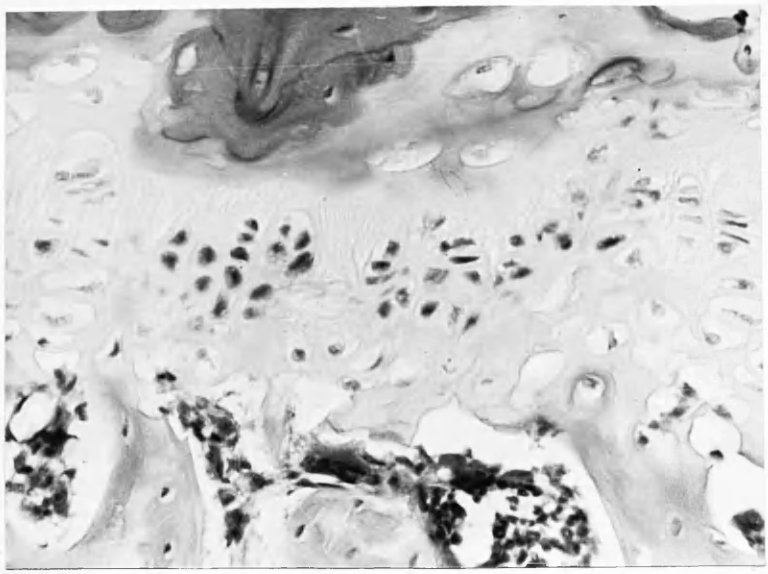

Fig. 114. As for Fig. 113. In the epiphyseal cartilage there is moderate disorder of cells of the zone of maturation.

H. & E. x 230.

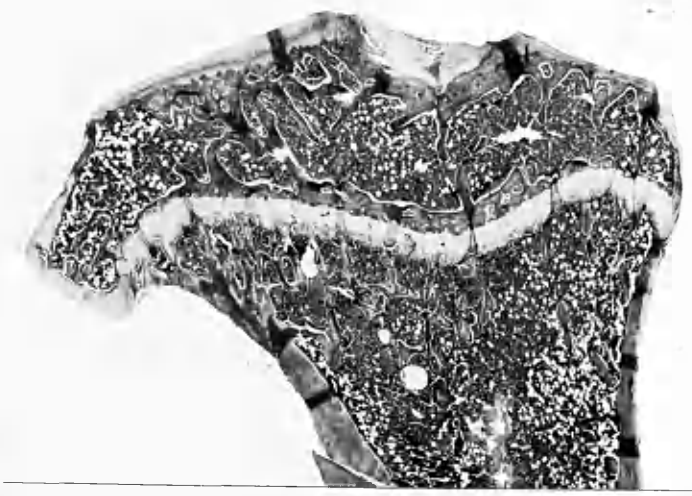

Fig. 115. Group 3A. Histological section of upper end of tibia showing epiphyseal cartilage of average width with marked reduction in numbers and length of subepiphyseal bone trabeculae.

H. & E. x 17.

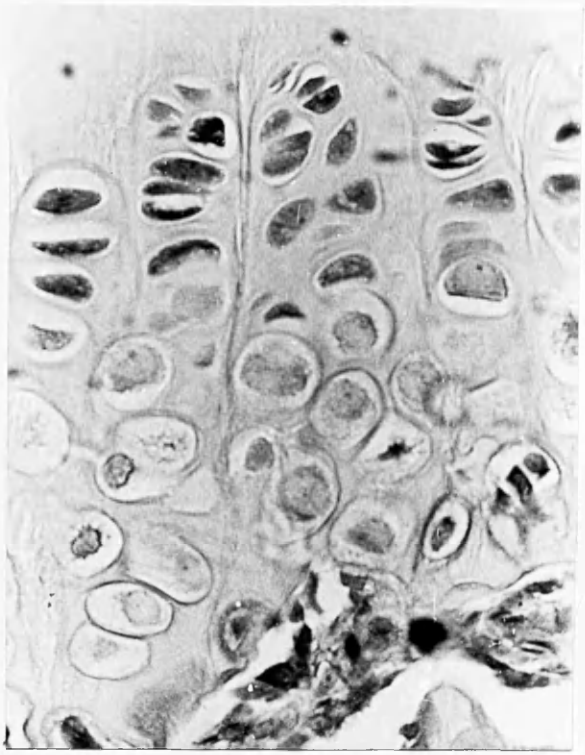

Fig. 116. As for Fig. 115. In the epiphyseal cartilage there is moderate disorder of cells of the zone of maturation.
H. & E. x 500.

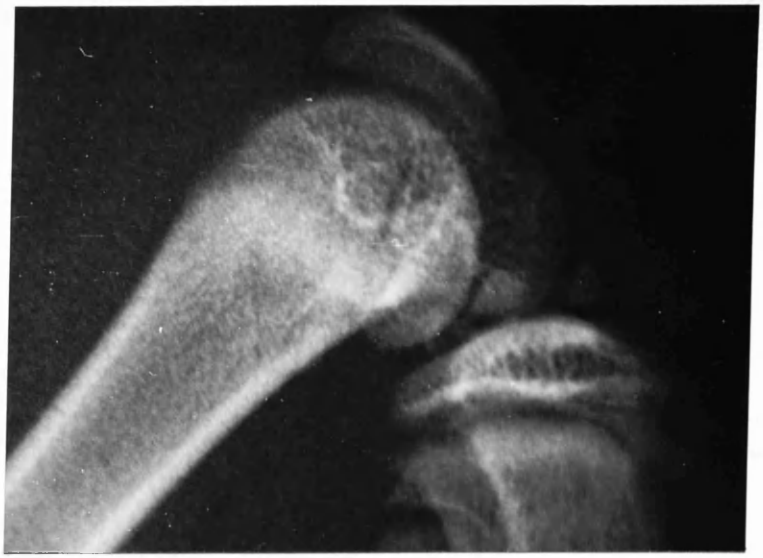

Fig. 117. Group 4. (Operated untreated control group). Photographic enlargement of a radiograph of knee region immediately prior to operation showing normal bone density. x 7.

