BONE GROWTH: A STUDY OF THE GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF THE MOUSE

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INTRODUCTION

(*a*) The use of mutant genes in developmental studies

The use of mutant genes in replacing operative techniques, especially in mammalian experimental embryology, is now a well-established procedure. The method as outlined by Grüneberg (1948*a*) requires the tracing back of the ultimate manifestations of the genes through plausible channels of cause and effect to manifestations ever earlier in their first appearance—the 'pedigree of causes'. In no single analysis of this nature can complete faith be placed in the conclusions obtained, though the postulated pathways may be accepted if there is agreement in the interpretation of several syndromes.

In the present work, however, though the mutant genes are again used to replace alternative operative techniques, they are used in a different way. Only one of the manifold effects of the genes is selected for study—that which affects the growth of the skeleton. The anomalies are first proved to result from the retardation of accretion and from the lack of erosion, and are then translated into a description of the processes of accretion and erosion in skeletal growth.

(*b*) An account of the 'experimental' mice

The condition of the skeleton making possible the study of sites of bone erosion and accretion arose twice, by spontaneous mutation, in quite unrelated stocks of mice bred in genetical laboratories.

The skeletal condition was first discovered in the mouse by Grüneberg (1935, 1936, 1937, 1938) and is a distinctive feature of grey mice called grey-lethals (symbol *gl*/*gl*) which regularly die around weaning. Grüneberg attributed the skeletal anomalies to a 'general arrest of development' and to 'failure of the secondary modelling of the bone surface by absorption'. Skeletal retardation was not unexpected since the mice were much smaller than their normal litter-mates, and at three weeks the degree of ossification of their skeletons was at a stage reached by normal mice when only 10 days old. Failure of erosion was inferred:

(i) from the small size of intraosseous foramina (which can be enlarged only by erosion),

(ii) from the fact that the teeth do not erupt, but lie crumpled in their unenlarged sockets,

(iii) from the persistence of the *spongiosa*, and

(iv) from the excessive grossness of the zygomatic arch and other bones, and of the growing ends of long bones, which gross formations largely correspond to the sites

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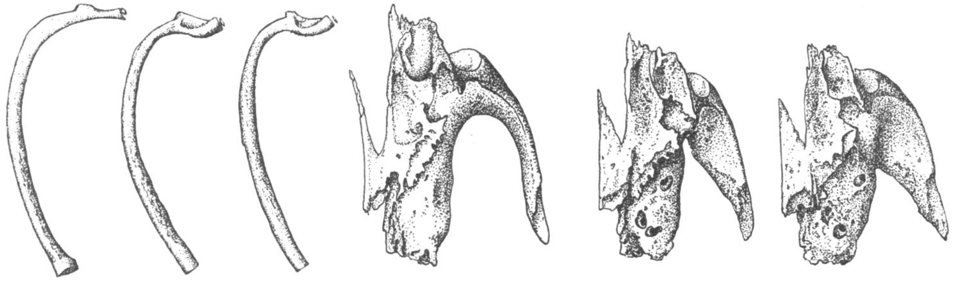
of erosion expected from Kölliker's work (1873). The erosional anomalies are no doubt related to the gigantic osteoclasts of the grey-lethals recorded by Barnicot (1947). Besides causing this extensive pathological syndrome and the grey coat colour, the grey-lethal gene irregularly produces kinked vertebrae near the tip of the tail.



Text-fig. 1. Drawings of a cartilage bone (the seventh rib) and a membrane bone (the maxilla) of normal, microphthalmic and grey-lethal mice respectively; demonstrating the striking similarity of the mutants' skeletons.

An almost identical condition of the skeleton was discovered again by Grüneberg (1948*b*) in mutant mice originally bred by Hertwig (1942*a*, *b*). The skeletons are so alike it is impossible always to distinguish the microphthalmic skeletons from those of grey-lethals (Text-fig. 1), though they are generally less retarded and erosional failure is apparently not always so complete. At 3 weeks they resemble in the development of their skeletons normal mice of the same stock which are only 14 days old; and the consequences of erosional failure are so variable that some mice live till long after weaning; indeed, one male has survived to sexual maturity and has bred successfully (Hertwig, 1942*b*). Besides their anomalous skeletons and their rudimentary eyes (from which they get their name), the mice are white, their eyes pink, some of their whiskers are kinked near the tips, and, like grey-lethals, their tails too are irregularly kinked.

The heterozygotes (+/*mi*) can be recognized by the variable spotting on the head, belly and tail, singly, or in any combination, and by their reddish eye colour. The heterozygote's tail is more rarely kinked than the homozygote's. It is of interest that in spite of these heterozygous expressions, the present author could detect no heterozygous manifestation of the gene either on the rate of growth of the skeleton or on the inhibition of erosion. This remarkable independence of the pleiotropic effects of the microphthalmic gene was also indicated by selected lines in which the heterozygotes were characterized by quite different expressions of the gene. For example, in one line the heterozygote usually had light eyes but very little spotting; in another, light eyes, a large spot on the forehead but none on the belly; while another had dark eyes and a large head spot. In other words, the gene can be made to manifest well in one direction without necessarily manifesting well in another. The pleiotropy is being investigated by Prof. Hertwig and her associates.

In homozygous grey-lethals and microphthalmics the anomalies attributable to retardation and to erosional failure are so widespread that it is reasonable to suppose that no bone in the mutant animals escapes the genes' action. Moreover, it seems on first investigation (and subsequent work described here confirms early impressions) that all the regular skeletal anomalies of the mutants can be explained in terms of these anomalies. It was for these reasons that the two stocks of mice appeared to Dr Grüneberg as admirably suited for the study of accretion and erosion in bone; and it was in response to his request that the present author undertook this work.

All the mice required were kindly supplied by Dr Grüneberg. In all, the skeletons of fourteen grey-lethals and twenty microphthalmics were prepared, together with those of at least one normal litter-mate for each mutant animal.

(*c*) Details of preparing the skeletons for analysis of their anomalies

Although the anomalies of the mutant mice are likely to become increasingly pronounced in older material, rendering the analysis of the differences more easy, certain and subtle, the optimum age at which to prepare the skeletons was found to be 3 weeks, since only a small proportion of the mutant animals could be expected to live and grow much beyond this age. This was associated with a disadvantage, however, that some of the bones, for example, the Tympanohyoidea (Johnson, 1933), would not even have begun to ossify.

The larger bones (skull, lower jaw, girdles and larger long bones of the limbs) were prepared by maceration in boiling water. It was found quite easy to remove the flesh with forceps, scalpel and scissors after only 20 min. boiling. There was little danger of disarticulating the epiphyses or compound bones except when eviscerated animals had been stored in the refrigerator for some days. The brain was removed by jets of water from fine pipettes, the dura with the aid of forceps.

Isolated skull bones were prepared by papaine digestion (Luther, 1949), as even dilute solutions of potassium hydroxide were found to damage the bones. Whether macerated in boiling water or by papaine, the bones were then defatted in acetone and bleached with hydrogen peroxide.

For the remaining bones which were small or only partially ossified, neither of these techniques was found suitable. These bones were made available for examination by staining whole mice (skinned and eviscerated) with alizarin, and then clearing and differentiating (the method used was a modification of Johnson (1933)).

For examination, macerated bones were placed on plasticine so that they could be supported in unstable positions. Bones from transparencies were cut from the rest of the skeleton and placed in a glycerol bath. This consisted of a wax square made in three depth sizes, filled to overflowing and placed between a glass plate and microscopic slide. Distortion was thereby reduced to a minimum, and by moving the square on the glass disc, or the slide on the square, the bone could be orientated and held in almost any desired position.

The bones were then drawn, the extreme variation in their opacity rendering them quite unsuitable as photographic subjects. The outline was made accurately by camera lucida attachment to one eyepiece of a low-powered binocular microscope after which shading was added free-hand. Magnification of the original drawings was usually × 9½, but this has been reduced to × 6½ for publication. Moreover, as the similarity between grey-lethals and microphthalmics is very strong, only the former have been reproduced in the appended plates.

Diagrams are free-hand interpretations of the original drawings or of superimposed tracings of them. The arrows indicate directions of growth.

(*d*) TECHNIQUES OF STUDYING BONE GROWTH

The techniques of studying bone growth are many. The earliest techniques in which holes were bored and metallic marks were fixed near the ends of long bones (Stephen Hales, 1727) are of little more than historic interest, being the first to show that growth of long bones is localized terminally.

A more subtle technique was developed by John Hunter (1835). This was the feeding of madder which stains bone laid down during the period of madder-feeding a pinkish colour. Bone laid down subsequently is recognized by being white against the pink background. This method, which is applicable to the study of surface accretion in both long and flat bones, was used also by Flourens (1840), but the works of both these early writers are almost inaccessible. The latest workers in this field (Brash, 1934; Payton, 1932, 1933) have not described the growth of an entire skeleton between them. While the madder technique is limited to those animals which will eat it, a parallel technique of much greater scope has been developed by Hoffman & Schour (1940*a*). These workers replaced madder-feeding with the injection of Alizarin red S, a stain related to madder and one of the anthraquinone group. New bone is recognizable within 3 hr. of the injection and retains its colour for at least 4 months. Recognition of the sites of bone accretion at successive injections would be facilitated if a different anthraquinone stain, having a contrasting staining reaction (Brash, 1939), were used for each injection.

A quite different technique has been evolved by Harris (1933). He noticed that bone formed during severe illness is extremely dense and can be recognized in radiographs many years afterwards. The extent to which these 'lines of arrested growth' are capped by later formed bone therefore affords a precise measurement of the extent of accretion. The dense lines are always formed adjacent to the conjugation cartilages and periosteum and therefore identify these as the sites of bone accretion. Boerema (1942) has made similar use of zones of dense bone formed as a result of seasonal consumption of phosphorus drugs. He met with special difficulty, however, in applying this method to the study of surface accretion in the vault bones of the skull.

Yet another technique is the use of radiophosphorus (Leblond, Wilkinson, Bélanger & Robichon, 1950). This substance is incorporated by newly formed bone and autoradiographs can be obtained from sectioned material. This technique is especially useful in studying histological aspects of bone accretion.

The rates of growth at the ends of long bones has also been estimated by counting the number of cells in the ranks of the conjugation cartilages (Harris, 1933).

In none of these studies was it possible to observe directly the extent of bone erosion, though several authors have inferred erosion from their observations on accretion. Kölliker alone (1873) attempted a simultaneous study of accretion and erosion, but his accounts of the two aspects of bone growth did not relate to the same species and were treated in different sections of his book. Kölliker described the *sites* of erosion from the occurrence of Howship's foveolae (formed by the osteoclasts) or of the osteoclasts themselves in thick tangential sections of softened bone. His method, however, was unable to provide information on the *rates* of erosion, since it seems that osteoclasts vary tremendously in their activity, and mere numbers afford no guide to the intensity of erosion. Thus not only did he observe osteoclasts before the formation of bone, but he also figures bone surfaces for which it is difficult to imagine osteoclasts more crowded together, and others which, although pot-marked all over with Howship's foveolae, present hardly any osteoclasts (compare his figs. 3, 6 and 9 with his fig. 5). Moreover, Ruth (1937) reports that he and Kawata (1924) observed comparable amounts of erosion in the pelvic symphysis of the guinea-pig during pregnancy but very different numbers of osteoclasts. Lastly, Barnicot (1947) described a massed migration of osteoclasts across the parietal bone of the mouse, although it is unlikely that there was any change in the sites of erosion.

The grey-lethal-microphthalmia technique used here has one major advantage over all the preceding methods—for it provides direct information on the rates of erosion. It displays the sum total of accretion and erosion over a long period, and unlike Kölliker's method, never depicts erosion which is only temporary and occurs at the time of death. Owing to the sublethal action of the mutant genes the technique is necessarily confined to very young animals, but this has resulted in the provision of some novel contributions to our concepts of bone growth. The method is also applicable to almost every bone in the skeleton.

Such an attractive method is not, of course, without its handicaps. In the first place it is not possible, as with the other techniques, to use a bone as its own 'control'; in fact, it is necessary to make comparisons between drawings of two bones. Thus error can be introduced into the analysis through ascribing differences which may occur between any two bones to the actions of the segregating genes. In the second place, the condition which makes the study possible is limited as a regular occurrence to the mouse and rat. In the rat the condition is inherited as a simple recessive (Bhaskar, Weinmann, Schour & Greep, 1950) and is only temporary. Recovery begins at about 30 days, and the skeleton is often completely normal by 150 days. Nevertheless, those authors have used the condition in a microscopic study of the growth processes of the tibia and humerus of the rat. It may be noted in passing that the Sirenia (Dugong and Manatee) exhibit a skeletal condition which may be regarded as a physiological grey-lethal. However, although the spongiosa persists, it seems that erosion of the external surfaces of the bones is in normal quantities. Sporadic cases of the condition have occurred in the rabbit and in man where it is known as osteopetrosis. Albers-Schönberg (1907) gave the first account, and there have been subsequent reports by Elliot Smith & Wood Jones (1910), by Suk (1929) and by Lightwood & Williams (1940). Ingalls & Grossberg (1932) have described it in a 'unique' pair of femora but failed to see the significance of the anomaly and did not mention whether other parts of the skeleton were affected.

(*e*) THE ANATOMICAL ATLAS

The present method of recognizing the sites of accretion and erosion necessarily entailed comparing camera lucida drawings of normal and mutant bones, and the drawings of the normal bones have been extensively labelled to serve as an atlas of the mouse skeleton (see Pls. 1–20). Although some precedent has been set by Greene (1935) in her *Anatomy of the Rat*, her account of the skeleton is inadequate for many anatomical purposes.

In this atlas the author has used the latinized veterinary nomenclature of Ellenberger & Baum (1926) which is preferable to the related BNA nomenclature (Jamieson, 1916), since it is adapted to four-footed animals. But where example has been lacking in Ellenberger & Baum the author has found it necessary to use BNA terms and even to invent new ones (recognizable in the text by the initials N.B. in parentheses).

The latinized nomenclatures may be a source of difficulty for the English reader but have the advantages of being the most complete applicable nomenclature and therefore the least likely to require the addition of new terms, and also of being the most standardized and universally recognized.

(*f*) THE PROBLEM OF SUPERFICIAL AND INTERSTITIAL GROWTH OF BONE

Unlike the madder technique, which reveals only the superficial component of bone growth and is independent of the occurrence of interstitial growth, the method used in the present work is based on the assumption that bone growth is entirely a surface phenomenon; and the conclusions presented in the subsequent pages would be quite invalidated if any interstitial growth of bone occurred. Further, unlike Harris's or Boerema's techniques where the lines of dense bone can be shown in successive radiographs to have remained at their original distances apart, the present method is incapable of providing complete proof of the absence of interstitial growth. It is therefore necessary carefully to examine these alternative theories as to the method of bone growth.

It is unnecessary to review in detail the case for the superficial growth of bone. Each of the diverse techniques described in an earlier section of this paper provides independent evidence for the existence of superficial bone growth. Further evidence is supplied by a series of embryological experiments performed by Lacroix (1942–3, 1946*b*) which involved the transplantation of pieces of conjugation cartilage and periosteum and proved that both are centres of bone production. The existence of bone erosion is itself evidence for surface accretion, since it is impossible for the complex shapes of most bones to be maintained merely by differential rates of accretion. The widespread occurrence of bone erosion recorded by Kölliker's classic work (1873) is proof of the widespread nature of surface accretion. Lastly, the skeletal deformities whose detailed investigation forms the bulk of this account can be explained quite simply on the assumption that surface accretion is retarded and that no erosion takes place. On the other hand, anyone believing solely in the interstitial growth of bone would find it extraordinarily difficult to explain the anomalies.

In spite of the weight of evidence for the superficial nature of bone growth, the concept has met with recurrent resistance from many osteologists. Their objection seems to be largely philosophical, regarding 'meristematic' growth as the sole property of the botanical world. Yet 'meristems' are found in the skin and in the ovary, and the bones of the limbs and girdles of the chick are delineated from the meristematic apical cap of the limb-bud (Saunders, 1948).

Compared with the experimental evidence for superficial growth that for interstitial growth is inadequate. None was available until 1929 when Kornew claimed to have demonstrated it. He surrounded the ulnar and fibular metaphyses of rabbits with metallic rings (i.e. at each end of the bone on the highly concave part near the conjugation cartilages). The rings moved farther apart as the bones grew in length, although they were placed behind the sites which are usually regarded as the regions where superficial accretion takes place. However, unless the rings were deeply embedded in the bone substance they were more likely to trace the growth of the periosteal membrane than of the bone itself, and it seems highly probable that Kornew's experiments merely demonstrated the interstitial growth of the periosteum!

Bisgard & Musslemen (1940) made unilateral bone grafts between exposed cancellous surfaces of the vertebral centra of month-old goats. They succeeded in getting bony continuity between four contiguous vertebrae in two cases and between two vertebrae in one other case. Even 10 months after the operation the ankylosed regions in no case showed any sign of bending, for the grafts themselves had grown. Although the authors had just demonstrated that the centra (corpora vertebrae) grow at equal rates at their two ends by ossification of the conjugation cartilages, they nevertheless concluded that the grafts had grown interstitially. But Lacroix (1946*a*) has suggested that the grafts underwent a series of profound histological changes, which do not occur in normal growth, involving decalcification followed by redeposition of the bone, this time at new surfaces.

Boerema (1942), who was using dense layers of bone produced by seasonal drugging with phosphorus compounds to show up the pattern of growth of the human skeleton, concluded that interstitial growth of the vault bones of the skull was not excluded, since these bones, in contrast to those of the limbs and girdles, showed no osteosclerotic lines. However, if their growth were superficial, there would have to be so much bone erosion and replacement that it is most probable that the original osteosclerotic lines would not sufficiently survive the passage of years as to be visible in radiographs.

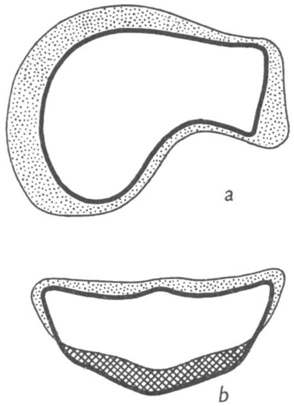
Lastly, as late as 1946, Leveuf used in evidence of interstitial growth failure of melanic exostoses (bony protuberances from the sides of the diaphyses) to become more distant from the growth (conjugation) cartilages. However, Lacroix considers this also inconclusive, because nowhere does Leveuf state that the exostoses are not remodelled, and that growth at the diseased end has not ceased. Either remodelling or cessation of growth at the diseased end could account for the situation in spite of superficial growth.

It is essential to make a clear distinction between interstitial *accretion* and *erosion* (erroneously believed to be directly involved in bone growth) and interstitial changes of *deposition* and *decalcification*. Both the latter are regular, but only accessory features of superficial bone growth, being necessary components of the histological changes from spiculate (endochondral) and from dense (periosteal) bone to the canalated (endosteal) bone of Haversian systems. While these processes confer on bone a certain plasticity of structure, the changes occur within the rigid framework of the outer layers of bone and cannot be directly involved in its growth. Interstitial decalcification results from osteolytic action of the cells contained within the spicules (Lacroix, 1942–3).

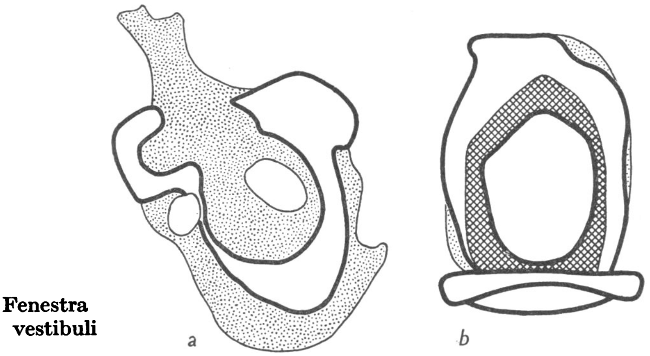
It can therefore be concluded that the present technique rests securely on the establishment of superficial accretion and erosion as general phenomena and on the banishment of interstitial growth to, at most, only rare expression.

(*g*) SOME BASIC PATTERNS OF BONE GROWTH

Although in bone growth we are concerned with the two opposed forces of accretion and erosion, they are, in fact, two intimately related and complementary processes, and the need is felt for substituting previous authors' static terms of 'site of accretion' and 'site of erosion' by a terminology which attempts to conjure up a picture of a co-ordinated system of movement in place of a picture of haphazard scattering of static sites of accretion and erosion. In the remaining part of this section eight theoretical interrelations of the sites of accretion and erosion are described, each of which has actually been observed in practice. Each is given



Text-fig. 2. (*a*) External accretion in the caput humeri. (*b*) External erosion in the os interparietale.



Text-fig. 3. (*a*) Internal accretion in the pars petrosa between birth and 3 days. (*b*) Internal erosion in the stapes.

Key. In figures 2–48 the growth processes differentiating older or normal bones (thin outlines) from younger or mutant bones (heavy outlines) are shown as follows:

Bone deposited by accretion ▒ Bone removed by erosion ▩

Bone which is first deposited and then eroded ▨

Bone substance common to old and young, or normal and mutant, is left unshaded.

Thus outlines of older or normal bones enclose unshaded and stippled areas; and outlines of younger or mutant bones enclose unshaded and cross-hatched areas.

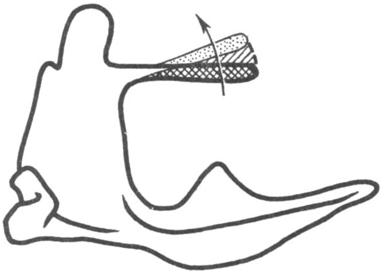
Arrows indicate directions of growth revealed by the mutants, and are lines of reference in superimposing the outlines of normal on mutant bones.

Only those processes visible in optical section are shown.

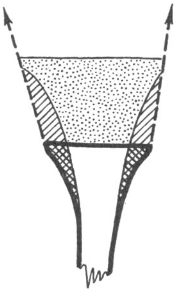
a descriptive name so that it may be remembered the more easily, as it will be referred to many times in subsequent sections.

First let us consider those movements which result from only one growth process. Accretion and erosion, the two processes concerned in bone growth, can each have two situations in relation to the bone (viz. on the external and internal surfaces). Hence there are four basic movements in growth. These are:

(i) *External accretion* (Text-fig. 2*a*). External accretion is characteristic of the head of the humerus (caput humeri). New bone is deposited at different rates over the surface of the existing bone. Alone, it suffices to maintain and to develop the bone's shape.



Text-fig. 4. Unilateral growth of the manubrium mallei.



Text-fig. 5. Diaphyseal growth.

(ii) *External erosion.* External erosion does not in itself result in growth and is probably always associated with some accretion. In the interparietale (Text-fig. 2*b*) it is the dominant process, so that the bone actually becomes increasingly shorter after 8 days from birth.

(iii) *Internal accretion* (Text-fig. 3*a*). In internal accretion, bone is deposited on the inner surface of the hollow bone, causing closure of the cavity, canal or foramen enclosed by the bone. It is of transitory nature. The fenestra vestibuli of the perioticum is an example of it, having at birth three times its final diameter (the perioticum grows also by external accretion).

(iv) *Internal erosion.* Intraosseous foramina are enlarged by internal erosion. Text-fig. 3*b* shows how the foramen stapedis is enlarged by internal erosion.

These four basic patterns may be combined in pairs to form four other known growth patterns of varying complexity, occurring sufficiently frequently to be described.

(v) *Unilateral growth.* In unilateral growth the first and second patterns are combined such that accretion is restricted to one side of the bone with erosion of the opposite and oldest side of the bone (Text-fig. 4).

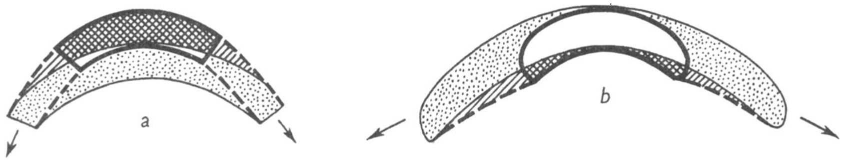
In this way the manubrium mallei is bent away from the processus longus. In this case, unilateral growth is inferred from the difference between normal and mutant mice in the angle between the two processes. The thickened process theoretically expected to arise in the mutants with this growth pattern has not been formed. This additional anomaly—complete failure of accretion—is special justification for regarding this pattern sometimes as a unit process, and not as a combination of two more or less independent activities.

On the other hand, the two processes (external accretion and external erosion) are independent of one another in the processus zygomaticus of the maxilla. Here, in the mutants, failure of erosion on the medial side does not totally inhibit accretion to the lateral side and the process becomes exceptionally thick (Pl. 13).

Reversed unilateral growth. When a foramen migrates it is said to do so by the 'reversed unilateral' pattern of growth because *internal erosion* leads the way and *internal accretion* follows behind—a double reversal of the normal 'unilateral' process in which the patterns are external and in which the sites of accretion and erosion are interchanged.