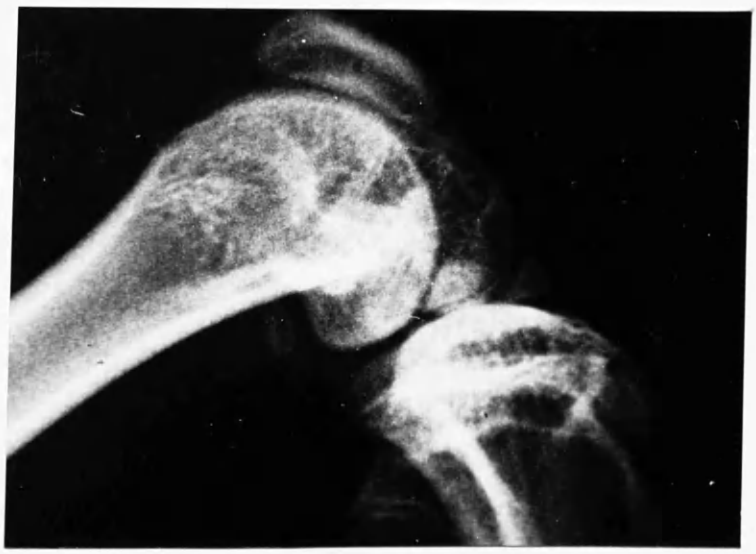

Fig. 118. As for Fig. 117, 100 days after operation showing + + + + osteoporosis. x 7.

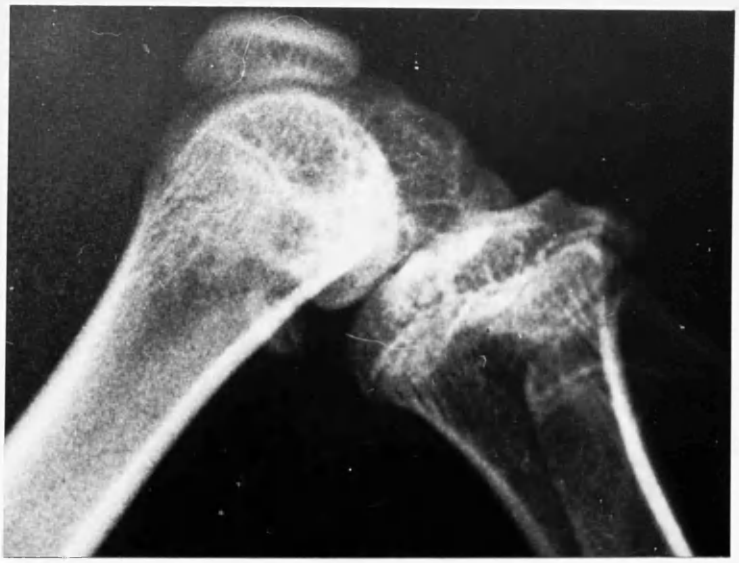

Fig. 119. As for Fig. 118, 130 days after operation showing + + + + osteoporosis. x 7.

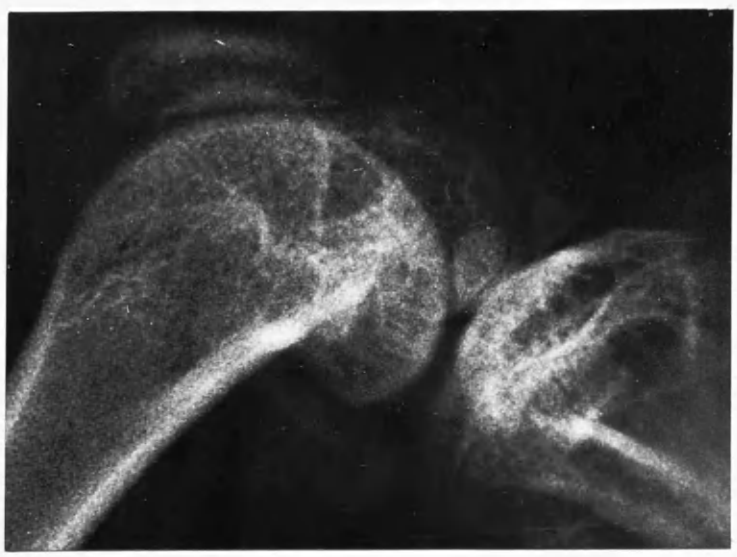

Fig. 120. As for Fig. 119, 160 days after operation showing + + + + osteoporosis. x 7.

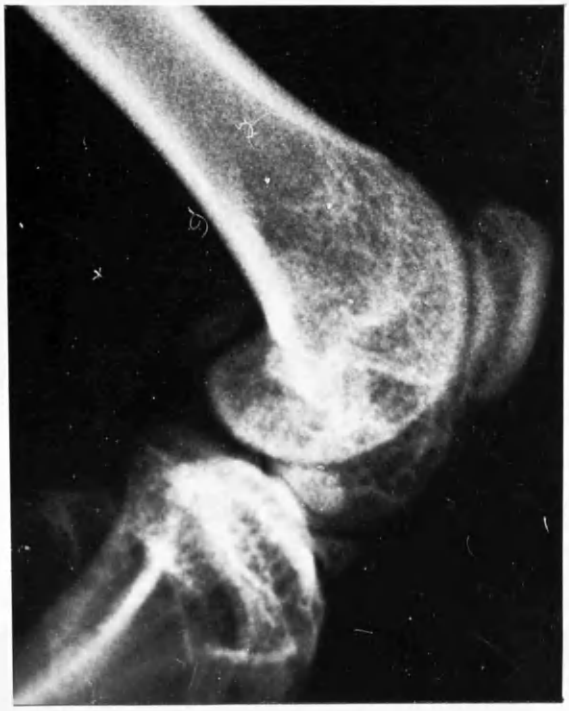

Fig. 121. As for Fig. 120, 175 days after operation showing + + + + osteoporosis.
x 7.

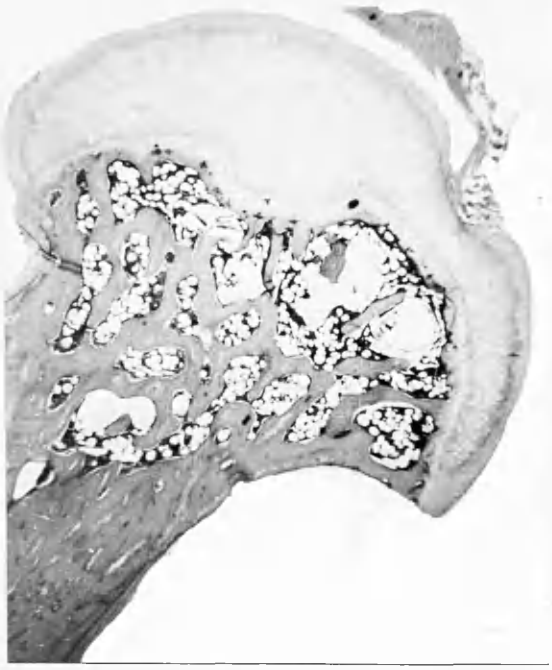

Fig. 122. Group 4. Histological section of upper end of femur showing regular bone pattern but marked osteoporosis.
H. & E. x 25.

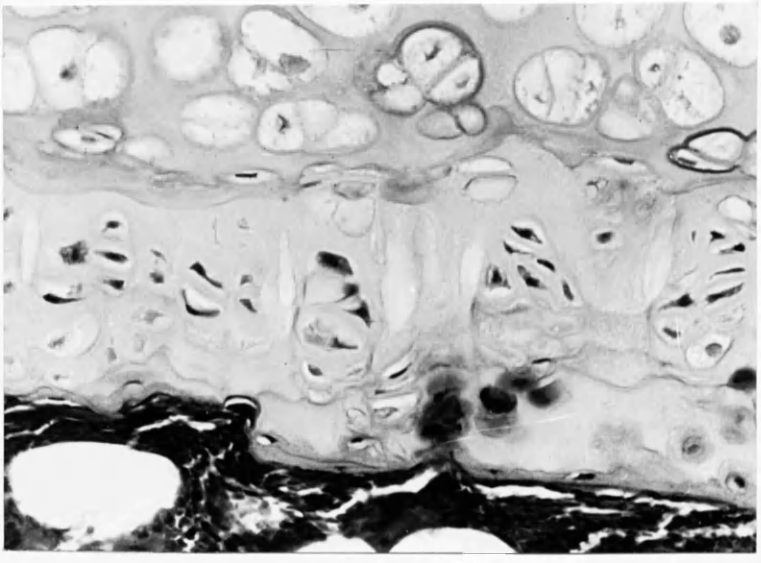

Fig. 123. As for Fig. 122. The epiphyseal cartilage is narrow and there is marked reduction in numbers of cells of the zone of maturation.
H. & E. x 230.

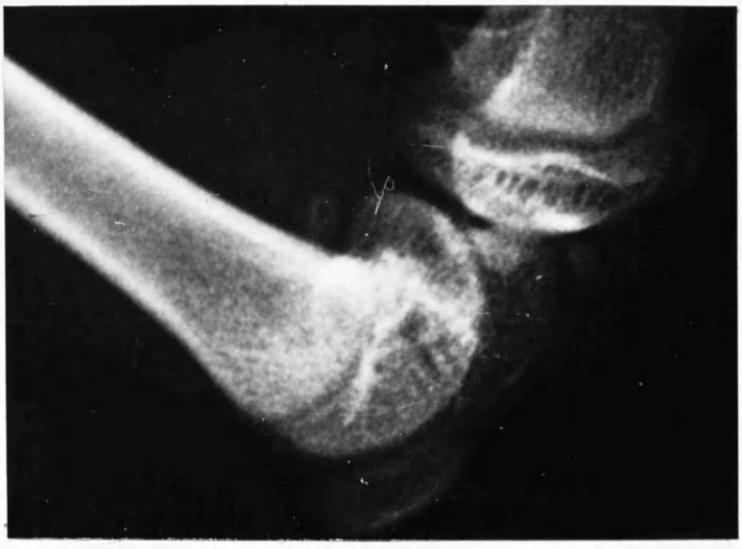

Fig. 124. Group 5. (Operated saline maintained control group). Photographic enlargement of a radiograph of knee region immediately prior to operation showing normal bone density. x 7.

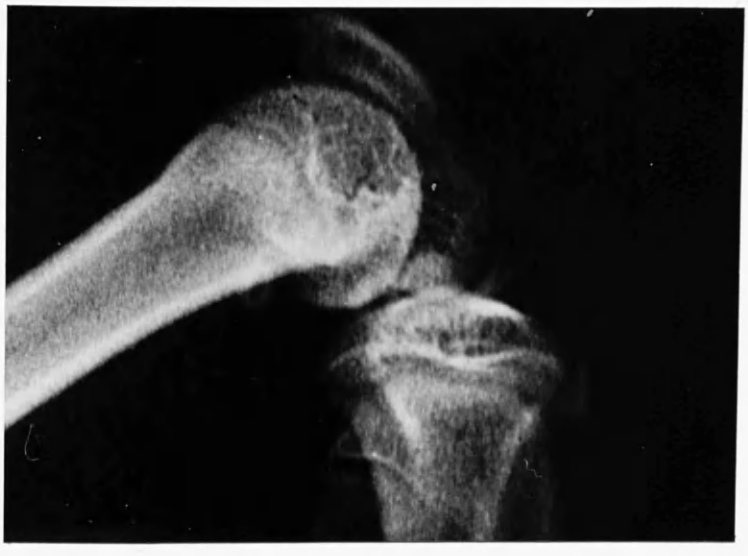

Fig. 125. As for Fig. 124, 100 days after operation showing + + + osteoporosis. x 7.