(vi) *Diaphyseal pattern.* Both accretion and erosion are external as in unilateral growth, but, unlike it, the erosional component tends to act at right angles to, and not opposite, the site of accretion; so that the newest, and not the oldest bone, is eroded (Text-fig. 5). The pattern is typical of the rapidly growing ends of all long bones.

The last two patterns to be considered are concerned with the growth of curved bones which, unlike the stapes, are not complete rings or cylinders; and they are alternative to the joint activities of external accretion with internal erosion already described for the stapes. In both, growth is primarily by peripheral accretion, but they differ radically in their secondary, remodelling processes.



Text-fig. 6. (*a*) Centripetal growth: ultra-diagram of an arcus vertebrae.
(*b*) Centripetal growth: ultra-diagram of the os parietale.

(vii) *Centripetal growth.* In many arcus vertebrae (neural arches), before their fusion with the centra and with each other, the remodelling process takes the form of accretion to the internal, concave surface, while erosion is external. The name centripetal growth has been thought appropriate to this pattern, since there is a steepening gradient in the extent of remodelling from the ends of the arch to its centre (Text-fig. 6*a*). (Although an arched bone has been taken as our example, the pattern is equally applicable to a bone which forms part of the shell of a sphere, in which case Text-fig. 6*a* represents a diametrical section across the bone.)

(viii) *Centrifugal growth.* In this case the secondary remodelling processes are most intense peripherally and migrate outwards as the bone grows. Also in contrast to the last pattern, accretion is external while erosion is internal (Text-fig. 6*b*). Centrifugal growth plays an important part in the growth of the parietale and frontale.

It should be made quite clear that these eight patterns are not hard and fast classifications of types of bone growth, but merely classifications of convenience, helping to make the description of growth vivid. The choice of pattern to describe a process is often arbitrary. For example: enlargement of the incisura lacrimalis of the maxilla (p. 237) can be regarded as resulting:

(1) from 'internal erosion' (of the borders of the incisura),

(2) from 'unilateral erosion' (when the site of erosion is regarded as on the posterior

wall of the lamina infraorbitalis and account is taken of the accretion to its anterior margin), or

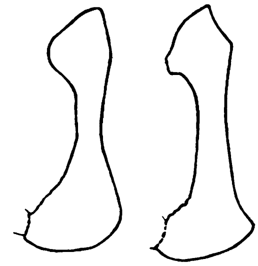
(3) from 'diaphyseal erosion' (when account is taken of accretion to the upper border of this lamina).

Several similar instances occur when the bones are similarly complex in shape, but the author does not consider that these situations will cause confusion.

There are of course many other combinations and interactions, but it is felt that these are too complicated and too rare to be usefully described here.

(*h*) THE METHOD OF ANALYSIS

The method of interpreting the anomalies of the mutant mice in terms of accretion and erosion consists first in recognizing in these anomalies the characteristic malformations resulting from any of the above basic patterns of growth. In some instances those discoveries may serve to display the direction in which particular features of the bone are growing, and if there are sufficient of such lines of reference, the outlines of the mutant and normal bones may then be superimposed so that all growing points coincide along the same lines. These lines of reference are indicated in the text-figures by arrows. At other times, it is only possible to arrange the superimposition so that none of the known features of the bone's growth is excluded. At all times the superimposition is only regarded as correct when there is total agreement as to the sites of accretion and erosion whichever mutant (grey-lethal or microphthalmic) is used in the analysis. It is noteworthy that substantial differences in the form of the two mutant bones such as occur for the pubis (Text-fig. 7) in no way affect the conclusion as to the manner of *normal* bone growth. This implies that variations in the form of identical bones occur within the same general pattern of growth and are not due to differences in the patterns themselves. (The causes for these variations are treated more fully in the Discussion.)



Text-fig. 7. Outlines of the pubic bones of a microphthalmic mouse (left) and of a grey-lethal (right). These substantial differences in shape do not affect the results of analysis as they occur within the same and normal pattern of growth. They are due to differences in the time and rate of ossification.

When the superimposition is made, and for as many angles of viewing as seems desirable, all the growth processes become detectable, and these are then described in terms of the patterns of growth detailed above.

Only in one instance has there been an unforeseen and serious but unavoidable discrepancy in the results of the analysis based first on the grey-lethal bone and then on the microphthalmic; but in this case, independent evidence was later obtained which demonstrated that a change of growth pattern did occur between 10 and 14 days, which are the developmental ages of the two different mutant animals used in the analysis.

GROWTH OF MOUSE BONES

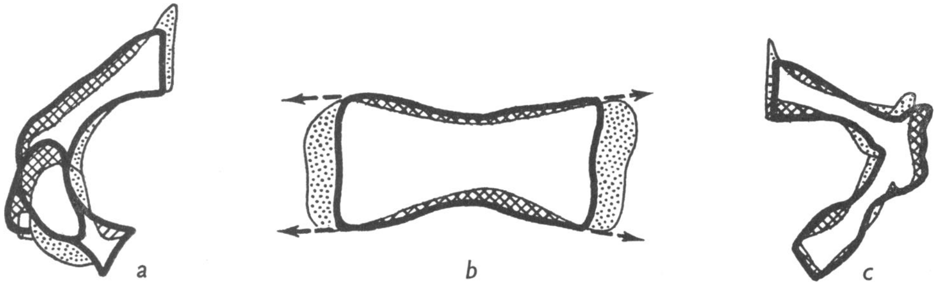
(*a*) Vertebral column (Pls. 1–5)

During their early development the vertebrae change from three-piece to unit construction—through the fusion of the two arcus vertebrae with the corpus. The transition occurs between 1 day after birth (for the caudal vertebrae) and 14 days (for the cervical vertebrae), so that each vertebra presents a compound picture of two entirely different and successive patterns of growth which cannot be distinguished in any individual case. But thanks to their repetitive nature of construction and a postulated repetition in their manner of growth, it is possible if not entirely legitimate to sort out the information gathered throughout the vertebral column to reveal the two patterns of growth. The following account is exemplified only by those vertebrae which were drawn, they being chosen to demonstrate the variation in vertebral form. (The names of bones in brackets give the origin of the evidence for the statement which precedes it.)

The early phase of growth

(i) *Arcus vertebrae* (Text-fig. 8*a*, *c*)

During the early period of growth the arcus vertebrae grows 'centripetally' usually with 'diaphyseal' growth at the initially wide synchondroses at both the interneural and neuro-central sutures (atlas, epistropheus (axis), sixth cervical, and second and seventh thoracic for the former; and second and seventh thoracic for the latter). While the direction of growth of these 'diaphyseal' components varies considerably between vertebrae (contrast the epistropheus and second thoracic), it is general for this component to contribute more to the breadth than to the height of the bone; a tendency which is corrected by the 'unilateral' component of the 'centripetal' pattern.



Text-fig. 8. Centripetal growth of the vertebral arches of the epistropheus (left) and seventh thoracic vertebrae (right). The middle figure shows diaphyseal growth of the vertebral body of a caudal vertebra.

The failure of this 'unilateral' accretion results in excessive breadth of the foramen vertebrale in the mutant atlas, epistropheus, seventh thoracic and lumbar vertebrae. And the failure of the corresponding 'unilateral erosion' is seen in the excessive breadth of the arcus in the epistropheus and second and seventh thoracic vertebrae.

The foramina transversaria move inwards by 'reversed unilateral' growth. The processus transversi grow at their bases by *erosion* of the surrounding region of the arcus with less rapid *erosion* of their tips. The failure of the latter causes the measurement across the vertebrae between the tips of the processus sometimes to excel those for corresponding normals.

The processus spinosi ossify late. That of the second thoracic vertebra* does not begin to ossify until the second phase of vertebral growth. On the other hand the spine of the epistropheus begins to form while the bony arcūs are still separated by cartilage. The latter spine develops by dorsal extension of the terminal growth zone (Lacroix's 'ossification ring') and, at this stage, there is 'diaphyseal erosion' of the more lateral parts of its base.

The method of analytical superimposition cannot be applied to the study of the growth of the arcus in the antero-posterior axis, for slight differences in the angles from which the bones are viewed can cause large differences in the outline drawings. The observations on the growth are therefore restricted to those limited portions of the bones' circumference which can supply independent information.

Anterior accretion occurs in the atlas in the region of the foramen vertebrale laterale. This foramen is formed by the flow of bone around it. In 7-day-old mice it is still open; and in the much retarded grey-lethal closure has just taken place and the foramen is very near the anterior border. But in the less retarded microphthalmic, failure of 'reversed unilateral growth' by which it normally migrates forward, has left it too far from the anterior edge.

In a similar way, the foramen transversarium of the epistropheus indicates that there is posterior accretion in this region. The posterior border is also the more important site of accretion in the region of the foramen alare ventrale of the atlas. This foramen is formed early in the ossification of the atlas, and is a mere pinprick in the mutants too far from the posterior border.

The other vertebrae afford no direct clues as to the surfaces to which accretion occurs. However, since the corpora (*vide infra*) grow at both ends, it is improbable that the arcus vertebrae differ widely in this respect.

(ii) *The corpus* (Text-fig. 8*b*)

The corpora of the more posterior caudal vertebrae and of the second, third and fourth sacral vertebrae grow in the typical 'diaphyseal' manner at about equal rates at each end. 'Diaphyseal' erosion, however, is limited to the sides and ventral surfaces. In all other vertebrae the waist of the corpus is obliterated by the replacement of 'diaphyseal erosion' by 'external accretion'—thus maintaining their typically rectangular shape.

(iii) *The arcus haemales* (*chevron bones*)

The ragged appearance inside the V of the mutant arcus haemales is certain indication that this is a site of erosion. In consequence there must be accretion to the outside of the V. It is possible that the arcus haemales also grow by terminal accretion to the dorsal ends of their limbs.

The second phase of vertebral growth

The processes so far considered in relation to the diametric growth of the vertebrae depend on their separation, apart from the arcus haemales, each into

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* This spine is absent in the grey-lethal mouse figured in Pl. 2. Grüneberg (1950) has noticed that this spine is absent in about half the animals in the stock.

three more or less independently growing pieces—the two arcus vertebrae and the single centrum. When fusion occurs between these pieces, growth is by no means completed, and profound changes in the patterns of growth are induced. The arcus now grows upwards and outwards by 'external accretion' while 'internal erosion' (caudal vertebrae and atlas) enlarges the foramen vertebrale—a kind of 'unilateral growth' on a curved surface. The processus articulares and transversi also grow upwards but by a 'unilateral' component behind the 'diaphyseal' growth by which they now increase in length. The spine of the epistropheus no longer grows by an extension of the 'diaphyseal' growth of the arches but merely by 'external accretion' to its tip. It is not until the second phase that the processus spinosus of the second thoracic vertebrae develops, and here, too, it is by 'external accretion'. The corpora continue to grow in length by 'diaphyseal' growth, and their neural surfaces are still not eroded. Thus their growth does not contribute to increasing the diameter of the foramen vertebrale.

(*b*) THE STERNUM

The sternum has three regions, together composed of seven segments (Pl. 5). The most anterior region is represented by the manubrium sterni which articulates with the clavicula and the first cartilago costalis. Then follows the corpus, constituted by a series of four small sternebrae which are more or less alike in their form and growth. The remaining costal cartilages articulate with them. The processus xiphoideus comprises the last region; it supports the cartilago xiphoidea.

(i) *The manubrium sterni* (Text-fig. 9*a*)

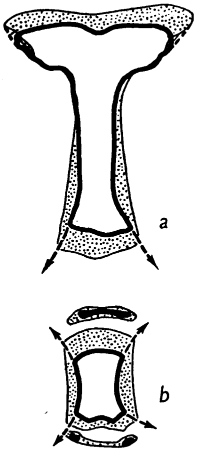
The manubrium sterni of the mouse has a distinct 'capitulum' (N.B.) and an elongated 'corpus' (N.B.). The capitulum articulates both with the claviculae and with the first cartilagines costales; the corpus carries a median longitudinal ventral crest. Growth is 'diaphyseal' anteriorly and posteriorly; 'diaphyseal erosion' of the ventro-lateral side of the corpus shapes the crest.

The grey-lethal mice do not exhibit the lack of 'diaphyseal erosion' in the capitulum, because at their stage of development (10 days) they have only just completed the exceedingly rapid ossification of the perfect cartilaginous model of the capitulum whose onset is deferred until the eighth day after birth. Erosional anomalies are, however, visible in the microphthalmic mice.

(ii) *The second to fifth sternebrae*

Growth of these sternebrae (Text-fig. 9*b*) is similar to that of the presacral corpora vertebrae—by 'external accretion' from an ever widening conjugation cartilage, while circumferential 'external accretion' fills out the waist into rectangular form.

The paired origin of the sternebrae is reflected in the paired ossification centres which form in the epiphyses around the eighth day. For the moment, at least, epiphyseal growth is by 'external accretion' alone.



Text-fig. 9. (*a*) The manner of growth of the manubrium sterni, and (*b*) of a small sternebra.

(iii) *The processus xiphoideus*

From the broad, anterior, ossified portion of the processus at birth, ossification spreads posteriorly by 'external accretion' over an ever decreasing surface up to the sixth or seventh day. But subsequently, the posterior extremity widens and 'diaphyseal growth' with erosion especially of the dorsal surface becomes apparent.

(*c*) THE RIBS (COSTAE) (Pl. 5)

The growth of the seventh rib is described first as being the most typical; those of the first and twelfth follow as showing the extent of variation in growth. (The thirteenth rib in these stocks is uncommon, and often only unilaterally represented or asymmetrically foreshortened.)

(i) *The seventh rib* (Text-fig. 10*a*)

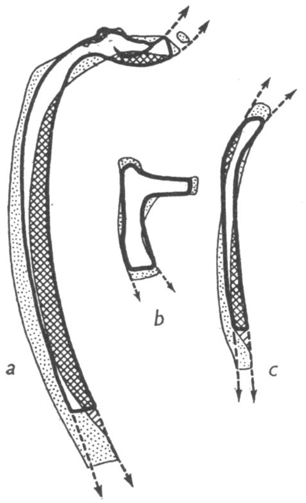
Distal growth is about seven times as important as proximal growth. The original curvature of the bone, as seen in the mutants, is due to graded rates of terminal accretion between the lateral and medial surfaces but is highly modified by three zones of 'unilateral' movement. Of these, two (one for the collum; the other extending practically throughout the whole length of the corpus as far as the distal 'ossification ring') are directed laterally, while the third (between the other two) is directed medially. Together, these three 'unilateral processes' serve to accentuate the curvature of the rib and so to enlarge the thorax.

(ii) *The first rib* (Text-fig. 10*b*)

The terminal rates of growth are probably much more equal than for the seventh rib; and while erosion plays an insignificant role, the accretional components of the 'unilateral' movements persist.

(iii) *The twelfth rib* (Text-fig. 10*c*)

In spite of its very different shape, the twelfth rib grows in the same manner as the seventh. Analysis of this rib's antero-posterior growth was made possible by the slightness of its lateral curvature which allowed it to be held in the glycerol bath for viewing from the medial aspect. Analysis indicated expansion of the middle of the corpus by accretion to both edges—a feature probably characteristic of rib growth.



Text-fig. 10. The growth of the seventh (*a*), first (*b*) and twelfth ribs (*c*). Note the importance of the unilateral pattern.

(*d*) THE SKULL (Pls. 6–13)

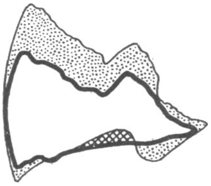
Although the skull is a compound bone, so that the growth processes of the individual bones must be neatly interrelated, it is unnecessary to give any general account of its growth.

It may, however, be remarked that unlike the vertebrae, sternebrae and ribs, no two bones of the skull (unless paired left and right) grow in a similar manner. When of complicated form, e.g. maxilla, the concept of a 'punctum fixum' is invalidated unless it is extended to encompass a position somewhere in the spaces between the bone's several processes and laminae. The squama occipitalis, sphenoidale orale and lacrimale may be cited for exemplifying the dependence of some bones on the normal growth of their neighbours for their own normal growth. Lastly, the occipitale and tympanicum may be cited for displaying similar age changes in their growth patterns to those encountered in the vertebrae.

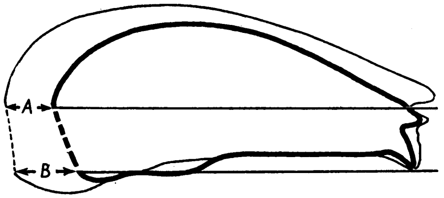
(i) *Occipitale: pars basilaris* (Pl. 7)

'External accretion' occurs at all borders but especially at the synchondroses with the partes laterales and with the sphenoidale aborale. Erosion occurs only on the inferior surface, where, on either side of the mid-line, just in front of the posterior margin, it brings out into relief part of the tuberculum pharyngeum.

Pars lateralis (Pls. 7, 8 and 9). There is 'external accretion' at the synchondrosis with the pars basilaris and along the margin of the foramen magnum. In pace with this growth, the canalis hypoglossi migrates medially by 'reversed unilateral growth'. The material is unsuited for further analysis.



Text-fig. 11. Growth of the squama occipitalis.



Text-fig. 12. The foramen magnum. Differential rates of growth of the floor and roof of the skull cause the foramen magnum to assume a more upright position.

Squama occipitalis (Pls. 6 and 8 and Text-fig. 11). In both its horizontal and vertical planes growth is 'centrifugal'. Failure of the proper co-ordination of the growth processes of adjacent bones in the mutants results in the corrugation of the anterior growing region by the strains exerted on it by the backwardly growing squamosum; and the overriding of the squama by the interparietale owing to erosional failure in the latter.

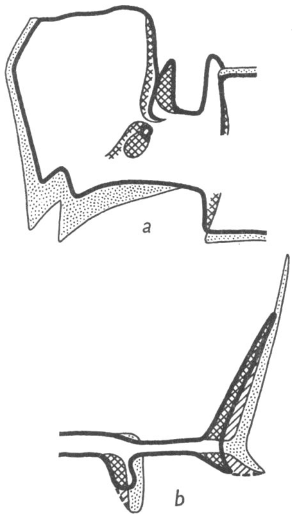
Foramen magnum. The growth processes intrinsic to the occipital bones do not solely account for the gradual assumption of a more vertical position by the foramen magnum. Part of this new orientation is due to the different rates of growth which affect the roofing bones as a whole compared with the basal bones as a whole (Text-fig. 12).

The squama occipitalis fuses with the partes laterales at about 17 days, and the occipitale becomes a single bone at about 20 days. While the present material is too young to demonstrate the new growth pattern there is little doubt that 'centrifugal' growth of the upper margin of the squama contributes most to the height of the bone, while 'centrifugal' growth in the horizontal plane, and 'external accretion' at the synchondrosis spheno-occipitalis, contribute to its growth in length.

(ii) *Sphenoidale aborale* (Pls. 7–9 and Text-fig. 13*a*, *b*)

The corpus grows at probably equal rates backwards and forwards by 'diaphyseal' growth in which the erosional component is strictly limited. The alae temporales grow outwards and upwards by 'external accretion' to their lateral and posterior margins, while their roots are more widely separated by a graded 'unilateral' movement which falls away towards the upper growing margins. Similarly, the ossa pterygoidea grow obliquely downwards and outwards by ventral 'external accretion', while their roots migrate 'unilaterally' so as to maintain the vertical position of each bone while broadening the soft palate. The processus pterygoidei move apart by laterally directed 'unilateral growth' which also enlarges the foramen orbitorotundum. The foramen ovale and canalis alaris grow outwards and backwards by 'internal erosion' and enlarge very considerably as they do so.

In adult mice the corpus thickens enormously to contain sinuses, and the canalis pterygoideus is developed. They are absent in 3-week-old mice.



Text-fig. 13. Growth of the sphenoidale aborale; (*a*), cerebral aspect; (*b*), vertical section.

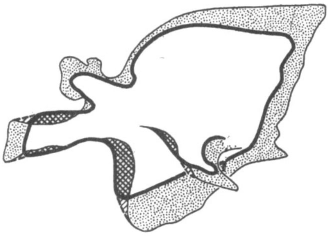
Text-fig. 14. Growth of the sphenoidale orale; cerebral aspect.

(iii) *Sphenoidale orale* (Pls. 7–9 and Text-fig. 14)

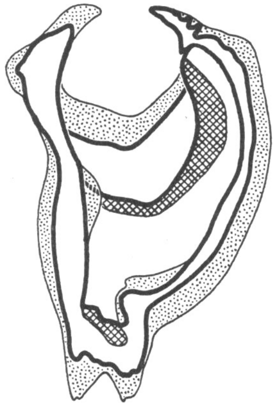
As mentioned when describing the changing orientation of the foramen magnum, the skull is not symmetrical in its growth processes. Similar asymmetry in the rates of growth of the bones in the base of the skull behind and in front of the sphenoidale orale cause this bone to leave the position it occupies at birth—the globular part of the cranium—and to move forward into the narrow part of the cranium. So that, besides growth processes which lead merely to the bones' enlargement, there are others which alter the bones' shape to suit the new environment.

The corpus is lengthened by 'diaphyseal growth' at each end. Erosion of the sides of the back half of the corpus enlarges the foramen orbitorotundum, while in front it maintains the shape of the foramen opticum. 'Diaphyseal erosion' of the upper surface of the corpus, anteriorly, helps to flatten the bone out from the curved infantile condition.

At birth, the alae orbitales consist each of two rami which grow out in the 'diaphyseal' pattern from the corpus. Erosion of the sides bordering the foramen opticum results in the latter's enlargement. Normally at the third day of post-natal development the rami fuse laterally to enclose the foramen opticum. Thenceforward, alar growth is by 'external accretion' and 'internal erosion'. These processes continue right down the roots of the alae so that the roots continue to separate. In changing to their new position the anterior and posterior rami are brought to the same level by 'unilateral' growth in the vertical plane; while the inter-alar angle is reduced by graded rates of 'unilateral' growth which increase from their medial to their lateral margins. Owing to the first of these trends the foramen opticum comes to occupy a more horizontal plane which necessitates heavy erosion of all its margins so that the nervus opticus may continue to pass freely out of the skull.



Text-fig. 15. Growth of the squamosum; lateral aspect.



Text-fig. 16. Growth of the tympanicum; superior aspect.

A quite frequent feature of the mutants is that they have open optic foramina, in which respect they are comparable to 2-day-old normal mice. This condition may be due to inhibition of accretion owing to failure to enlarge the incisura sphenoidalis of the frontale, again suggesting the interdependence of bones for their normal growth.

(iv) *Squamosum* (Pls. 6–9 and Text-fig. 15)

The squamosum grows by 'external accretion' over almost all its margins, but particularly along the antero-inferior border. However, the processus postglenoidalis grows by 'diaphyseal growth' and the lower posterior border of the squamosum is heavily eroded for enlarging the fissura squamotympanica.*

Erosion of the cerebral surface is confined peripherally and is associated with the extension of sutural overlaps with the parietale, frontale, ala temporalis and

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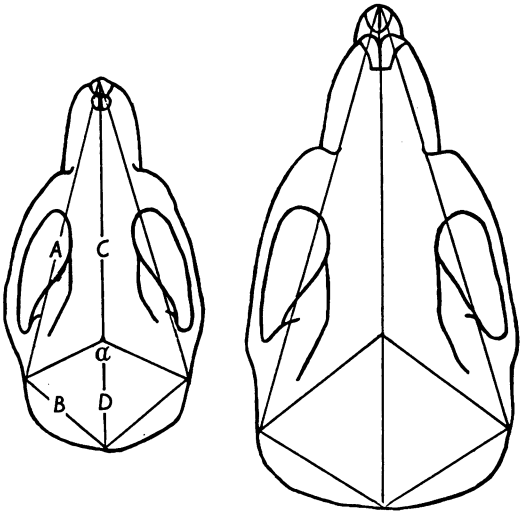
* Greene's (1935) 'postglenoid foramen'.

also with the 'diaphyseal growth' of both the processus postglenoidalis and processus caudalis.

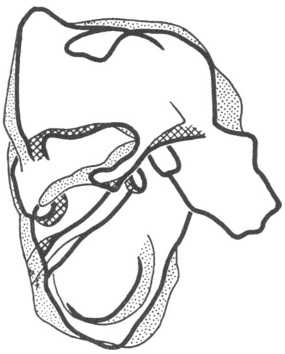
Although passively maintaining its level on the squamosum the processus zygomaticus is carried up the side of the skull, partly by the relatively greater accretion along the inferior border of the squamosum, and also by 'external accretion' to the alae temporales of the sphenoidale aborale. The forward migration of the processus zygomaticus, however, is produced by its own 'unilateral growth'.