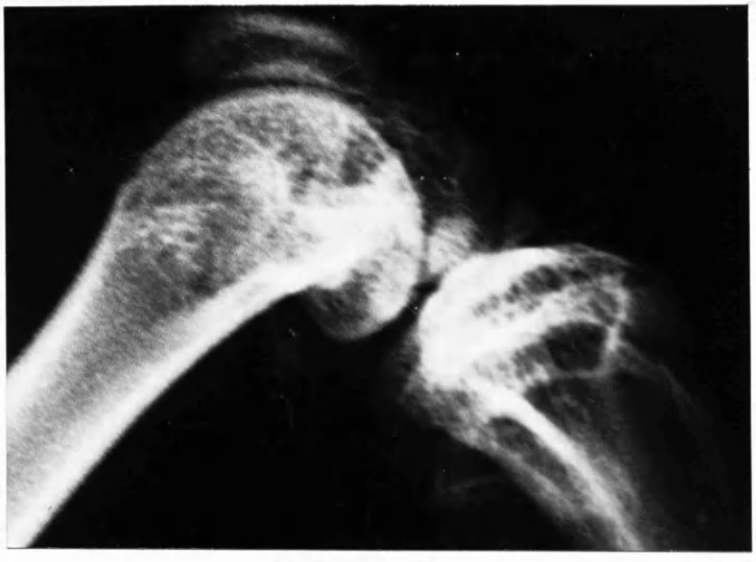

Fig. 126. As for Fig. 125, 130 days after operation showing + + + osteoporosis. x 7.

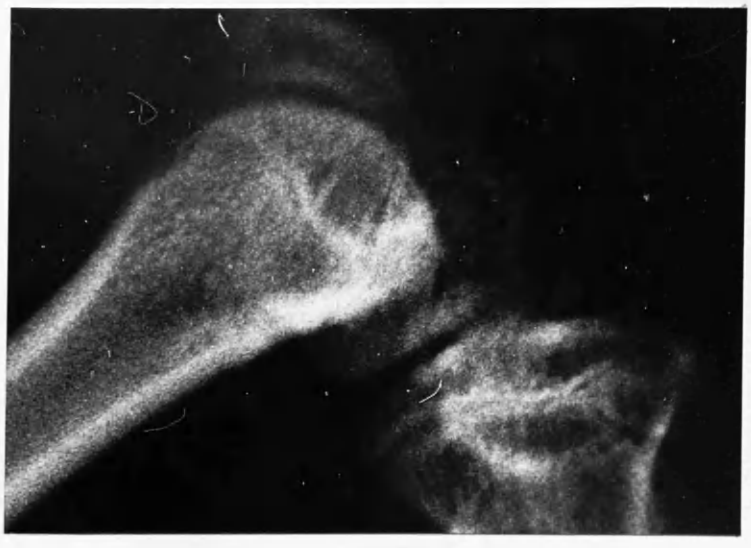

Fig. 127. As for Fig. 126, 160 days after operation showing + + + osteoporosis. x 7.

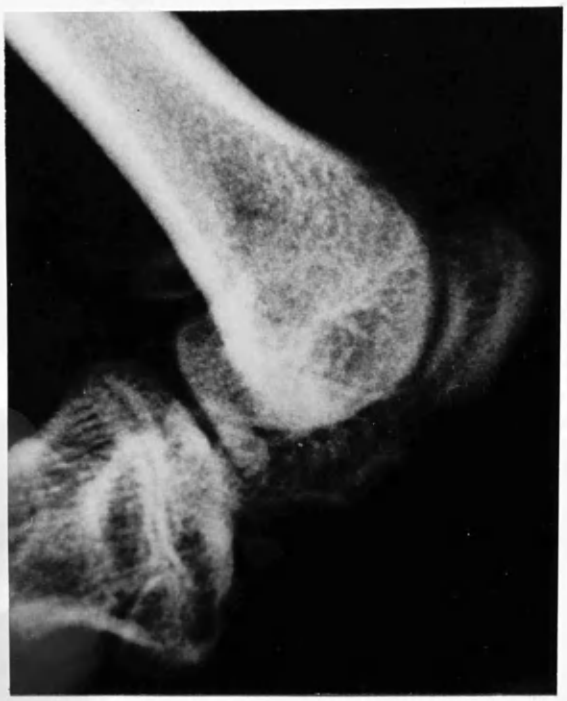

Fig. 128. As for Fig. 127, 175 days after operation showing + + osteoporosis. x 7.

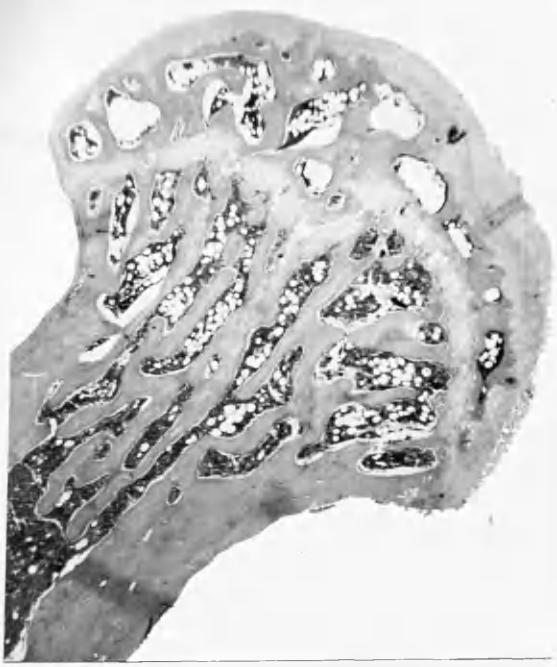

Fig. 129. Group 5. Histological section of femoral head region showing slight osteoporosis. H. & E. x 25.