(v) *Tympanicum* (Pls. 7–9 and Text-fig. 16)

The bulla tympanica bulges ventrally, posteriorly and ventro-medially by 'external accretion' and 'internal erosion' which are particularly active in these directions. The tuba auditiva ossea (Eustachii) grows medially by 'external accretion'.



Text-fig. 17. Diagram showing how rotation of the bullae tympanicae (i.e. reduction of the angle α) is brought about by differential rates of growth fore and aft, both laterally and medially (i.e. of A/B compared with C/D).



Text-fig. 18. Growth of the perioticum; inferior aspect.

On the seventh and tenth days after birth ossification spreads by 'internal accretion' from the posterior and anterior corners of the annulus tympanicus respectively into the porus acusticus externus. The porus of the retarded mutants is therefore exceptionally wide and has rough margins due to the infiltration of the blastemic tympanicum by bone spicules. When its ossification is completed the porus grows by 'internal erosion'.

The bulla is rotated in the course of its development, not from intrinsic processes, but by differential rates of growth between the mid-lines and the sides of the floor of the skull, in front of and behind the bulla (Text-fig. 17).

(vi) *Perioticum* (Pls. 7, 8 and 11 and Text-fig. 18)

'External accretion' occurs over almost all the visible surfaces of the perioticum. But as it is a hollow bone of great complexity, especially within the cochlea, 'internal erosion' must play a highly important part. Erosion is visible externally also; the base of the processus stylomastoideus moves posteriorly by 'unilateral growth', while its tip grows rapidly after delayed ossification by 'external accretion' within the perfectly modelled cartilage. The lateral margin of the foramen stylomastoideum is subject to 'internal erosion', and the posterior end of the sulcus stapedius is subject to superficial 'unilateral growth', the tegmen tympani grows downwards by 'unilateral growth', and the fossa parafloccularis is enlarged by 'internal erosion'. The fenestrae cochleae and vestibuli are first formed in bone by the flow of ossification around them followed by 'internal accretion', but this temporary phase is already over by 10 days, after which they are enlarged by the opposite process of 'internal erosion'. The very variable shape, but not necessarily the size, of the fenestra flocculi (N.B.) is determined by the extent of deficient initial ossification, which is only just completed in this region at 10 days.

(vii) *Malleus* (Pls. 8 and 10 and Text-fig. 19)

The mouse malleus is an extraordinarily large bone in which, relative to man, the size of the collum and processus longus (anterior) are enormously exaggerated. At 21 days the processus longus is firmly ankylosed with the tympanicum.

The growth of the malleus is characterized by a number of local torsions of one part of the bone relative to another. 'Unilateral growth' results in the twisting of the manubrium away from the processus longus in both ventral and lateral directions, and in the lateral twisting of the ventro-posterior part of the collum relative to its dorsal part. Medial accretion to the capitulum with medial erosion of the root of the processus longus (whereby it is converted into the collum) result in a fourth torsion. 'Diaphyseal growth' is involved in the anterior extension of the processus longus, in the antero-posterior broadening of the collum, and in the ventral growth of the processus muscularis.

(viii) *Incus* (Pl. 10 and Text-fig. 20)

The corpus grows by 'external accretion' and by the incorporation of the roots of the crura longum and breve. The crus longum grows ventrally by 'diaphyseal growth' with a mild, anteriorly directed 'unilateral' migration of its middle region. The lenticulare and its supporting processus lenticularis maintain their position at right angles to the tip of the crus longum by downwardly directed 'unilateral growth'. The crus breve grows obliquely downwards and posteriorly by 'external accretion', but the crest on its lateral side is bent into an S-shape by 'unilateral' movements in opposed directions at its two ends.

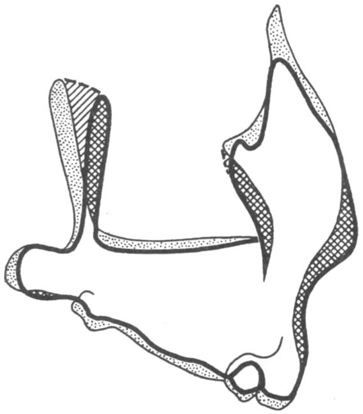
(ix) *Stapes* (Pl. 10 and Text-fig. 21)

The stapes grows by 'external accretion' at more or less equal rates around its periphery; and 'internal erosion' of the foramen stapedis (N.B.) which maintains the thin crescent-like section of the crura and basis. The oddity of the shape of the mutant bone follows entirely from the gradual spread of ossification without erosion from the basis to the capitulum.

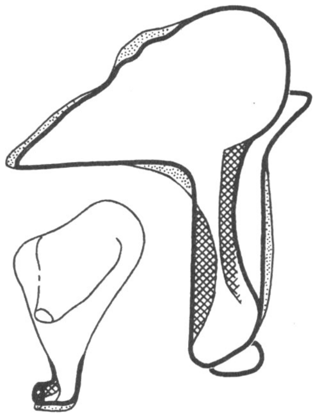
(x) *Interparietale* (Pls. 6 and 8 and Text-fig. 2*b*)

Growth of the interparietale in the transverse axis of the skull is 'centrifugal'. There is also 'external accretion' at its anterior margin and 'external erosion' at its posterior margin. In the mutants, failure of erosion may cause it to override the squama occipitalis at its ventro-posterior corners.

A remarkable feature of this bone's development is the inversion of the usual relationship between the rates of accretion and erosion at opposite margins, so that the interparietale may be larger in the long axis of the skull at 8 days than it is when adult. In this bone, therefore, 'external *erosion*' proceeds more rapidly than 'external *accretion*'. Table 1 shows the relative dimensions of the interparietale and the skull in two normal mice, one of them 3 weeks old, the other adult. The measurement in the younger mouse is taken as the unit for the corresponding measurement in the older. It is seen that the changes in the relative sizes of the skull and the interparietale are brought about more by the diminution of the interparietale than by the excess growth of the skull.



Text-fig. 19.



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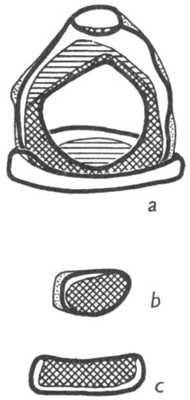


Text-fig. 21.

Text-fig. 19. Growth of the malleus; medial aspect.

Text-fig. 20. Growth of the incus; lateral aspect. Inset, growth of the processus lenticularis; posterior aspect.

Text-fig. 21. Growth of the stapes, (*a*) medial aspect, (*b*) cross-section of the crus anterium, (*c*) cross-section of the basis. (Horizontal shading indicates deep erosion of the surface seen in optical section.)

Table 1. *Relative lengths in older and younger normal mice of the skull and interparietale*

	Length of	
	Skull	Interparietale
Normal: 3 weeks	1	1
Normal: adult	1·21	0·69

(xi) *Parietale* (Pls. 6, 8 and 12 and Text-fig. 22)

The parietale grows entirely by the 'centrifugal pattern'. In so doing it gradually changes from the highly convex bone which extends well down the sides of the young animal's skull into the older animal's much flatter bone which is confined almost entirely to the roof of the skull. According to the balance at its periphery between the opposing processes of accretion and erosion within this pattern so the bone changes its outline. At its periphery erosion predominates:

anteriorly on the medial side, so as to make way for the backward extension of the frontale,

on the whole of the lateral side to make way for the upward growth of the squamosum,

and posteriorly near the middle, in relation to remodelling of the sutura lambdoidea.

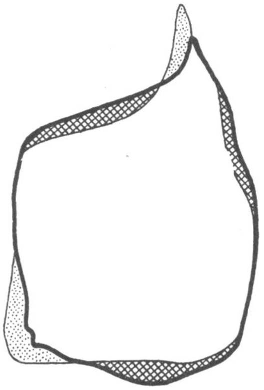
On the other hand, accretion is dominant:

posteriorly, at the medial corner, and

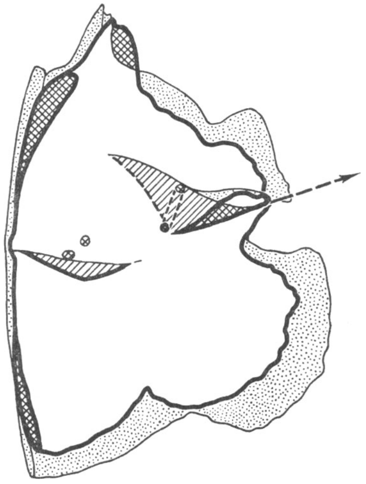
anteriorly, on the lateral side of the processus frontalis (N.B.).

There is no medial growth at the suture with the other parietal bones.

In the mutants, inhibition of this growth pattern sets up such stresses that mutant skulls burst with high frequency in the parietal region during their preparation.



Text-fig. 22. Growth of the parietale; internal or external aspect.



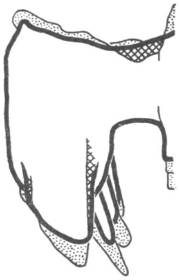
Text-fig. 23. Growth of the frontale; interna aspect.

(xii) *Frontale* (Pls. 6–8 and 12 and Text-fig. 23)

The pars nasofrontalis, or horizontal lamina of the frontale, is lengthened by ‘centrifugal growth’, i.e. by peripheral accretion to the external surface of its anterior and posterior margins, with peripheral erosion of the cerebral surface in these regions. Along the sutura coronalis the rate of backward accretion increases towards the mid-line, to some extent compensating for the erosion of the parietale in this region. As part of this ‘centrifugal pattern’ the arcus superciliaris is raised and sharpened by accretion to the outer, dorsal surface with compensating erosion of the cerebral surface. The processus zygomaticus grows forwards, sideways and upwards and bears the arcus zygomaticus with it away from the side of the cranium. This growth is associated with ‘diaphyseal erosion’ of the posterior border of the processus zygomaticus whence the arcus superciliaris is modelled. There is no medial growth, but lateral accretion in the middle of the arcus superciliaris both widens the frontale and reduces the curvature of the orbit.

The pars orbitotemporalis (or vertical lamina) grows by 'external accretion' to all its borders with the exception of a little erosion of the anterior border of the incisura sphenoidalis; the latter otherwise being enlarged by the regression of ventral accretion away from its mouth. The crista ethmoidalis (N.B.) grows obliquely downwards and forwards, while 'diaphyseal erosion' scoops out the lower end of its anterior wall, and 'unilateral growth' whisks the upper 'tail' of the crest forwards. The foramina frontale (N.B.) and ethmoidale move forwards in the 'reversed unilateral pattern'; and migration of superficial localized sites of accretion and erosion, following accretion along the anterior margin, moulds the fossae frontales. Failure to enlarge the foramina frontale and ethmoidale may be partly responsible for the death of the mutants through constriction both of the veins supplying a transverse blood sinus of the brain, and of the nervus ethmoidalis.

Text-fig. 24. Growth of the interfrontale; lateral aspect.



Text-fig. 25. Growth of the ethmoidale; dorsal aspect.

(xiii) *Interfrontale* (Pls. 6 and 9 and Text-fig. 24)

The interfrontale is an ephemeral bone in the mouse though a constant feature of both the microphthalmic and grey-lethal stocks. According to Keeler (1933), on inadequate data, it is probably inherited as a single recessive. It consists of a stout caput (N.B.) articulating with the ethmoidale below, and a dorsoventrally flattened pars caudalis (N.B.).

In young mice the bone is flexed in accordance with the flexure of the skull roof in this region, and it becomes straightened out by upwardly directed 'unilateral growth' of the pars caudalis. The caput grows forwards in the 'diaphyseal pattern'.

(xiv) *Ethmoidale* (Pl. 12 and Text-fig. 25)

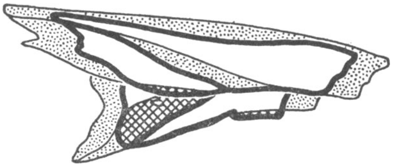
After fusion of the labyrinthi and lamina perpendicularis growth is in general by 'external accretion' and 'internal erosion', but with other small-scale processes maintaining the finer details of construction of the labyrinthi. Before fusion, the stalks of the labyrinthi grow towards the lamina pependicularis by 'diaphyseal growth'. More detailed description of the ethmoidale's growth is impossible owing to the great variation in its form. (The cellulae ethmoidales are opened by fragmentation on disarticulating the skull.)

(xv) *Lacrimale* (Pls. 6, 8 and 11)

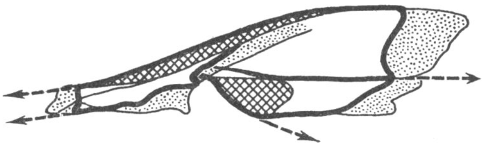
The capitulum (N.B.) grows upwards by 'diaphyseal accretion', and there is intensive 'diaphyseal erosion' of the anterior and ventral sides of the 'collum' (N.B.). The growth of the squama, however, cannot be described because of the infliction in the mutants of largely extrinsic deformities by its constriction within the unenlarged canalis infraorbitalis.

(xvi) *Nasale* (Pls. 6, 8 and 11 and Text-fig. 26)

The nasale grows in length by 'centripetal growth'; the 'unilateral' component in the middle of the bone leading to its progressive specialization as a roofing bone by converting it from a quartered cylinder (the shape determined by its terminal growth anteriorly) into a flatter and more horizontal sheet. This component also carries the sutural articulation for the incisivum (premaxilla) forwards so that it maintains its position relative to the growing tip.



Text-fig. 26. Growth of the nasale and concha dorsalis; lateral aspects.



Text-fig. 27. Growth of the vomer; lateral aspect.

(xvii) *Concha dorsalis* (Pl. 11 and Text-fig. 26)

By analogy with the nasale with which it ankyloses at 9 days, the concha dorsalis grows forwards by 'external accretion'. In addition, there is 'diaphyseal growth' posteriorly. The intensive 'diaphyseal erosion' of the lateral side transfigures the solid posterior block into the dainty longitudinal anterior lamella. The foramen perforating this lamella is carried backwards by 'reversed unilateral growth'.

(xviii) *Vomer* (Pl. 11 and Text-fig. 27)

The mouse vomer consists of a basis (N.B.) which rests on the processus palatini of the maxillae, and two large alae vomeris. These comprise (i) the alae septales (N.B.) which are situated immediately above the basis and lie vertically on either side of the cartilaginous septum nasi; and (ii) the horizontal alae ethmoidales (N.B.) which are the posterior extensions of the alae vomeris behind the basis. They cup the labyrinthi of the ethmoidale and fragment on their separation. Hence their ragged appearance.

The basis grows forwards and downwards by 'unilateral growth'. The alae septales grow forwards by 'external accretion', the alae ethmoidales backwards and downwards, also by 'external accretion'. Because the labyrinthi do not separate to any great extent, the angle between the elongating alae ethmoidales becomes progressively smaller. This is achieved by erosion of the medial sides of the roots of the alae, accompanied by lateral accretion. The backs of the alae septales are converted into the roots of the alae ethmoidales by 'unilateral' growth processes in the horizontal plane.

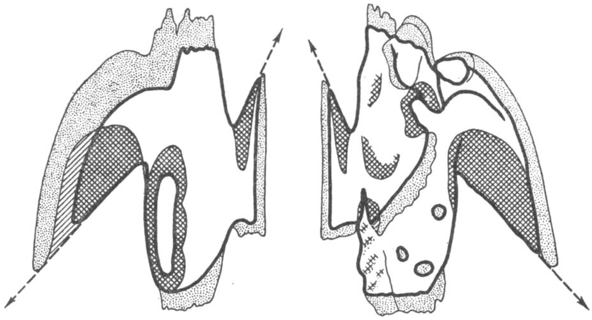
(xix) *Incisivum* (Premaxilla) (Pls. 6–8 and Text-fig. 28)

Posteriorly, the incisivum gains length and height by 'external accretion' to the processus nasalis and palatinus, and to the corpus. While anteriorly there is no increase in length, there is a further contribution to the height of the bone, such that the bone more than compensates for the removal of the nasale from the side of the skull (*vide supra*) and adds considerably to the height of the skull in this region. There is no medial growth.

The only complications visible externally are the backward migration by 'diaphyseal growth' of the crest demarcating the anterior and medial limit of the canalis infraorbitalis; the enlargement of the mouth of the alveolus incisivus superior by 'internal erosion' of its anterior, lateral and posterior, but not medial margins; and the backward extension of the corpus at the expense of the fissura palatina, by 'internal accretion'.

Of the processes affecting the interior of the bone, only those affecting the eruption of the incisor tooth are discussed.

Text-fig. 28. Growth of the incisivum; inferior aspect.



Text-fig. 29. Growth of the maxilla; left, inferior aspect; right, superior aspect.

Eruption of the dens incisivus. While in the mutant mice the incisor tooth is trapped in the corpus of the incisivum, in normal mice extremely extensive 'internal erosion' of the alveolus allows the root of the tooth to grow backwards, right out of the incisivum and into a special cup developed by the maxilla. This backward growth of the tooth is associated with a considerable thickening of the lateral wall of the incisivum by 'external accretion' to both its medial and lateral surfaces. Moreover, anteriorly 'internal erosion' enlarges the diameter of the alveolus by removing part of its lateral, posterior and anterior walls, thus enabling the tooth to erupt through the gums.

(xx) *Maxilla* (Pls. 6–8 and 13 and Text-fig. 29)

The main trends of growth in the maxilla are upwards and laterally; there is little medial growth and negligible accretion to the under side.

While the tip of the processus zygomaticus grows backwards, sideways and slightly upwards simply by 'external accretion', the more anterior regions of the processus shift sideways and also slightly upwards by 'unilateral growth'. It is noteworthy that some parts of the processus must be eroded and rebuilt many times before the bone doubles in thickness. The vertical flange below the most anterior parts of the processus zygomaticus is cut out of it by superficial 'external erosion' of the crista facialis. The flange forms the side wall of the canalis infraorbitalis, which is enlarged by the 'unilateral growth' of the flange beside it; and of the processus frontalis above it. There is no erosion of its ventral or medial walls.

The lamina orbitalis grows backwards and upwards and the lamina infraorbitalis grows forwards and upwards, both by 'external accretion' to their periphery. Between them, the incisura lacrimalis is formed by 'diaphyseal erosion' of their adjoining borders. Their basal portions are shifted sideways by 'unilateral growth' which also causes the expansion of the fossae maxillares (N.B.).

The delicate cup which houses the tip of the root of the upper incisor grows by accretion to all but its lateral surfaces while being deeply hollowed from in front by 'internal erosion'. Failure of the latter may account for the flatness of this portion of the alveolus in the mutants (cf. inhibitory action in the manubrium mallei (p. 220)).

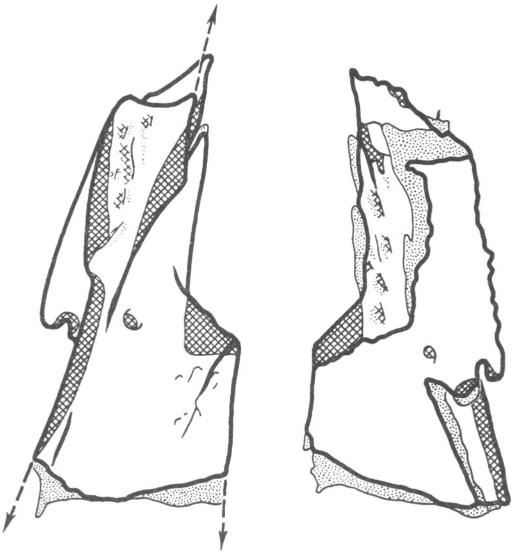
There is some medial accretion to the processus palatinus, while 'diaphyseal erosion' enlarges the fissura platina in both medial and posterior directions. The lateral border of the fissura, however, is not eroded, while enlargement of its anterior regions is cared for by the incisivum. Growth of the processus in length is by 'centripetal growth', the 'unilateral' component of which lowers the roof of the palate. The notch for the foramen palatinum majus is widened by 'internal erosion' of the medial border of the processus palatinus and of the base of the lamina orbitalis. 'Internal accretion' takes part in its backward migration.

On the limbus alveolaris, which grows at equal rates forwards and backwards, the 'rough' for articulation for the 'palatinum' is probably maintained by backwardly migrating alternating zones of superficial erosion and accretion. In the mutants, failure of erosion in the palatinum probably accounts for the mal-development of the crests by accretion to the opposite surface of the maxilla, and vice versa.

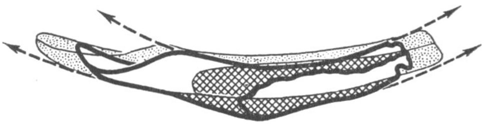
Eruption of the molar teeth. The molar teeth are at first largely passive in their eruption, which results from processes involving the maxillary bone about them. First, the foramina above the roots of the teeth are closed by 'internal accretion' thus completing the process of ossification of the maxilla. Then the incurved walls of the alveoli are eroded so that the teeth can be carried out of the crypt by accretion within and by the continued growth of their own roots. The roof of the crypt is lowered by 'external erosion', and with the passage of the bulky crowns from the body of the maxilla the limbus alveolaris becomes much shallower and narrowed down. These processes continue into old age so that in old mice the teeth are rooted in a very broad and shallow bed which encroaches on the palate to a quite considerable extent.

(xxi) *Palatinum* (Pls. 7 and 13 and Text-fig. 30)

The palatinum grows in length equally at each end. This growth is 'diaphyseal'. Anteriorly, 'diaphyseal erosion' cuts out the anterior border of the foramen palatinum majus and remodels both sides of the processus orbitalis; posteriorly it enlarges the foramen sphenopalatina by cutting down the processus sphenopalatinus, and shortens the lamina pteryopalatina. The meatus nasalis is widened by 'diaphyseal accretion' to the medial border of the pars horizontalis and by 'unilateral erosion' of the base of the pars perpendicularis; it is heightened by 'diaphyseal erosion' of the nasal surface of the pars horizontalis and by 'unilateral erosion' of the pars orbitalis. The pars horizontalis is also widened by accretion to its lateral border. The 'roughs' for articulation with the maxilla, sphenoidale orale and alae temporales are maintained by the migration of alternating zones of very superficial accretion and erosion. The foramen palatinum minus migrates obliquely backwards and laterally by 'reversed unilateral growth'.



Text-fig. 30. Growth of the palatinum; left, superior aspect; right, inferior aspect.



Text-fig. 31. Growth of the zygomaticum; lateral aspect.

(xxii) *Zygomaticum* (Pls. 6–8 and Text-fig. 31)

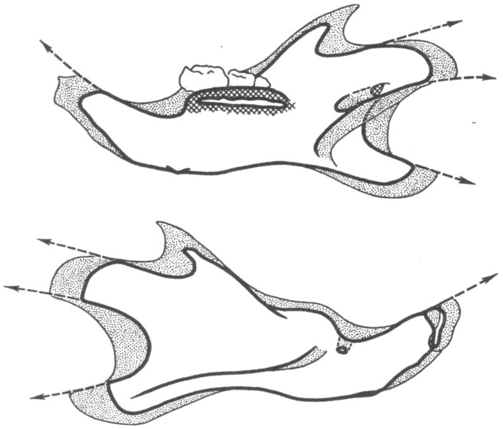
Growth of the zygomaticum is 'centripetal'. Accretion at its ends progresses at about equal rates; and the erosional component not only reduces the curvature of its inferior border, but also extends the surface for articulation with the maxilla at the expense of the anterior limit of the corpus. As this trend is coupled with posterior accretion to the corpus this appears to slide backwards in relation to the processus maxillaris. There is some medial erosion in this region of the processus temporalis. Accretion to the lateral surface is the chief cause for growth in thickness.

In the mutants, the anterior end of the processus maxillaris is strongly flexed medially, but there is no sign either in mutants or in normals of medial erosion in this region. It is therefore concluded that this is an extrinsic phenomenon imposed on the mutant zygomaticum by the anomalous growth of the processus zygomaticus of the maxilla with which it is here in intimate contact.

(*e*) THE LOWER JAW (Pl. 14 and Text-fig. 32)

In spite of the complexity of its shape, erosion enters surprisingly little into the growth of the mandibula. As seen from the side, its shape is almost entirely maintained by differential rates of peripheral accretion. Generally the rate of growth anteriorly is less than that posteriorly; and while almost the whole of the upper border is a site of upward growth, growth downwards is limited to the posterior end. Apart from the merest suspicion of erosion over a small section of the margo interalveolaris, the remaining borders are either accretional or 'indifferent'.

In the backward growth of the processus condyloideus there is 'diaphyseal erosion' of the lateral side of the collum above its mid-line, and on the whole of its medial surface. The foramen mandibulare is carried backwards by accretion to its 'trailing' edge though a little erosion of its leading edge is also required. By accretion to its ventro-posterior border the processus condyloideus incorporates the root of the processus angularis.

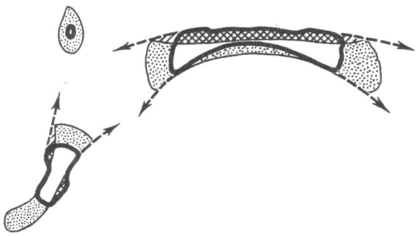


Text-fig. 32. Growth of the mandibula; medial aspect above and lateral aspect below.

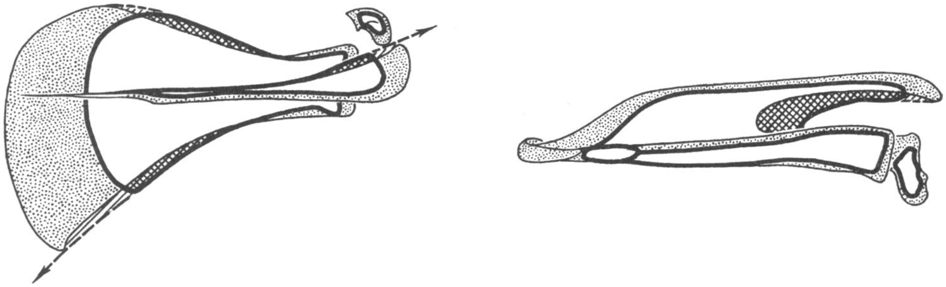
Erosion is not manifest elsewhere except in the enlargement of the alveoli of the teeth. However, for the molar alveoli it is only transient, for after the release of the teeth by removal of the incurved walls of the alveoli, the alveolar margins become sites of active accretion, while there is even more accretion to the floor of the alveoli. Thus the crowns of the teeth are pushed above the level of the jaw, a remarkable process which has been observed both by Kölliker (1873) in the ox and by Brash (1934) in the pig. At the same time the molar teeth with their alveoli slide slowly forwards and laterally by the latter's 'unilateral growth'. Perhaps no better account of eruption of the mandibular molars can be found than in Hoffman & Schour's study (1940*b*) in the rat. From their study it can be seen that the growth of the tooth root by accretion of both dentine and secondary cementum is also important in bringing about occlusal movement of the tooth throughout life. The potential increment to the 'clinical crown' of the tooth is, however, exactly balanced by attrition if occlusion is normal. In the alveolus incisivus inferior 'internal erosion' is very important; for by its activity the incisor tooth can extend from its confines in the corpus (its location at birth) right under the dentes molares, almost to the foramen mandibulare. In so doing, the tooth becomes very much wider and there is consequently much 'internal erosion' even at the mouth of the alveolus. So great is the pressure of tooth growth in the mutants that their incisor roots often force their way into the canalis mandibularis and thence out of the foramen mentale. Here they form a calcified misshapen lump (Grüneberg, 1935) round which the jaw grows, so leaving a 'crater' which is a frequent characteristic of both microphthalmic and grey-lethal mice.

The reader interested in the growth of the teeth themselves is referred to Hoffman & Schour (1940*b*) and Grüneberg (1937).

The lateral side of the jaw is the site for greatest contribution to growth in breadth, although, as the incisor root tunnels through the pars molaris there is medial accretion here too. The crista masseterica is moved forwards slightly by accretion to its more anterior slopes, while the foramen mentale changes the direction of its opening from sideways to upwards by lateral accretion directly below it.



Text-fig. 33. Growth of the hyoideum; inferior or superior aspect.



Text-fig. 34. Growth of the scapula.

(*f*) Hyoid (Pl. 11 and Text-fig. 33)

(i) The basis of the hyoideum grows in a slight dorso-ventral curve and with a much stronger antero-posterior curve by 'centripetal growth'.

(ii) Proximally, the cornu majus grows 'diaphyseally', while posteriorly it is at 3 weeks still infiltrating its cartilaginous model by 'external accretion'.

(iii) At about 10 days, ossification of the cartilaginous cornu minus begins; at 3 weeks it is only just completed. The manner of its later growth is not known though it is probably by 'external accretion' too.

(*g*) FORE-LIMBS AND GIRDLE

(i) *Scapula* (Pl. 15 and Text-fig. 34)

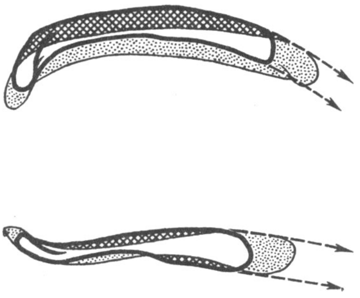
The most rapid growth in the scapula occurs along its margo vertebralis. So triangular is the blade that there is little 'diaphyseal erosion' of either the margo cervicalis or margo axillaris; but on its lateral surface 'diaphyseal erosion' on either side of the spina scapulae hollows out the vertebral limits of the fossae supra- and infra-spinata. On the facies medialis 'diaphyseal erosion' plays a very unimportant role. There is a little accretion near the cavitas glenoidalis.

The spina scapulae grows 'unilaterally', the erosional component enlarging the incisura spinoglenoidalis while the un-eroded parts become the root of the acromion.

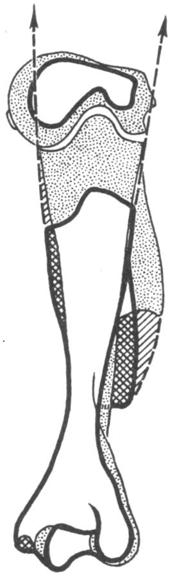
The acromion ossifies later than the collum and grows by 'diaphyseal accretion' to its ventral border at a rate intermittent between those for the margo vertebralis and cavitas glenoidalis. Only its cervical margin is eroded.

The scapula increases in height by accretion to the free edge of the spina scapulae and margo axillaris, and by unilateral growth of the acromion.

The coracoideum, which fuses with the scapula at 18 days, grows only by external accretion.



Text-fig. 35. Growth of the clavicula; above, posterior view; below, lateral view.



Text-fig. 36. Growth of the humerus; posterior aspect.

(ii) *Clavicula* (Pl. 15 and Text-fig. 35)

In the mouse, the extremitas acromialis (N.B.) which represents the 'acromial end' and 'conoid tubercle' of man, meets the corpus at the angulus claviculae (N.B.).

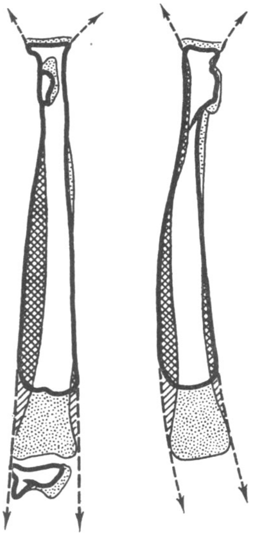
The clavicula grows in the 'centripetal' pattern with 'diaphyseal growth' of the extremitas sternalis.

(iii) *Humerus* (Pl. 17 and Text-fig. 36)

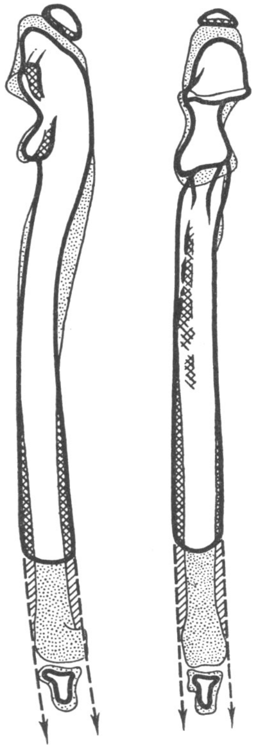
Practically all increase in length of the humerus results from 'diaphyseal growth' at the proximal end of the corpus associated with 'unilateral growth' of the crista humeri and tuberositas deltoidea. Towards its lower end the corpus is thickened by accretion to its antero-lateral surface, just as accretion to the free (antero-lateral) edge of the crista adds to its height.

The distal end contributes almost nothing to the bone's length, but it is generally enlarged. The crista epicondyli lateralis and its proximal continuation, the margo lateralis, become increasingly prominent by accretion to their antero-lateral edges; and the former is thrown into greater prominence by slight 'diaphyseal erosion' of the anterior surface of the root of the epicondylus lateralis. The epicondylus medialis grows medially with 'diaphyseal erosion' of its distal border.

The caput and trochlea grow by 'external accretion' alone. The latter fuses with the diaphysis (corpus) at about 7 days.



Text-fig. 37. Growth of the radius; left, ventral view; right, medial view.



Text-fig. 38. Growth of the ulna; left, medial view; right, dorsal view.

(iv) *Radius* (Pl. 16 and Text-fig. 37)

Unlike the humerus, the radius grows much faster (eight times as fast) at its distal end than at its proximal end. Growth is 'diaphyseal', but the erosion is almost entirely limited to the dorso-medial surface and produces a curvature of the bone which does not result directly from its terminal accretion. This curvature is enhanced by 'unilateral' growth near the proximal extremity. The tuberositas radii (which contains the 'punctum fixum') enlarges by 'external accretion' to all its surfaces. The small amount of growth at the proximal end of the corpus is 'diaphyseal'. Neither epiphysis suffers erosion; the proximal epiphysis fuses to the collum at about 14 days.

(v) *Ulna* (Pl. 16 and Text-fig. 38)

In the ulna, proximal growth is a little more important, though distal 'diaphyseal growth' is still the chief contributor to growth in length. Erosion is more symmetrical than in the radius, though the distal end is eroded chiefly from its ventro-lateral side (again in contrast to the radius where the most intense erosion was from the dorso-medial side). Lateral erosion is important in shaping the tuberositas ulnae. Near the incisura semilunaris the sharp juvenile dorso-ventral flexion of the bone is reduced by 'external accretion' which is greater on the ventral side than on the dorsal surface.

The lips of the incisura grow upwards and away from one another with 'diaphyseal erosion' reforming the corpus behind their recurved margins.

Growth of the processus olecrani is by 'external accretion', while the olecranon (its epiphysis) is unilaterally eroded from its diaphyseal side.*

Text-fig. 39. Growth of a typical metacarpal or metatarsal bone.

Text-fig. 40. Growth of a typical proximal phalanx; from above.

Text-fig. 41. Growth of a typical terminal phalanx; from the side.

(vi) *Manus* (Pl. 17)

The drawings of the manus were made from alizarin clearances of the intact hand; and as it was impossible to orientate all of the bones correctly with regard to the other members of the comparative series, the following analyses have been essentially rule-of-thumb. However, as such (provisional) analyses have previously been borne out by the conclusions from the more precise method of superimposing outline drawings, considerable confidence may be placed in the present series.

Carpus. Ossification of the cartilaginous carpals does not begin until the third day after birth and then proceeds by 'external accretion' alone. No mutant carpale shows any anomaly which might be attributed to erosional failure. It is not known for what period this simple method of growth is maintained.

Metacarpus (Text-fig. 39). With the exception of the metacarpale primum whose growth is more typical of a phalanx, the metacarpales grow by 'diaphyseal growth' of the distal end of the corpus; with 'external accretion' to the capitulum and basis, neither of which contribute greatly to the bone's growth in length.

Phalanges proximales (Text-fig. 40). (Phalanges prima and secunda of digiti 2–5; and phalanges prima only of digitus 1.) 'Diaphyseal growth' of the *proximal* end

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* This is the only occasion in which 'unilateral growth' of epiphyses (a general phenomenon in the pig (Payton, 1933)) was observed in the present work.

of the corpus; with 'external accretion' to the capitulum and the basis, which fuses with the corpus at about 15 days after birth. There is very little 'diaphyseal erosion' of the plantar surface of the corpus.

Phalanges terminales (Text-fig. 41). (Phalanx tertia of digiti 2–5; and phalanges secunda of digitus 1.) Ossification spreads proximally from the tuberositas unguicularis and growth continues by 'external accretion' to the articular surface with the capitulum phalangis proximalis. The foramen transversarium phalangis (N.B.) migrates backwards by 'reversed unilateral growth'; and the distal root of the bony spicule which bridges it is also eroded.

Ossa sesamoidea. The growth of these bones is by 'external accretion' alone.

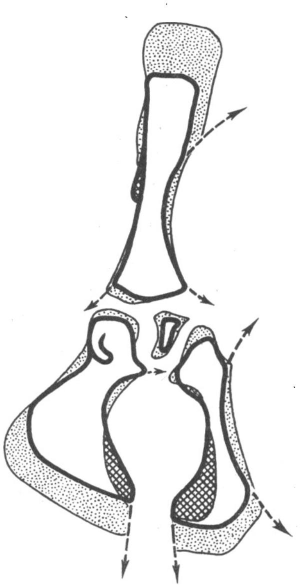
(*h*) HIND-LIMBS AND GIRDLE

(i) *The pelvis* (Pl. 18 and Text-fig. 42)

The right and left halves of the mouse pelvis, the ossa coxae, are readily separated. In adult male mice a few spicules extend across the symphysis pelvis (symphysis ossis pubis) (Ruth, 1936) and in 3-week-old mice the symphysis is still cartilaginous. In pregnant females it is represented by a ligament which may be as much as 2–3 mm. wide. In virgin and in non-pregnant females the ligament is much narrower, but there is never bony communication.

Each os coxa itself consists of four bones. Three are large, the ossa ilium, pubis and ischii, but the fourth, the os acetabuli, is extremely small. These four bones are more or less separate structures until they fuse at about 3 weeks in the acetabular region; the pubis and ischii fuse posteriorly about 10 days after birth. The four bones are treated individually in this account.

The rate of growth of the three major bones is much less at their acetabular extremities than at their ends distal to the acetabulum. In the course of their development, the acetabular sutures are remodelled from a tiradiate figure, Y, into a ƔY-like figure, associated with a slight overlapping of the bones.



Text-fig. 42. Growth of the pelvis (os coxa); lateral view.

Ilium. (While retaining the veterinary term tuba coxae (Ellenberger & Baum, 1926) for the ventral edge of the ala ossis ilium, the author has shifted the positions of the spina iliaca anterior superior and the spina iliaca anterior inferior caudally so as to conform with their muscle attachments in man, and has translated these terms into forms more suitable for four-footed animals. The translations are as follows:

> the spina iliaca anterior superior becomes spina iliaca ventralis cranialis, and
> the spina iliaca anterior inferior becomes spina iliaca ventralis caudalis;

and to bring their analogues on the dorsal side of the ala into line,

> the spina iliaca posterior superior becomes spina iliaca dorsalis cranialis, and
> the spina iliaca posterior inferior becomes spina iliaca dorsalis caudalis.)

While the growth at both ends of the ilium is 'diaphyseal', the erosional component is limited to the outer surface (facies glutea) anteriorly, and to the inner surface (facies pelvina) posteriorly. Anteriorly, the 'diaphyseal' erosion helps to cut out the tuber sacrale which migrates forwards in the 'unilateral pattern'. Along the ventral margin of the bone, however, accretion is directed both downwards and forwards, so that the 'unilateral pattern' is not involved in the forward migration of the spina iliaca ventralis caudalis.

The rate of growth at the acetabular extremity is never great and falls off until its cessation with the ossification of the acetabular sutures at 3 weeks.

Pubis. Like the ilium, the pubis grows at very unequal rates at its two ends, the slower growth at the acetabular end ceasing altogether at 3 weeks. Growth at each end is 'diaphyseal', but the erosion is restricted to three sites, one to each end of the obturator border, the third to the facies pelvina near the junction of the rami acetabularis and symphysicus. The bone is deepened by 'external accretion' to its ventral border, so obliquely directed that neither the eminentia iliopectinea nor the tuberculum pubicum involves 'unilateral growth' in their divergement movement in the antero-posterior axis of the bone.

Owing to the opposition of accretional and erosional sites in the pubis the ramus symphysicus can be regarded as growing 'unilaterally' backwards; and the ramus acetabularis as growing 'unilaterally' downwards.

Ischii. Like the other pelvic bones, the ischium grows very little at its acetabular end. Distally, growth is 'diaphyseal' with heavy erosion of the acetabular border and some mild erosion of the facies pelvina of the ramus symphysicus. At the acetabular end there is accretion to both the ilial and pubic sutures (contributing to growth in length and breadth respectively) with 'diaphyseal erosion' of the buttress for the facies lunata and of the pelvic surface. Between the two sites of 'diaphyseal growth' the corpus is thickened by accretion to both dorsal and obturator borders.

Acetabuli. The os acetabuli, which forms the pubic wall of the acetabulum, grows only by 'external accretion'.

(ii) *Femoris* (Pls. 18 and 19 and Text-fig. 43)

Four-fifths (80%) of the growth in length is contributed by the 'diaphyseal growth' of the distal extremity of the corpus. Erosion is very intense on the ventral surface where it removes the ridges on either side of the fossa intercondyloidea, producing the flat planum popliteum.

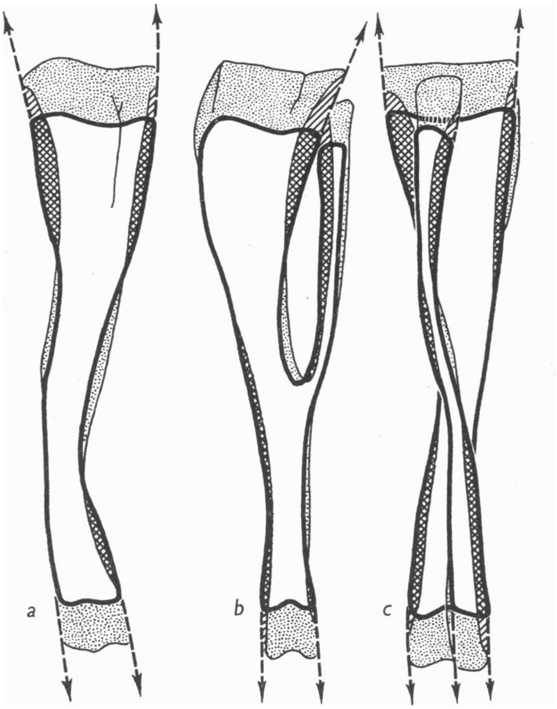
With the exception of the obliquely medial and upward 'diaphyseal growth' of the collum femoris, growth at the proximal end of the bone proceeds by 'external accretion'. The distal migration of the trochanter tertius is obtained without erosion, by discrepant rates of accretion to its distal and proximal edges.

(iii) *Ossa cruris* (*tibia and fibula*) (Pl. 19 and Text-fig. 44)

In the tibia and fibula terminal growth is slightly faster proximally than distally. This growth is 'diaphyseal', but in the fibula it interacts with strong 'unilateral' trends. The crista tibiae is enlarged by accretion to its anterior and lateral edge, so that in very old animals it becomes curved back upon itself. On either side of the crista tibiae 'diaphyseal erosion' spreads with increasing intensity to the posterior side of the tibia, where it serves to enlarge the spatium interosseum cruris. This is also enlarged by the bowing out of the fibula posteriorly by a 'unilateral' trend which almost obliterates 'diaphyseal erosion' of its posterior surface.

At the distal ends of the bones 'diaphyseal erosion' is limited to the medial side of the tibia and to the lateral side of the fibula. And the fibula gradually becomes incorporated into the tibia by the building up of the posterior side of the tibia to the level of the fibula and by erosion of the posterior surface of the fibula.

Text-fig. 43. Growth of the os femoris; dorsal view.



Text-fig. 44. Growth of the tibia and fibula; (*a*) anterior, (*b*) medial, and (*c*) posterior view.

The juvenile flexions of the bones are softened by changes in their middle regions. In the tibia there is accretion to posterior, lateral and medial surfaces with some erosion anteriorly. In the fibula there are 'unilateral' movements directed medially towards the extremities and laterally in the intervening region.

The only pattern involved in the growth of the epiphyses is 'external accretion'.

(iv) *Patella* (Pl. 19)

The bony patella grows by 'external accretion' to the centre of ossification in the sesamoid cartilage which precedes it.

(v) *The pes* (Pl. 20)

(Unlike the domesticated animals and man, the mouse has three in place of two proximal tarsal bones. Rather than regard the supernumerary bone as an unparalleled entity, the author agrees with Greene (1935) in believing this to be the os tarsi tibiale, the 'talus' to be the os tarsi intermedium, and the 'calcaneum' to be the os tarsi fibulare).

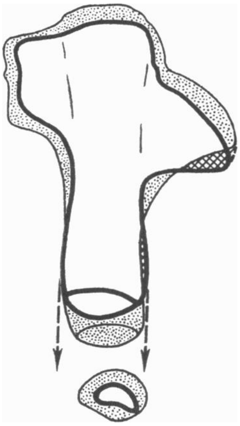
The tarsus. The growth of only the os tarsi fibulare (calcaneum) (Text-fig. 45) is more complicated than by 'external accretion' alone.

With the exception of the sustentaculum tali in whose forward growth the 'unilateral pattern' is involved, growth of the anterior end of the fibulare is by 'external accretion'. 'External accretion' also widens the girth of the middle of the bone; while, posteriorly, 'diaphyseal growth' contributes a further increase in length. Erosion of the lateral side is obliterated by accretion which introduces a slight curvature of the body of the fibulare. The tuber calcanei grows by 'external accretion'.

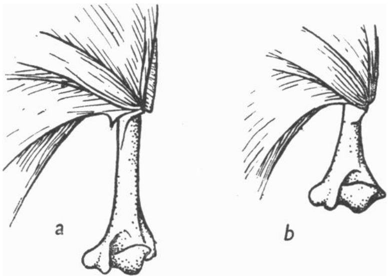
Metatarsus and digiti pedis. The growth patterns of the metatarsus, phalanges and ossa sesmoidea are precisely similar to those of the manus and require no further comment.

(*i*) PENIS BONE

The os priapis grows at its proximal end by 'diaphyseal growth'.



Text-fig. 45. Growth of the fibulare; ventral view.



Text-fig. 46. Dissections of the forearm of a normal mouse (*a*), and a microphthalmic mouse (*b*) at 21 days, showing the migration of the deltoid muscles along the crista humeri. Is the consequent release of muscular tension the normal stimulus for erosion of the crista?

(*j*) COMPARISON WITH OTHER AUTHORS' RESULTS

Providing there is a reasonable similarity in their form, there is, in general, a striking resemblance in the manner of growth of the bones of the mouse, described above, and of other animals (rat, cattle, pigs, man) described by other authors. In fact, the resemblance in the growth of the lower jaws of the pig, ox and mouse transcends their resemblance in shape.

However, there are some more serious discrepancies. In particular, while 'unilateral growth' has been found for only one epiphysis in the mouse (olecranon), Payton (1933) found it in all the twelve pigs' epiphyses which he studied; and in one epiphysis the effect of no less than four-fifths of the accretional increment was simultaneously removed by erosion of the opposite surface. These differences may be related to age changes in the manner of growth of the bones.

Kölliker (1873) described additional sites of erosion on the medial side of the processus jugularis of the occipitale; on the upper surface of the processus zygomaticus of the squama temporalis, and on the medial side of the lacrimale. He also found anterior growth in the incisivum where the present author found only backward growth. In describing the growth of the humerus, Kölliker may have erred in failing to recognize the possibility of the migration of the deltoid muscles. Dr Grüneberg (unpublished) first noted the migration of these muscles up the crista humeri in sections of grey-lethal mice, and the present author has confirmed this in dissections of microphthalmic mice (Text-fig. 46).

Lastly, it may be remarked that the relative rates of growth at the ends of the six major long bones of the limbs vary from species to species, and in every case the rates described here for the mouse differed from those of the pig (Payton, 1932). The equality of growth at the two ends of the tibia and fibula, and the lack of growth at the distal extremity of the humerus, in mice, are, however, quite compatible with the manner of growth of these bones in humans as obtained by the analysis of Harris's radiographs (1933).

DISCUSSION

SOME GENERAL PROBLEMS IN BONE GROWTH

Having dealt with the details of the growth of individual bones, it is now time to discuss some more general problems of bone growth, and, in particular, those problems upon which the present work may throw additional light. It is intended chiefly to discuss the relative importance of inherent and extrinsic determination of bones and of bone growth, while also touching on the significance of the osteoclast and of the epiphysis.