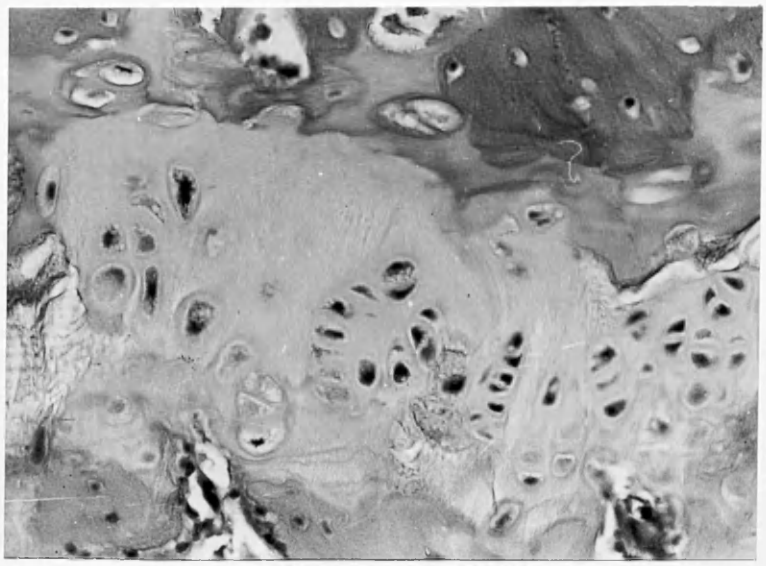

Fig. 130. As for Fig. 129. In the epiphyseal cartilage there is moderate diminution in numbers of cells mainly in the zone of maturation.

H. & E. x 230.

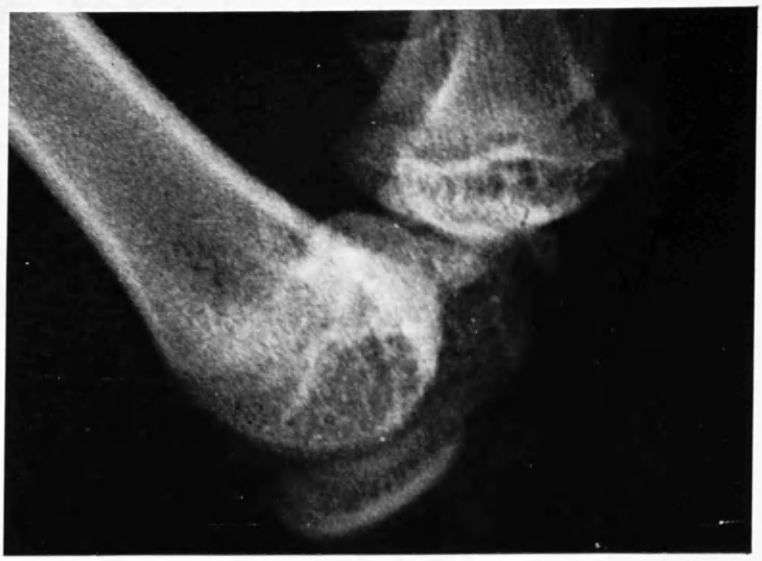

Fig. 131. Group 6 (Unoperated cortisone-treated control group). Photographic enlargement of a radiograph of knee region immediately prior to the time of operation showing normal bone density.

x 7.

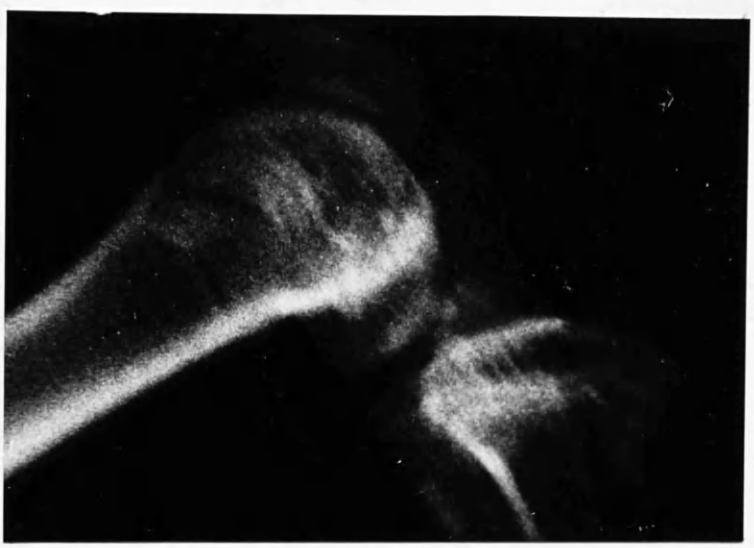

Fig. 132. As for Fig. 131, 100 days later showing normal bone density on a hard plate.

x 7.

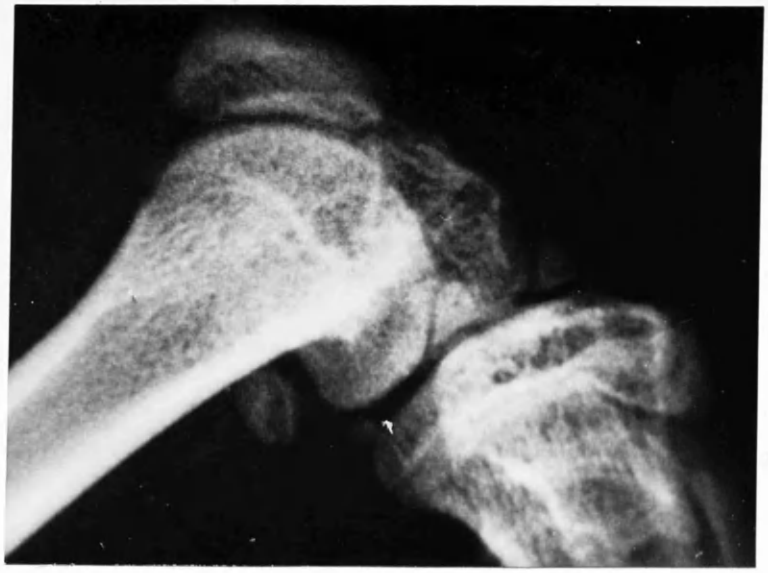

Fig. 133. As for Fig. 132, 130 days later showing normal bone density.

x 7.