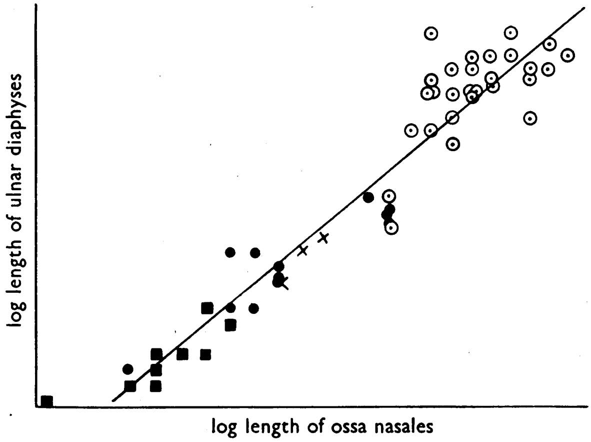
(*a*) *Embryonic determination of bones*

The modern concept of bone growth is vastly different from the theories of the early mechanists who believed bone to be laid down along lines of stress (the trajectorial theory) and erosion to be a necrotic process; and it is now known that bones have an identity quite apart from their normal environments. Thus Saunders (1948) proved that the bones of the chick's limbs were finally determined immediately following their serial proliferation from the apical ectodermal cap of the limb-buds. And in earlier experiments (notably those of Murray & Huxley (1925), Fell (1928) and especially Fell & Robison (1929)—all reviewed by Murray (1936)) the enormous power of explanted blastemic bones to self-differentiate in tissue cultures, even when stripped of their musculature, was demonstrated. Lastly, in a series of papers (1942–7) Lacroix has published accounts of the osteogenic properties of mere fragments of bone, and even of bone membrane (periosteum) and conjugation cartilage which could produce bone of normal histological appearance growing in a manner typical of the bone from which the pieces had been taken. Pfeiffer (1948) has demonstrated a similar property of marrow cells. On the other hand, Hauschka (1951) has gone too far in claiming to have found differentiated bones from mouse embryo mince injected intraperitoneally into adult mouse hosts, for while the shapes of the 'bones' were comparable to normal bones, their manner of growth was not, and it would be better to interpret the objects as nothing more than osteomata. This type of research culminated in Lacroix's discovery (1947) that even an alcoholic extract of conjugation cartilage had osteogenic properties and was capable of inducing an osteoma growing in a more or less typical 'diaphyseal' manner.

These facts leave little scope for the play of normal environmental forces occurring within the developing animal in the determination of normal bone growth.

(*b*) *Accretion and extrinsic factors*

In spite of the degree of self-determination it is generally believed that the sites of accretion and erosion are not so independent of extrinsic factors (Murray & Selby, 1930) and come under the influence of specific stimuli. It is rarely possible to put the supposed stimuli for accretion to independent test, and in the one case where this was possible, a non-specific factor was found responsible. Grüneberg had once suggested (1935, and quoted by de Beer, 1940) that the snout of the mouse required a stimulus from the growth of the incisor tooth to attain its proper length, and that the shortness of the grey-lethal snout was due to the mal-development of the incisor tooth. This idea is now definitely invalidated, for a plot of the length of the nasal bones (in lieu of the snout) against an 'unrelated' body measurement (corpus ossis ulnae) for grey-lethal, microphthalmic and 2- and 3-week-old normal mice (Text-fig. 47) shows that the regression line relating these measurements to each other is common to all the four groups. Thus the shortness of the grey-lethal snout is due merely to the general retardation of development and not to any circumstance peculiar to the region of the snout.



Text-fig. 47. Graph showing the constancy of the allometry of the length of the snout to an 'unrelated' body measurement in normal and mutant mice of 20 and 21 days and in three normal mice of only 14 days of age. ⊙ $+/+$, $+/gl$ and $+/mi$ at 21 days of age; × $+/+$ at 14 days; ■ gl/gl at 20 and 21 days; ● mi/mi at 21 days.

It is not at all clear to what extent other stimuli might be responsible for individual sites of accretion. But in one case at least it appears that the extrinsic factors have a more passive role: to provide *conditions* for general development, not an array *stimuli* for specific features of development. Thus in a remarkable teratoma taken from a girl and containing the three middle digits of a 'hand', perfection in development was graded disto-proximally (Nicholson, 1937). The most proximal structure bore no resemblance to any known bone. This was followed by two lumps of bone and the three 'metacarpals' thinly ossified and fused at their proximal ends. The distal ends of the 'metacarpals' and the phalanges were almost normal and the terminal phalanges were lenticulated. It seems as if the existence of ever more proximal structures assisted towards the provision of more normal conditions for the distal structures subsequently delineated from the apical cap.

Now the importance of intrinsic factors in bone accretion does not mean that the skeleton is an anarchy of individually governed bones. On the contrary, such almost perfect correlations as between the measurements of the two bones above (Text-fig. 47) are not usually met in studies of qualitative characters (such as weight and tail length) and betray the existence of a factor which rigidly controls the rate of growth of all bones in the skeleton and is not intrinsic to any single bone.

(c) *Extrinsic factors affecting erosion*

While the extrinsic factors affecting accretion evade discovery, those affecting erosion are more apparent. Jores (1920) caused erosion of the processus spinosi of thoracic vertebrae in guinea-pigs and rabbits by exerting a pressure on them by tying little mercury-filled bags to them, or by strapping water bags under the skin. And Loeschke & Weinnoldt (1922) have recorded instances in man of erosion of the inner tables of the skull leading to exposure of the diploë and even to fenestration, due to abnormally high internal pressures caused by hydrocephaly, brain tumours and premature synostosis of the skull. They also showed that erosion ceased and may be replaced by accretion when the pressure is removed. It is, however, natural that the bones surrounding such a delicate organ as the brain should be responsive to the organ's variations in form; and Loeschke's and Weinnoldt's findings cannot necessarily be extended to other parts of the skeleton.

Indeed, it may well be that the release of tension is sometimes the stimulus for erosion. Thus, in sectioned material of grey-lethals Grüneberg (unpublished) found that the deltoideus and pectoralis muscles had migrated away from the tip of the crista humeri, whereas in normal mice their insertion coincides with the tip of the crista. (The present author has confirmed this anomalous situation in dissection of microphthalmic mice, and one of such dissections, with a normal for comparison, is figured in Text-fig. 46.)

Enthusiasm for investing pressure with the responsibility of causing erosion must be tempered by consideration of Payton's work (1933) concerning the rates of accretion and erosion at opposite surfaces of the articular epiphysis of the long bones of the limbs of the pig. In all of the twelve epiphyses he studied he observed the 'unilateral' pattern of growth, and although the pressure at articular and diaphyseal surfaces of each epiphysis must have been approximately equal, erosion of the diaphyseal surface in one epiphysis was four-fifths as great as accretion to the articular surface. Nor was there an obvious correlation between the rates of erosion of adjacent epiphyses. In other words, pressure cannot be the *cause* of erosion. At most it can only be a stimulus to which a given bone or part of a bone may or may not respond; and emphasis is again laid on the intrinsic properties of the individual bones.

It was mentioned in the Introduction that the skeletons of grey-lethal and microphthalmic mice were not quite identical and that the microphthalmics frequently showed less obvious signs of erosional failure. And it was once thought (Grüneberg, 1948*b*) that a comparative study of the skeletons of these mice might furnish further information on the problem of the control of erosion. Alas, it is now clear that a semblance of erosion in these mutants can be produced merely by differences in the time of onset and in the rate of ossification. For, in the mutants, remodelling progresses normally until the 'bone' becomes calcified. Thus, if ossification of the stapes is delayed, the size of its foramen stapedis is more normal; and if ossification, once initiated, is rapid, the mutant stapes will present fewer of its characteristics which result from the slow progress of ossification from basis to capitulum. On the other hand, the slow progress of ossification in the tibia will produce more gracefully shaped metaphyses which might be interpreted as indicating a partial success at erosion. It would, therefore, seem impossible to draw any reliable conclusion concerning effective stimuli for erosion from the intended study.

As for the intrinsic control of erosion, this seems to lie with the conjugation cartilage for, whereas dead bone is totally absorbed on transplantation (Hancox, 1947), the presence of conjugation cartilage (or its contaminants), as in Lacroix's experiments, leads to regulated erosion. But how this influence of the conjugation cartilage is transported throughout the bone is not known. Hancox (1949) has suggested that the occurrence of erosion is determined by trophic gradients; but here it should be noted that, whereas in 'diaphyseal growth' much of the youngest bone is absorbed, in 'unilateral growth' it is the oldest.

(*d*) *Plasticity in patterns of growth*

The topic of the changeability, or plasticity, of the manner of growth of bones during their development is especially applicable to the problem of extrinsic versus intrinsic determination of sites of both accretion and erosion. There are few bones indeed (some carpal, tarsal and sesamoid bones) which adhere throughout their life to a single pattern of growth ('external accretion');* and even in these, shortly after ossification has begun, internal changes occur in the bones which are not visible from the exterior and are, therefore, not considered in this paper. But in every other bone even the externally visible patterns of growth change from time to time.

Thus in the arcus of the vertebrae, 'external accretion' (during the ossification of the original cartilaginous model) is soon replaced by 'centripetal growth' which is itself replaced by a tertiary pattern ('external accretion' with 'internal erosion') when the elements of the vertebra fuse into a single unit. Similar radical alterations in patterns of growth must always occur whenever bones are involved in bony fusions—as when the occipital bones, or the sacral vertebrae, or the pelvic bones, or the tympanicum and perioticum and malleus, or epiphyses and their diaphyses unite.

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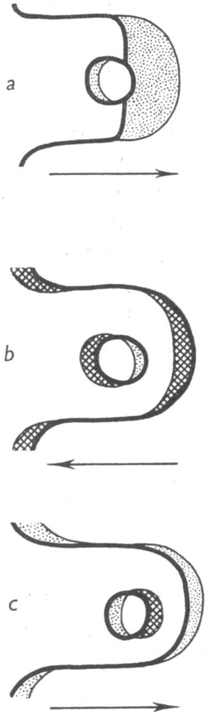
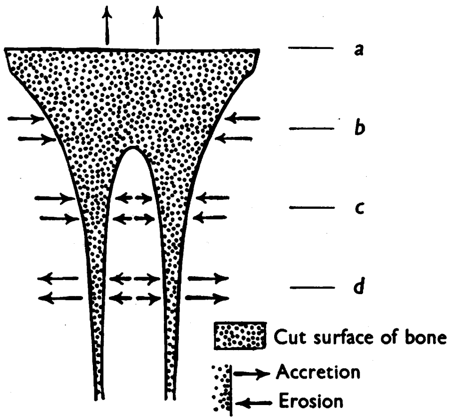
* Schour & Massler (1940) and Massler & Schour (1951) have also noted that there are two phases of growth: the first of generalized accretion, followed by a second and longer period of only localized accretion. The first period of growth lasted up to 60 days in the rat. This conclusion, however, may be erroneous, since it appears that these authors did not realize that while alizarin (which they were using to record the bones' manner of growth) stains only the growing bone in old skeletons, it stains bone formed long previous as well as just after its injection in young animals (Cameron, 1930).

In the tympanicum the tertiary pattern is a reversal of the secondary. 'External accretion' from the annulus tympanicus is followed by 'internal *accretion*' in the porus acusticus externus; but when the ossification is completed (by 14 days after birth) the porus is enlarged like the rest of the bulla, by 'internal *erosion*'.

The foramen transversarium likewise undergoes a reversal of secondary and tertiary patterns (Text-fig. 48*a–c*). Its ossification is accomplished by 'internal accretion' (*a*), which is replaced by medially directed 'reversed unilateral growth' as part of the 'centripetal growth' of the arcus vertebrae (*b*); but when the vertebral elements fuse and their growth is by 'external accretion' with 'internal erosion', the direction of 'reversed unilateral growth' of the foramen is also reversed (*c*).



Text-fig. 48. Plasticity of bone growth. I. The three successive patterns of the processus transversus.



Text-fig. 49. Plasticity of bone growth. II. The four successive patterns of growth affecting a piece of a long bone initially at (*a*).

It is instructive to consider the fate of a single disc of bone adjacent to the conjugation cartilage of a long bone (Text-fig. 49). As it is carried towards the middle of the bone by continued calcification of the conjugation cartilage, it may be subjected to no less than four successive patterns of growth. At first (*a*) it is subjected to 'diaphyseal accretion' by ossification of the matrix of the conjugation cartilage (*endochondral* ossification). Then when the disc comes to occupy a position a little behind the cartilage its circumference is 'diaphyseally' eroded (*b*). This 'diaphyseal erosion' is accompanied by a continuous *endosteal* reformation of the wall of the metaphysis between the endochondral spicules (Leblond *et al.* 1950).

A little later (*c*) it is also internally eroded for the extension of the marrow cavity. And, lastly (*d*) when it is very near the middle of the bone it partakes in the widening of the diameter of the diaphysis by 'external accretion' (*periosteal* ossification), while 'internal erosion' continues to enlarge the marrow cavity. Thus, in a long bone these four processes and these three types of bone formation may be depicted as migrating away from the punctum fixum towards the growing ends, each successive phase tending to encroach on each previous phase—a dynamic picture truly reflecting the plasticity of bone growth.

It seems impossible to attribute such radical alterations in the patterns of growth of adjacent regions at the same time, and of the same region at adjacent times, to equally fundamental changes in the local environmental factors which are sometimes presumed to control the bone's growth. And it seems preferable to turn our attention away from supposed (extrinsic) stimuli for growth and erosion towards the study of the bone's (intrinsic) reaction to its environment. But extrinsic factors are not entirely banished from influencing bone growth: to them are bequeathed those abnormalities of development which allow, for example, a skilled anatomist to recognize the skeleton of a cobbler; a bone to form ball-and-socket joints on either side of an unsplinted fracture; and the cessation or arrest of growth with severe illnesses.

But if difficulty is encountered in attributing the plasticity of bone growth to an ever-changing array of environmental factors determining local processes, then a similar difficulty must be encountered if this array is substituted merely by an array of *inherent* factors. In short, it is probable that the inherent determination is not of local growth processes but of whole patterns of growth. Thus the conjugation cartilage does not only produce endochondral bone, but also controls the extent of metaphyseal erosion, the extension of the marrow cavity, the formation of marrow, and the deposition of periosteal bone (Lacroix). Similarly, fracture of the shaft of a bone is not associated merely with the local deposition of new bone at the site of repair, but stimulates the deposition of bone over almost the entire surface of the shaft (Brooks, 1917).

(*e*) *The function of the osteoclast*

Now bone accretion and bone erosion are not just opposite processes in the same reversible reaction, but are quite distinct processes. Bone accretion is a function of cartilage and periosteal cells and a third type of cell called osteoblasts; but erosion is the function of a fourth type of cell called the osteoclast—though, as the relation between osteoclast and erosion has been open to doubt, it will be discussed here. In the light of the present contribution it is no longer permissible to accept the indecisive conclusion which Hancox (1949) reached after a comprehensive review of published research on the osteoclast—that in relation to bone absorption 'osteoclasts must still be regarded as enigmatical structures'.

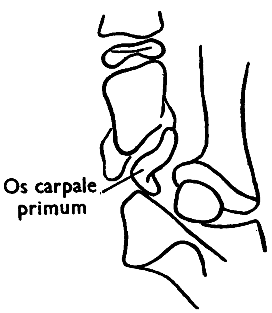
In the first place it is no longer possible to regard the osteoclast as unrelated to the process of bone absorption, since the sites of erosion described by the present author closely tally with the sites of osteoclasts described by Kölliker (1873).

Next, the osteoclast cannot be a by-product of erosion (an opinion held as late as 1937 by Wilton), since it occurs with almost normal frequency in the grey-lethal mouse (Barnicot, 1947) in which there is no bone erosion at all. Moreover, Kölliker has reported seeing osteoclasts even before ossification centres had been formed.

But are we to believe with Jaffe (1933) that the osteoclast merely removes bone cells, or that it actually erodes the bone salts? Of this there can be no final proof without microcinematographic recording of the action of the osteoclast (Hancox, 1949). However, the wide fluctuations in the population of osteoclasts mentioned in the Introduction (p. 216) allow no store to be placed in Ruth's objection (1937) that the number of osteoclasts which he observed at the symphyseal tables of the ossa pubes of the pregnant guinea-pig was too small to account for the extent of erosion there. On the other hand, the close correspondence in size between the foveolae of Howship and the osteoclasts which they contain is strong evidence of the erosive action of osteoclasts on bone. Since no evidence of bone phagocytosis has been obtained (McClean & Bloom, 1941) it is almost certain that this dissolution is by the application of extracellular osteolytic enzymes.

(*f*) *The phylogenetic determination of bones*

In spite of the lack of ontogenetic plasticity in bone determination there is still widespread adherence to Broom's (1930) belief in the phylogenetic transmutation of bones. Broom believes the os carpale primum to represent the metacarpale primum of therapsid reptiles, and the metacarpale primum of living mammals to be a transformed phalanx. On the other hand, owing to the close resemblance (see Text-fig. 50) of the 10-day-old os carpale primum to a typical proximal phalangeal epiphysis (basis phalangis), the transformation could be argued differently. But any theory which attempts to explain the distribution of the epiphyses in the hands and feet in such a manner presupposes two postulates: first, that the ossified elements in the hands and feet are permanently represented throughout phylogeny; and secondly, that the distribution of the epiphyses is determined by extrinsic factors varying along the proximo-distal axis of the limb. Neither of these postulates can be considered proven.



Text-fig. 50. The os carpale primum of a 10-day-old normal mouse. Note its resemblance to a typical phalangeal epiphysis

No living tetrapod can supply evidence concerning the first postulate, as it is characteristic of this whole group for the first digit, when present, to have at least one element less than the others. But the evidence of the fossil, mammal-like reptiles, stresses the ephemerality of the elements of the hands and feet, for whole segments of the digits and carpus have disappeared in the course of evolution without being retained as transformed bones; and conversely, they have appeared without being converted from an already existing bone. Thus the Pelycosaurs have three more carpal bones than the mouse and four supernumerary phalanges; the Gorgonopsids are similar but lack the os carpale 5 and some intermediate phalanges are greatly shortened; while the Dicynodonts, though having the typical mammalian phalangeal formula, lack the os carpale 5 but possess the mammalian os carpale accessorium. Moreover, the ephemerality of epiphyses is clearly demonstrated by reports in human pathology. For Burke (1930), Ogilvie (1931*a*), Pryor (1936) and Brailsford (1948) have all observed cases of first or second metacarpal bones with proximal and distal epiphyses in place of the normal single epiphysis. Brailsford has also noted phalanges with two epiphyses, and Ogilvie (1931*b*) records a case of a bilaterally symmetrical bipartite os carpale radiale. None of these authors comments on or figures correlated deficiencies in the rest of the skeleton of the hand so that it is clear that epiphyses can be created *de novo*.

Pryor's observations (1936) on the bifurcated and completely split thumbs of two children suggest that the epiphyses of the metacarpal bones are determined, not by a proximo-distally graded field (the necessary second postulate for accord with the theory of evolutionary transmutability of the elements of the hands), but by a transversely graded field. For in the case of bifurcation, the postaxial process (that nearest the metacarpale secundum) had a distal epiphysis as well as the common proximal one; while the preaxial process was without the distal epiphysis. Similarly, in the case of complete separation, the postaxial segment had no proximal epiphysis, while the preaxial segment had. (The radiographs were not clear enough to determine the disposition of distal epiphyses.)

However, Carter (1951) notes instances in the mouse of a splitting and even complete bifurcation or trifurcation of the big toe associated with the gene luxate, in which it is the *preaxial* digits which exhibit triphalangy and have a distal epiphysis. Carter regards the extra digits as prehallucal. In view of this evidence, if the distribution of epiphyses (and the number of phalanges) is determined simply by a transverse gradient, then it would appear that the gradient originates in the true hallux itself.

It may, nevertheless, be concluded that the similarity of the first metacarpal and metatarsal bones to phalanges, both in regard to the position of their epiphyses and to the manner of their growth, is not an indication of their common phylogeny.

(*g*) *The significance of epiphyses*

The epiphysis is an anatomical entity about whose evolutionary significance there has been much confused thought owing to the misconception that it is peculiar to mammals. Haines (1938) has shown that epiphyses are present as cartilaginous structures in bony fishes and Amphibia, and that the evolution of ossified epiphyses has occurred independently in reptiles and mammals (Haines, 1941). Theories which are concerned with the appearance of epiphyses in mammals only, are therefore discredited.

We have just seen in the special case of the mammalian hand and foot that the epiphyses are unlikely to represent pre-existing bones in phylogeny ('atavistic' epiphyses of Parsons, 1903), and we must therefore turn to their possibilities as functional entities. Epiphyses occur only at the growing ends of cartilage bones and transmit either articular pressure or muscular traction to the shaft (Parsons, 1904, 1905). In situations where there is neither mobile articulation nor muscular traction, as at the costo-chondral junctions or symphysis spheno-occipitalis, no epiphyses are formed. Haines (1940) has discounted Parson's theory that traction epiphyses are derived from sesamoid structures, and Appleton (1922) has shown that, unlike sesamoid bones, these epiphyses develop independently of their musculature. It appears, then, that all epiphyses are formed by the separation of the growth cartilage into epiphyseal and conjugation cartilage, the latter becoming subterminal and retaining its contribution to growth (Harris, 1933). What advantages does this confer?

While traction epiphyses quite obviously present an economy in the amount of migration of muscle attachments which would be necessary were the growth cartilage terminal, the significance of pressure epiphyses is not as clear. From his study of the epiphyses of primitive forms Haines (1938) believed that the division of labour of the growth cartilage allowed the elaboration of the articular surface without interfering with the development of an efficient trabecular pattern in the shaft which demanded a flat growth cartilage. However, in higher animals the conjugation cartilage has become so infolded (Text-fig. 51) that it is questionable whether this advantage has been maintained. For the higher animals a more plausible suggestion is due to Nicholson (1937), that the situation of the growth cartilage behind the elaborately modelled and enlarged head presents a great saving in the extent of bone remodelling. This theory cannot apply, however, to the simple epiphyses of primitive forms; and it therefore appears that the advantages gained by the development of epiphyses have changed in the course of their evolution.

Text-fig. 51. Oblique view of the distal end of the shaft of the femur to show the infolding of the conjugation cartilage.

(*h*) *Conclusions*

It can be seen from the foregoing discussion that there still remain many unsolved problems in bone growth. These have been sufficiently stressed, but it is proposed to bring this subject to a close by drawing together some of the more significant positive conclusions which have been attained.

Bones are considered to be determined almost entirely independently of extrinsic control; yet the co-ordination in their rates of growth implies the existence of a common extrinsic growth-controlling factor. They appear to possess an extraordinary lack of variability both in ontogenetic and in phylogenetic determination; the distribution of the epiphyses of the metacarpale and metatarsale primum is not considered evidence of their evolution from phalanges, nor is the carpale primum regarded as a modified metacarpal bone or phalangeal epiphysis. The value of the epiphysis seems to have changed in the course of its evolution.

Patterns of growth of individual bones in the mouse may change no less than four times (often with severe contrasts between successive patterns) in the course of the first 3 weeks of the bone's formation. This, too, reflects the almost total lack of extrinsic control of bone growth. The conjugation cartilage is the centre of control of 'diaphyseal growth' of long bones, and the osteoclast is demonstrated the agent of erosion.

Murray & Selby's conclusion that extrinsic factors become of increasing importance in advanced development of the bone, is modified; to extrinsic factors is now relegated the control only of abnormal growth.

SUMMARY

1. The uses of gene actions to replace operative technique in experimental embryology are discussed; and the two mutant stocks of mice used for the present study are described.

2. Methods of studying bone growth are discussed, and a new method is described and is used in the present study. This is due to Grüneberg (1948), and involves the interpretation of the condition of hereditary pathological skeletons.

3. The superficial versus interstitial nature of bone growth is considered.

4. The growth of the mouse skeleton in terms of patterns of accretion and erosion at normally visible surfaces is described. Eight patterns are described as fundamental in bone growth. The general results of earlier workers in this field are confirmed.

5. The relative importance of extrinsic and intrinsic factors in bone determination and bone growth are discussed. Extrinsic factors are considered responsible only for abnormal growth.

6. The distribution of epiphyses in the metacarpus and metatarsus is shown to be due to a transversely graded field and cannot indicate the evolution of the metacarpale primum or metatarsale primum from a proximal phalanx.

7. In spite of the rigidity of developmental and evolutionary determination of bones, their manner of growth is immensely plastic and many change four times within the early development of the bone.

8. While the significance of the epiphysis is still uncertain, the osteoclast is proved to be the agent of erosion.

The anatomy of the normal skeleton of the mouse at the age of three weeks is depicted in a series of half-tone, camera lucida drawings. Standard latinized nomenclature is used throughout.

The author is deeply indebted to Dr Hans Grüneberg for suggesting this problem and for the interest he has shown throughout its investigation. He is especially grateful to Mr E. D. Roberts for tuition in drawing, and for the elaborate care he has employed in labelling these drawings, and for assistance with the text-figures. He wishes to thank Dr D. S. Falconer for much helpful advice on writing the text; and Prof. C. H. Waddington for granting him permission to continue this work in Edinburgh. Part of the cost of publication of the plates has been met by a grant from the Carnegie Trust which is gratefully acknowledged.

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EXPLANATION OF PLATES

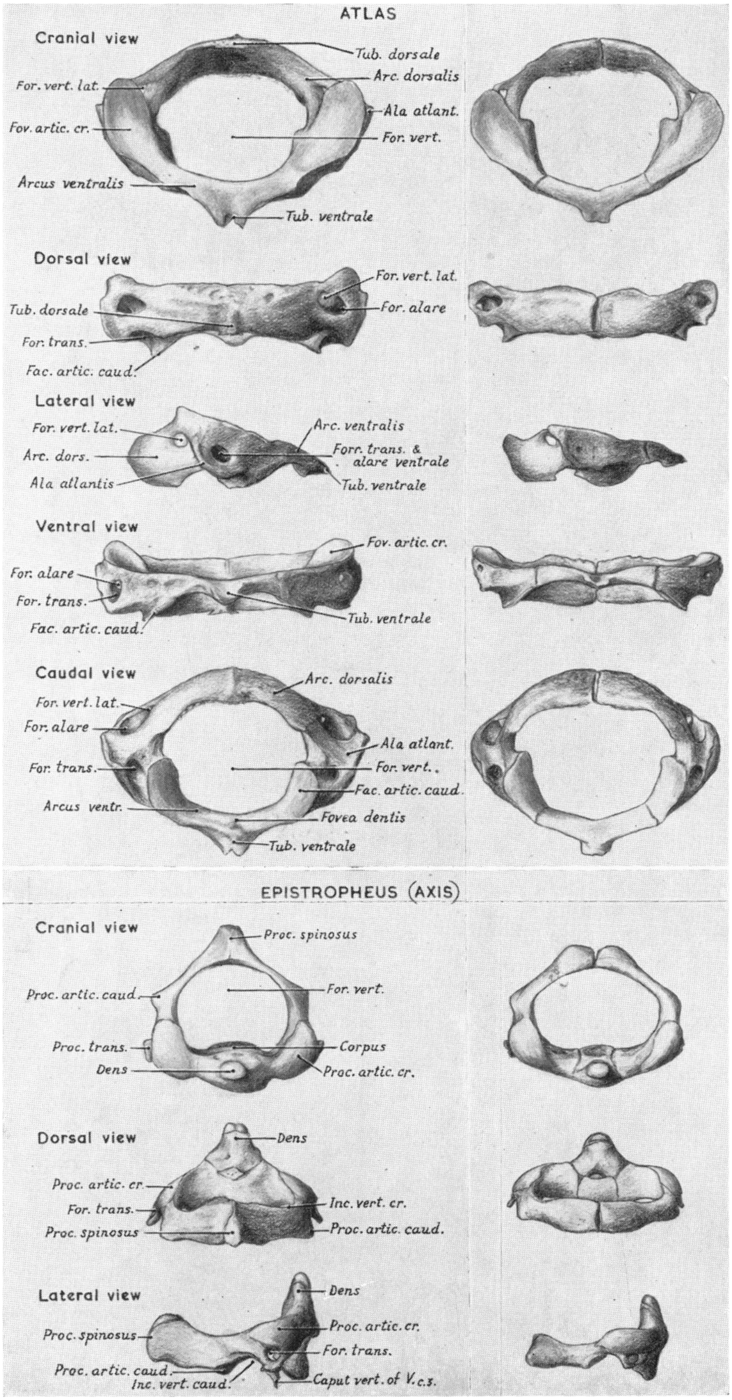
The skeletal anatomy of normal and grey-lethal mice at 3 weeks of age: features of normal bones are labelled. Original drawings made by camera lucida at standard magnification of × 9·5, with the exception of vertebrae coccygeae, palatinum, lacrimale and patella (magnification of × 19), and auditory ossicles (× 30). All drawings reduced in reproduction to two-thirds, i.e. to × 6·5, 13 or 20.

Index of abbreviations used in the plates

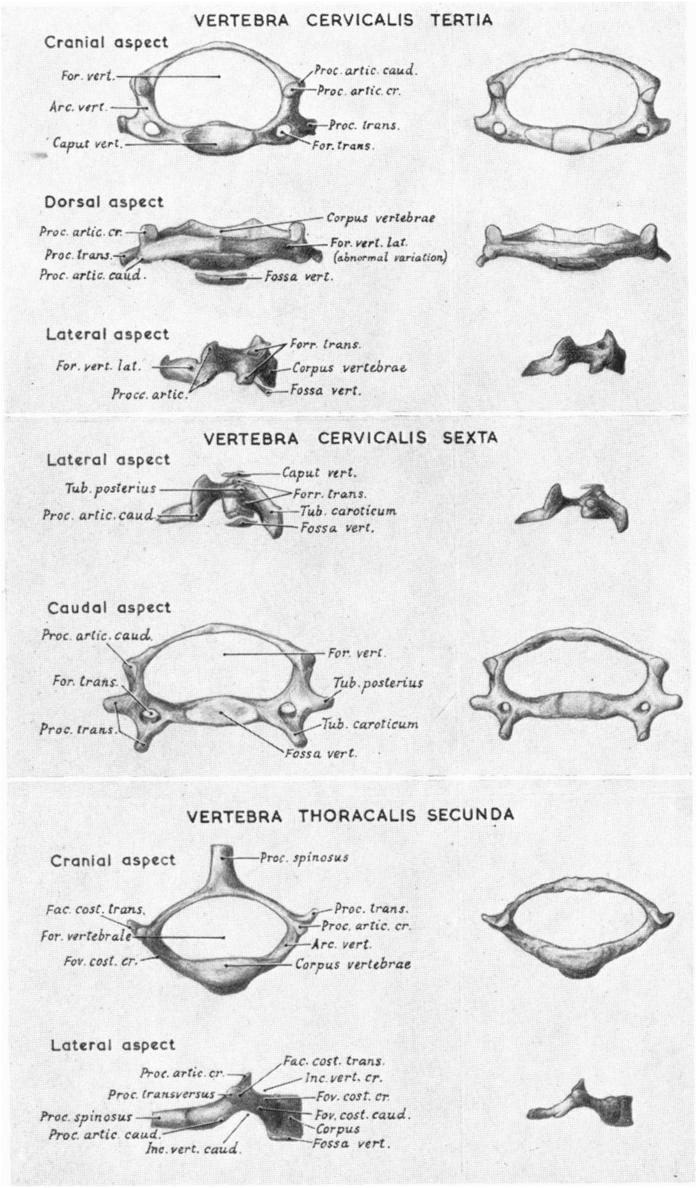
Ala atlant.	Ala atlantis
sac.	sacralis
temp.	temporalis
Alv. inc. sup.	Alveolus incivus superior
Arc.	Arcus
dors.	dorsalis
vert.	vertebrae
Arcus ventr.	ventralis
Can.	Canalis
infr.	infraorbitalis
pteryg. (*ant. opening*)	pterygoideus (anterior opening)
Capit.	Capitulum
cost.	costae
mand.	mandibulae
Caput vert. (*of* $V._{c.3}$)	Caput vertebrae (of vertebra cervicalis tertia)
Cartil. scap.	Cartilago scapulae
Coch. tali prox.	Cochlea tali proximalis
Coll.	Collum
Cond. med.	Condylus medialis
lat.	lateralis
Corpus vert.	Corpus vertebrae

Cris.	Crista
epic. lat.	epicondyli lateralis
eth.	ethmoidalis
sac. art.	sacralis articularis
sac. lat.	sacralis lateralis
tub. min.	tuberculi minoris
D. m. tertius	Dens molaris tertius
Emin. iliopect.	Eminentia iliopectinea
intercond.	intercondyloidea
Epic. lat.	Epicondylus lateralis
med.	medialis
Extr. acr.	Extremitas acromialis
dist.	distalis
st.	sternalis
vert.	vertebralis
Fac. artic. caud.	Facies articularis caudalis
auric.	auricularis
cost. trans.	costalis transversarium
Fen.	Fenestra
Fiss. pal.	Fissura palatina
For.	Foramen
intervert.	intervertebrale
lac.	lacerum
nutr.	nutricium
obt.	obturatum
pal. maj.	palatinum majus
sac. dors.	sacrale dorsale
sac. vent.	sacrale ventrale
stylomast.	stylomastoideum
trans.	transversarium
trans. phal.	transversarium phalangis
vert.	vertebrale
vert. lat.	vertebrale laterale
Forr.	Foramina
fr. int.	frontalia interna
pal. min.	palatina minora
trans.	transversaria
Foss. max.	Fossae maxillares
Fossa infrsp.	Fossa infraspinata
mand.	mandibulae
mass.	masseterica
supratr.	supratrochlearis
vert.	vertebrae
Fov. artic. cr.	Fovea articularis cranialis
cost.	costalis
cost. caud.	costalis caudalis
cost. cr.	costalis cranialis
Inc.	Incisura
acet.	acetabuli
clav.	clavicularis
lacr.	lacrimalis
sem.	semilunaris
sph.	sphenoidalis
spinoglen.	spinoglenoidalis
vert. caud.	vertebralis caudalis
vert. cr.	vertebralis cranialis
Incc.	Incisurae

Lam.	Lamina
infr.	infraorbitalis
pt-pal.	pterygopalatina
Lin. anc.	Linea anconaea
intertr. post.	intertrochanterica posterior
Linea trans.	Linea transversa
M.	Margo
Os c.	Os carpi
c. acc. pr.	carpi accessorium primum
c. acc. sec.	carpi accessorium secundum
pteryg.	pterygoideum
ses. ph. tertiae	sesamoideum phalangis tertiae
sph.	sphenoidale
tarsi inter.	tarsi intermedium
Ossa sess. ph. pr.	Ossa sesamoidea phalangis primae
Pars horiz.	Pars horizontalis
nasofr.	nasofrontalis
orb.	orbitotemporalis
perp.	perpendicularis
Pect. o. pubis	Pecten ossis pubis
Ph.	Phalanx
Proc.	Processus
acc.	accessorius
artic. caud.	articularis caudalis
artic. cr.	articularis cranialis
condyl.	condyloideus
corac.	coracoideus
cost.	costarius
mam.	mamillaris
palat.	palatinus
pteryg.	pterygoideus
sphenopal.	sphenopalatinus
stylomast.	stylomastoideus
trans.	transversus
zyg.	zygomaticus
Procc. artic.	Processus articulares
R. acet.	Ramus acetabularis
sym.	symphysicus
Radix arc. vert.	Radix arcus vertebrae
Sp. il. dors. caud.	Spina iliaca dorsalis caudalis
il. dors. cr.	iliaca dorsalis cranialis
il. ventr. caud.	iliaca ventralis caudalis
il. ventr. cr.	iliaca ventralis cranialis
nas. ab.	nasalis aboralis
Spatium inteross. cruris	Spatium interosseum cruris
Sulcus stap.	Sulcus stapedeus
Sut.	Sutura
Sym.	Symphysis
p.	pubis
Syn.	Synchondrosis
intersph.	intersphenoidalis
T. isch.	Tuber ischiadicum
Tab. isch.	Tabula ischiadica
Tub.	Tuberculum
cost.	costae
Tubs.	Tuberositas
delt.	deltoidea
infragl.	infraglenoidalis
supragl.	supraglenoidalis

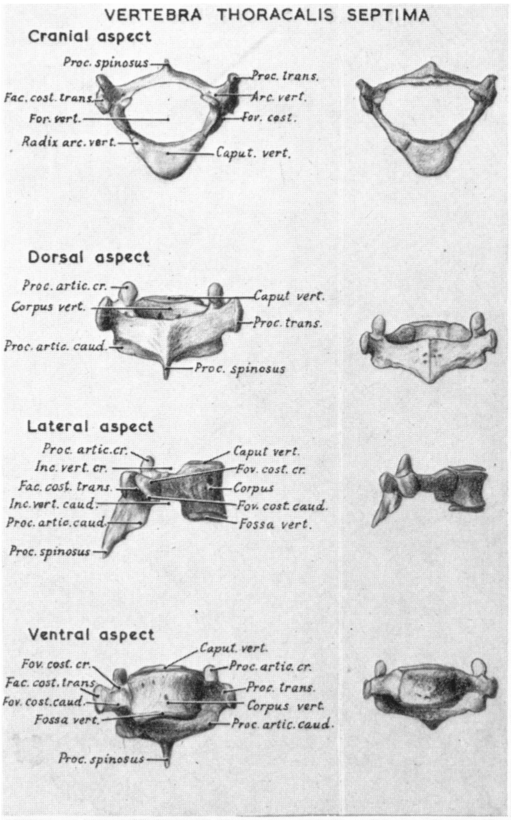


BATEMAN—BONE GROWTH: GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF MOUSE

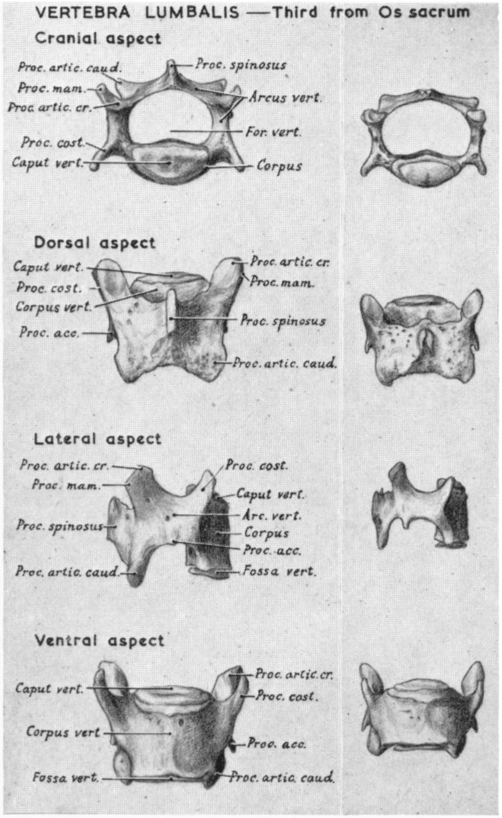


BATEMAN—BONE GROWTH: GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF MOUSE

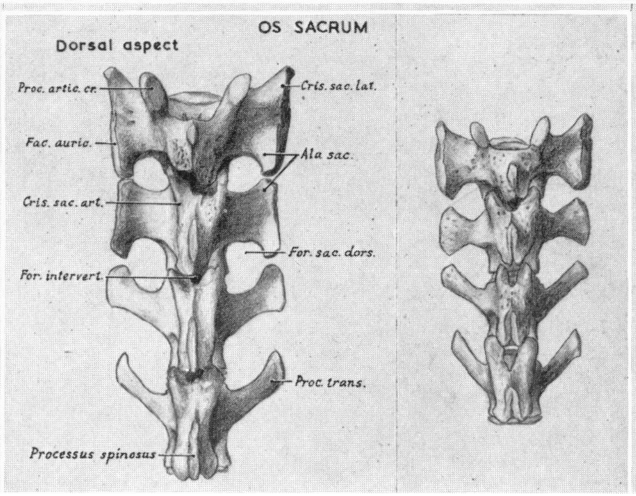
VERTEBRA THORACALIS SEPTIMA



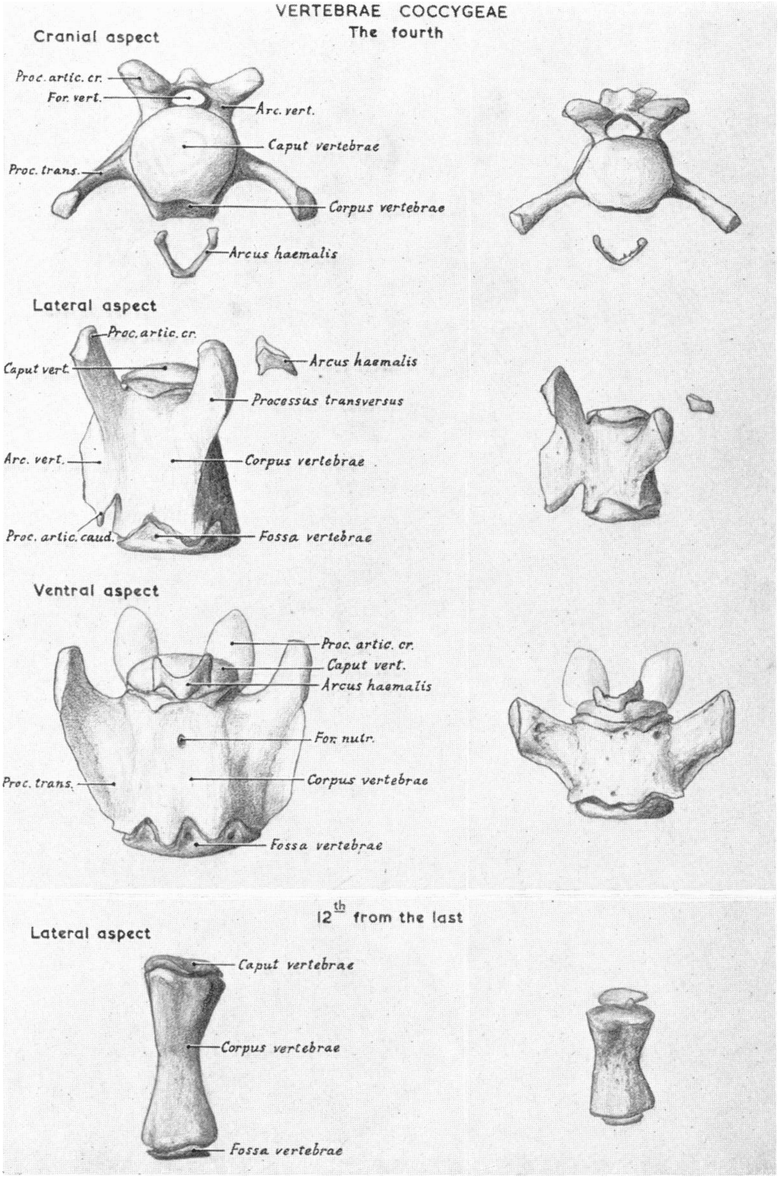
VERTEBRA LUMBALIS —Third from Os sacrum



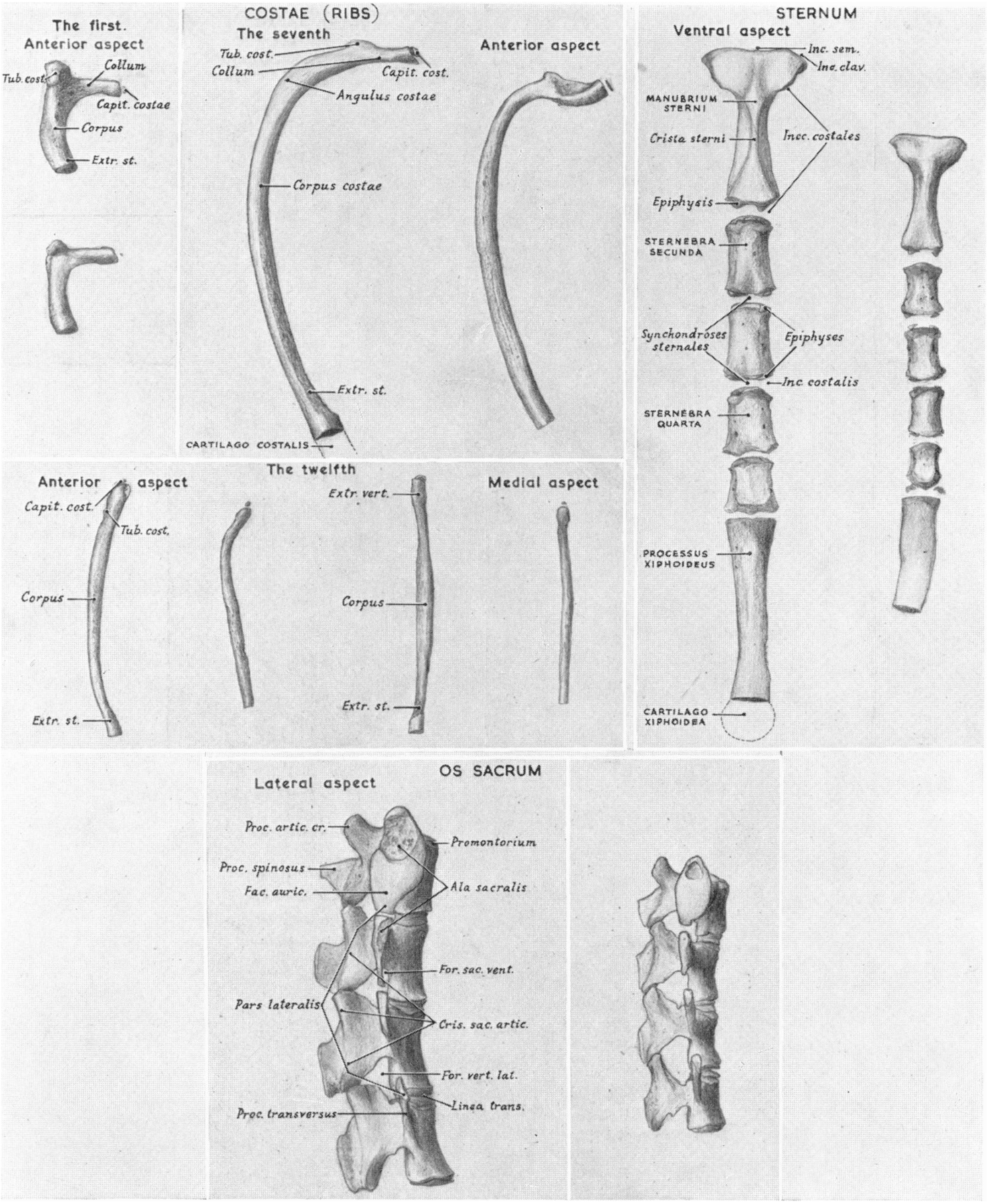
OS SACRUM



BATEMAN—Bone growth: grey-lethal and microphthalmic mutants of mouse



BATEMAN—BONE GROWTH: GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF MOUSE



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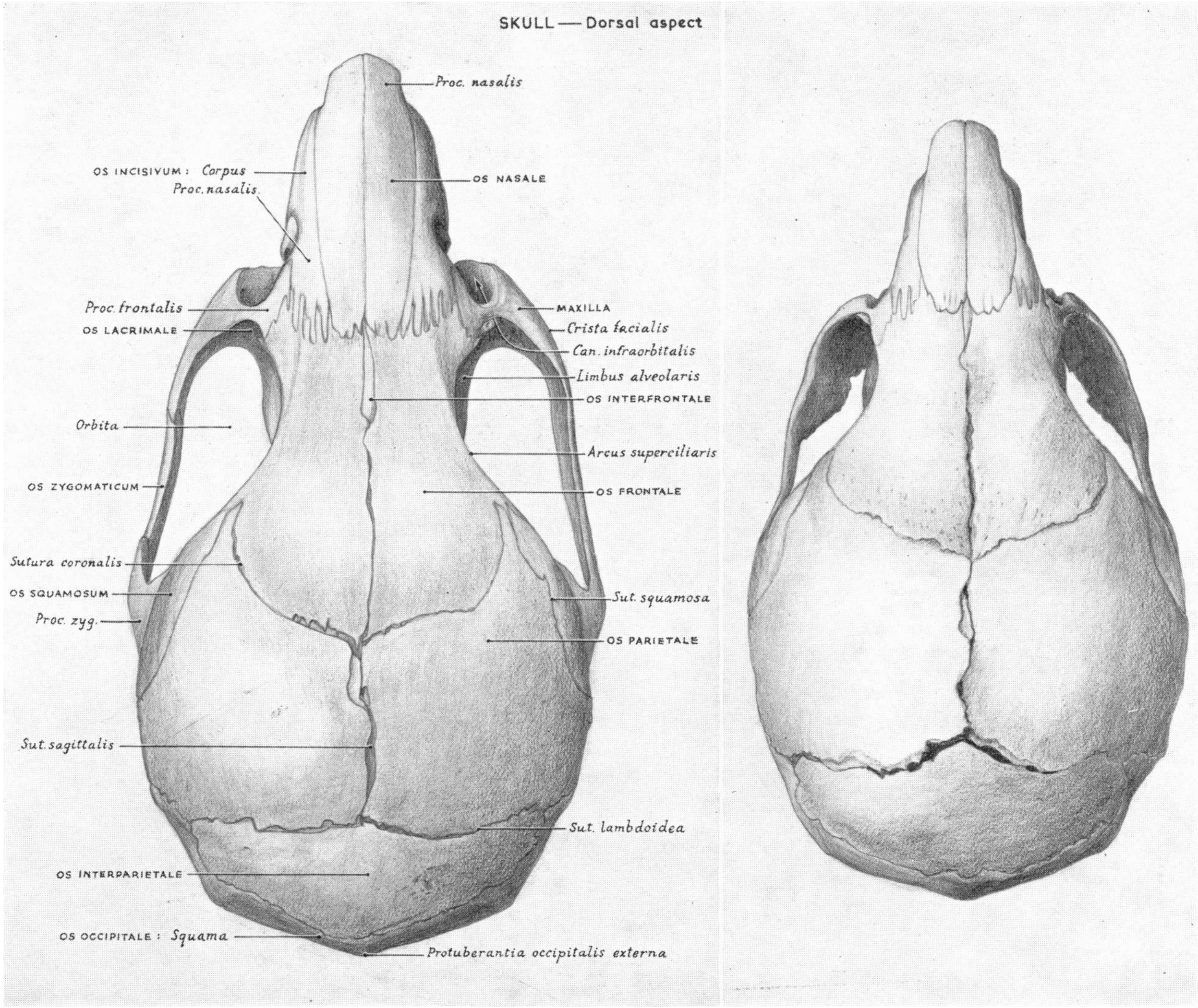