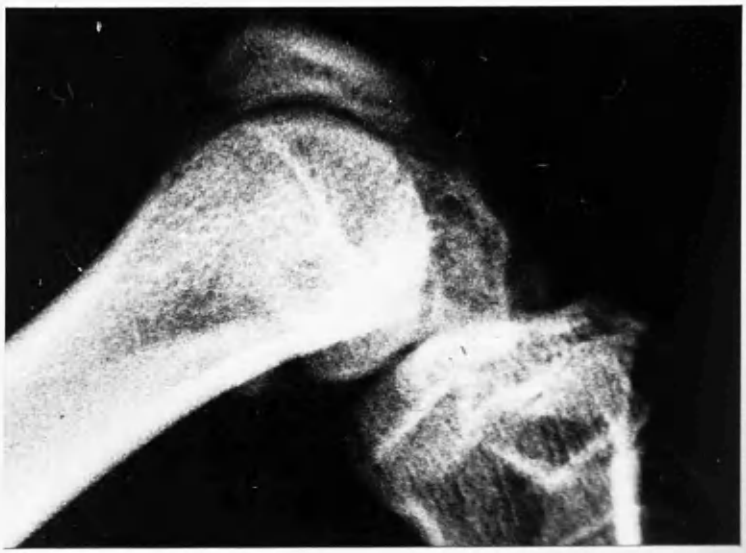

Fig. 134. As for Fig. 133, 160 days later showing normal bone density.

x 7.

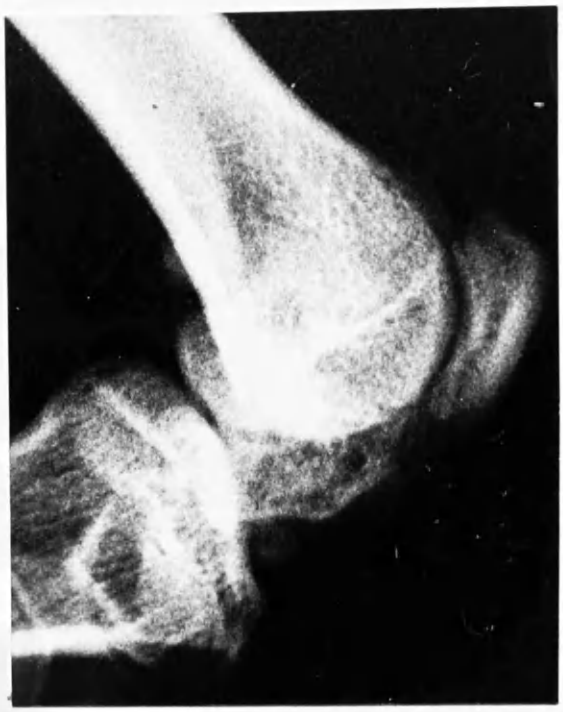

Fig. 135. As for Fig. 134, 175 days later showing normal bone density. x 7.

Fig. 136. Group 6. Histological section of femoral head region showing normal bone structure. H. & E. x 25.

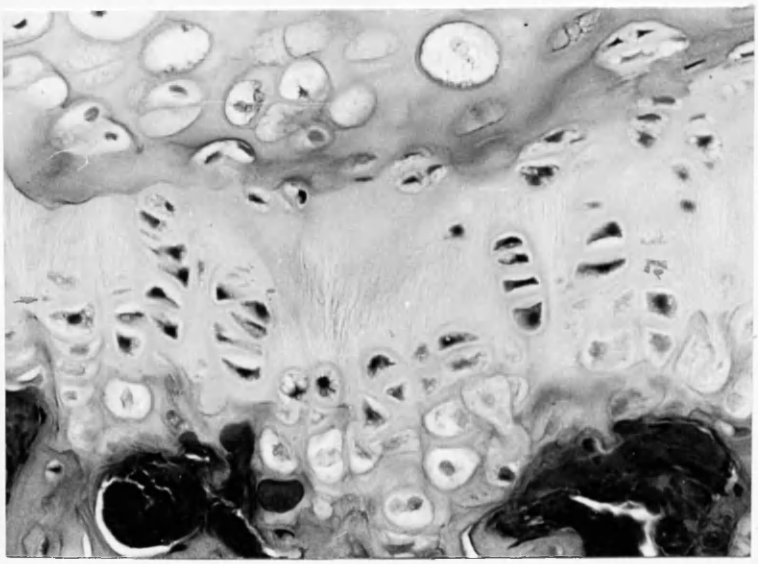

Fig. 137. As for Fig. 136. The epiphyseal cartilage is of normal width. The cells of the zone of maturation are plump and conspicuous.
H. & E. x 230.

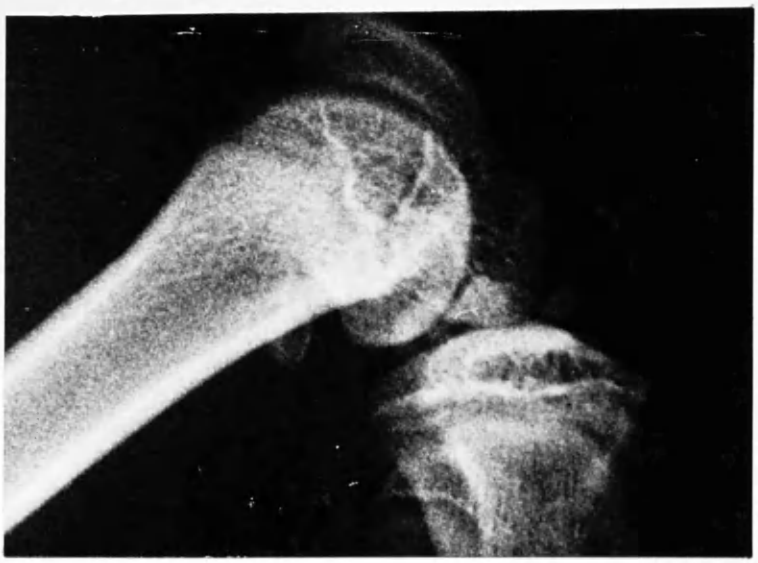

Fig. 138. Group 7 (Unoperated normal control group). Photographic enlargement of a radiograph of knee region immediately prior to time of operation showing normal bone density.

x 7.