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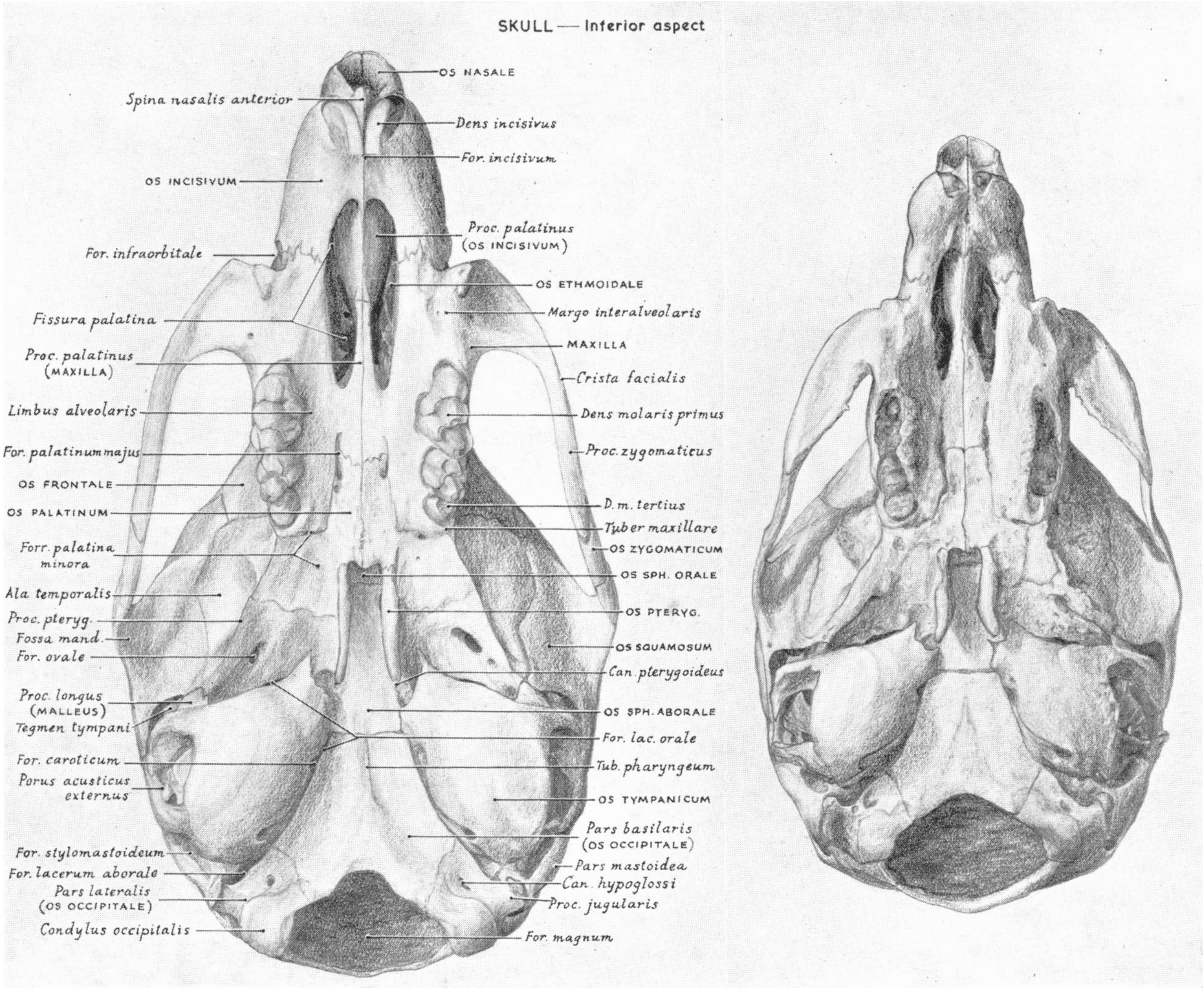
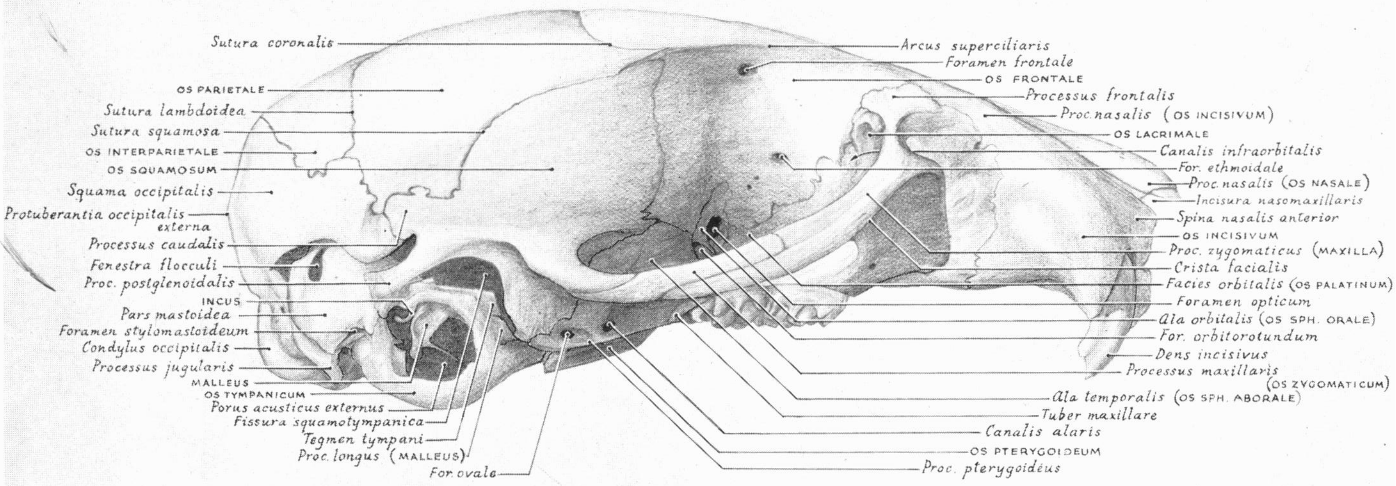
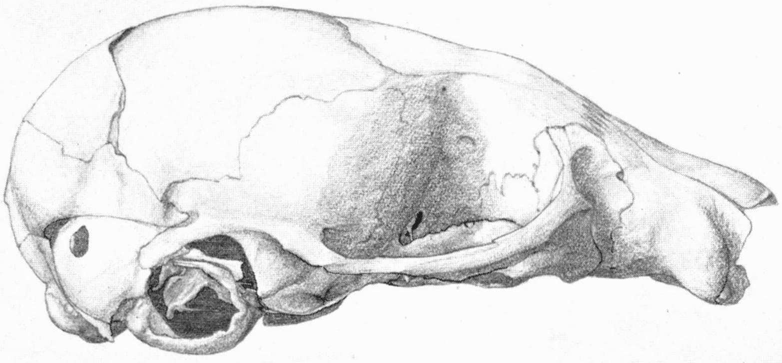


BATEMAN—Bone growth: grey-lethal and microphthalmic mutants of mouse


SKULL — Lateral aspect



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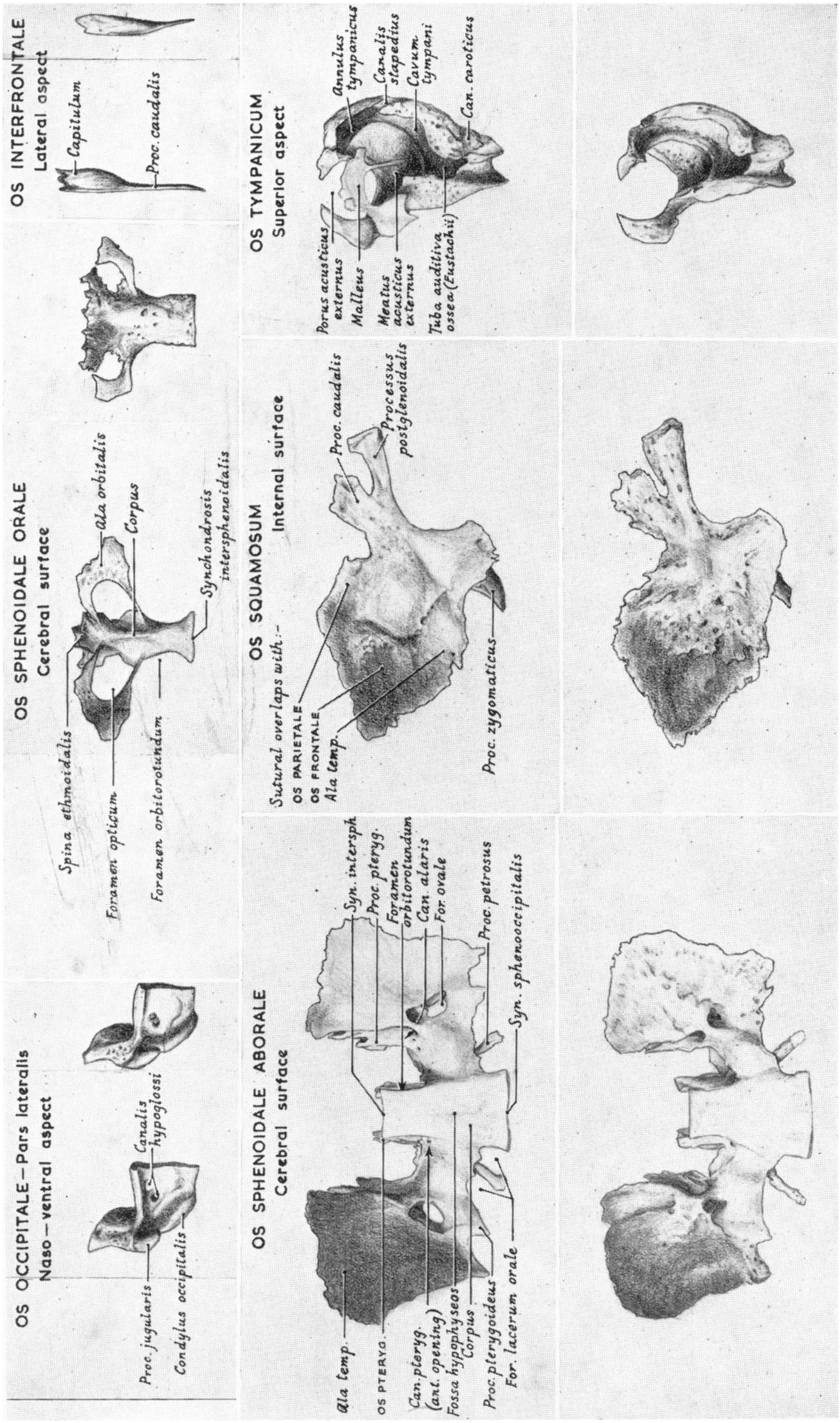
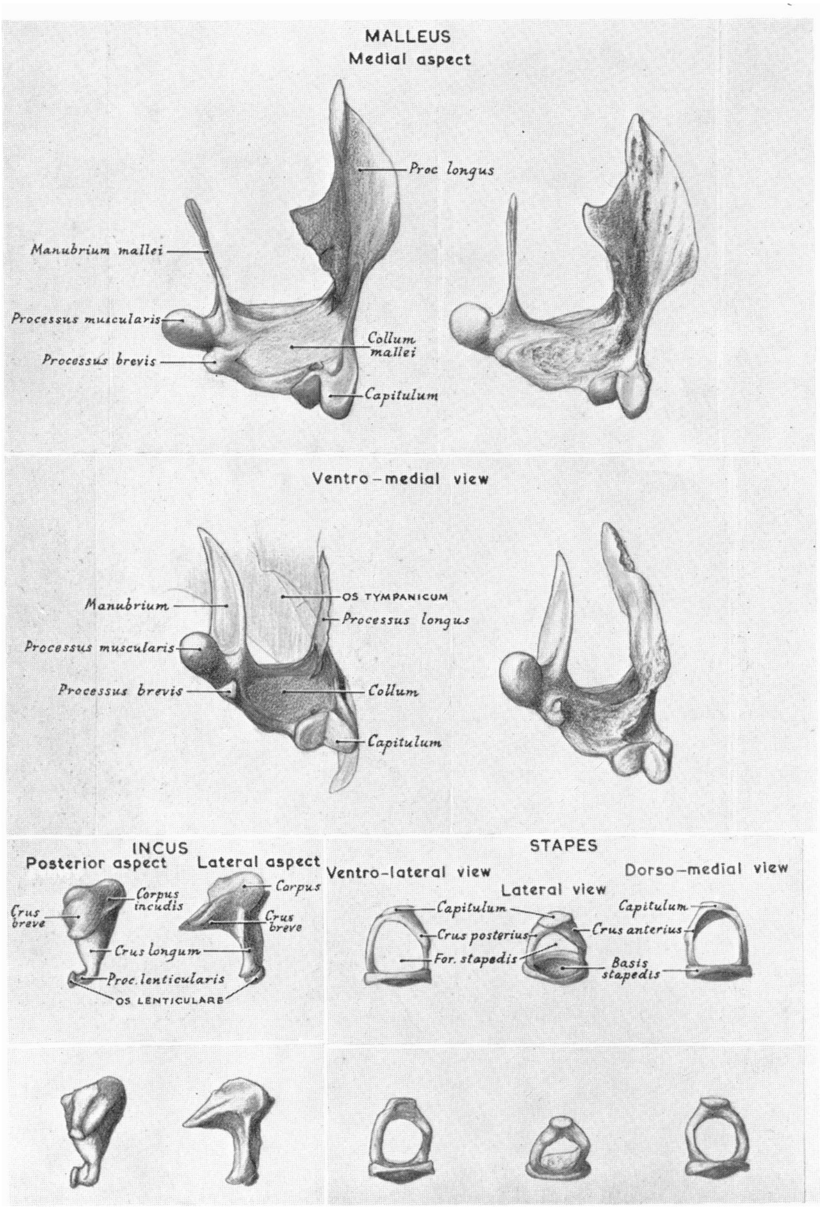
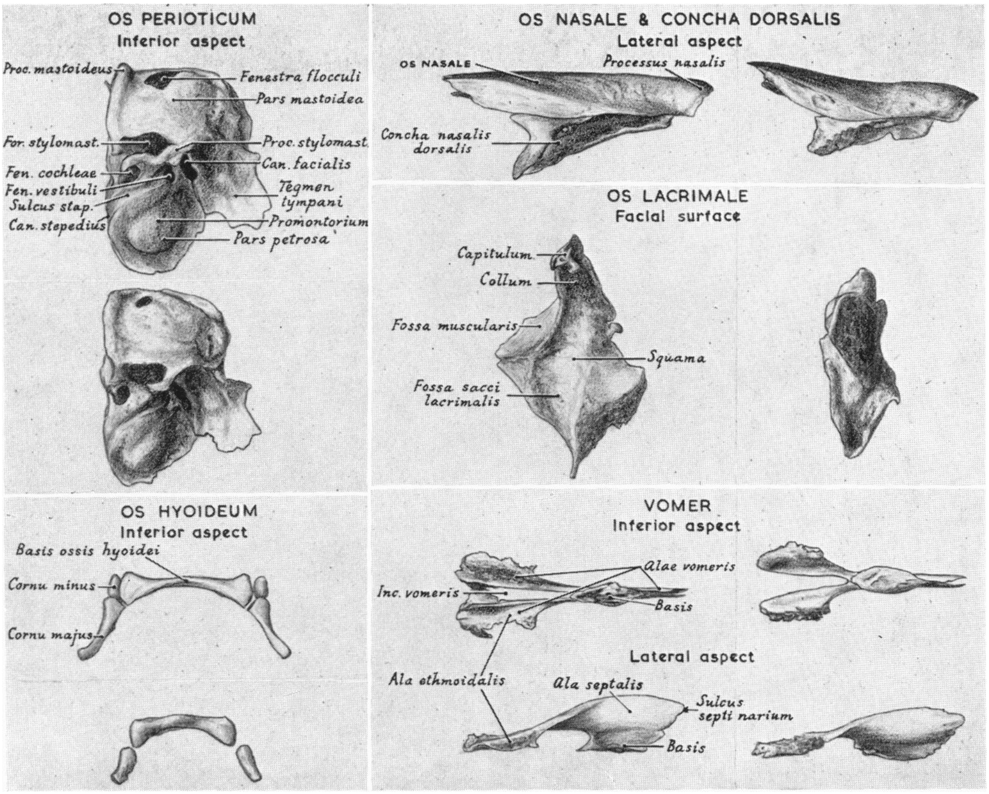


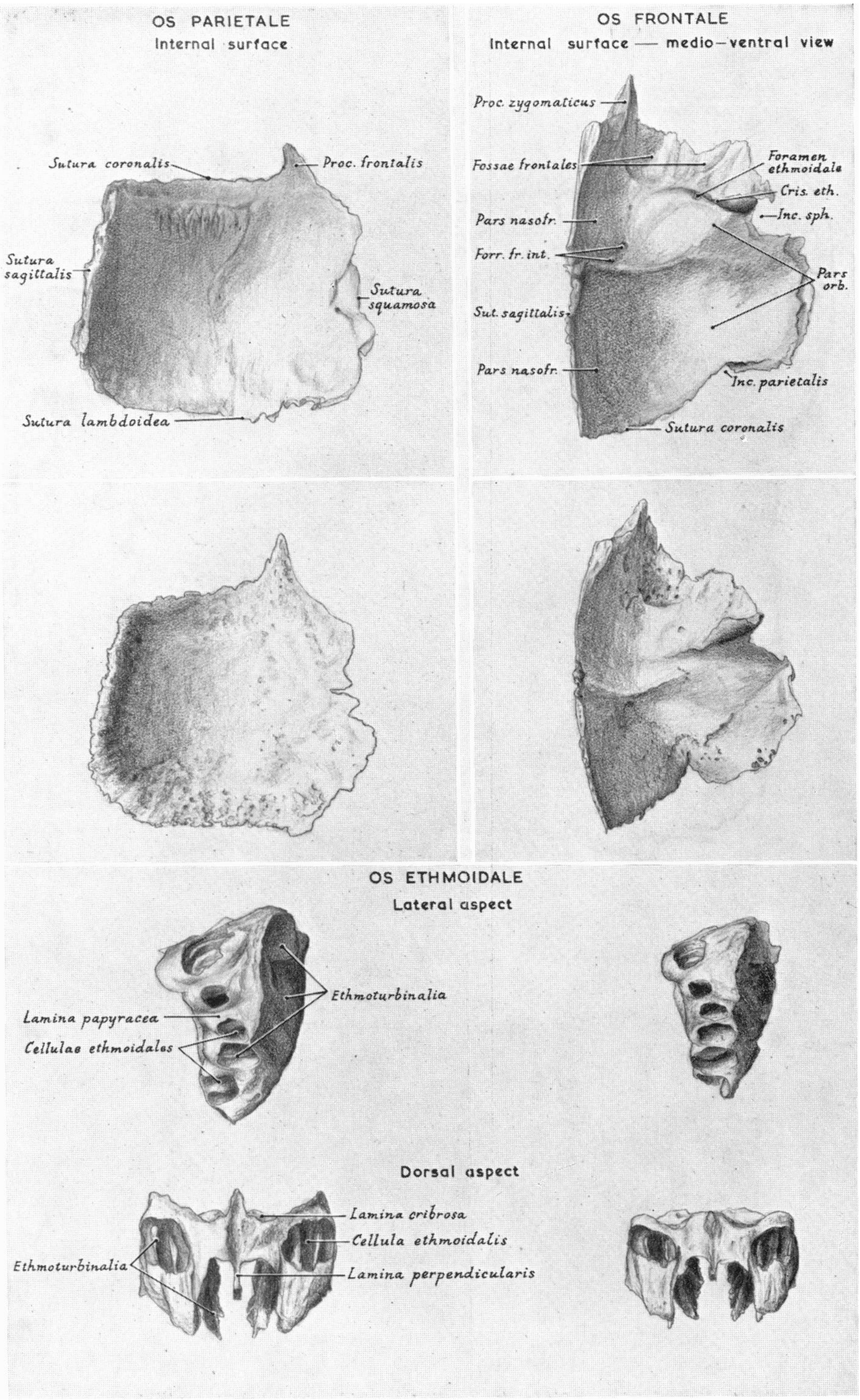
BATEMAN—BONE GROWTH: GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF MOUSE



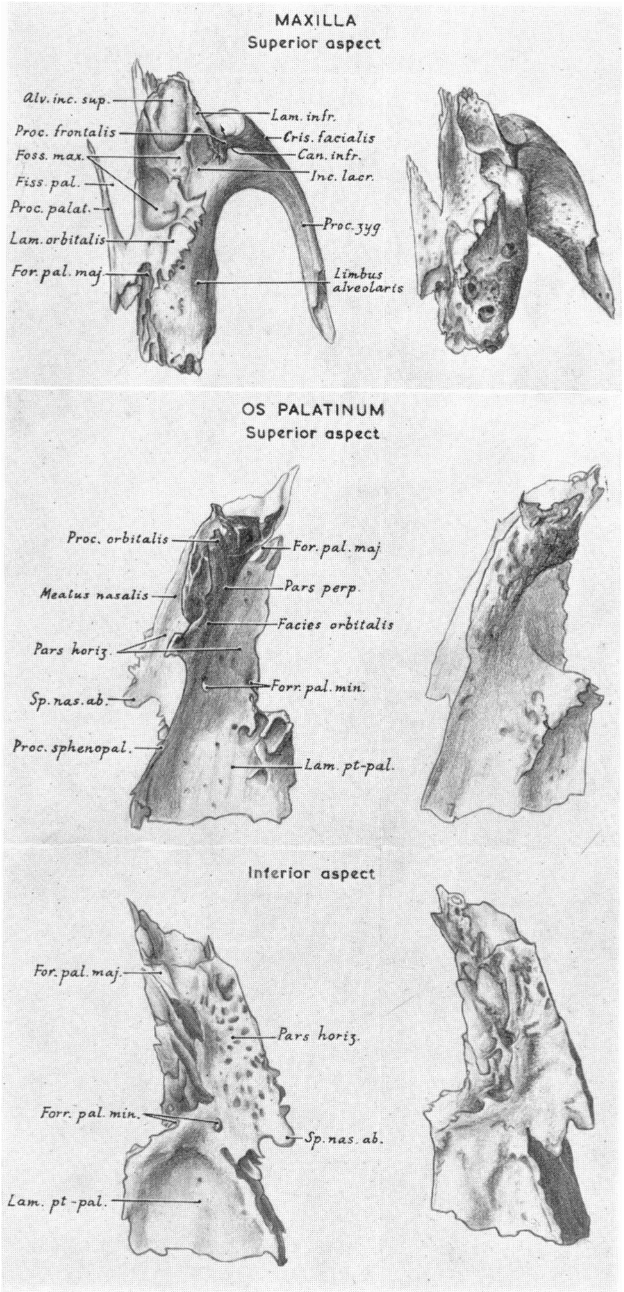
BATEMAN—Bone growth: grey-lethal and microphthalmic mutants of mouse



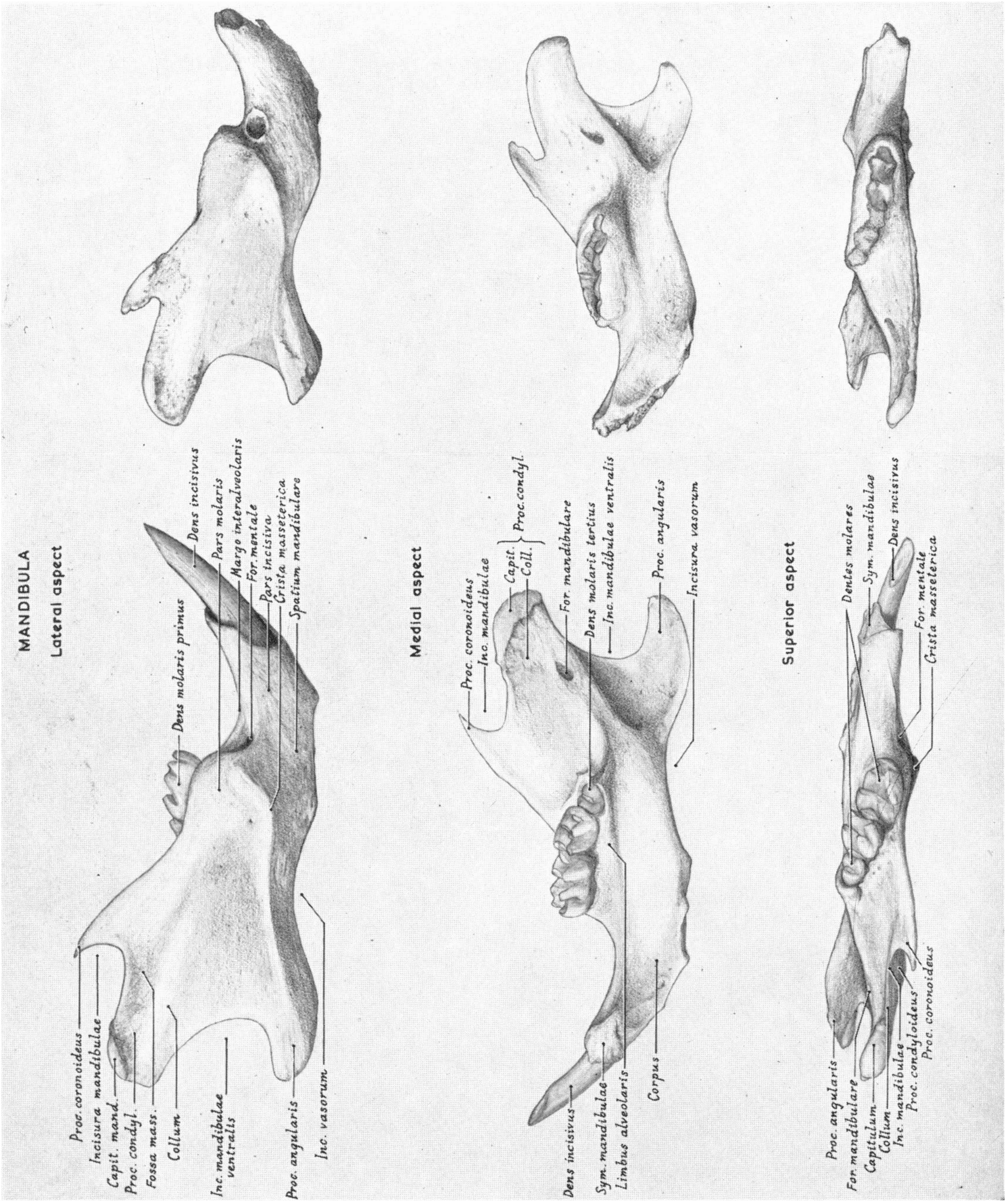
BATEMAN—Bone growth: grey-lethal and microphthalmic mutants of mouse



BATEMAN—Bone growth: grey-lethal and microphthalmic mutants of mouse



BATEMAN—BONE GROWTH: GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF MOUSE



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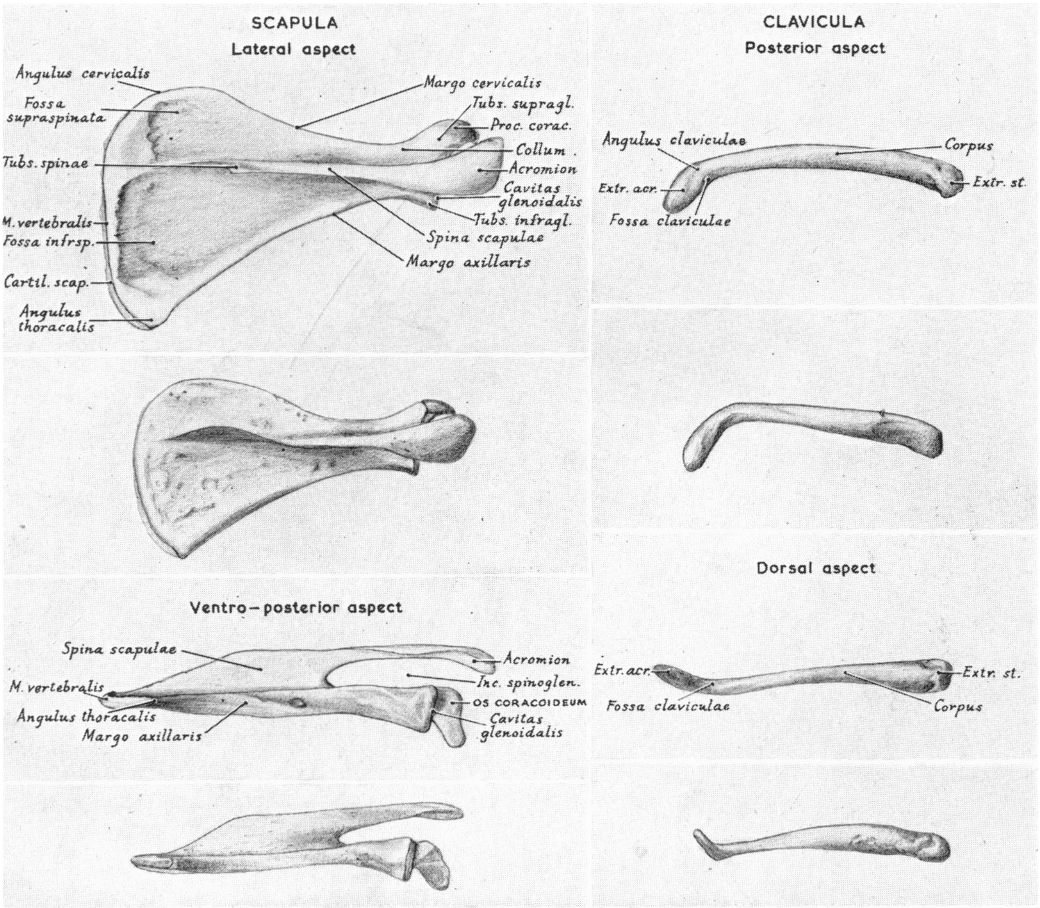
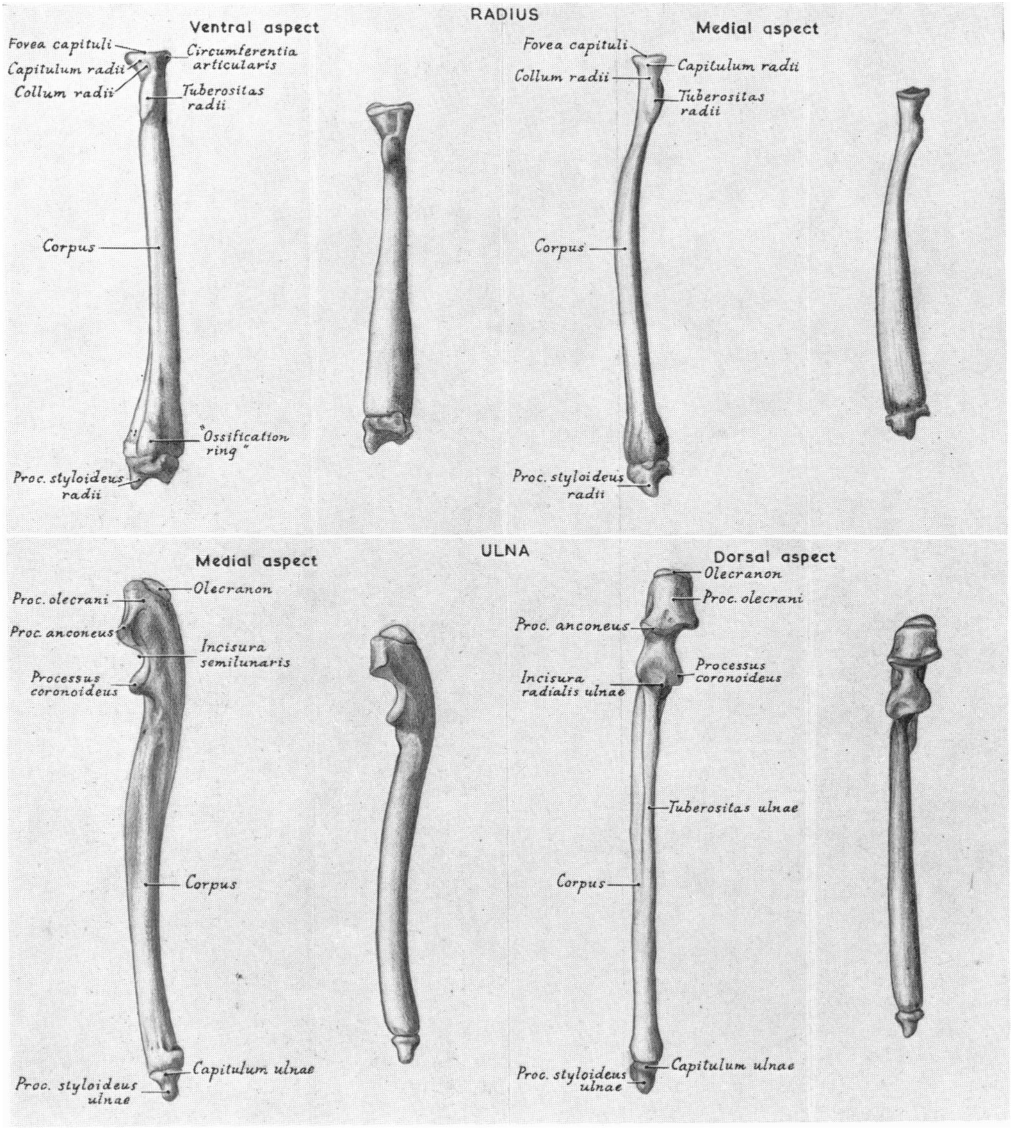


BATEMAN—BONE GROWTH: GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF MOUSE



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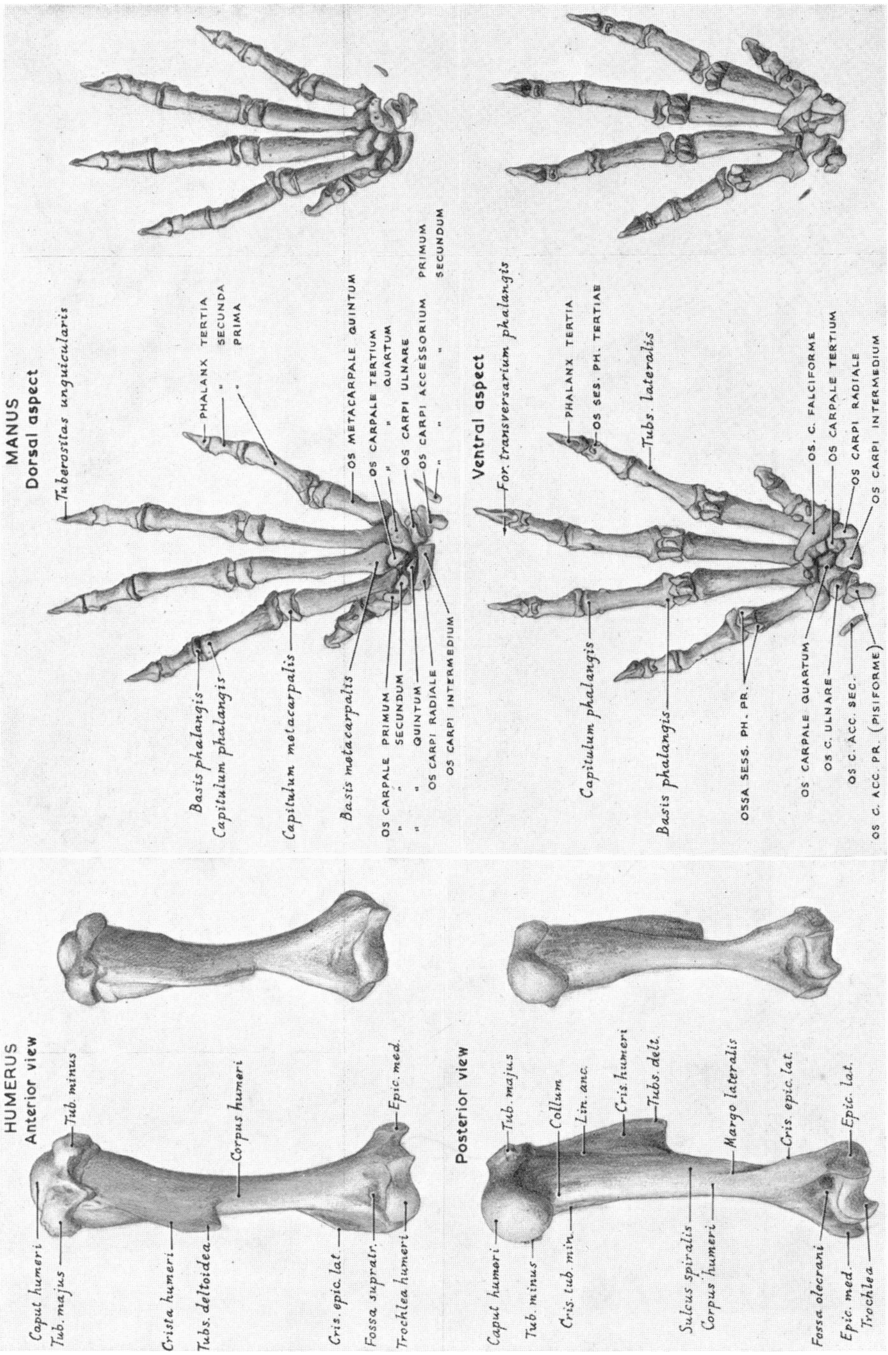

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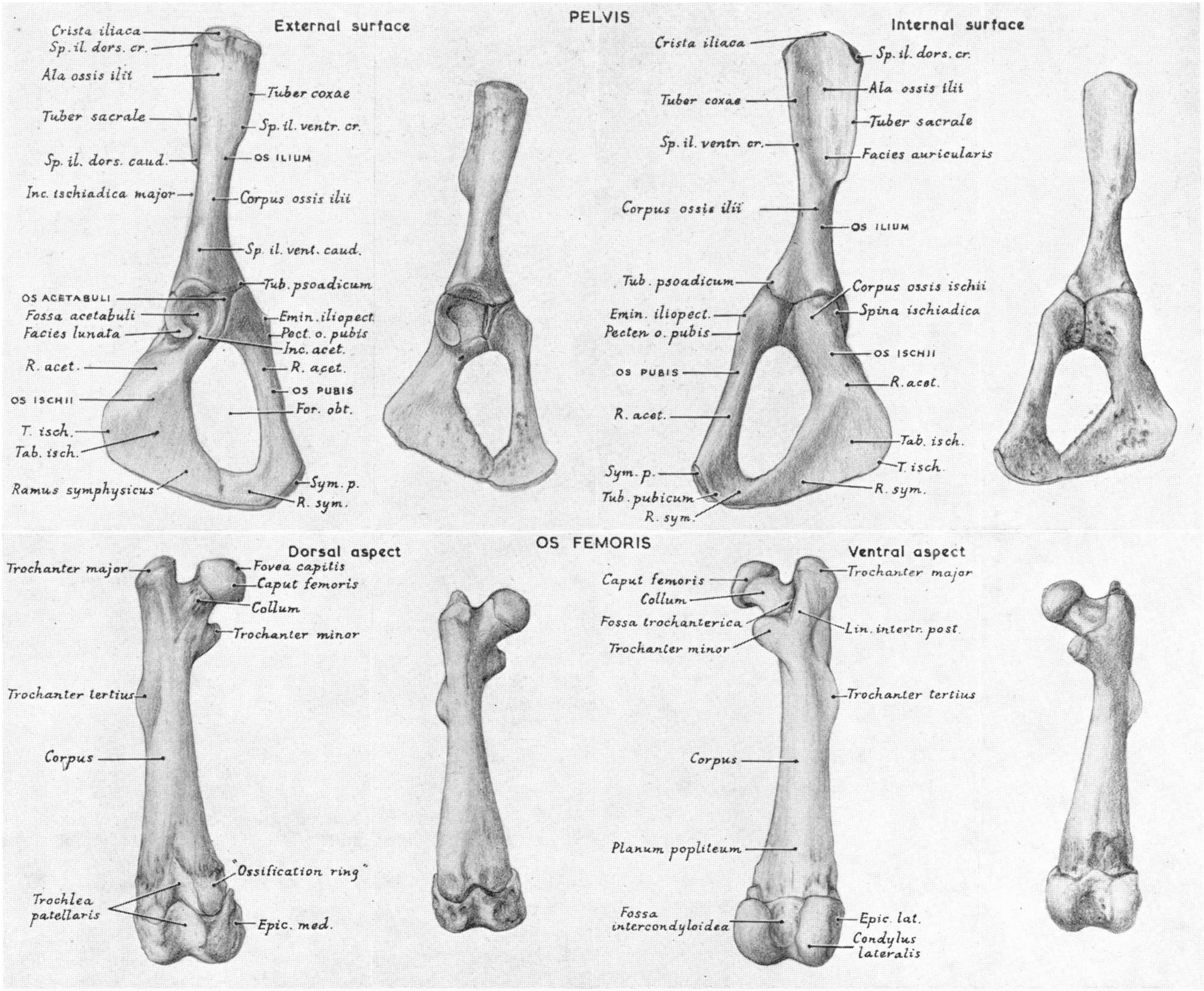

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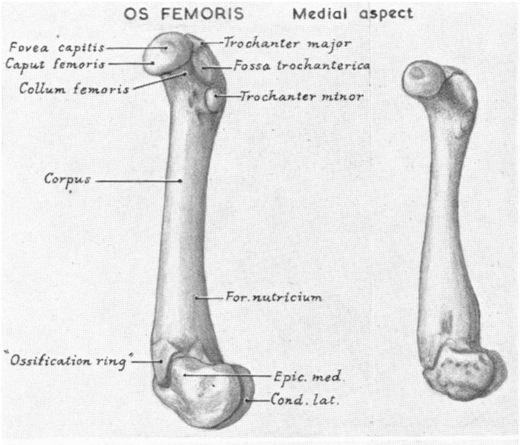

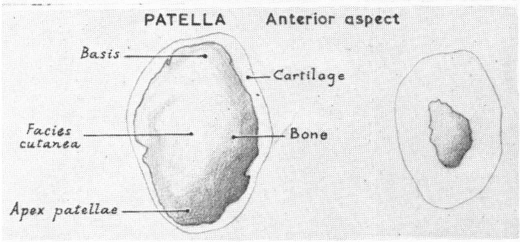

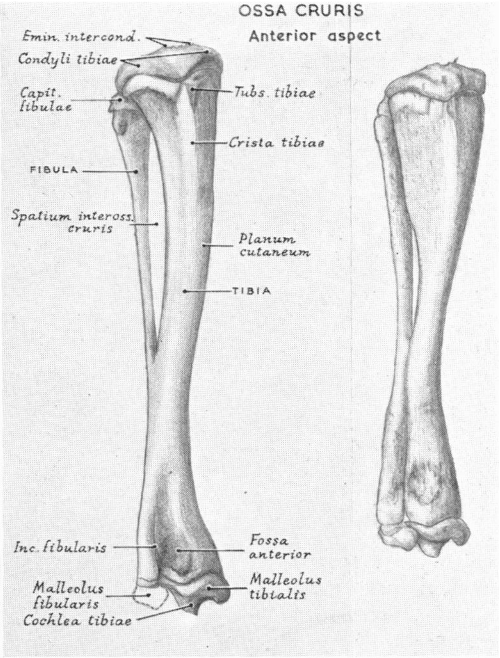

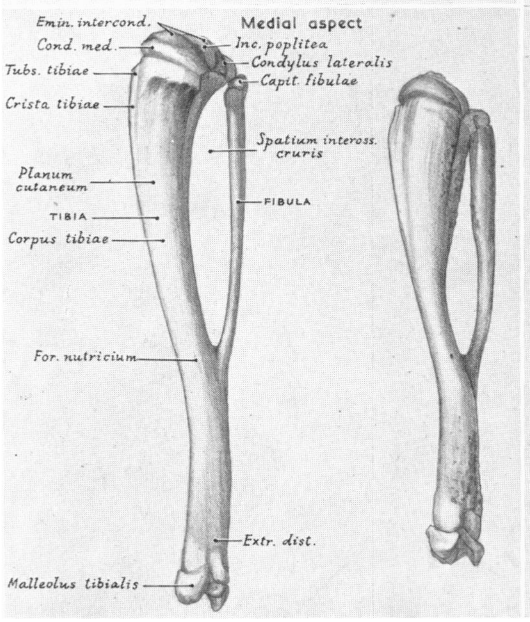

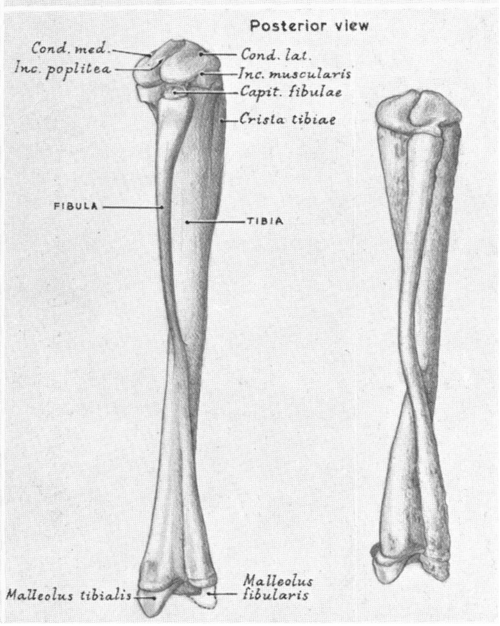

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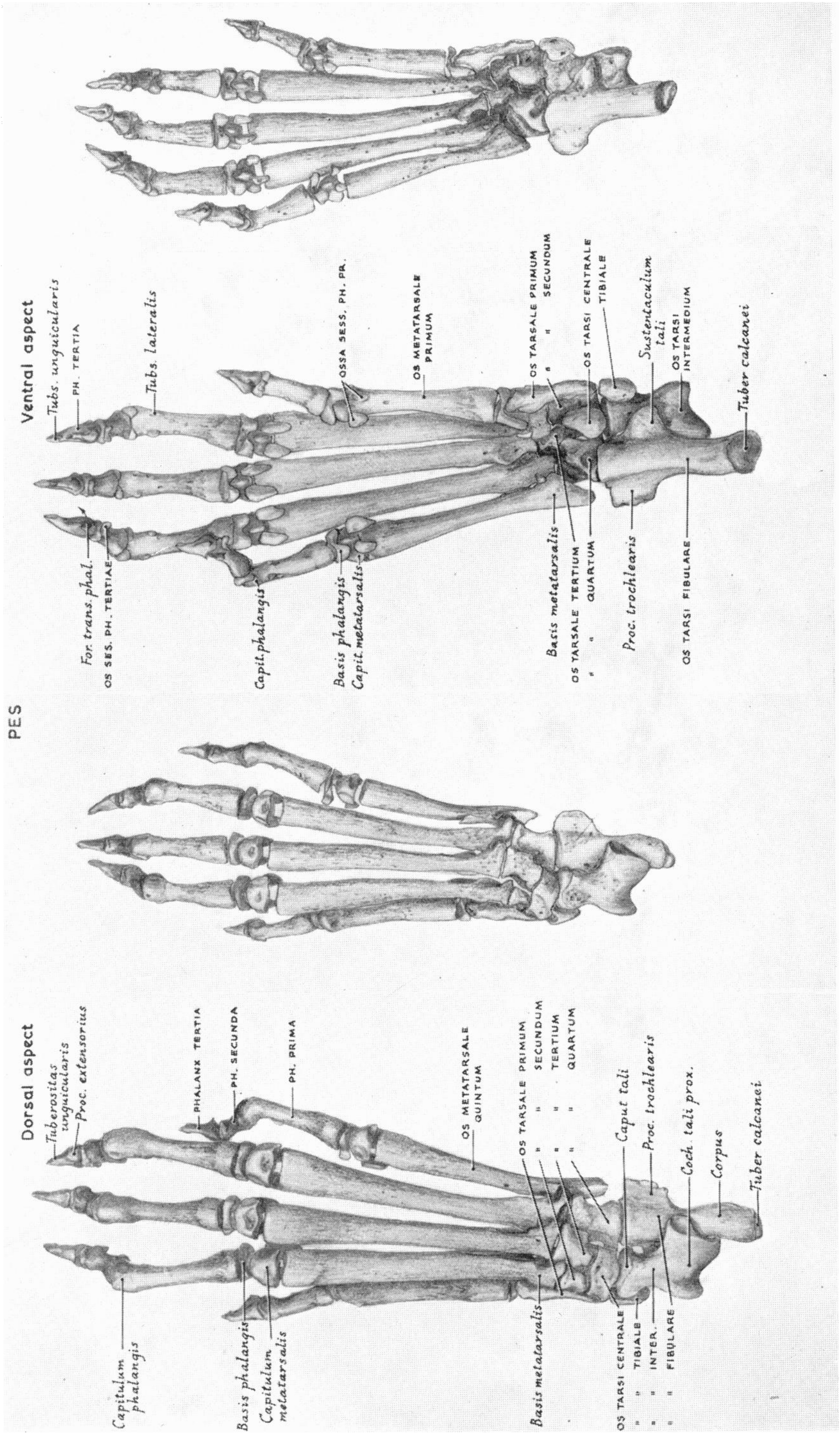

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