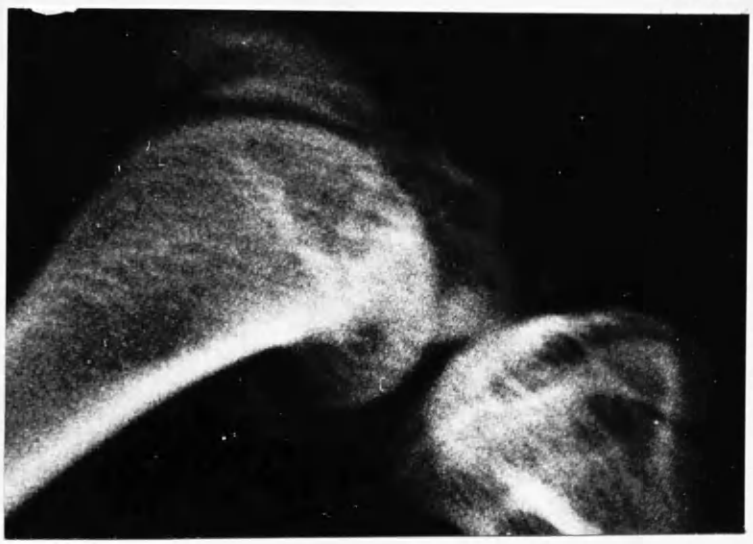

Fig. 139. As for Fig. 138, after 100 days showing normal bone density.

x 7.

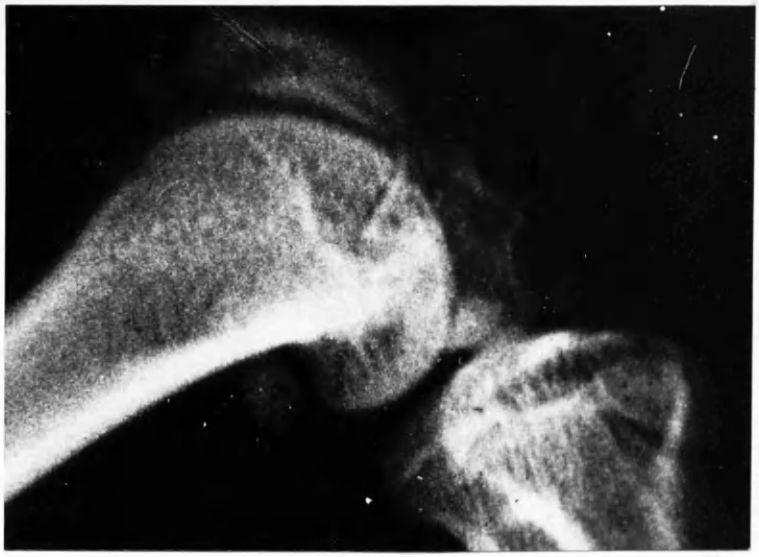

Fig. 140. As for Fig. 139, after 130 days showing normal bone density.
x 7.

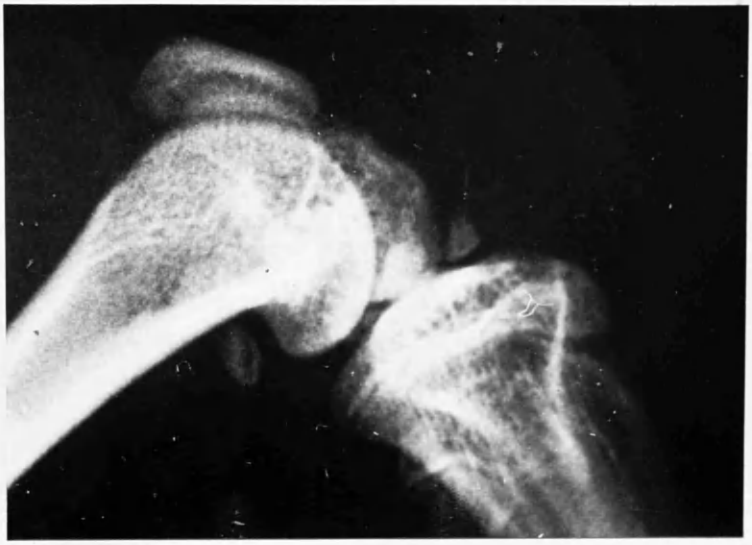

Fig. 141. As for Fig. 140, after 160 days showing normal bone density.
x 7.

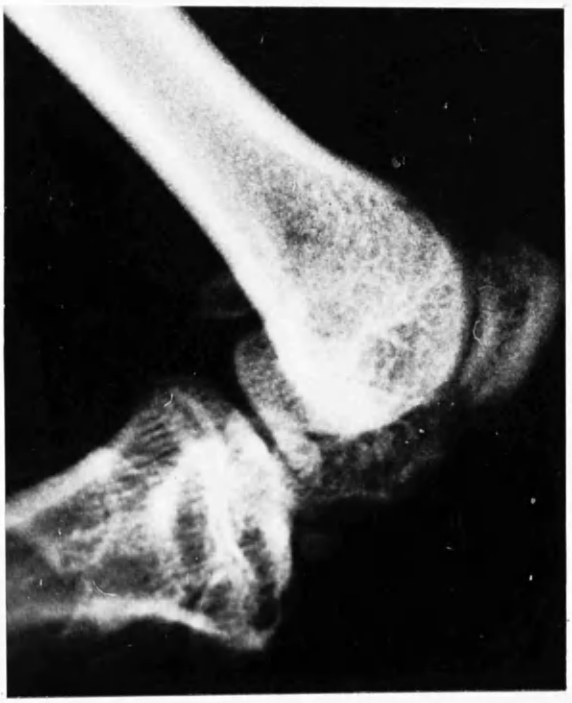

Fig. 142. As for Fig. 141, after 175 days showing normal bone density.
x 7.

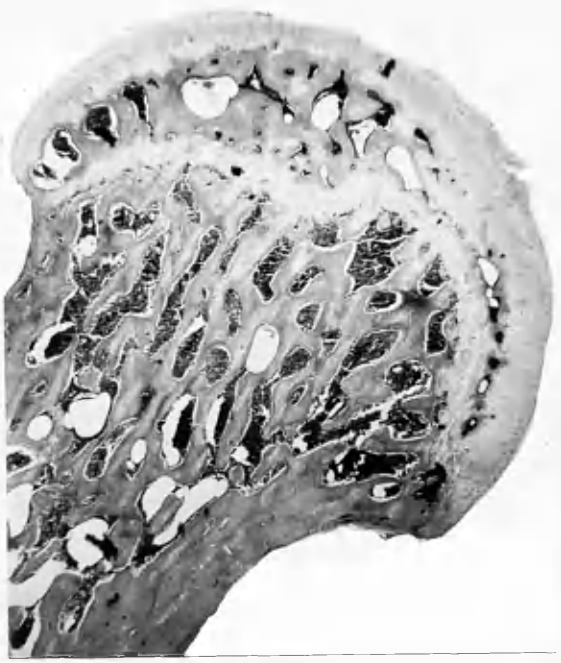

Fig. 143. Group 7. Histological section of femoral head region showing normal bone structure.
H. & E. x 25.

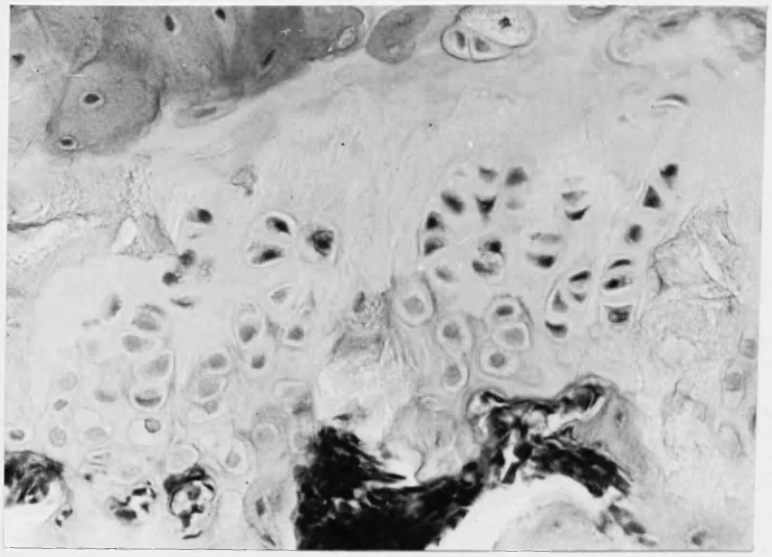

Fig. 144. As for Fig. 143. In the epiphyseal cartilage the cells of the zones of proliferation, and maturation show the normal appearance for rats of this age. H. & E. x 230.

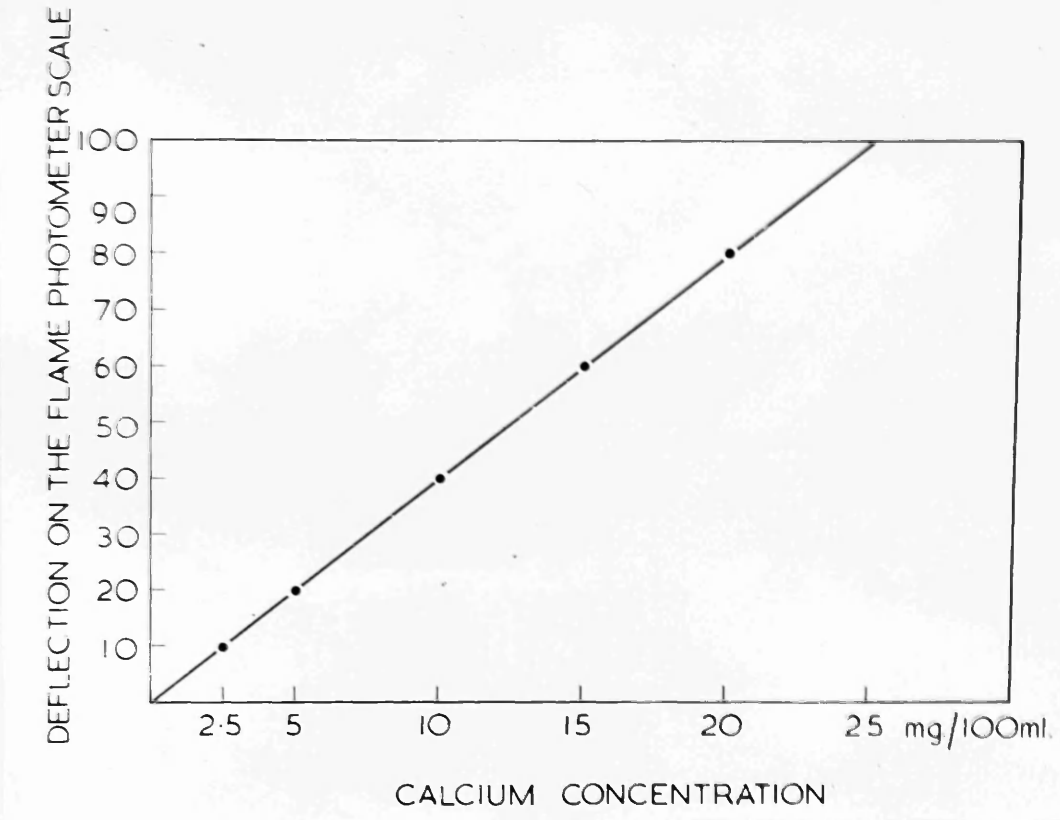

Fig. 145. The estimation of calcium by flame photometry. There is a straight line relationship between the flame photometer readings and the calcium content of various standard solutions.